MYOCARDIAL PHARMACOKINETICS AND PHARMACODYNAMICS IN THE SHEEP

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TABLE OF CONTENTS - GENERAL

TABLE OF CONTENTS - SECTION I

TABLE OF CONTENTS - SECTION II

TABLE OF CONTENTS - SECTION III

TABLE OF CONTENTS - SECTION IV

ABSTRACT

The myocardium is an important site for the therapeutic and toxic effects of many drugs; however, methods used to date to predict myocardial drug concentrations have been flawed. In this thesis, myocardial pharmacokinetic profiles were defined using mass balance principles and were correlated with simultaneously measured myocardial pharmacodynamic profiles. Left coronary artery blood flow was measured by a Doppler method, the myocardium perfused by this vessel was defined, and frequent arterial and coronary sinus blood samples were taken so that the rapid uptake and prolonged elution of drugs from the myocardium could be quantitated. The calculated myocardial drug concentrations correlated well with the measured haemodynamic effects. The importance of measuring blood flow for mass balance calculations was subsequently confirmed.

Because the "mass balance" method does not directly measure tissue drug concentrations, some of the underlying assumptions necessary for its use were validated. Rates of drug diffusion from the surface of organs and rates of drug transported by lymph were shown to have negligible effects on the mass balance, findings which also have implications for the understanding of mechanisms of drug disposition in poorly perfused tissues and the interstitial space. A rapid blood sampling method was developed because previously available methods were shown to be inadequate for applying mass balance principles. It was also found that the i.v. injection of hypotonic solutions had deleterious effects on myocardial function in sheep. This has significant implications for the formulation of i.v. drugs.

The myocardial pharmacokinetics and pharmacodynamics of i.v. bolus doses of lignocaine and pethidine were investigated in conscious, unrestrained sheep. Subconvulsive doses of these drugs significantly decreased myocardial contractility in a dose dependent manner, with only the highest dose of pethidine inducing apparent central nervous system (CNS) effects, suggesting that cardiovascular system may not in fact be more tolerant to local anaesthetic toxicity than the CNS. The time - courses of the myocardial depressant effects of both drugs were better related to

their concentrations in the myocardium than in arterial or coronary sinus blood. Threshold myocardial drug concentrations for causing a 10% reduction in myocardial contractility were 4.5 ± 2.4 μg/g and 8.2 ± 3.8 μg/g for lignocaine and pethidine, respectively. Net drug uptake lasted only a minute, and equilibrium between myocardial and blood drug concentrations was not attained in these studies, casting doubt on the validity of some widely made assumptions in pharmacokinetic studies. The elution of pethidine from the myocardium was more rapid than that of lignocaine, possibly due to its greater lipophilicity.

Analogous studies in various tissues and regions using drugs with a wide range of physiochemical properties under different pathophysiological conditions will allow better prediction of site - specific drug concentration - effect relationships, and may ultimately provide a scientific basis for the design of drugs and drug dosing regimens.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of my knowledge and belief, it contains no material previously published or written by another person, except where due reference is made in the text.

I consent to the thesis being made available for photocopying and loan if applicable if accepted for the award of the degree.

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Papers

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CHAPTER 1: A LITERATURE REVIEW OF STUDIES OF MYOCARDIAL PHARMACOKINETICS AND PHARMACODYNAMICS.

1.1 Introduction

Several groups of commonly used drugs exert effects on the heart either through receptors on the surfaces of the myocardial cells or by intracellular mechanisms (Goldstein et al., 1974; Hondeghem and Datzung, 1984; Clarkson and Hondeghem, 1985). For example, the myocardial toxicity of antiarrythmic drugs is manifested by conduction disturbances, hypotension or even cardiac standstill (Harris et al., 1952; Epstein, 1953; Lewis 1983). For other drugs such as analgesics, barbiturates, local anaesthetics and some antineoplastic agents, myocardial toxicity is apparent as myocardial depression, hypotension, arrythmia or myocardiopathy (Gonway and Ellis, 1969; Strauer, 1972; Pacciarini et al., 1978; Bowman and Rand, 1980; Jaenke et al., 1980; Legha et al., 1982; Kotelko et al., 1984; Reiz and Nath, 1986).

Such myocardial side effects greatly increase the risks associated with the use of these drugs. Clinical experience and literature reports show that it is possible to devise administration regimens of these drugs whereby the desired therapeutic effects can be achieved while toxic effects on the heart are minimised (Giardina et al., 1973; Lima et al., 1978; Mungall and Docktor, 1983; Zipes, 1985). However, few experimental studies have addressed the scientific basis of these problems and their empirical solutions in an integrated manner. While pharmacokinetic studies aim to describe or predict the time - courses of drug concentrations in blood or some other site, they often do not include the study of the effects of the drugs. Conversely, pharmacodynamic studies have often measured the therapeutic or toxic effects of drugs, but often have correlated these effects to drug doses or the drug concentration at a site removed from the site of action of the drug. A central tenet of this thesis is that integrated pharmacokinetic - pharmacodynamic studies are necessary to properly correlate the time - courses of drug concentrations at their sites of action and their effects at those sites of action.

An important feature of such integrated studies is the need to measure the concentration of the drug at a site as close as possible to, or in equilibrium with, the concentration at the site of drug effect. However, the vast majority of the pharmacokinetic studies reported in the literature involve the study of the time - courses of drug concentration in "systemic" arterial or venous blood (usually peripheral venous blood) and therefore describe the "systemic pharmacokinetics" of the drug. Recently, "regional" pharmacokinetics has been defined (Upton et al., 1988a; Upton, 1990) as the study of the time - courses of drug concentrations in organs or regions of the body. With respect to the heart, it has been proposed that the myocardial concentrations of drugs will to a large degree determine the magnitude of their therapeutic or toxic effects on the heart (Jaenke et al., 1980; Horowitz and Powell, 1986). This thesis examines the relationships between the myocardial pharmacokinetics and pharmacodynamics of drugs in a conscious, unrestrained sheep preparation. It is hoped that further understanding of these relationships may ultimately provide a scientific basis for the design of the administration regimens, molecular configuration and formulation of drugs with effects on the heart.

This chapter is a brief review of studies of myocardial pharmacokinetics and pharmacodynamics, with particular emphasis on those using mass balance principles and the drugs lignocaine, procainamide and pethidine which were chosen for study in the experimental sections of this thesis.

1.2 Methods for studying myocardial pharmacokinetics

Basic pharmacokinetic data can be obtained from either *in vitro* or *in vivo* experiments. In addition, modelling methods can then be used to describe these data, possibly for the purpose of predicting the pharmacokinetics under altered circumstances. These distinctly different methods are discussed in turn.

1.2.1 Experimental methods for studying myocardial pharmacokinetics

The experimental methods for regional pharmacokinetic studies in general (Upton et al., 1990a), and for studies of myocardial drug uptake have been

recently reviewed (Horowitz and Powell, 1986). These can be generally divided into two categories - *in vitro* and *in vivo*.

1.2.1.1 *In vitro* techniques

Cell fragments, cell culture and tissue preparations

Cell fragments from the myocardium (Potter, 1967; Dutta et al., 1968) and cultured myocardial cells (Horowitz et al., 1982) have been used for studies of subcellular or cellular drug uptake from the surrounding medium. Isolated myocardial tissues (either cardiac muscle and/or Purkinje fibres) have also been studied. They were usually superfused in physiological electrolyte solution with pH at 7.4 and a temperature of 37°C, bubbled with 95% oxygen and 5% carbon dioxide (Pruett et al., 1977; Lullmann et al., 1979; 1980; Pang and Sperelakis, 1983). Drugs, usually tritiated, were added to the culture solution and, after set time periods, the tissues were taken out, blotted dry, homogenised and assayed for drug. For these techniques, tissue drug uptake was usually expressed as a cardiac tissue to medium drug concentration ratio.

The use of cultured tissues or cells in the study of drug uptake allows strict control of the factors which influence myocardial drug uptake. For example, adjustment of the pH and electrolytes in the medium makes possible the study of the influences of the physicochemical properties of drugs on tissue uptake. Furthermore, it is possible to achieve drug concentrations in the myocardial tissues which would be impossibly high for *in vivo* studies. There is no redistribution of drug to other organs, drug clearance, or blood protein binding of drug in such studies.

Isolated perfused heart preparations

Myocardial drug uptake has also been studied using isolated perfused hearts from animals (e.g. the Langendorff preparation). The hearts were usually rapidly removed from the experimental animals, cannulated and kept under constant perfusion with Krebs - Henseleit bicarbonate buffer solution. They

were usually electrically stimulated so that they continued to beat (Dutta et al., 1968; Kim et al., 1983; Gillis and Kates, 1986). The drugs under study were added into the perfusate reservoir, and the concentrations of drug in both the efferent (from the coronary sinus) and afferent (from the aorta) perfusate were measured. The amount of myocardial uptake was calculated using mass balance principles (see 1.5) from perfusate flow rate and concentration differences between the afferent and efferent perfusate (Gillis and Kates, 1986).

A number of advantages of this preparation are apparent. Drug uptake into the isolated perfused heart preparation is not confounded by the redistribution that occurs during *in vivo* studies. It is possible to control the coronary perfusion rate, and the preparation is free from the influences of anaesthesia and the nervous system. Again, these factors are advantages only in studies of the basic mechanisms of myocardial drug uptake. The lack of both protein and red blood cells in the perfusion medium will almost certainly influence drug uptake, although these conceivably could be added to the perfusion medium.

Whether the features of the preparations described above are advantageous or disadvantageous depends on the purpose of the study. Further discussion of these issues is presented below (see 1.4.1).

1.2.1.2 *In vivo* techniques in animals

Several methods have been used to determine myocardial drug concentrations in animals.

Post - mortem myocardial biopsy

Myocardial tissue has been collected for drug assay after sacrificing animals to obtain the effective or toxic tissue concentrations (Dutta et al., 1963) under both normal and pathological conditions (Marcus et al., 1964; Doherty

and Perkins, 1966; Benowitz et al., 1974a; Lloyd and Taylor, 1975; 1977; Zito et al., 1980; Zito et al., 1981; Morishima et al., 1984).

The serial sacrifice of a number of animals at different pre - determined times is necessary to characterise the time - course of myocardial drug concentrations. This has been compared with the time - courses of blood drug concentrations (Wenger et al., 1978; Nattel et al., 1979; Wenger et al., 1980).

Serial myocardial biopsy

The serial biopsy of myocardial tissue in the same animal has been possible under anaesthesia in open - chested animal preparations with a high speed boring tool (Keefe and Kates, 1982; Halpern et al., 1984). Similarly, serial endocardial biopsies of the interventricular septum and the free wall of the ventricles (Cho, 1973; Selden and Neill, 1975; Anderson et al., 1980) have been collected and assayed to characterise the time - course of myocardial drug concentrations, and these have been compared with the time - course of serum drug concentrations.

Serial and post - mortem biopsy methods have similar advantages, but the post - mortem method is hampered by the need to use large numbers of animals to characterise the time - course of myocardial drug concentrations. However, it does not require anaesthesia. The principle advantage of both methods is that different areas of the myocardium can be sampled, thereby allowing the determination of the drug concentrations in different parts of the heart, such as the atria and ventricles, and in ischaemic and normal myocardium. Most importantly, both can be used to characterise the *in vivo* time - course of tissue drug concentrations.

Arterial and coronary sinus blood sampling

The short term uptake and subsequent elution of drugs in the myocardium have been determined using mass balance principles. The basis of this

method will be discussed subsequently (see 1.5). In all cases, the concentration differences between paired arterial and coronary sinus blood samples were used to calculate some parameters which reflected the movement of drug into and out of the myocardium (Selden and Neill, 1975).

1.2.1.3 Techniques in man

There have been few reports of studies involving measurements of the time - courses of myocardial drug concentrations in man, particularly with corresponding measurements of the time - courses of therapeutic or toxic drug effects. Myocardial tissue biopsies have been taken from patients during cardiac surgery, either before or after cardiopulmonary bypass. The biopsies were assayed to determine the myocardial drug concentrations for comparison with simultaneous serum drug concentrations (Coltart et al., 1972; Carroll et al., 1973; Gullner et al., 1974; Debbas et al., 1983; 1984; Padrini, 1985; Escoubet et al., 1986). These studies usually have been opportunistic, and have had constraints on the number and size of tissue samples that could be taken, and on the dose regimens of the drugs administered to the patient.

The calculation of myocardial drug uptake from paired coronary sinus and arterial blood samples is a promising method for investigations in humans (Marks et al., 1964; Selden and Neill, 1975; Hayward et al., 1983; Horowitz et al., 1986). It has been used in patients undergoing cardiac catheterisation procedures for diagnostic purposes (Selden and Neill, 1975; Horowitz et al., 1986). Unlike many other studies, in these studies the coronary blood flow rates were also measured so that myocardial drug uptake and elution could be determined (see 1.5).

In some instances, post - mortem sampling of tissues to study digoxin distribution has been reported (Doherty al., 1967; Maggioni et al., 1983; Brien et al., 1987). This method has limited use because it is relatively opportunistic and uncontrolled.

1.2.2 Modelling methods for studying myocardial pharmacokinetics

In view of the influence that pharmacokinetic models have had on the design and interpretation of studies of the myocardial kinetics of drugs, it is pertinent to briefly discuss their application to this field. Pharmacokinetic models can describe, and more importantly, predict the time - courses of drug concentrations in the body. The ability to predict drug concentrations has the potential to aid the clinical use of drugs by allowing clinicians to design different dose regimens to achieve the desired drug concentrations in particular tissues or organs in the body (Wagner, 1975; Lima et al., 1978). Pharmacokinetic models can be generally grouped into two types, systemic or regional. Each has quite different applications. These are discussed below.

1.2.2.1 Systemic pharmacokinetic models

Systemic pharmacokinetic models can be compartmental or physiologically based. In compartmental pharmacokinetic models, the whole body is represented as one (e.g. Boyes et al., 1970), two (e.g. Boyes et al., 1971; Galiazzi et al., 1976; Lima et al., 1979) or three (e.g. Dutcher et al., 1977; Kates and Jaillion, 1980) compartments. Commonly, the body is modelled as two compartments representing central and peripheral tissues. In the central compartment, tissues and organs are assumed to be well - perfused, and drug concentrations in the tissue and blood reach equilibrium with blood rapidly after drug administration (Goth, 1984a). Drug distribution and redistribution between the compartments are described by a few rate constant and volume of distribution terms. The myocardium and other very well - perfused organs such as the brain are usually presumed to be located in the central compartment.

Physiologically based pharmacokinetic models differ in that each important tissue or organ in the model is represented by regional physiological models, and these are interconnected by the circulatory system (Himmelstein and Lutz, 1979; Gerlowski and Jain, 1983). These regional models will be discussed subsequently (see 1.2.2.2). However, in the application of physiologically based

models to systemic pharmacokinetics, several observations are pertinent. Firstly, the regional models are simplified by the use of a number of assumptions. Secondly, the parameters for the regional models are often derived from the literature. Thirdly, physiological models are often used to study factors influencing the systemic blood concentrations of drugs. It should be apparent that the success of these systemic physiological models is highly dependent on the appropriateness of the regional models of which they are composed. These are discussed below.

1.2.2.2 Regional pharmacokinetic models

The aim of regional pharmacokinetic modelling (Upton, 1990; Upton et al., 1991a) is to represent the kinetics of a drug in a specific region of the body (a region has been defined as the organ(s) or tissue(s) between specified afferent and efferent blood vessels). Studying a particular region has the advantage of reducing the number of factors influencing the measured drug concentrations, but more importantly is closer to the study of the concentration of drugs at their site of action. There are a number of regional pharmacokinetic modelling methods which have been, or have the potential to be, applied to the uptake and elution of drugs by the myocardium. These have been discussed in detail (Upton et al., 1991a), and will be introduced briefly under the following headings:

"Black box" methods

Black box methods assume that no information is available about the region other than the time - courses of the arterial and venous blood drug concentrations. They are generally based on statistical moment analysis, and the processes of convolution and deconvolution. It is now becoming apparent that these methods could be useful for empirically predicting the "response" of regions which behave in a linear manner to different dose regimens or time - courses of arterial drug concentrations.

The use of mass balance principles in regional pharmacokinetic studies is also a "black box" method, but will be discussed subsequently (see 1.5).

Single compartment, flow - limited models

These are by far the most common regional pharmacokinetic models, and they are the basis of many of the systemic physiological pharmacokinetic models discussed in the literature (Himmelstein and Lutz, 1979; Gerlowski and Jain, 1983). A region is represented as a single compartment, and it is assumed that drugs mix instantaneously with the tissues of the region. Equations to describe this are therefore based on two assumptions: Firstly, that the rates of increase and decrease of tissue drug concentrations are limited by regional blood flow. Secondly, that the tissue drug concentrations are proportional to the regional venous blood drug concentrations. The validity of these assumptions will be discussed below (1.4).

Multi - compartment models

In these models, the region is usually divided into up to three subcompartments: vascular, interstitial and intracellular. Differential mass balance equations are written for each compartment represented by inflow, outflow, accumulation and disappearance of drugs (Gerlowski and Jain, 1983; Himmelstein and Lutz, 1979). These models have a large number of physiological parameters. In this way the factors which influence drug disposition, such as anatomical (organ volumes and tissue sizes), physiological (blood flow rates and enzyme reactions) and thermodynamic (drug protein binding and transport) parameters which were often used for modelling (Bischoff and Dedrick, 1968; Chen and Andrade 1976; Igari et al., 1982) can be included as parameters of the model.

These multi - compartment regional pharmacokinetic models have yet to be widely used, perhaps because of the difficulty in experimentally determining the values of the parameters of the model.

1.3 Methods for simultaneously studying myocardial pharmacokinetics and pharmacodynamics

Like pharmacokinetic studies, pharmacodynamic studies can be performed in both *in vitro* and *in vivo* experimental preparations. For *in vitro* experiments, drug effects can be correlated with drug concentrations in either the tissue being studied or the culture medium (which is assumed to be in equilibrium with the tissue concentration). For *in vivo* experiments, drug effects can be correlated with tissue or blood drug concentrations.

1.3.1 Experimental methods

The *in vitro* and *in vivo* methods for studies of myocardial pharmacodynamics are discussed separately as follows.

1.3.1.1 *In vitro* studies

Myocardial cells from guinea pig atrium (Inomata et al., 1989) and chicken embryo heart (Howowitz et al., 1982), and Purkinje fibres (Pruett et al., 1977) from the dog have been used to study the relationships between drug concentrations in the culture medium or the tissue itself and drug effects such as electrolyte channel blockade and antiarrhythmic actions. Isolated perfused hearts of rabbits have been used to study myocardial drug uptake (calculated by mass balance principles, see 1.5) and drug effects such as those on the electrocardiograph, myocardial contraction velocity and amplitude, oxygen consumption and coronary blood flow rate (Gillis and Kates, 1986; Nielsen - Kudsk et al., 1988; Nielsen - Kudsk et al., 1990). The fact that the cultured tissues or cells are free from both autonomic and somatic nerve supply and the influences of endogenous biochemical substances, such as catecholamines (Kim et al., 1983), excludes the influence of such exogenous variables on the correlation between drug uptake and effect.

1.3.1.2 *In vivo* studies

Myocardial biopsy (Keefe and Kates, 1982; Vogt et al., 1988) and mass balance principles (by measuring arterial and coronary sinus blood concentration

differences, Hayward et al., 1983) have been used for the measurement or calculation of myocardial drug concentrations which were then correlated to drug effects such as conduction rates, prolongation of ventricular effective refractory period (in infarcted myocardium) and myocardial contractility.

1.3.2 Pharmacodynamic models

These models describe the relationships between drug concentration and drug effect without considering the time - courses of either drug concentration or effect. Such models are therefore pertinent for studies of steady state drug concentration - effect relationships, such as those that occur after the long term administration of drugs, or in most *in vitro* experiments. The various pharmacodynamic models for describing such systems have been reviewed (Holford and Sheiner, 1981; Schwinghammer and Kroboth, 1988). Those models relevant to myocardial studies are briefly described below.

1.3.2.1 Linear and log linear pharmacodynamic models

For linear models, the relationship between drug concentration and effect is represented by a straight line, and the parameters of the model can be obtained by the process of simple linear regression (Holford and Sheiner, 1981; Schwinghammer and Kroboth, 1988). This model can be expressed as:

$$E = S \times C + E_0 \qquad \ldots(1.1)$$

where E is drug effect, S the slope of the linear relationship, C the drug concentration and E_0 the magnitude of the effect when no drug is present. An advantage of this model is that it predicts there will be no effect in the absence of drug. However, this model predicts that there is no maximum drug effect, which is physiologically impossible for many biological systems.

The log linear pharmacodynamic model differs from the linear model only in that drug concentrations are expressed as the logarithm of the drug concentration (log C). For the log linear model, the relationship between

concentration and effect between 20% and 80% of the maximum value is approximately linear. However, intrinsic to the mathematics of this model is that it can not describe the drug effect when the drug concentration is zero.

The myocardial pharmacokinetics and pharmacodynamics of propafenone and verapamil have, respectively, been described by linear and log linear pharmacodynamic models (Keefe and Kates, 1982; Gillis and Kates, 1986).

1.3.2.2 E_{max} models

These models can describe drug concentration - effect relationships over a wide range of drug concentrations up to the maximum effect for most drugs. They take the following mathematical form:

$$E = \frac{E_{max} \times C}{EC_{50} + C} \qquad \ldots(1.2)$$

E_{max} is the maximum drug effect and EC_{50} is the drug concentration which produces 50% of the maximum drug effect. Modifications of this model can describe the inhibitory effects of drugs (Holford and Sheiner, 1981; Schwinghammer and Kroboth, 1988). For the effective use of this model, the maximum effect of a drug needs to be determined, although this is sometimes impossible for *in vivo* studies.

The relationship between systolic time intervals and plasma concentrations of timolol have been described by the E_{max} model (Singh et al., 1980). Relationships between blood timolol concentrations and the percentage of heart rate change were also described by this model (Bobik et al., 1979).

1.3.2.3 Sigmoid E_{max} models

Sigmoid E_{max} models describe "S" shaped concentration - effect relationships and take the following mathematical form:

$$E = \frac{E_{max} \times C^N}{EC_{50}{}^N + C^N} \qquad (1.3)$$

N is an arbitrarily determined constant which affects the slope of the curve. The physiological meaning of N in most experiments has not been determined.

The *in vitro* myocardial pharmacodynamics of bepridil and the dopamine re - uptake inhibitor, GBR 12909 (Nielsen - Kudsk et al., 1988 and 1990) have been described by the sigmoid E_{max} model.

The selection of a steady state pharmacodynamic model is generally influenced by the experimental data, and the nature and requirements of the experiments (Schwinghammer and Kroboth, 1988).

1.3.3 Pharmacokinetic - pharmacodynamic models

These models are commonly used to describe experimental data with changing time - courses of drug concentrations and drug effects, such as those which occur after the short term use of drugs. In such circumstances, there is often a time difference between the drug concentration and drug effect which can be detected by plotting the drug concentration against the drug effect at each time point. Any time difference between concentration and effect will be apparent as hysteresis on these plots (see Figs. 1.1 and 1.2) such that the magnitude of drug effect when the drug concentration is increasing is different to that when the drug concentration is decreasing. If the drug effect lags behind the drug concentration, an anti-clockwise time order of the hysteresis loop is observed. If drug concentration lags behind drug effect, a clockwise hysteresis loop is observed.

The reasons for hysteresis in concentration - effect plots are dependent on the nature of the experimental system. Generally, the time lag is attributed to the lack of equilibrium between the drug concentration which is measured at a site distant to the site of drug effect. For example, in studies of the relationships between the pharmacokinetics and pharmacodynamics of cardioactive drugs such

as antiarrhythmics (Binnion et al., 1969; Anderson et al., 1980; Eichelbaum et al., 1980; Hashimoto et al., 1984) and anaesthetics (Coetzee et al., 1989), it has often been found that effects on the myocardium lagged behind the blood drug concentrations. Thus, when blood drug concentrations were plotted against drug effects, the time sequence of these points showed anti-clockwise hysteresis.

Various models have been used to compensate for this lack of equilibrium between the measured drug concentrations and drug effects (Hull et al., 1978; Holford and Sheiner, 1981; Christensen et al., 1982; Stanski and Maitre, 1990).

1.3.3.1 Compartmental pharmacokinetic - pharmacodynamic models

Drug effects can be correlated with drug concentrations in any compartment of a compartmental pharmacokinetic model (see 1.2) to remove the time differences between the drug concentrations (usually arterial or venous blood drug concentrations) and effects. For example, the QT interval prolongation induced by intravenous use of procainamide was found to lag behind the time - course of drug concentrations in the central compartment of a compartmental model, but correlate well with a compartment which was represented by the saliva (Galeazzi et al., 1976). Similarly, when such a model was used to study digoxin blood concentrations and effects on the heart, a good relationship was found between the calculated drug concentrations in a slowly distributing peripheral compartment and the effects of digoxin (Kramer et al., 1979).

1.3.3.2 Effect compartment pharmacokinetic - pharmacodynamic models

A typical effect compartment pharmacokinetic - pharmacodynamic model is the parametric pharmacokinetic - pharmacodynamic model (Sheiner et al., 1979), which describes drug concentration time - course by one of the compartmental pharmacokinetic models (see 1.2.2.1) and concentration - effect relationship by one of the pharmacodynamic models (see 1.3.2) The kinetics and dynamics are linked by a "first order" drug elimination constant (K_{eo}) of a hypothetical effect compartment which receives drug input (negligible mass) from the plasma compartment, and K_{eo} is adjusted until the hysteresis between effect

compartment drug concentrations and drug effects disappears. It was used to study the correlation between the force of thumb adduction and venous blood concentrations of d - tubocurarine (Sheiner et al., 1979). Unlike the procainamide study (Galeazzi et al., 1976), the effect compartment dose not necessary have anatomical definition.

1.3.3.3 Semiparametric and nonparametric pharmacokinetic - pharmacodynamic models

In the semiparametric model, the pharmacokinetics is described by the pharmacokinetic compartmental model, with a hypothetical effect compartment linked to the plasma compartment by a first order rate constant which is adjusted so that concentrations in the effect compartment and effect shows no hysteresis, without using a pharmacodynamic model (Hull, et al., 1978). The nonparametric models only assume that effect compartment drug concentrations lag behind plasma concentration by a rate constant K_{eo} (Fuseau and Sheiner, 1984; Unadkat et al., 1986), without using either the pharmacokinetic nor the pharcodynamic model. These models have been used for studies of the CNS effects of thiopentone (Unadkat et al., 1986; Stanski and Maitre, 1990)

1.4 Some limitations of existing methods

A discussion of the limitations of existing methods used in studies of myocardial pharmacokinetics and pharmacodynamics is helpful both to understand and interpret previous experiments, and to design future experiments.

1.4.1 Differences between in vitro and in vivo data

Although *in vitro* studies of drug uptake have provided a great deal of insight into the factors influencing tissue drug uptake (such as drug lipophilicity, pH, energy requirements and electrolyte concentrations), *in vitro* pharmacokinetic data often can not be directly related to *in vivo* conditions, or used directly as a guide to the clinical use of drugs. There are many reports which show that *in vivo* drug uptake occurs at a much faster rate than that observed using *in vitro* preparations. For

example, the *in vitro* rate of uptake of propranolol into guinea pig atrium was found to be relatively slow, requiring 3 hours for the tissue/medium ratio to reach a constant value (Potter, 1967). However, in an *in vivo* dog model the myocardial propranolol concentration peaked well before 10 min after injection (Pruett et al., 1977). Similar discrepancies between the *in vitro* and *in vivo* rates of drug uptake were found for verapamil. *In vitro*, verapamil took 2 hours to reach a tissue to medium ratio of 20 to 30 :1 in the resting and stimulated beating left auricles of guinea pig hearts superfused with Tyrode's solution (Lullmann et al., 1979). Similarly, the slow rate at which verapamil reduced the contraction (induced by potassium) of rabbit aortic rings suggested a slow rate of diffusion of verapamil into the tissue (Mars and Sperelakis, 1981). However, in an *in vivo* study (Keefe and Kates, 1982) in which verapamil was given intravenously to anaesthetised dogs, it was found that distribution equilibrium between the plasma and myocardium was achieved within 10 min of administration. The maximum P - R prolongation occurred 20 min after administration. This rapid action of verapamil was shown in other studies in dogs. P - R prolongation on the electrocardiogram occurred within 2 min after intravenous verapamil administration (McAllister et al., 1977), and maximum P - Q interval prolongation appeared 1 min after an intra - coronary verapamil infusion (Neugebauer, 1978).

Furthermore, different *in vitro* experimental preparations have given differences in the time required to reach steady - state drug effect or drug concentration. For example, propafenone is a class I antiarrhythmic agent with an additional effect on slow calcium channel and cardiac beta adrenergic receptors (Ledda et al., 1981). In the guinea pig papillary muscle superfused with Tyrode's solution containing 3×10^{-5} M propafenone (Kohlhardt and Seifert, 1980), the time needed to reach steady state inhibition of the maximum velocity of muscle contraction was 35 to 45 min. In an isolated perfused rabbit heart model (Gillis and Kates, 1986), this time was reported to be 112 min. It has been suggested that the longer time reported in the latter study represents the time required for propafenone to diffuse across the capillary walls.

These differences between preparations can be rationalised to some extent by considering the integrity of the relevant organ or tissue. In studies using subcellular preparations, the drugs will not need to pass through capillary walls and cell membranes, although this occurs in isolated perfused organs and whole body systems. Therefore, drugs which exhibit a strong affinity for cellular or subcellular components *in vitro* may reach these components only slowly *in vivo* (Junod, 1976). Kinetic constants from subcellular preparations would therefore over - estimate *in vivo* values.

Tissue slice preparations have no blood flow and therefore the diffusion into the tissue may be poor compared with *in vivo* conditions. Furthermore, it has been reported that even oxygen diffusion into tissue slices is poor (Bingmann and Kolde, 1982; Lipinski, 1989). If the interior of the slice is hypoxic, this may influence the activity of enzymes and the integrity of cells within the slice (Lullmann et al., 1988)

Even isolated perfused organ systems can be far removed from normal physiological conditions. The perfusate is usually an oxygenated "physiological" salt solution (Gillis and Kates, 1986), although the importance of plasma protein binding on drug distribution has long been emphasised (Tucker et al., 1970; McNamara et al., 1979; Burch and Stanski, 1983). For pharmacodynamic studies, the normal nervous and hormonal control of processes affected by the drug are absent, and this may have a large quantitative and qualitative influence on the time - course of drug effect.

Although for the clinical use of drugs it is desirable to understand both their *in vivo* pharmacokinetics and pharmacodynamics, *in vivo* studies are not as well suited to eliciting specific mechanisms influencing drug disposition and drug effect as *in vitro* studies (see 1.2.1). Thus, an understanding of the limitations of different experimental approaches provides a rational basis for their appropriate application.

1.4.2 Effects of anaesthesia

It is now well recognised that anaesthesia causes profound biochemical and physiological changes. Although this is most obvious with some of the general anaesthetics such as halothane and barbiturates (Gonway and Ellis, 1969; Ty Smith et al., 1978; Warren and Stoelting, 1986), almost all anaesthetics can, to different extents, influence drug kinetics (Runciman and Mather, 1986).

1.4.2.1 General anaesthesia

General anaesthesia can alter haemodynamics, typically by reducing cardiac output and the fraction of cardiac output received by each organ. Thus, the kinetics of drugs whose distribution or clearance are flow - limited may be altered (Runciman et al., 1984b; 1984c; 1985; 1986; Mather et al., 1986a; 1986b; Runciman and Mather, 1986) and this may also influence the effects of the drugs (Thomson et al., 1971; Stenson et al., 1971).

Halothane was reported to cause a dose dependent decrease in mean arterial pressure and cardiac output in rabbits and sheep (Halpern et al., 1984; Upton et al., 1990b). In sheep, halothane caused significant decreases in renal and hepatic blood flow (to 50% and 71% of control values, respectively; Mather et al., 1986b). Furthermore, halothane anaesthesia can have effects on drug kinetics independent of effects due to changes in haemodynamics. For example, halothane reduced the hepatic extraction of chlormethiazole to 82% of the control values, and abolished the lung extraction and clearance of chlormethiazole (Runciman et al., 1986). In similar studies, halothane anaesthesia decreased hepatic clearance of pethidine to 60% of control, and the renal extraction of pethidine was abolished. In the same preparation, halothane anaesthesia reduced the renal extraction of cefoxitin to 48% of control values (Runciman et al., 1985). Halothane has also been found to influence drug binding in blood (Gordon et al., 1987).

Moderate doses of barbiturates have been reported to cause myocardial depression in animal preparations (Gonway and Ellis, 1969). Even doses of

thiopentone in rabbits which were too low to cause respiratory arrest were associated with hypotension, marked dilatation of the heart, increased central venous pressure and reduced aortic blood flow (Gordh, 1964). Similarily, doses of 20 mg/kg of thiopentone to dogs reduced the contractile force of the left ventricle by 15% (Cotten and Bay, 1956). Nevertheless, studies of drug distribution in animals under barbiturate anaesthesia have been reported for digoxin (Roverge et al., 1970), lignocaine (Branch et al., 1973; Patterson et al., 1982), procainamide (Weily and Genton, 1972), bupivacaine (Irestedt et al., 1976; 1978) and bretylium (Anderson et al., 1980).

Apart from their depressant effect on the myocardium, barbiturates can also impair the circulatory compensatory reflexes by depressing the brain stem centres and impairing ganglionic transmission (Bowman and Rand, 1980). Under barbiturate anaesthesia, some local anaesthetics in subconvulsive doses can cause marked myocardial depression and significant circulatory changes (Priano et al., 1969; McWhirter et al., 1972; Blair, 1975). It was reported that four hours after administration, when the blood levels of the barbiturate were expected to have decreased significantly, depressed cardiovascular function had not recovered (Bowman and Rand, 1980). This would have implications for studies of both drug distribution and drug effect. However, the cardiovascular effects of lignocaine (Austen and Moran, 1965; Liu et al., 1982; Zito et al., 1981), bupivacaine (Hotvedt et al., 1985), verapamil (Mangiardi et al., 1978), procainamide (Austen and Moran, 1965), nitrous oxide and pethidine (Stanley et al., 1977) have all been studied under pentobarbitone or thiopentone anaesthesia. The results from these experiments would be expected to be different to those in conscious animals (Scott et al., 1971).

Furthermore, it has been reported that barbiturate anaesthesia can alter the proportions of intravascular and interstitial volumes within an organ (expanding the intravascular and decreasing interstitial volumes). Again, this is likely to affect drug distribution in these two spaces (Quin and Shannon, 1976),

and may alter the effects of the drug, depending on whether the drug acts in the interstitial or intracellular space.

In view of the pharmacokinetic and haemodynamic effects of general anaesthesia, it seems likely that animals under general anaesthesia will have different patterns of drug distribution to conscious animals, with high drug concentrations in organs with relatively preserved perfusion (such as the myocardium and brain) and low drug clearance due to the decreased perfusion and extraction in the organs of drug elimination such as the liver and kidney. This would be qualitatively similar to the effects of mild haemorrhagic shock, after which high concentrations of digoxin were found in the heart (Lloyd and Taylor, 1975), the effects of congestive heart failure and the effects of drugs which induce haemodynamic changes on the pharmacokinetics of antiarrhythmic agents (Branch et al., 1973; Woosley et al., 1986).

General anaesthesia makes certain procedures much easier, such as serial tissue samples in pharmacokinetic studies, but the haemodynamic effects of general anaesthesia on drug disposition should be considered. Studies in conscious, preferably unrestrained animals are necessary if information is required on drug disposition under normal physiological conditions.

1.4.2.2 Local anaesthesia

Local anaesthetics can reach relatively high blood concentrations following infiltration for nerve blocks. During pharmacokinetic studies this can cause problems if the local anaesthetic competes for binding sites on plasma proteins or in tissues such as lung and myocardium (Post et al., 1979; Lullmann et al., 1980; Horowitz et al., 1986). This may also compound drug effects. For example, plasma concentrations of lignocaine in the antiarrhythmic range (2.9 to 7.1 μg/ml) caused a decrease in cardiac output and a rise of central venous pressure in patients and dogs under nitrous oxide and halothane anaesthesia (Scott et al., 1971; McWhirter et al., 1973).

In another study, although cardiac output was not changed by the intravenous use of lignocaine, the distribution of cardiac output to different organs and tissues was altered (Benowitz et al., 1974a).

1.4.3 Drug administration and the frequency of blood sampling

The duration of intravenous drug administration, and the frequency of blood or tissue sampling greatly influences the results of pharmacokinetic and pharmacodynamic experiments (Chiou, 1980). Indeed, it has been standard practice in many studies of bolus kinetics to allow a period of several minutes after the bolus and before the first blood sample for "initial mixing" (Chiou, 1979; Horowitz et al., 1986). These studies only examine the elution and elimination of the drug, and ignore the important and rapid tissue uptake of the drug in the first few minutes immediately after the bolus (Hayward et al., 1983). The influence of these factors on studies of myocardial drug uptake are discussed below.

1.4.3.1 Drug administration regimen

In the pharmacokinetic literature, intravenous drug administration is often described as an intravenous bolus, but the exact duration and rate of injection in many cases is not specified. It is known that drug injection rate is one of the determinants of peak blood drug concentrations following intravenous injection (Crawford, 1966; Upton et al., 1990b). Variations in injection rate will result in differences in the time - courses and profiles of arterial drug concentrations, and in turn differences in myocardial drug disposition with attendant differences in pharmacodynamics. This fact has been demonstrated by studies that have designed dose regimens for antiarrhythmic and myocardial inotropic drugs (Lucchesi et al., 1966; Roverge et al., 1970) and by studies of the central nervous system toxicity of local anaesthetics (Malagodi et al., 1977; Rutten et al., 1989). Therefore, the drug injection rates within studies should be kept as constant as possible.

There has yet to appear in the literature an exact definition of the term " bolus". The reported rates of "bolus" injection varied from so called "within seconds" to

a few minutes (Hashimoto et al., 1984; Kates and Jaillion, 1980), or are not reported at all (Mangiardi et al., 1978; Seltzer et al., 1980; Halpern et al., 1984). In fact, it may be preferable to consider a bolus as a very short infusion, and in all pharmacokinetic studies drug injection rates and volumes, as well as the drug dose, should be stated. This would greatly aid the comparison of different "bolus" studies.

1.4.3.2 Frequency of blood sampling

A common feature of most studies of drug uptake and elution by the heart is the need to characterise transient drug concentration - time "peaks" entering or leaving the heart. An inadequate frequency of blood sampling could cause two potential problems. Firstly, a lack of understanding of the nature of the drug uptake and elution process means that a sampling regimen can completely miss these transient "peaks" which are important in determining the myocardial pharmacodynamics immediately after drug injection. Secondly, if a peak is recognised, the frequency of blood or tissue sampling may be inadequate to accurately describe the true time - course of the "peak", leading particularly to errors in the maximum concentration and area under the curve of the peak (Selden and Neill, 1975). In theory, the blood sampling frequency needed to accurately describe such peaks could be determined using well - understood chromatographic data acquisition principles.

These problems are illustrated by the following examples. In one study of methyldigoxin pharmacokinetics in the human myocardium, the first arterial and coronary sinus blood samples were taken 10 min after a 5 min intravenous infusion of methyldigoxin. It was concluded that the rapid myocardial uptake of methyldigoxin could not be determined using the sampling regimen used (Hayward et al., 1983). In another study in patients and dogs using the same method, the paired blood samples were taken at 15 sec intervals. The myocardial uptake of ouabain was found to have two phases (Selden and Neill, 1975), but the calculated myocardial drug concentrations were lower than the

measured myocardial drug concentrations from myocardial tissues obtained by biopsy. It was suggested that the process of estimating the blood ouabain concentrations prior to the start of blood sampling using back extrapolation may have under - estimated the area under the ouabain blood concentration time - curve. Similarily, in studies of the time - course of myocardial drug concentrations using a high speed boring tool, it was found that by the time the first myocardial biopsy was obtained, the concentrations of the drugs in the myocardium had fallen below the peak level (Nattel et al., 1979; Keefe and Kates, 1982).

The importance of matching the frequency of blood sampling to the characteristics of the uptake and elution process is also illustrated by studies of drug uptake by the lung, which is extremely rapid (Junod, 1976).

1.4.4 Intravascular transit

Intravascular transit time through a tissue or an organ (i.e. the time taken for blood to pass through the organ) can influence some studies which use arterio - venous drug concentration differences to determine drug uptake and elution. When the uptake and elution is extremely rapid, the intravascular transit time may cause an arterio - venous drug concentration difference which is not due to drug uptake or elution (Upton et al., 1988a). This influence of the intravascular transit time on myocardial drug uptake seems only to have been considered in two studies (Selden and Neill, 1975; Upton et al., 1988a) to date.

1.4.5 Assumptions in pharmacokinetic modelling

The description of drug disposition in the body is complicated by the fact that there are huge differences in the anatomy, physiology and biochemistry of organs and tissues within the body. For the purpose of pharmacokinetic modelling, this problem is overcome by making assumptions about the behaviour of drugs in the body in order to construct a simplified representation of the body. Unfortunately, some of the assumptions that have been made to model myocardial drug uptake

are so oversimplified that they are not of use, have not been tested experimentally, or even worse are at odds with experimental data.

1.4.5.1 Systemic pharmacokinetic models

Although compartmental models (see 1.2.2) have been successfully used to design dose regimens in clinical medicine, a number of general criticisms are appropriate. Firstly, the compartments and parameters of the model have no anatomical or physiological reality (Gerlowski and Jain, 1983; Hull, 1990). Functionally, this means that virtually any convex or concave concentration time - curve can be represented by a compartmental model - without any ability to interpret what the model means. Secondly, the selection of the number of compartments used in the model is not determined by biological considerations, but often by the blood sampling regimen used (McAllister et al., 1977). Thirdly, the assumption of a homogeneous blood pool with no differences between arterial and venous blood is not valid (Price, 1960; Benowitz et al., 1974a; Hinderling and Garret, 1976; Christensen et al., 1982; Chiou, 1979). Indeed, it has been shown that even Evans blue dye requires as long as 10 min in order to achieve complete mixing in blood after bolus injection (Broadbent and Mood, 1954; Chiou, 1989a and 1989b).

The apparent volume of initial distribution is calculated by dividing the bolus drug dose by the extrapolated plasma concentration at time zero. This is considered to be the highest plasma concentration attainable after drug administration. However, at time zero, the actual drug concentration at the sampling site should be zero (Broadbent and Mood, 1954; Chiou, 1979; Huang et al., 1991). The peak concentrations of intravenously administered drugs are usually achieved not instantaneously, but rather relatively gradually. The initial concentration peaks actually measured may be much higher than those estimated from data obtained a few minutes after dosing (Selden and Neill, 1975). Thus the "true" apparent initial volume of distribution is usually overestimated using these calculations.

More specific problems become apparent when compartmental models are applied to the myocardium. The heart is generally considered to be highly perfused and in rapid equilibrium with blood in a central compartment, but during cardiac ischaemia it was found to function as a series of peripheral compartments (Wenger et al., 1978; 1980; Zito et al., 1981; Horowitz et al., 1986). Even with the normal myocardium, time is needed for the drug concentrations in blood and myocardial tissue to reach equilibrium, even after short - term intravenous drug administration. For example, after the intravenous administration of bretylium, pseudo - equilibrium between the drug concentrations in blood and the myocardium was not reached even 12 - 24 h after administration (Anderson et al., 1980).

As stated previously, the success of systemic physiologically based models is highly dependent on the suitability of the regional pharmacokinetic models of which they are composed. These are discussed below.

1.4.5.2 Regional pharmacokinetic models

Most regional pharmacokinetic models can generally be grouped into two categories: single compartment flow - limited models or membrane limited models. The former is by far the most commonly used model in the absence of evidence suggesting the presence of membrane limited kinetics (Gerlowski and Jain, 1983; Himmelstein and Lutz, 1979).

Single compartment, flow - limited models

In these models it is assumed that drug transport across the capillary wall and cell membranes of the region is much faster than the rate that blood flow supplies drug to the region. Thus, a region is represented as one compartment. The drug concentration in the compartment is determined by the volume of the compartment, and a partition coefficient relating the tissue drug concentration to the regional venous drug concentration.

Generally it is assumed that all highly lipophilic drugs behave in this way. For example, such models have been established for lignocaine and digoxin distribution in different organs and tissues in the body such as lung, brain, muscle, liver, heart, kidneys and the gastro - intestinal tract (Benowitz et al., 1974a; Harrison and Gibaldi, 1977). Despite this wide - spread assumption, there is no substantial experimental evidence for these models in the literature and, indeed, the single compartment approach may not be applicable in such circumstances. For example, it has been reported that intra - tissue concentration gradients can be large, as reported for some solid tumours (Jain et al., 1979). Similarly, lignocaine and bupivacaine concentrations in the fat of the hindquarters of sheep were not in equilibrium with blood drug concentrations 180 min after the start of constant rate drug infusions (Upton et al., 1991b).

Multi - compartment models

In these models, each region is commonly represented as three compartments each with anatomical significance (usually vascular, interstitial and intracellular compartments). Physiological (e.g. tissue volumes, blood flow rates), physicochemical (e.g. binding, lipid solubility, ionization) and pharmacological (e.g. mechanisms of transport, sites of action) parameters are incorporated into these models to describe drug transport between compartments. This makes the model able to accommodate changes of physiological circumstances such as those which occur in disease states (Benowitz et al., 1974a; 1974b). Despite this, these multi - compartment models have been little used, and only one instance of their application to the heart could be found in the literature. In the report, a conceptual model was proposed, but its solution was not derived mathematically (Lullmann et al., 1979). In the model, drug could be associated with either the interstitial space, the plasmalemma, cell water or cellular binding sites. That such a model is necessary is demonstrated by a study of ouabain disposition in the heart using the indicator - diffusion

method (Duran and Yudilevich, 1974). It was found that the ouabain rapidly distributed into the interstitial space, while the cell wall barrier limited drug distribution into the myocardial cells.

1.4.6 Limitations of pharmacokinetic - pharmacodynamic models

It has been proposed recently that as some of the assumptions made as part of compartmental pharmacokinetic modelling of drug kinetics after intravenous bolus drug injections (see 1.4.5.1) are invalid, these compartmental models are not appropriate as investigative tools for pharmacokinetic - pharmacodynamic studies (Hull, 1990). For the same reason, myocardial drug concentration - effect studies should be based on data from actual experiments to provide a solid background for any myocardial pharmacokinetic and pharmacodynamic modelling.

1.5 Mass balance principles in the study of regional pharmacokinetics

The "mass balance" approach represents an application of the Law of Conservation of Matter. While some applications of mass balance principles are fundamental to most pharmacokinetic methods, the direct application of mass balance principles to regional pharmacokinetics has been less common. However, over the last 30 years it has become apparent that the use of mass balance principles provides a simple, empirical method of calculating the uptake and elution of drugs in tissues in a relatively noninvasive manner in both animals and humans (Upton et al., 1988a; Horowitz et al., 1986). This is usually of interest for one of two reasons. Firstly, the region in question may influence the whole body pharmacokinetics. For example, the liver, lung and hindquarter disposition of a drug (Upton et al., 1988a) may affect the time - course of its myocardial and brain concentrations. Secondly, the region may be a site of the therapeutic or toxic actions of the drug such as the heart or brain (Upton et al., 1985; Horowitz et al., 1986; Szeto et al., 1980). In the latter case, it has been proposed that there is some predictable relationship between regional drug concentrations and drug effects (Horowitz and Powell, 1986).

The application of mass balance principles in pharmacokinetic studies simply requires that the total mass of drug entering a region via the arterial blood will be

equal to the mass of drug leaving the region by the regional venous blood plus the mass of drug remaining in the region and the mass of drug metabolized in or eliminated from the region. At times when the arterial drug concentration is higher than the regional venous drug concentration, the net drug movement is from the blood into the region. When the venous drug concentration is higher than the arterial drug concentration, the net drug movement is from the region into the blood. A few definitions and concepts have been recently established for regional pharmacokinetic studies using mass balance principles (Upton et al., 1988a; 1990a). The rate, or the net drug flux (J_{net}), of this movement can be calculated by the following equation:

$$J_{net} = Q \times (C_a - C_v) \qquad \ldots(1.4)$$

Note that Q is the regional blood flow, and C_a and C_v are the representative drug concentrations in arterial and venous blood supplying and draining the region, respectively.

The total mass of drug remaining in the region after a given time (M_{net}) is the integral of the net drug flux over time:

$$M_{net} = \int_0^t J_{net} \cdot dt = \int_0^t Q \cdot (C_a - C_v) \cdot dt \qquad \ldots(1.5)$$

If the mass or volume of the tissue under study is known and no metabolism or elimination occurs in the tissue, the mean tissue drug concentration can be calculated by dividing the net drug mass by the tissue mass.

However, a number of important criteria need to be satisfied for any region before the mass balance principles can be applied (Runciman, 1982; Upton et al., 1988a; 1990a). These are briefly discussed below, with emphasis on factors pertaining to studies of myocardial pharmacokinetics.

1. The contribution of the intravascular transit time in the organ or region being studied must make a negligible contribution to the arterio - venous blood drug concentration difference across the region. It has been reported that the mean intravascular transit times for the myocardium (from the ascending aorta to the coronary sinus) of both dog and sheep were 1.2 - 7 sec and 4 sec respectively (Selden and Neill, 1975; Upton et al., 1988a). This intravascular transit time is much shorter than the time difference between equal arterial and venous blood drug concentrations in most of the studies of myocardial drug uptake and therefore will make a negligible contribution to the measured arterial and coronary sinus blood concentration differences.

2. All the unchanged drug must leave the organ via the venous blood vessels. Thus, the direct diffusion of drug between adjacent organs or tissues, or the removal of drug by way of lymphatic drainage must be negligible. Gas transfer between organs by direct diffusion (Perl et al., 1965), and drug transfer through different layers of skin tissue (Bogaert, 1987; Singh and Roberts, 1990) have been reported. The transfer by direct diffusion of other substances from the left ventricular myocardium into adjacent ischaemic myocardium and from the myocardium into the surrounding medium have also been reported (Watanabe et al., 1963; Jennings et al., 1964; Myers and Honig, 1966; Watkinson et al., 1979; Hanley et al., 1983). This suggests that the influence of this phenomenon on mass balance calculations should be determined before mass balance calculations are used in any particular application.

The influence of the removal of drug from regions by lymph can be estimated from studies of drug concentrations in the lymph (Verwey and Williams, 1962; Brown, 1964; Garlick and Renkin, 1970). Again, these concentrations and lymph flow rates are sufficient to suggest that the kinetics of a given drug in lymph should be determined if the drug is to be studied using mass balance principles. However, given the slow flow rate of myocardial lymph (Quin and Shannon, 1976; Milnor, 1980), it would be expected that the rate of drug transport from the heart by lymph would be negligible. However, this has not been confirmed experimentally.

3. The afferent and efferent blood samples must be representative of blood entering and leaving the region. Attention must be paid to the anatomy of the blood vessels of the organ studied when different animal species are used, e.g. the hemiazygos vein drains into the coronary sinus in several animals, including the sheep (May, 1964; Nancarrow, 1986). Methods for investigation of the tissue blood supply include perfusing the specific vessels with pigmented gelatin or india ink (Bond et al., 1973; Downing et al., 1973)

For calculating regional drug concentrations, the tissue mass perfused and drained by the blood vessels selected for blood sampling should be identified. However, this myocardial tissue mass appears not to have been defined in previous studies of myocardial drug uptake.

4. The arterial and regional venous blood concentration - time curves must be described using an adequate frequency of blood sampling. This should match the rate of change of the blood drug concentrations, which is in turn determined by the drug injection site and rate and the cardiac output (Crawford, 1966; Upton et al., 1990a). In myocardial pharmacokinetic studies after intravenous bolus injections, the rapid myocardial drug uptake will not be demonstrated by relatively infrequent blood sampling immediately after drug administrations (Chiou, 1980; Hayward et al., 1983). Methods for rapid blood collection from deep vessels such as the pulmonary artery and aorta are available (Bertler et al., 1978; Roerig et al., 1989). However, studies of dye dilution curves (Dow, 1958; Sheppard et al., 1959) suggest that the adequancy of these methods for characterising rapid changes in blood drug concentrations needs to be questioned.

5. For the calculation of regional drug distribution using mass balance principles, the regional blood flow must be measured rather than assumed to be a fixed fraction of cardiac output. In relation to myocardial pharmacokinetic studies, coronary artery blood flow must be measured.

6. For the correct use of mass balance principles in studies of regional tissue drug concentrations, there should be neither metabolism nor elimination of drug in this region, or, if present, the rate of any such process should be measured.

1.6 Myocardial pharmacokinetics and pharmacodynamics of lignocaine, procainamide and pethidine *in vivo*

1.6.1 Lignocaine

Lignocaine is a Class Ib antiarrhythmic drug for the treatment of ventricular arrhythmias due to acute myocardial ischaemia (Harrison et al., 1963; Gianelly et al., 1967; Grossman et al., 1968; Carson et al., 1979; Lucchesi and Patterson, 1984; Frumin et al., 1989), and is also a widely used local anaesthetic agent. At relatively high blood concentrations, lignocaine can prolong cardiac conduction times and depress myocardial contractility (Austen and Moran, 1965; Blair, 1975; Block and Covino, 1981; Liu et al., 1982). Extensive clinical use has shown wide variations in inter - individual responses to lignocaine after intravenous administration. This has led many researchers to correlate the onset of effective antiarrhythmic action or myocardial toxicity with blood or myocardial lignocaine concentrations in order to provide objective criteria for the choice of dose regimen for different situations.

1.6.1.1 Pharmacokinetics and pharmacodynamics

Like many pharmacokinetic - pharmacodynamic studies, studies on lignocaine have shown hysteresis between the myocardial effects of the drug and its concentration in blood. For example, following an intravenous bolus and infusion of lignocaine in dogs, it was found that the time - course of the elevated fibrillation threshold after premature ventricular contraction in normal hearts was parallel to the arterial blood concentrations of lignocaine, but the peak effects appeared "a few minutes" after peak blood concentrations of the drug (Gerstenblith et al., 1972). In another study, after intravenous bolus injections of lignocaine (200 mg and 400 mg) to dogs, it was found that there was no close temporal relationship between the magnitude of the negative

inotropic effect and plasma lignocaine levels and that the depressant effects of lignocaine on myocardial contractility were found to lag behind blood drug concentrations (Binnion et al., 1969).

There are also inconsistencies in the blood concentrations at which lignocaine is reported to first have its antiarrythmic effect. In the above report (Gerstenblith et al., 1972), it was shown that the increase in ventricular threshold in the normal heart occurred simultaneously with the appearance of lignocaine in blood (over the range of 0 - 6 μg/ml). However, in a study of the effects of lignocaine infusion on the ventricular fibrillation threshold after a premature heart beat in dogs, it was found that there were no measurable effects when the plasma drug concentrations were below 1.3 - 4 μg/ml (Schnittger et al., 1978). It was also found that individual animals showed wide variability in the plasma concentrations at which the onset of measurable effects on the ventricular threshold were observed. At a given plasma concentration of the drug, some animals had a considerable increase in the ventricular threshold, while others had little or no increase.

In another study, lignocaine given to dogs as an intravenous bolus and followed by an intravenous infusion prolonged the conduction time and the effective refractory period in the infarcted myocardium at steady state blood concentrations between 3.1 - 3.3 μg/ml (Kupersmith et al., 1975). This did not occur in the normal myocardium. The greatest prolongation of the effective refractory period lagged well behind the peak serum lignocaine concentration, although the conduction time changes were closely related to the serum lignocaine concentration. It was suggested that this difference was due to differences in the time the drug took to reach different parts of myocardial tissues, such as Purkinje fibres, deep tissue or the infarcted myocardium.

The experiments cited above suggest that different concentrations of lignocaine might be needed to produce different actions on the myocardium. That is, conduction changes occur at lower myocardial lignocaine concentrations, while

an increase in the effective refractory period requires higher concentrations. Furthermore, it seems that there may be differences between lignocaine concentrations in normal and infarcted myocardium.

This viewpoint is supported by reports that the antiarrhythmic effect of lignocaine was dependent on the lignocaine concentrations in ischaemic myocardium. By comparing venous, arterial, normal and ischaemic myocardial lignocaine concentrations in dogs and pigs with ligated left anterior descending coronary arteries, it was shown that the antiarrhythmic effects of lignocaine were related only to ischaemic myocardial lignocaine concentrations (Davis et al., 1979; Vogt et al., 1988).

The disposition of lignocaine in ischaemic myocardium and the lateral border zone was studied in relation to regional blood flow (Patterson et al., 1982). Ten minutes after the injection of 75 mg of lignocaine to dogs, the myocardial lignocaine concentration was 0.56 ± 0.12 μg/g in normal, and 0.40 ± 0.08 μg/g in the ischaemic zone, while their respective blood flows were 1.1 ± 0.45 ml/g/min and 0.56 ± 0.12 ml/g/min. Although in this study there appeared to be a poor correlation between regional blood flow and lignocaine concentrations in the ischaemic myocardium, this conclusion may be different if the time - course of the blood flow to the region was related to the uptake and elution characteristics of the drug in different regions of the heart. However, even this relationship appears to be complex. In a study relating the initial distribution of lignocaine into the myocardium after a bolus injection with simultaneous measurement of regional blood flow, it was found that the rate of uptake was proportional to regional blood flow during the initial 1 - 3 min after the bolus with correlation coefficients between blood flow and myocardial drug concentration of 0.81 and 0.85 for ischaemic and normal regions, respectively. The rate of elution from the ischaemic myocardium was to some degree related to regional blood flow (Zito et al., 1981). It was also found that the ischaemic

myocardium reached its peak lignocaine concentration later, and had a slower rate of elution, than normal zones (Zito et al., 1981).

Another important effect of blood flow on lignocaine distribution was shown in studies in patients with heart failure, liver disease and renal failure. It was found that there was an inverse relationship between cardiac index and lignocaine blood levels. This was attributed to the redistribution of blood flow in favour of the heart and brain, and the higher lignocaine concentrations in blood due to decreased hepatic blood flow (and therefore decreased lignocaine clearance) under these circumstances. Such pathological conditions may result in accumulation of the drug in the heart and brain which would alter the therapeutic or toxic effects on these sensitive organs (Thomson et al., 1971; Stenson et al., 1971).

It can be concluded that conventional monitoring of therapeutic efficacy based solely on plasma or serum lignocaine concentrations, especially after short term intravenous use, would not provide insight into blood flow related regional pharmacokinetic phenomena (Horowitz and Powell, 1986). However, such phenomena are particularly important to the delivery of antiarrhythmic drugs such as lignocaine to critically ill patients with arrhythmias from ischaemic heart disease.

The observation that the efficacy of antiarrhythmic drugs in reverting tachyarrhythmias is likely to be correlated with drug concentrations within various regions of the myocardium or mean myocardial concentrations, has precipitated studies correlating global myocardial lignocaine concentrations with myocardial effects.

Studies of the peak extraction of lignocaine in the myocardium of pigs after bolus administration of either 6 mg/kg or 12 mg/kg doses were found to be dose independent and elution from the heart commenced relatively soon after injection (Reiz and Nath, 1986).

The short term myocardial uptake of lignocaine was measured in a study in patients undergoing diagnostic catheterisation procedures (Horowitz et al., 1986). By comparing arterial and coronary sinus blood concentration differences, an initial phase of net lignocaine uptake into the myocardium followed by one of net elution was shown. The latter phase was not identical to the simultaneously measured venous lignocaine concentrations, but rather appeared to correlate well with the relative duration of action of lignocaine. Compared with conventional pharmacokinetic methods, the mean period of net lignocaine uptake by myocardium was considerably shorter than the "distribution half - life" of 1.5 min (Boyes et al., 1971; Benowitz et al., 1974a), and the initial drug efflux was considerably more rapid than the "elimination half - life". On the basis of these findings, lignocaine was suggested to be a preferred agent for the initial emergency management of ventricular tachyarrhythmias.

1.6.2 Procainamide

Procainamide is an effective Class Ia antiarrhythmic drug for the control of ventricular arrhythmias after myocardial infarction or open heart surgery (Mark et al., 1951; Harris et al., 1952; Lucchesi and Patterson, 1984; Frumin et al., 1989). In its early years of experimental and clinical use, there were some reports of cardiotoxicity, including ventricular stand - still and sudden hypotension, although it was regarded as a promising "suppressor" agent for use in severe ectopic ventricular tachycardia (Berry et al., 1951; Harris et al., 1952; Epstein, 1953).

The results of early studies on the dose regimen of procainamide were conflicting and showed a wide variety of inter - subject responses. For example, while one group reported their safe use of the drug at the rate of 200 mg/5 min intravenously until either the arrhythmia was interrupted or a total of 1 g had been used (Giardina et al., 1973), another recommended that procainamide should not be given intravenously at rates greater than 50 mg/min (Epstein, 1953). Based on a two compartment model for procainamide disposition, a loading and

maintenance dose regimen was recommended (Lima et al., 1978). Furthermore, the administration of procainamide as an intravenous bolus or a rapid infusion has been associated with a number of troublesome or even dangerous side effects, including hypotension, tachycardia, A - V block and myocardial depression (Berry et al., 1951; Epstein, 1953; Kayden et al., 1957).

1.6.2.1 Pharmacokinetics and pharmacodynamics

It has been recognised that the antiarrhythmic efficacy of procainamide depends in part on the rapid achievement and maintenance of therapeutic serum concentrations (Giardina et al., 1973; Lima et al., 1978). In particular, its apparent narrow therapeutic range, especially after intravenous use, has led many investigators to examine the range of plasma procainamide concentrations that controlled ventricular arrhythmias without undesirable cardiovascular effects. Unfortunately, the results of these studies have been conflicting (Beelet et al., 1952; Kayden et al., 1957; Bigger and Heissenbuttle, 1969; Koch - Weser et al., 1969; Lima et al., 1978). Some studies refute the notion that the plasma concentrations of procainamide always reflect the concentrations of the drug at its site of action (Scheiman et al., 1974; Elson et al., 1975). For example, when testing a dose regimen developed on the basis of clinical experience, intravenous procainamide (100 mg) was given over 2 min and the dose repeated every 5 min until the arrhythmia was abolished, 1 g of the drug was given, or untoward drug effects appeared (Giardina et al., 1973). Although this initially produced a linear increase in the procainamide plasma concentrations after each dose, and a graded decrease in premature ventricular depolarizations, the effective plasma procainamide concentrations varied from 4 to 10 μg/ml and the arrhythmias recurred at concentrations between 1.0 and 8.7 μg/ml. It was considered that the differences in the plasma concentrations at arrhythmia abolition and at recurrence were probably related to hysteresis between plasma and myocardial concentrations observed in other studies (Scheiman et al., 1974).

Another investigation compared the time - courses of venous plasma and saliva procainamide concentrations and QT interval changes after a 30 min intravenous infusion of 500 mg of procainamide in healthy volunteers. A significant degree of hysteresis was found between the QT interval changes and the plasma procainamide concentrations, but no hysteresis was found for the saliva procainamide concentrations (Galeazzi, 1976). The delay of the pharmacological effect of procainamide with respect to its peak plasma concentration was found to be characterised by a half - time of approximately 7 min. This delay was assumed to be the time required for the procainamide to penetrate to its site of action in the heart. In another study, the effect of procainamide on QRS prolongation on the electrocardiogram was found to lag behind blood procainamide concentration by 3 to 18 min (Liem, 1988). As a result, the conventional concept of an effective plasma concentration was challenged and proposed to be meaningless unless defined with respect to time.

Some workers have recognised that a true understanding of the basis of this hysteresis can only be reached by studying the relationship between the tissue (in particular myocardial) distribution of procainamide and its pharmacological effect. However, some aspects of the tissue distribution of procainamide were documented almost as soon as the drug was introduced (Mark et al., 1951). One hour after the intravenous infusion of 1.25 g of procainamide to dogs, it was shown that procainamide concentrations in most organs and tissues were still considerably higher than plasma drug concentrations. The myocardial drug content was 81 mg/kg when the blood concentration was 32 mg/L. Studies of procainamide pharmacokinetics by compartmental modelling showed that procainamide had a rapid distribution half - life of about 5 min and that it was extensively bound in organs such as the heart, kidney, liver and lungs (Graffner et al., 1975; 1977).

Other workers have studied the relationships between physiological variables, in particular myocardial blood flow, and myocardial procainamide

concentrations - principally to determine whether the decreased myocardial blood flow following myocardial infarction reduces the myocardial procainamide content and procainamide efficacy. In the normal canine heart, it was found that the myocardial procainamide concentration normalised for the plasma concentration was not related to myocardial blood flow (Strauss et al., 1978) after intravenous infusion. The administration of procainamide by intravenous infusion to dogs following the ligation of their left circumflex coronary arteries (Wenger et al., 1978; 1980) showed no significant decrease in the myocardial procainamide concentrations until the blood flow was compromised to 31 - 40% of control. Even when the myocardial blood flow was $\leq$ 0.1 ml/min/g, (approximately 8% of control flow) the myocardial procainamide concentration was 39 ± 4% of control. This was attributed to the extensive uptake into and slow elution of procainamide in the ischaemic myocardium. In control regions, the time - course of the myocardial procainamide concentrations followed the time - course of the plasma procainamide concentrations, and reached a peak 30 sec after administration. In the ischaemic region, the relationship was less marked. The accumulation of procainamide in the ischaemic area was related to the regional myocardial blood flow, and it was also found that the rate of decline of the procainamide concentrations was slower in this region. It was concluded that the ischaemic parts of the myocardium would have significantly different procainamide kinetics to normally perfused myocardium, and could behave like a "peripheral compartment" in compartmental pharmacokinetic terms.

1.6.3 Pethidine

Pethidine is a synthetic opioid analgesic (Jaffe and Martin, 1980). Although its respiratory depressant effect is equal to that of morphine when the two drugs are compared in equi - analgesic doses, its cardiovascular depressant effect is much greater (Strauer, 1972; Bovill et al., 1984). This was illustrated in a study in which the effects of high doses of fentanyl, pethidine and naloxone on the cardiovascular system were compared in dogs (Freye, 1974). Pethidine was given intravenously in

bolus doses of between 2.5 and 20 mg/kg. Pethidine reduced left ventricular pressure, coronary sinus blood flow, mean pulmonary artery blood pressure and central aortic blood pressure. Higher doses caused greater depression of the measured haemodynamic parameters. Following doses of greater than 10 mg/kg, reflex tachycardia appeared frequently, and if the dogs had not previously received lower doses of pethidine, this dose led to asystole with a sudden drop in mean pulmonary artery, central aortic and left ventricular pressures.

Similarly, in another study it was shown that when pethidine was given intravenously to dogs (8 mg/kg over 10 min and followed by infusion at the rate of 4 mg/kg/h for 20 min), it produced a marked decrease in stroke volume, cardiac output, mean blood pressure, right atrial pressure, mean pulmonary artery pressure and systemic vascular resistance, but no significant change in heart rate (Stanley et al., 1977).

The influence of pethidine on myocardial contractility in intact dogs both before and after sympathetic blockade and atropinisation has also been investigated (Sugioka et al., 1957). Pethidine at a dose of 5 mg/kg reduced myocardial contractility by 26% after sympathetic blockade. It was found that the circulatory response to intravenously administered pethidine was proportional to the dose and the rate of administration.

In a study in patients anaesthetised with N_2O and pethidine (2 mg/kg for induction and 1 mg/kg intravenously for maintenance), a marked reduction in cardiac output of 49%, decreased stroke volume, blood pressure and an increase in pulmonary vascular resistance was observed (Stanley and Liu, 1977).

However, recently a positive inotropic effect of pethidine on the myocardium was reported (Helgesin and Refsum, 1987; Helgesin et al., 1990); no mechanism was proposed.

1.6.3.1 Pharmacokinetics and pharmacodynamics

Many pharmacokinetic studies have been focused on the clinical pharmacokinetics and analgesic efficacy of pethidine (Mather and Meffin, 1978; Glynn and Mather, 1982). Two compartment pharmacokinetic models have been used to described pethidine distribution in young and old dogs (Waterman et al., 1990). It was found that after intravenous administration, blood drug concentrations declined in two phases with a rapid distribution half - life of approximately 2 min and a terminal elimination half - life of approximately 60 min. Pethidine pharmacokinetics were also studied in the pregnant rat and described by a physiological flow model (Gabrielsson et al., 1986). It was found that pethidine was rapidly distributed into highly perfused organs such as the brain and lungs.

In vitro studies suggest that the magnitude of myocardial depression caused by pethidine is proportional to the concentration of pethidine acting directly on the myocardium. For example, right ventricular papillary muscles from cats in solutions of 0.1, 1, 10, and 100 μg/ml of pethidine showed depression of a number of measurements of muscle mechanics at pethidine concentrations greater than 1 μg/ml. The depressant effects were concentration dependent, and at equi - analgesic doses, pethidine had a 100 - 200 fold greater effect in depressing the contractility of the myocardium than morphine, fentanyl and piritramide (Strauer, 1972).

Pethidine distribution in the tissues of different species has been reported. In man, dog and rat, the organs with the highest concentrations are the lungs, liver, kidneys and brain (May and Adler, 1962). The partition coefficient of pethidine between heart and blood of the near term sheep fetus was found to be approximately 2, and was significantly different from that in the immature fetus (Morishima et al., 1984). There were no toxic effects on blood pressure or heart rate when pethidine plasma concentrations were below 100 μg/ml.

In another study, it was found that after intravenous administration of pethidine (2.5 mg/kg) to pregnant sheep, a positive arterio - venous pethidine concentration gradient was present across the fetal brain for approximately 10 min (Szeto et al., 1980). This time required for plasma - brain equilibration may explain the poor correlation between pethidine concentrations in the umbilical cord and fetal respiratory depression.

In general, although pethidine can have quite profound effects on the myocardium, little is known about the kinetics of its uptake and elution in the heart.

1.7 Aims of the research

The central hypothesis of this thesis is that the time - course of myocardial drug concentrations is a major determinant of the time - course of the magnitude of myocardial drug effects. There have been few *in vivo* studies in either animals or man which have appropriately addressed this hypothesis. From the literature review it is apparent that the existing methods for *in vivo* myocardial drug pharmacokinetic and pharmacodynamic studies and modelling have involved many factors which influence myocardial drug disposition and drug effect, and have used many over - simplified assumptions. The most successful studies to date have used methods based on arterial and coronary sinus blood sampling and mass balance principles to determine myocardial drug uptake and elution (Selden and Neill, 1975; Horowitz et al., 1986).

Methods based on mass balance principles for the study of myocardial pharmacokinetics are relatively non - invasive and thus permit the simultaneous study of pharmacodynamics in subjects whose physiological status has not been altered excessively by interventions such as anaesthesia. The overall aim of the research work described in this thesis was to define the myocardial pharmacokinetics of selected drugs and to correlate these with simultaneously determined pharmacodynamics in conscious, unrestrained sheep.

The specific aims were:

1. To validate the method for regional pharmacokinetic studies by experimentally examining some prerequisites for the use of mass balance principles;

2. To use mass balance principles to study myocardial pharmacokinetics and the relationship between myocardial pharmacokinetics and pharmacodynamics after the short term intravenous use of drugs in conscious, unrestrained sheep. Three drugs, lignocaine, procainamide and pethidine were chosen for study because of their wide clinical use (1.6) as well as their physiochemical properties. Lignocaine is a basic amine with a pKa of 7.9 and intermediate lipophilicity; procainamide is also a basic amine with a pKa of 9.2 and low lipophilicity and pethidine has a similar pKa to procainamide but a high lipophilicity.

Figure 1.1 The effect of time differences between the measured drug concentration and the measured drug effect when the concentration - effect relationship is linear. For the purposes of illustration, a hypothetical time - course of drug concentrations was simulated in which the drug concentrations rapidly rose and fell in a manner similar to that which occurs in organs such as the heart after an intravenous bolus of a drug. In this instance a symmetrical peak was produced based on a binomial distribution, although *in vivo* organ drug concentrations after an intravenous drug bolus often rise at a greater rate than they fall (Upton et al., 1988a; 1990b).

Five alternative relationships between the time - course of drug effect and the drug concentrations described above were simulated. The simplest was a direct linear relationship between drug concentration and drug effect (relationship C), with the units of drug effect arbitrarily assigned as one half of the units of drug concentration. In relationships A and B, in addition to this linear relationship the drug concentrations lagged behind the drug effect by a long and short time interval, respectively. In relationships D and E, the drug effect lagged behind the drug concentrations by a short and long time interval, respectively.

The upper panel of this figure shows the time - courses of the drug concentrations and the 5 relationships governing drug effect. The lower panels marked A - E show plots of drug effect versus drug concentration for the 5 relationships described above, respectively. These are summarised below:

(A) Linear relationship - long lag between effect and concentration.

(B) Linear relationship - short lag between effect and concentration.

(C) Linear concentration - effect relationship.

(D) Linear relationship - short lag between concentration and effect.

(E) Linear relationship - long lag between concentration and effect.

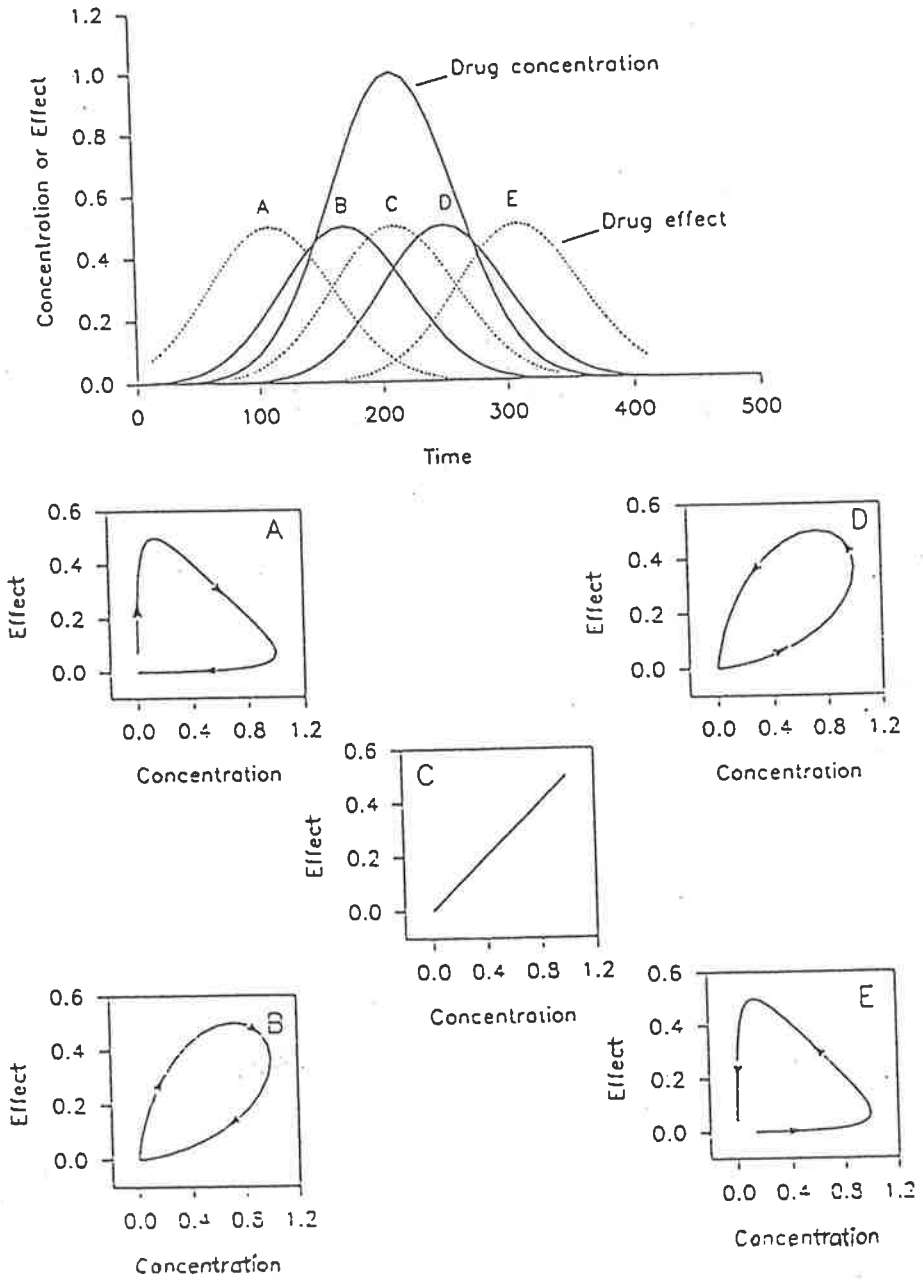

Concentration or Effect
Time
Drug concentration
Drug effect
A
B
C
D
E
Effect
Concentration

Figure 1.2 The effect of time differences between the measured drug concentration and the measured drug effect when the concentration - effect relationship is nonlinear.

As in Figure 1.1, a hypothetical time - course of drug concentrations was simulated in which the drug concentrations rapidly rose and fell. Five alternative relationships between the time - course of drug effect and the drug concentrations described above were simulated. In relationship C there were no time - differences between the drug concentration and drug effect, but the concentration - effect relationship was based on a maximum effect model:

$$\text{Effect} = E_{max} \times C / (E_{50} + C)$$

where E_{max} was the maximum drug effect (arbitrarily assigned as one half of the units of drug concentration in this case), E_{50} was the drug concentration at which the effect was half of E_{max}, and C was the drug concentration. In relationships A and B, in addition to this nonlinear relationship the drug concentrations lagged behind the drug effect by a long and short time interval, respectively. In relationships D and E, the drug effect lagged behind the drug concentrations by a short and long time interval, respectively.

The upper panel of this figure shows the time - courses of the drug concentrations and the 5 relationships governing drug effect. The lower panels marked A - E show plots of drug effect versus drug concentration for the 5 relationships described above, respectively. These are summarised below:

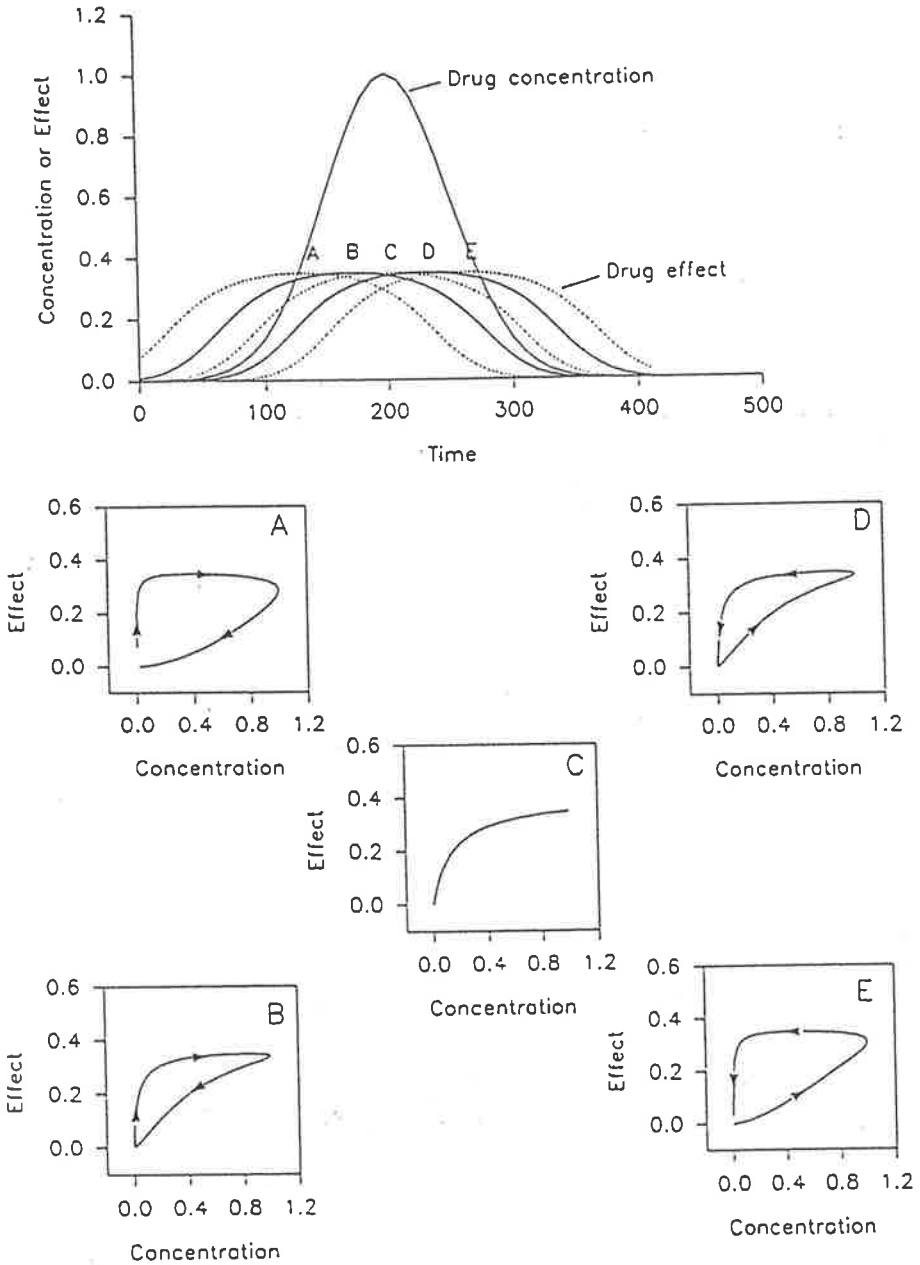
(A) Nonlinear relationship - long lag between effect and concentration.

(B) Nonlinear relationship - short lag between effect and concentration.

(C) Nonlinear concentration - effect relationship.

(D) Nonlinear relationship - short lag between concentration and effect.

(E) Nonlinear relationship - long lag between concentration and effect.


Concentration or Effect
Drug concentration
Drug effect
A B C D E
Time
Effect
Concentration
A
B
C
D
E

SECTION II: VALIDATION OF THE USE OF MASS BALANCE PRINCIPLES FOR REGIONAL PHARMACOKINETIC STUDIES IN THE SHEEP.

Introduction

From Chapter 1, it is apparent that the use of mass balance principles constitutes an indirect method for calculating regional drug disposition and requires a number of assumptions or criteria to be met (Upton et al., 1988a). In this section of the thesis, two previous inadequately validated assumptions were tested experimentally: 1. that all drug enters and leaves the region via vascular transport. 2. that after intravenous bolus drug administration, the time - course of the afferent and efferent drug concentrations of a region may be accurately characterised by a rapid blood sampling technique.

The general materials and methods used for studies in sheep and for the drug assays are presented in Chapter 2. The first assumption described above was tested in Chapters 3 and 4. In Chapter 3, the rate of non - vascular drug transport from the surfaces of organs of anaesthetised sheep was determined. In Chapter 4 the rate of drug transport from a region by the lymphatic system was determined, and the use of drug concentrations in lymph to provide insight into interstitial concentrations of drugs was discussed. The second assumption was tested in Chapter 5 in which the ability of several commonly used rapid blood sampling methods to characterise rapidly changing blood drug concentrations was examined, and a new rapid blood sampling method was described.

CHAPTER 2: GENERAL MATERIALS AND METHODS FOR STUDIES IN THE SHEEP.

2.1 Animal selection

Sheep were used because they were readily available, could be easily tamed and are relatively large compared to other commonly used experimental animals. The chronically catheterised sheep preparation was used as developed by Runciman et al., (Runciman, 1982; Runciman et al., 1984a; 1984b; 1984c) and as used for studies of systemic pharmacokinetics and regional pharmacokinetics and pharmacodynamics (Runciman et al., 1984b; 1984c; Nancarrow et al., 1987; Upton et al., 1988b; Rutten et al., 1990). Sheep have proven to be tolerant of the anaesthesia and surgery necessary for the catheterisation of multiple blood vessels, and it has been found that catheters can be satisfactorily maintained for up to three months, allowing multiple studies to be carried in conscious, unrestrained animals. Furthermore, sheep can be easily approached during experiments for drug administration, blood sample collection and the measurement of physiological parameters, and have sufficient blood volume for repeated blood sample collection during pharmacokinetic studies without significant decreases in their haemoglobin levels or alterations in their haemodynamic or pharmacodynamic status.

Whenever animals are used for experimentation, question arises as to what extent the pharmacokinetic and pharmacodynamic data (and conclusions) can be directly extrapolated to the human situation. Although the rates and routes of drug elimination differ between sheep and other species (Kao et al., 1978; 1979), it may nevertheless be argued that the basic mechanisms of drug distribution are a function of physiological processes (e.g. perfusion, diffusion and binding) that are essentially the same in all vertebrates. These processes are sufficiently poorly understood to justify detailed basic research in animals such as sheep. A solid knowledge of these basic mechanisms will provide a strong foundation from which to plan further research in humans, and may ultimately allow prediction of aspects of drug disposition in one species from data of another, or even from *in vitro* studies.

2.2 Preparation and catheterisation of sheep

Healthy, parasite free, female Merino sheep between 1 and 2 years old and weighing between 40 - 50 kg were supplied from a constant breeding stock by Mortlock Farm of the University of Adelaide. Prior to purchase, the haemoglobin types of the sheep were determined using the gel electrophoresis technique (Nancarrow, 1986). Only sheep of haemoglobin type A were used because these sheep have very similar oxyhaemoglobin dissociation characteristics to humans (Huisman and Kitchen, 1968).

Sheep were placed in mobile metabolic crates (Fig. 2.1) 1 to 2 days before the initial surgery, and were fasted on the day before surgery.

2.2.1 Anaesthesia

Procedures for anaesthetising sheep were the same for both the surgical placement of long - term blood vessel catheters and the acute experiments. The method used has been reported previously in detail (Runciman et al., 1984a). Briefly, anaesthesia was induced with sodium thiopentone (20 mg/kg) via a left jugular vein cannula (1) placed percutaneously just before the induction. Sheep were then intubated with a cuffed endotracheal tube (2). Anaesthesia was maintained with 1.5% halothane in 40% oxygen (balance nitrogen). Artificial respiration was kept at 20 breaths/min using a Mark 7 ventilator (3) and a circle system. The end expired carbon dioxide was monitored by an infrared carbon dioxide analyser (4) and maintained between 4 and 4.5%. An intravenous infusion of 0.9% saline (approximately 100 ml/h) was maintained through the left jugular vein cannula during the entire period of anaesthesia and surgery.

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1 14G, Intracath, Deseret Medical Inc., Sandy, UT, USA

2 Size 9; Portex Ltd., Hythe, England

3 Bird, Palm Springs, CA, USA

4 Normocap, Datex Instrumentarium Corp, Finland

2.2.2 Catheterisation

Catheters were placed for the purposes of drug administration, blood sample collection and the measurement of myocardial function and haemodynamic status. The general method of blood vessel catheterisation has been described in detail previously (Runciman, 1982; Runciman et al., 1984a). A brief description is given below. More details of specific methods for the catheterisation of blood vessels, such as the aorta, inferior vena cava, right atrium, pulmonary artery, and coronary sinus, will be described in the methods sections of the appropriate chapters.

Briefly, the neck was shorn and the right carotid artery and jugular vein were exposed using sterile technique through a right - sided longitudinal neck incision. Up to 3 catheters of various sizes, depending on the requirements for different studies, were inserted into the carotid artery using the Seldinger technique and advanced under fluoroscopic control until their tips were approximately 2 cm above the aortic valve. The same method was used to introduce catheters into the jugular vein, from which their tips were further advanced into one of the vessels of the venous system described below as appropriate: the inferior vena cava, midway between the level of entry of the renal veins and the confluence of the iliac veins (IVC catheter); the right atrium (RA catheter); the pulmonary artery (7F balloon tipped thermodilution catheter (5)). This catheter was used in conjunction with a cardiac output computer (6) for the measurement of cardiac output and the blood temperature of the sheep.

In all cases, the positions of the catheter tips were confirmed by fluoroscopic monitoring, often with the aid of the injection of radio - opaque dye (7) down the catheter to visualise the catheterised blood vessel.

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5 Swan - Ganz, Edwards Laboratories, Irvine, California, USA

6 Model 9520A, Edwards Laboratories Inc., Irvine, California, USA

7 Conray 420 (70% iothalamate), May and Baker Ltd., Dagenham, UK

In chronically catheterised sheep, catheters were secured using the method of Runciman et al., (1984a; 1984b). After the sheep had recovered from anaesthesia, they were extubated and transferred into their metabolic crates (Fig. 2.1). The catheters were connected through three - way stopcocks (8), extension lines and continuous flushing devices (9) to a gas powered flushing system which allowed each catheter to be flushed with heparinised 0.9% saline (5 i.u./ml) at a rate of 3 ml/hr. This prevented the blocking of the catheters due to local blood coagulation at their tips (Runciman et al., 1984a). This dose of heparin did not systemically heparinise the animals (Runciman, 1982). Methadone 10 mg in 1 ml was given subcutaneously for post - operative pain management. The chronically catheterised sheep were usually able to stand in their crates and begin eating and drinking 1 - 2 hr after surgery. Their food and water consumption usually returned to pre - catheterisation values 2 - 3 days after surgery.

2.2.3 Sheep maintenance and handling

After catheterisation, sheep were inspected at least once a day. They had free access to food and water, except during experiments. Their food and water consumption and urine output (separated from faeces by a wire mesh and collected in containers beneath the crate (Fig. 2.1)) were recorded. The flushing system was checked and catheters were given rapid flushes with heparinised 0.9% saline daily via the fast flush devices of the pressure infusors (Fig. 2.1 and Runciman et al., 1984a). Most catheters remained patent during the catheterisation period. If a catheter was found to be blocked, it was repeatedly flushed with sterile heparinised 0.9% saline using a 1 ml syringe (10). If this failed to unblock the catheter, a guide wire (11) was inserted down the lumen of the catheter, using sterile technique, until it was estimated that 1 - 2 cm of the wire

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8 Viggo Products, Helsingborg, Sweden

9 Intraflo, Abbott Laboratories, North Chicago, USA

10 Terumo, Melbourne, VIC, AUS

11 TSF - 38 - 145 - X, Cook Inc., Bloomington, IN, USA

had emerged from the tip of the catheter. If this failed, the sheep was no longer used for studies and was given a fatal dose of pentobarbitone intravenously.

Healthy sheep ate, on average, 1 kg of lucerne chaff a day and were alert. If an animal became ill, as evidenced by a drop in food intake and urine output and by a lethargic, miserable appearance or by a blood temperatures over 40°C, a blood sample was taken for bacterial culture. Arterial blood (10 ml) was collected using sterile technique and 5 ml of blood was injected into Bactec NR 6A and 7A bottles (12) for blood *in vitro* aerobic and anaerobic bacterial culture by the Department of Microbiology, Flinders Medical Centre. Sheep with positive blood cultures that failed to respond quickly to selective antibiotic therapy were also given a fatal dose of pentobarbitone intravenously.

Except for acute studies, chronically catheterised sheep in their metabolic crates were brought into the laboratory on the experimental days. Before any experiment, at least 20 min were permitted for them to adapt to the laboratory environment. It was shown in previous investigations (Runciman et al., 1982; 1984a) that they did not appear to be distressed during studies, generally showing no change in cardiac output, heart rate or blood pressure.

2.3 Drug studies

Lignocaine, procainamide and pethidine were selected for study. They were chosen mainly on the basis of their physicochemical properties and their therapeutic or toxic effects on the heart (see Chapter 1).

Drugs in their commercial forms for intravenous (i.v.) use (1% lignocaine hydrochloride (13), 10% procainamide hydrochloride (14) and pethidine hydrochloride (15)) were obtained from the Pharmacy Department of Flinders

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12 Becton Dickinson Diagnostic Instrument Systems, Towson, MD, USA

13 Xylocard, Astra Pharmaceuticals Pty Ltd, Sydney, NSW, AUS

14 Pronestyl, E.R Squibb and Sons, Noble Park, VIC, AUS

15 David Bull Laboratories, Mulgrave, VIC, AUS

Medical Centre. They were diluted in sterile mixing bottles to the desired concentrations with 0.9% NaCl for i.v. injection (16) immediately before drug administration and then withdrawn into 50 ml glass syringes (17). The syringes were connected to the infusion catheter using a 3 - way stopcock (18) and a 75 cm extension line (19) in acute studies. In chronically catheterised sheep, the extension lines were arterial pressure tubing (20) and a 75 cm extension line (19).

A Harvard single channel infusion pump (21) was used for all drug infusions. Before the start of an infusion, the catheter dead space was filled with the drug solution with a 2 ml syringe (10). After each experiment, the exact infusion rate was calculated from the total volume of drug solution delivered and the infusion time.

2.4 Blood sample handling

Blood samples from different sites were collected through the appropriate intravascular catheters. In acute studies, a three - way stopcock (22) was connected to the catheter. At the time of sampling, 2.5 ml of blood was withdrawn into a 5 ml syringe (10) to account for 1.2 ml of catheter dead space. Next, 0.5 ml of blood for drug assay was taken using a 1 ml syringe. Depending on the requirement of the assay, this blood was transferred into 1.5 ml Eppendorf microtubes (23) or 10 ml soda glass tubes (24) both of which contained 25 µl of heparin (1000 i.u./ml).

For studies in chronically catheterised sheep, blood samples were drawn from the intravascular catheters through a 75 cm extension line (19) and double stopcock (the

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16 Travenol Laboratories Pty. Ltd., NSW, AUS

17 "Top" Brand, Tokyo, Japan

18 Viggo Products, Helsingborg, Sweden

19 50 - 305, Tuta Laboratories, Sydney, NSW, AUS

20 91 cm, Sorenson Research, 4455 Atherton Drive, Salt Lake City, Utah, USA

21 Series 902A, Harvard Apparatus, Millis, MA, USA

22 Cobe Laboratories, Lakewood, CO, USA

23 Micro Test - tubes 3810, Eppendorf, Hamburg, FRG

24 GS10/W/Z/C, Johns Products, Oakleigh, VIC, AUS

"two stopcock" method). A volume (5 ml) was taken to account for the catheter and extension tube dead - space (2.5 ml). Blood samples for drug assay were taken from the proximal stopcock using a 1 ml syringe.

The development of a rapid sampling method for characterising the changes in blood drug concentration after i.v. bolus drug injection will be described in Chapter 5.

2.5 Drug assays

All samples were stored at -20°C until assayed.

2.5.1 Analytical equipment

Lignocaine and pethidine were assayed using an Hewlett Packard Model 5710A gas chromatograph (GC) with a nitrogen - phosphorous detector (25). Procainamide was assayed using an high pressure liquid chromatograph (HPLC) with a Waters Model 6000A pump, a Waters U6K injector, a Waters C18 reverse phase column, and either a Waters Model 450 variable wavelength UV detector (26) or a Beckman 160 UV absorbance detector (27).

Either a Hewlett Packard Integrator Model 3380A integrator or a Rikadenki chart recorder Model R - 02 were used to record the chromatograms.

2.5.2 Calibration

In each experiment, 10 ml of blood (blank blood) was collected from the sheep before drug administration and stored at 0°C. At the time of drug assay a standard curve was prepared by transferring 0.5 ml of this blank blood into five Eppendorf microtubes or glass tubes (as appropriate). To these tubes drug solution was added (less than 100 µl volume) to produce a range of final drug concentrations. A zero drug concentration sample was always used. These "standard curves" were always prepared to cover the anticipated range of drug

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25 5710 AGC, Hewlett Pakard, Palo Alto, CA, USA

26 Milipore Waters, Milford, MA, USA

27 Linear Instruments, Reno, Nevada, USA

concentrations. Drugs used as the internal standards for the assays (see 2.5.3) were diluted in water and added to the standard tubes in volumes of less than 50 µl. The reproducibility of assays was routinely assessed by assaying 10 samples from a pool of blood "spiked" to a known concentration. The coefficients of variation were always less than 5%. The standard curves were accepted only when the linear regression of a five concentration curve produced a r^2 value higher than 0.997 for any assay.

2.5.3 Assay methods

2.5.3.1 Lignocaine

Mepivacaine was used as the internal standard and the samples were collected in Eppendorf tubes. After the samples had thawed, 200 µl of 0.1N NaOH and 200 µl of toluene were added to the tubes. The tubes were mixed on a vortex mixer (28) for 1 min and the samples were centrifuged (29) at 15,000 rpm for 3 min. 0.5 to 2 µl of toluene was drawn off the sample using a 10 µl syringe (30) and injected into the gas chromatograph. The column was 2 m long by 2 mm internal diameter containing 3% OV17 on 100/120 mesh Gas - Chrom Q (31). The carrier gas was nitrogen at a flow rate of 30 ml/min. The oven, injector and detector temperatures were 230°C, 250°C and 300°C, respectively. The assay sensitivity was 0.005 µg/ml and the coefficient of variation was less than 5%.

2.5.3.2 Pethidine

Lignocaine was used as the internal standard and the samples were collected in Eppendorf tubes (23). The assay was similar to that used for lignocaine but the oven temperature was 220°C. The assay sensitivity was 0.005 µg/ml and the coefficient of variation was less than 5%.

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28 Thermolyne MaxiMix, Thermolyne Corp, IO, USA

29 Model 5412, Eppenforf, Hamburg, FRG

30 Scientific Glass Engineering, Melbourne, VIC, AUS

31 Alltech Australia Pty. Ltd., Homebush, NSW, AUS

2.5.3.3 Procainamide

N - proprionyl procainamide was used as the internal standard. The samples were collected into 10 ml soda glass tubes (24). 1 ml of carbonate buffer (pH 10.5) and 5 ml of diethyl ether were added to blood. The tubes were mixed on a vortex mixer (28) for 1 min and centrifuged (32) at 3,000 rpm for 5 min. The tubes were frozen in a dry ice - methanol bath and the organic layer decanted into tubes containing 200 µl of 0.5% w/v acetic acid. These tubes were mixed again and centrifuged and the organic layer discarded. 20 µl of the remaining acetic acid containing the drug was injected into the HPLC. The mobile phase was methanol : pH 5.5 acetic acid buffer (35:65) and the UV detector was set at 280 nm. The assay sensitivity was 0.02 µg/ml and the coefficient of variation was less than 5%.

The major metabolites of lignocaine, pethidine and procainamide, which have effects on the cardiovascular system, i.e. monoethylglycelxylidine (MEGX), norpethidine and N - acetylprocainamide, respectively, can also be detected by these assay methods if they present in the blood samples.

2.6 Data handling

Drug concentration - time data and other experimental parameters were entered into a spreadsheet program (33) run on an IBM(R) compatible personal computer. This was used to manipulate and calculate all data. Graphs were prepared and plotted using the Sigma - Plot™ (34) and a NEC Silentwriter 2 laser printer (35). Statistical analysis was performed by transferring the data to an interactive statistical analysis program, Statistix (36), also run on the IBM(R) compatable computer.

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32 Model GLC - 2B, Sorvall, DuPont Instruments, Newtown, CT, USA

33 SuperCalc 4, Computer Associates International Inc, San Jose, CA, USA

34 Jandel Scientific, 65 Koch Rd., Corte Madera, CA 94925, USA.

35 NEC Corporation, Tokyo, 183 Japan.

36 NH Analytical Software, MN, USA

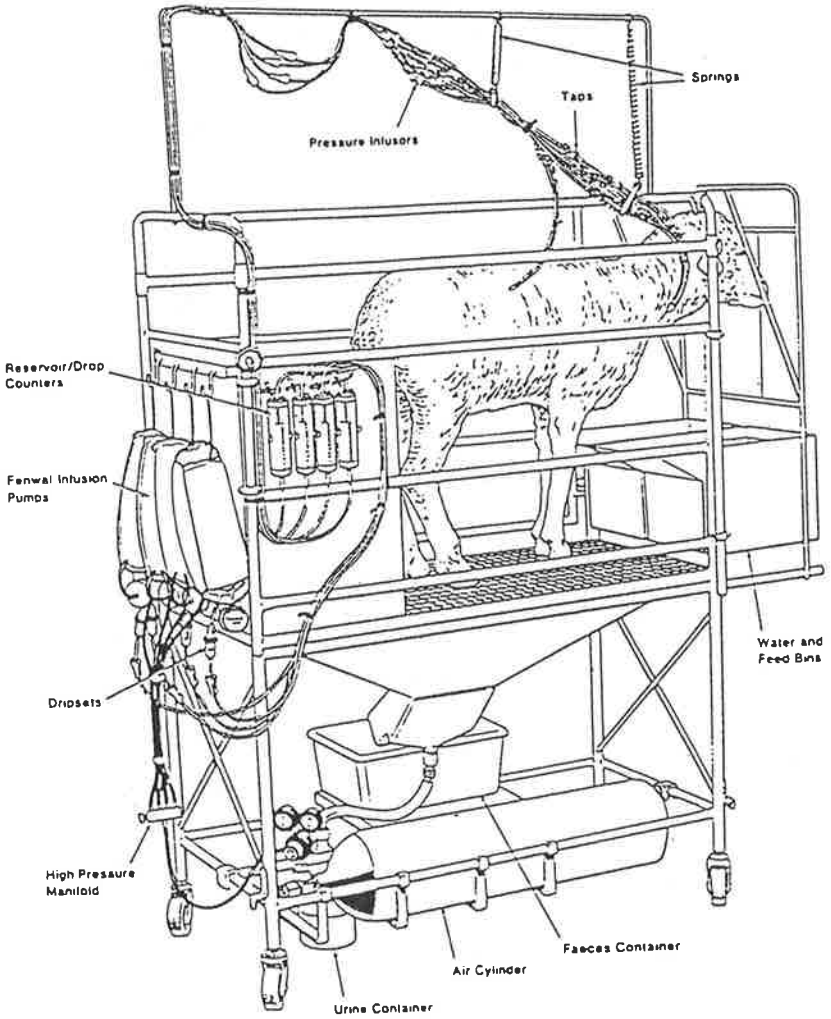

Figure 2.1

A chronically instrumented sheep and catheter flushing system in a metabolic crate.

CHAPTER 3: THE RATE OF DIFFUSION OF DRUGS FROM THE SURFACES OF ORGANS.

3.1 Introduction

The representation of organs as compartments is a method fundamental to pharmacokinetic modelling (Himmelstein and Lutz, 1979; Goth, 1984a). In most cases, compartments are defined as homogeneous pools of drug from which all molecules have an equal probability of entering or leaving (Segre, 1982). In physiologically based pharmacokinetic models, different organs are represented by compartments that are linked together in a manner determined by the circulatory system, and drug disposition is described by differential mass balance equations between compartments. Intrinsic to this use of compartments is the assumption that drug only enters or leaves the compartment in the afferent and efferent blood, respectively. This assumption is also implicit in regional pharmacokinetic studies that use mass balance principles (Horowitz et al., 1986; Upton et al., 1988a; Roerig et al., 1989). Although these methods do not require that the organ under study behaves like a compartment with respect to a drug, they do use differences between the mass of drug entering and leaving organs via afferent and efferent blood to calculate the mass of drug remaining in the organ. It is assumed that the movement of drugs between the surfaces of adjacent organs or tissues is negligible compared with that transported by blood. Thus, in both of these cases, it is assumed that the anatomical boundary of the organ is also the boundary of the organ with respect to drug movement.

However, there is some evidence that drugs are able to diffuse directly between adjacent organs. For example, the movement of highly diffusible gas between adjacent tissues has been documented (Perl et al., 1965), and the diffusion of lignocaine and glyceryl trinitrate down a concentration gradient between tissues has been shown *in vivo* (Bogaert, 1987; Singh and Roberts, 1990). Electrolytes and Rb^{86} have been reported to diffuse from the normally perfused myocardium into ischaemic myocardium and from the ventricular cavity into the myocardium (Jennings et al., 1964; Myers and Honig, 1966). Adenosine has be found to be able

to diffuse from the left ventricular wall into the surrounding medium (Watkinson et al., 1979; Hanley et al., 1983).

With respect to studies in this thesis, the quantification of the rate of diffusion of drugs from the surfaces of organs and tissues *in vivo* is therefore necessary for the valid use of mass balance principles. This is particularly so in studies of drug concentrations in the heart following the rapid intravenous injection of drugs because under these circumstances it would be expected that the concentration gradient between the surfaces of well and poorly perfused tissues (such as the myocardium and pericardial adipose tissue) would be large.

The aim of this study was to quantitate the rate and extent of the diffusion of drugs from the surfaces of two representative well perfused organs, the heart and kidney, under conditions which maximised the concentration gradient at the surface of the organs. To do this, methods were devised such that the heart and kidney could be surrounded by drug free saline during an intravenous drug infusion designed to achieve relatively constant blood and tissue drug concentrations. Thus, increases in drug concentration in the saline could be assumed to be due to the direct diffusion of drug out of the organ.

3.2 Methods

3.2.1 Anaesthesia

The experiments were acute, non - survival studies performed on anaesthetised sheep. Anaesthesia was induced and maintained as described in Chapter 2.

3.2.2 Catheterisation of blood vessels

Catheters were placed via the right carotid artery and jugular vein with their tips in the right atrium (7F, 60 cm polyethlene catheter(1)) and the ascending aorta (7F, braided angiographic catheter(2)) for drug administration and arterial blood

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1 William A. Cook, Sydney, NSW, AUS

2 Multi - purpose A1, Cordis Corporation, Miami, FL, USA

sampling, respectively. Details of the method of catheterisation have been presented in Chapter 2.

3.2.3 "Enveloping" the heart and kidney

The heart and the left kidney were "enveloped" so that they could be surrounded with a pool of saline that could be exchanged and sampled. For the kidney this envelope was a polyethylene bag which does not absorb drugs, while for the heart the pericardium was used as a natural envelope after ligating all blood vessels supplying the pericardium.

An incision on the left side of the abdominal wall, approximately 10 cm below and parallel to the costal margin was made to expose the left kidney. The perinephric fat was carefully dissected from the kidney. Damage to the kidney as evidenced by bleeding from its surface was carefully avoided. The kidney and a 5F polyethylene catheter (1) were placed into the polyethelene plastic bag and the open end of the bag was sealed using the bag's "Snap - lock(R)" seal around the pedicle of the kidney (Fig. 3.1 B).

The heart was exposed through bilateral thoracotomies at the 4th intercostal spaces. The pericardium was dissected from the retrosternal tissues and the diaphragm. All blood vessels of the pericardial tissue were ligated and a 5F polyethylene catheter (1) was inserted into the pericardial space through a small cut on the pericardium. A purse - string suture was then used to secure the catheter and seal the pericardial cut (Fig. 3.1 A).

3.3 Study design

Lignocaine, pethidine and procainamide were used as marker drugs. All are tertiary amines of similar molecular weight, but differ in pKa and lipophilicity (Upton et al., 1987). Each drug was studied in three different sheep.

3.3.1 Drug infusions

Lignocaine hydrochloride or pethidine hydrochloride were infused into the right atrium at 2.7 mg/min using the method described in Chapter 2. Procainamide

hydrochloride was infused into the right atrium at 5 mg/min. All drugs were infused for 300 min in each study.

3.3.2 Sample collection

At approximately 230 min after the start of the drug infusion, the pericardial and perinephric spaces were washed with 20 ml of 0.9% saline which was flushed through the catheters into the pericardial and perinephric spaces and then was drawn back and discarded. This was repeated until the aspirated saline was apparently clean. At 240 min after the start of the drug infusion, 15 ml of 0.9% saline was injected into the pericardial and perinephric spaces (designated time 0). This saline will be referred to as the artificial pericardial or perinephric fluid in subsequent discussion. Immediately after the addition of the saline, 0.5 ml of arterial blood and 0.5 ml of the artificial pericardial and perinephric fluid were collected simultaneously using 1 ml syringes (3). Further samples were collected at 5, 10, 15, 20, 25, 30, 45 and 60 min. The 0.5 ml samples were taken after first removing 5 ml of fluid to allow for the catheter dead space (2.5 ml), and the dead space was replaced after each sample collection. In addition, the arterial catheter was flushed with an additional 2.5 ml of 0.9% heparinised saline (1 i.u./ml) to prevent blood clotting inside the catheter. Samples were transferred into Eppendorf microtubes or 10 ml glass tubes preheparinised with 25 i.u. heparin sodium (4) and stored until assayed (see 2.5.3).

At the end of the studies, the sheep were sacrificed with a fatal over - dose of intravenous pentobarbitone and the heart and kidney were examined to make sure that there was no gross damage to the surfaces of the organs.

3.3.3 Data analysis

The rate of entry of the drugs into the artificial pericardial and perinephric fluids at a given time (t_n) was calculated as follows:

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3 Terumo Pty Ltd, Melbourne, VIC, AUS

4 David Bull Laboratory Ptd. Mulgrave, VIC, AUS

$$\text{Rate of entry } (\mu g/min) = \frac{(C_f \times V_f) - (C_{f.pr} \times V_{f.pr})}{\Delta t} \quad \ldots(3.1)$$

where C_f and V_f are the drug concentrations in artificial pericardial or perinephric fluid (μg/ml) and the volume of fluid in the artificial pericardial or perinephric space (ml), respectively at, time t. $C_{f.pr}$ and $V_{f.pr}$ are the equivalent values for the previous time point t_{pr} and Δt (min) is the time interval between the two samples ($t - t_{pr}$).

The maximum amounts of drug that entered the artificial pericardial and perinephric fluids (A_{max}) were calculated as follows:

$$A_{max} = C_{peak} \times V_{peak} \quad \ldots(3.2)$$

where C_{peak} is the peak drug concentration in the artificial pericardial or perinephric fluids, and V_{peak} is the artificial pericardial or perinephric fluid volume at the time of the peak concentration.

The general methods of data handling have been described (see 2.6). Kruskal - Wallis One Way Analysis of Variance was used to compare the rate of diffusion between the different drugs and $P < 0.05$ was considered statistically significant.

3.4 Results

3.4.1 The time - courses of pericardial and perinephric drug concentrations

The coefficients of variation of the drug concentrations in arterial blood during the last 60 min of drug infusions when the pericardial and perinephric samples were collected did not exceed 11 - 12% for all three drugs (Table 3.1). The arterial blood drug concentrations were therefore relatively constant and essentially at "steady - state". In general, the drug concentrations in the artificial pericardial and perinephric fluids increased from zero during the sampling period and in some cases themselves reached a plateau. For example, the drug

concentrations in artificial pericardial fluid in all but one experiment reached steady - state by the last 30 min of the sampling period. The exception was one procainamide study for which the coefficient of variation of the drug concentrations in this period was 20%. The perinephric fluid increased continuously and did not reach steady - state during the sampling period. This is particularly apparent in Fig. 3.2 which shows examples of the time - courses of the concentrations of the three drugs in arterial blood and artificial pericardial and perinephric fluids.

3.4.2 The rate of entry of drugs into the artificial pericardial and perinephric fluids

The time - courses of the rates of entry of drugs into the artificial pericardial and perinephric fluids are shown in Fig. 3.3. It is apparent that the maximum rates of the three drugs entering into pericardial fluid were reached within the first 5 - 10 min after the introduction of drug free 0.9% saline into the artificial pericardial and perinephric spaces. The maximum rate of these drugs entering the perinephric fluid were reached at approximately 5 - 30 min. The maximum rates of entry of each drug are shown in Table 3.2. The ratios of the maximum rates of drug entry to the simultaneous arterial blood drug concentrations were used to compare the rates of entry between the three drugs (Table. 3.3).

3.4.3 The amounts of drug that entered the artificial pericardial and perinephric fluids

The maximum amounts of drug that entered the artificial pericardial and perinephric fluids are shown in Table 3.4. Also shown in Table 3.4 are the maximum amounts of these drugs that entered the artificial pericardial or perinephric fluids expressed as percentages of the amounts of drug delivered during the last 60 min of intravenous drug infusion.

3.5 Discussion

In this study an *in vivo* preparation with which to study the rate and extent of drug exchange between the surface of an organ and fluid surrounding the organ was developed. In this preparation it is likely that the entry of drug into the artificial

pericardial and perinephric fluids was due to diffusion. However, the pericardium secretes a small amount of fluid into the pericardial space under normal physiological conditions. It is also possible that some exudate or plasma from the heart and kidney, due to the surgical procedures or introduction of saline, could introduce drug into the artificial pericardial and perinephric fluids. In a pilot study, this preparation was examined for possible leakage of blood into the artificial pericardial and perinephric fluids. 10 ml of 0.9% saline was introduced into both the artificial pericardial and perinephric spaces for 20 min and then sampled. The red blood cells (RBC) in the samples were counted by a automatic blood cell counting machine (5). This study was repeated 10 times. The number of RBC in the artificial pericardial and perinephric fluids was between 0 and 30 cells/10 ml. The whole blood RBC count of the sheep was 5.58×10^6/ml. Therefore the largest possible amount of blood in the 0.5 ml samples of the artificial pericardial and perinephric fluids during the 20 min was 0.0003 μl. This is approximately 0.00006% of the volumes of the artificial pericardial and perinephric fluids, and contamination with this amount of blood would not contribute significantly to the drug concentrations measured in these fluids.

Further evidence that the entry of drugs into the artificial pericardial and perinephric fluids was due to diffusion is provided by the relationships between the rates of drug entry into the fluids and the lipophilicities of the drugs. The rank order of lipophilicities of the drugs is pethidine > lignocaine > procainamide (Upton et al., 1987). It is known that drugs with higher lipophilicities passively diffuse more rapidly through biological membranes (Cho, 1976). In this study, after corrections for the different arterial blood drug concentrations, the maximum rates of diffusion of pethidine (for both artificial pericardial and perinephric fluid) and lignocaine (for the artificial pericardial fluid) were significantly higher than that for procainamide (Table 3.3) and were in agreement with their lipophilicities. It was also observed that the maximum rates of drug entry into the pericardial fluids were at time 0 or 10

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5 Coulter Counter T 660, Coulter Electronic Inc., Hialeah, FL, USA

min when the concentration gradient between the myocardium and pericardial fluid would be expected to be the greatest. This is also consistent with drug diffusion along concentration gradients. This was not observed for drug entry into the perinephric fluid (Table 3.2), which may have been due to the initial washing of the perinephric space removing drug from the surface of the kidney prior to the experiment. Furthermore, the pH of the blood, tissue and the artificial fluid will influence drug diffusion, but they were not measured.

The *in vivo* preparation presented in this chapter is equivalent to the *in vitro* preparations in which excised tissues are surrounded by a fluid containing drug (Lullmann et al., 1988). However, such an *in vivo* preparation has the advantage of using tissues that have a perfused capillary bed. In comparison, the diffusion distances of *in vitro* preparations are extremely long (and diffusion rates are thereby correspondingly slow).

In this study, the drugs were infused to "steady - state" arterial drug concentrations (Table 3.1) in order to achieve constant myocardial and renal tissue drug concentrations. This maximized the initial drug concentration gradients between the surfaces of the organs and the surrounding fluids. Under such circumstances, drugs rapidly entered the artificial pericardial and perinephric fluids (Fig. 3.2) - the rates of entry reached their peak within the first 5 - 30 min of sample collection for all three drugs (Fig. 3.3, Table 3.2). During the last 30 min of sample collection, the rates of drug entry decreased or remained stable and were essentially minimal as the drug concentration gradients and therefore the driving forces for drug diffusion across the surfaces of the organs were reduced. Negative rates were even observed, which might suggest drug diffusion from the artificial fluid into other surrounding tissues.

The final concentrations of all three drugs in the artificial pericardial and perinephric fluids at the end of the sampling period were relatively high (Fig. 3.2). A fundamental implication of this finding is that areas of low or no perfusion within the body can ultimately achieve relatively high drug concentrations if they are adjacent to an area of high drug concentration. For example, this may provide a mechanism

by which drugs enter infarcted myocardium from the surrounding healthy myocardium (Zito et al., 1981), as reported for electrolytes and Rb^{86} (Jennings et al, 1964; Myers and Honig, 1966).

In pharmacokinetic terms, representing organs as compartments in which the anatomical boundaries of the organ also represent a barrier for drug movement, is not appropriate especially for poorly perfused organs or tissues. In practical terms, this has greatest implications for regions of low drug concentration and perfusion (represented by the artificial pericardial and perinephric fluids in this study) in which such direct diffusion may make a substantial contribution to the resultant drug concentrations.

The use of mass balance principles for well perfused organs such as the heart and kidney, and their representation as compartments in physiological models, is less affected by these observations. For example, the amounts of the three drugs that entered the artificial pericardial and perinephric fluids were only approximately 0.01% to 0.03% of the total amount of the drugs administered during the sampling period. Furthermore, it is known from our previous studies that the coronary artery and renal blood flow (one side) in anaesthetised sheep weighing 40 - 50 kg are approximately 140 and 200 ml/min, respectively (Runciman et al., 1985; Mather et al., 1986a; Chapter 8). The approximate rates that the drugs entered the heart and kidney via the arterial blood can be calculated from the product of coronary and renal blood flow and the arterial blood drug concentrations. The ratios of the maximum rates of diffusion of the three drugs into the artificial pericardial and perinephric fluids to those rates of drug delivery by arterial blood into these organs are shown in Table 3.5. These values range from 0.11 - 1.5 % and are essentially negligible. It is concluded that vascular transport is the predominant mechanism by which drugs enter and leave the heart and kidney, and that the use of mass balance principles to study drug uptake and elution in these organs is valid.

Table 3.1 The drug concentrations (μg/ml) in arterial blood, and in the artificial pericardial and perinephric fluids during the last 60 min of the drug infusions (mean ± S.D., n = 3).

	Study 1	Study 2	Study 3
Arterial blood			
Lignocaine	1.7 ± 0.1	2.5 ± 0.1	3.0 ± 0.4
Procainamide	7.4 ± 0.8	7.1 ± 0.6	12.8 ±1.2
Pethidine	1.2 ± 0.2	1.5 ± 0.1	1.5 ± 0.2
Artificial pericardial fluid			
Lignocaine	1.4 ± 0.1	1.9 ± 0.2	2.3 ± 0.3
Procainamide	4.6 ± 0.9	6.3 ± 0.8	11.5 ± 1.0
Pethidine	1.1 ± 0.0	1.3 ± 0.1	1.2 ± 0.1
Artificial perinephric fluid			
Lignocaine	1.0 ± 0.3	1.2 ± 0.2	1.3 ± 0.2
Procainamide	0.3 ± 0.0	5.9 ± 1.1	1.4 ± 0.5
Pethidine	0.9 ± 0.2	1.4 ± 0.0	1.0 ± 0.2

Table 3.2 The maximum rates of entry of the three drugs into the pericardial and perinephric fluids and the times at which these maximum rates occured (mean S.D., n = 3).

	Pericardial fluid		**Perinephric fluid**	
	Maximum rate (μg/min)	Time (min)	Maximum rate (μg/min)	Time (min)
Lignocaine	2.9 ± 0.9	5 ± 0	1.0 ± 0.8	23 ± 3
Procainamide	4.9 ± 2.2	5 ± 0	1.2 ± 1.3	7 ± 3
Pethidine	2.2 ± 1.0	7 ± 3	1.7 ± 1.3	15 ± 13

Table 3.3 The ratios of the maximum rate of drug entry into the pericardial or perinephric fluid and the simultaneously measured arterial blood drug concentrations (Mean S.D., n = 3).

	Pericardial fluid	**Perinephric fluid**
Lignocaine	1.27 ± 0.45[a]	0.41 ± 0.27
Procainamide	0.56 ± 0.23	0.15 ± 0.20
Pethidine	1.46 ± 0.48[a]	1.24 ± 1.01[a]

[a]Significantly higher than that of procainamide (Kruskal - Wallis One Way Analysis of Variance).

Table 3.4 The maximum amounts of the three drugs that entered the artificial pericardial and perinephric fluids (mean S.D., n = 3) during the last 60 min of the drug infusions, and these amounts expressed as percentages of the total amounts of drug infused (mean S.D., n = 3) in the same period.

	Pericardial fluid		Perinephric fluid	
	μg	% of infused	μg	% of infused
Lignocaine	21.5 ± 5.6	0.01 ± 0.00	13.5 ± 2.1	0.01 ± 0.00
Procainamide	86.0 ± 41.7	0.03 ± 0.01	28.8 ± 33.7	0.01 ± 0.01
Pethidine	13.8 ± 1.2	0.01 ± 0.00	12.6 ± 2.8	0.01 ± 0.00

Table 3.5 The maximum rates at which the drugs entered the artificial pericardial and perinephric fluids expressed as percentages of the calculated rates at which the drugs entered the organs via arterial blood* (Mean S.D., n = 3).

	Heart	Kidney
Lignocaine	1.17 ± 0.29%	0.16 ± 0.10%
Procainamide	0.66 ± 0.30%	0.11 ± 0.16%
Pethidine	1.50 ± 0.68%	0.59 ± 0.45%

*Based on the knowledge that coronary artery blood flow rate and renal artery blood flow rate (one side) in anaesthetised sheep are 140 and 200 ml/min, respectively (Runciman et al., 1985; Mather et al., 1986a; Chapter 8).

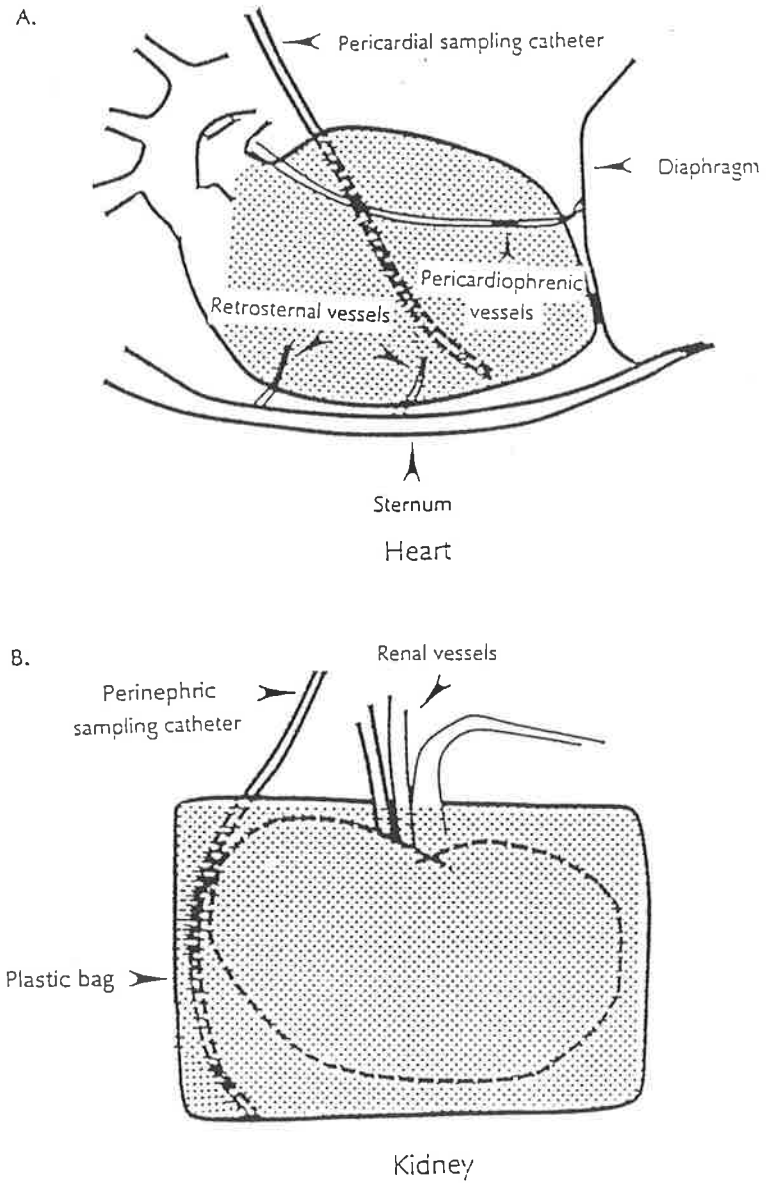


Figure 3.1

(A) The pericardial space inside the pericardium (shaded area). The blood vessels supplying the pericardium were ligated and a sampling catheter was inserted into the pericardial space as shown.

(B) The perinephric space (shaded area) created by putting the kidney and a sampling catheter into a polyethylene bag. The opening of the bag was sealed around the renal vessels.

Figure 3.2

Examples of the time - courses of the concentrations of lignocaine (A), procainamide (B) and pethidine (C) in arterial blood (open circles), artificial pericardial fluid (open triangles) and perinephric fluid (open squares) after the addition of drug free 0.9% saline into the pericardial and perinephric spaces at time 0.

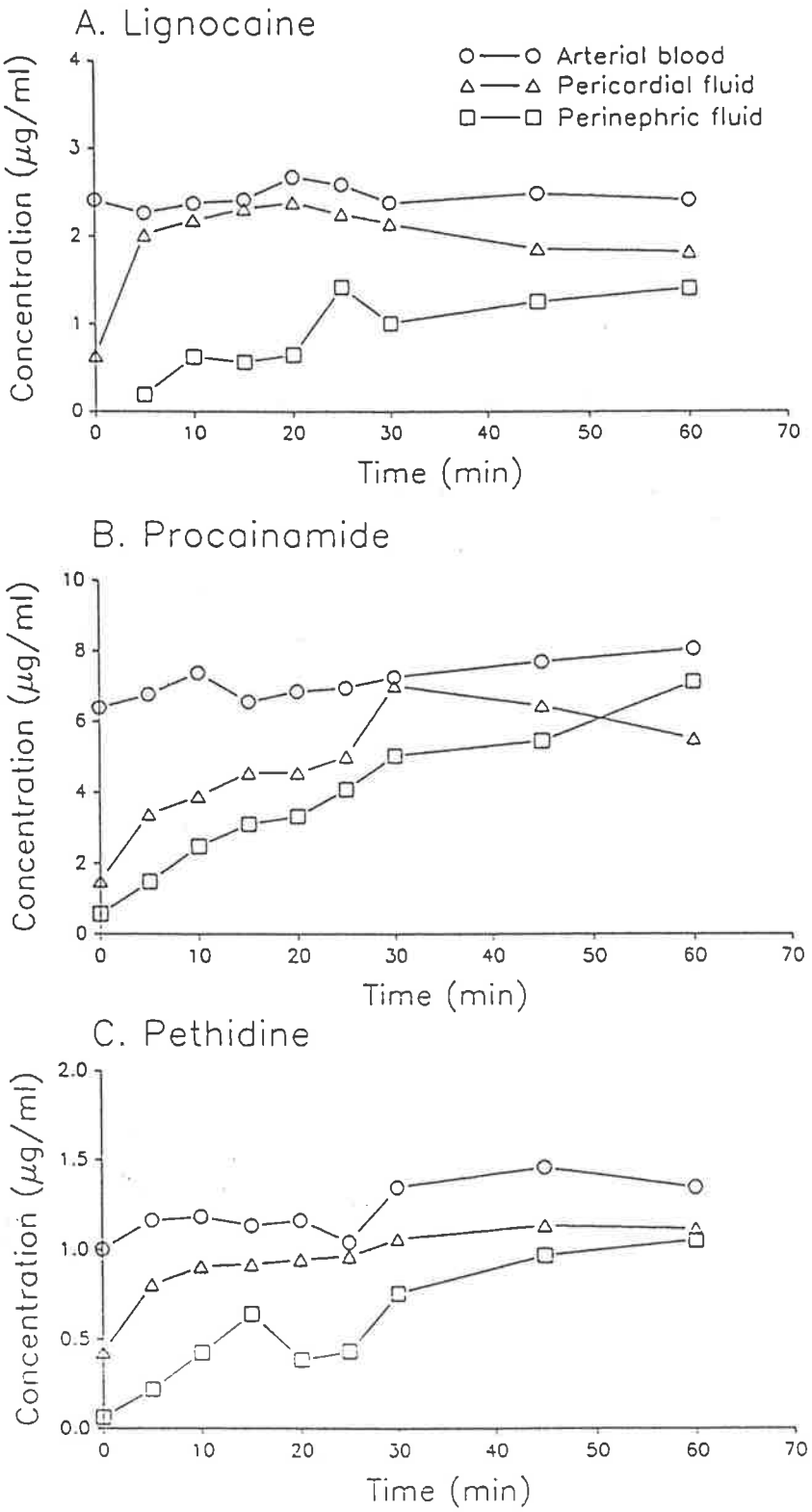
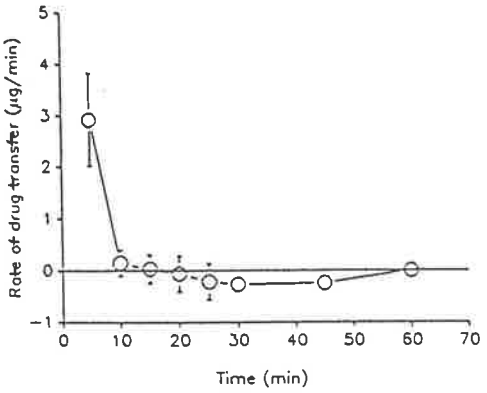

A. Lignocaine
Arterial blood
Pericardial fluid
Perinephric fluid
Concentration (μg/ml)
Time (min)
B. Procainamide
Concentration (μg/ml)
Time (min)
C. Pethidine
Concentration (μg/ml)
Time (min)

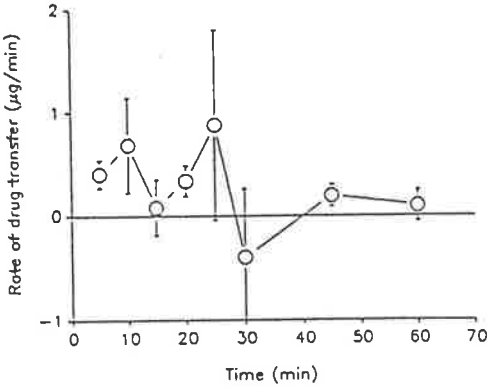
Figure 3.3

The time - courses of the mean (± S.D., n = 3) of rates of entry of lignocaine, procainamide and pethidine from the surface of the myocardium into the artificial pericardial fluid (A, B and C respectively) and from the surface of the kidney into the perinephric fluid (D, E and F respectively).

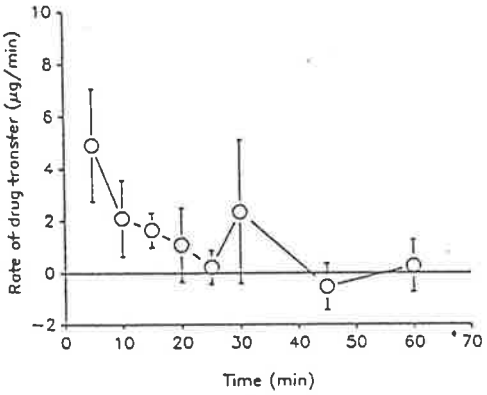
A. Lignocaine



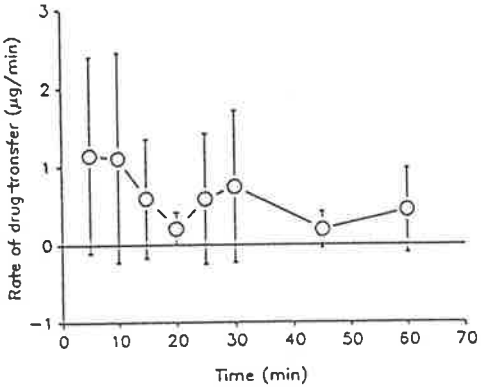
D. Lignocaine



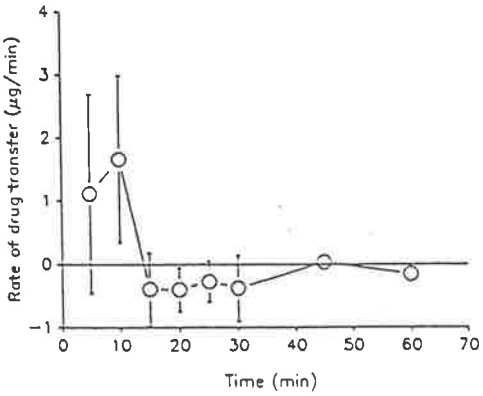
B. Procainamide



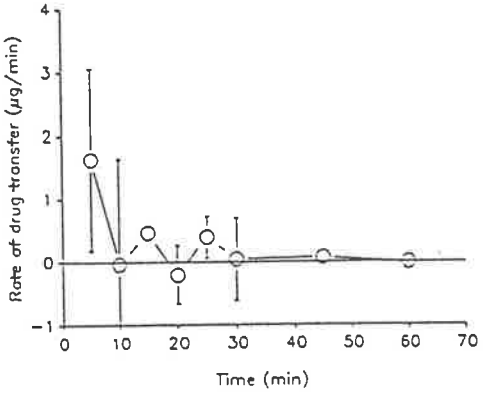
E. Procainamide



C. Pethidine



F. Pethidine



CHAPTER 4: DRUG TRANSPORT BY LYMPH AND LYMPH DRUG CONCENTRATIONS AS AN INSIGHT INTO INTERSTITIAL DRUG DISPOSITION.

4.1 Introduction

The lymphatic system exists in almost all tissues and organs of the body. Like the capillary and venous system, the lymphatic system starts with lymphatic capillaries from the interstitial space of the tissue (Fig. 4.1.) These lymphatic capillaries combine to form collecting lymphatics and then lymph trunks, and finally, by the right lymph duct and thoracic duct, lymph is carried from tissues and organs back to the blood circulation (Guyton, 1986a). Lymph flow is approximately one tenth of the effective flow of fluid into the interstitial space through the arterial capillaries, with the remainder being reabsorbed further "down stream" in the microcirculation. In a previous application of mass balance principles, it was assumed that the amount of drug carried by lymph from a tissue or an organ was negligible compared to that carried by venous blood (Upton et al., 1988a; 1988b). This was not unreasonable because lymph flow is much slower than blood flow, and the protein content of lymph is lower than that in blood. An aim of the studies reported in this chapter was to test this assumption experimentally.

However, the study of drug concentrations in lymph is also of interest because in physiologically normal tissues or organs, the composition of lymph closely resembles that of interstitial fluid. Interstitial drug concentrations are relevant to studies of both pharmacokinetics and pharmacodynamics. The interstitial space can be a large volume for drug distribution within the "tissue compartment" (Lullmann et al., 1979). More importantly, many drugs exert their effects by interacting with receptors on the surfaces of parenchymal cells (Goldstein et al., 1974) which are surrounded by interstitial fluid. The occupancy of these receptors and the effects of these drugs will therefore be a function of the interstitial drug concentrations.

It would seem that the difficulty of implementing methods for studying interstitial drug concentrations has, to date, precluded appropriate experimentation (Holm,

1978). Therefore a method was developed for cannulating the main lymph vessel draining the hindquarters of sheep (Upton et al., 1988b; Walsh, 1968), and this was used to study the kinetics and binding of lignocaine, procainamide and pethidine in the interstitial space of the hindquarters of sheep. These drugs were selected for the reasons given in Chapters 1 and 3.

The specific aims of the studies described in this Chapter were as follows: 1. To determine whether the rate of drug transportation by lymph is negligible compared to that transported by blood; 2. To determine the relationship between arterial and regional venous blood and lymph drug concentrations. 3. To determine the binding of these drugs in lymph and blood; 4. To determine the effects of general anaesthesia on the lymph and blood drug concentrations.

4.2 Methods

4.2.1 Definition of the hindquarters of sheep

The hindquarters of sheep is defined as the non - visceral region perfused by the abdominal aorta distal to the branching of the renal arteries, and drained by the IVC distal to the sites of entry of the renal veins. This is a 10 - 20 kg region, composed mainly of muscle, bone, fat and skin in the approximate proportions of 50, 20, 20 and 10% by weight, respectively (Upton et al., 1988b).

4.2.2 Catheterisation of blood vessels

Studies were conducted in both chronically catheterised conscious, unrestrained sheep, and in acutely prepared anaesthetised sheep. In both cases, sheep were initially anaesthetised and catheterised with an IVC catheter as described in Chapter 2 (see 2.2). An additional 7F multi - purpose A1 catheter (1) was placed in the abdominal aorta via a retroperitoneal incision with its tip located proximal to the branching of the iliac arteries. A modified 60 cm 7F polyethylene catheter (2) was placed in the right atrium through the right jugular vein (see 2.2) for drug

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1 Cordis Corporation, Miami, FL, USA

2 William A. Cook, Sydney, NSW, AUS

infusion in chronically catheterised sheep. For acute studies in anaesthetised sheep, a Vialon catheter (3) was used for this purpose.

4.2.3 Lymph vessel catheterisation and maintenance

At the time of surgery, a para - lumbar incision was made to expose the lumbar lymph vessel draining the hindquarters. This lymph vessel lies in close proximity and parallel to the abdominal aorta and IVC in the retro - peritoneal space. It was carefully dissected from the surrounding adipose tissue and then ligated proximally. If there were other tributary lymph vessels from the hindquarters present, they were ligated with silk ligatures. The main lymph vessel from the hindquarters was cannulated distally using the Seldinger technique with a 30 cm 4F pre - heparinised polyethylene catheter (2), below the level of the renal vessels and the chyle cyst. The catheter was tied to the lymph vessel after passing two silk ligatures round the vessel. In chronically catheterised sheep, a catheter clamp (4) was used to secure the catheter to the adjacent lumbar muscle. The incision was closed and the lymph vessel catheter was led out of the sheep through the incision. The correct placement of the catheter was confirmed by the absence of emulsified fat in the lymph and by the appearance of Patent Blue Dye (5) in the lymph, approximately 3 to 5 min after the injection of 3 ml of the dye into the leg muscles.

In chronically catheterised sheep, the lymph catheter was connected to 140 cm extension tubing (6). The lymph was allowed to flow continuously and was collected via the catheter into a sterile 1000 ml preheparinised, empty infusion bag which was hung outside the metabolic crate (Fig. 2.1), below the level of the abdomen. The collected lymph was re - infused into the IVC of sheep after each study. On some occasions, the lymph catheter was found to be blocked by lymph clots. Attempts were made to unblock the catheters using methods similar to

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3 14G, Intracath, Deseret Medical Inc., Sandy, UT, USA

4 Arrow International, PA, USA

5 Laboratorie Guerbet, Aulnay - Sous - Bois, CEDES, France

6 Tuta Laboratories, Sydney, NSW, AUS

those described for blood catheters in Chapter 2 (see 2.2), but these were not often as successful as those for blood vessel catheters.

4.2.4 Study design

Before each experiment 5 ml of lymph and arterial blood was collected for determination of protein content by the Diagnostic Laboratories of Flinders Medical Centre.

4.2.4.1 *In vivo* studies

Drug kinetics in anaesthetised sheep

With sheep under 1.5% halothane anaesthesia (see 2.2), right atrial infusions of lignocaine hydrochloride or pethidine hydrochloride (each at 2.7 mg/min), or procainamide hydrochloride at 5.0 mg/min, were given for 220 min. These infusion rates were used because they have been shown not to produce haemodynamic changes in conscious, unrestrained sheep (Upton et al., 1988b; unpublished data). Arterial and venous blood samples (0.5 ml each) for drug assay were taken from the abdominal aorta and IVC catheters every 20 min for the duration of the infusion. These samples were collected into 1.5 ml Eppendorf microtubes (7) or 10 ml glass tubes containing 25 i.u. of sodium heparin. Lymph flowed from the catheter spontaneously, and samples were collected during the first 10 min of each blood sampling interval into 10 ml pre-heparinised polypropylene tubes (8). The volume of lymph collected during the study period was recorded. Lymph flow rate was determined by dividing the collected lymph volume by the time of lymph collection. All samples were stored and assayed as described in Chapter 2 (see 2.5). Studies were performed in three different sheep for each of the drugs.

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7 Micro Test - tubes 3810, Eppendorf, Hamburg, FRG

8 Disposable Products, AUS

Drug kinetics in conscious, unrestrained sheep

For studies in conscious, unrestrained sheep, at least 2 to 3 days were allowed for these sheep to recover from surgery. Lignocaine and procainamide were infused as described above, but the infusion rate of pethidine was increased to 6.6 mg/min after a pilot study showed that the infusion rate used in anaesthetised sheep produced blood drug concentrations that were too low for accurate measurements. Arterial and IVC blood samples were collected, as described above, every 20 min until 180 min after the end of the infusion. As before, lymph samples were collected during the first 10 min of each blood sampling interval. The volume of lymph collected was recorded and the flow rate calculated. The samples were treated and assayed as described above. During the study periods, the sheep were standing in their metabolic crates and could move and eat freely. The three drugs were studied in each of three sheep. At least 36 hr were allowed to lapse between each study in each sheep.

4.2.4.2 *In vitro* drug binding in blood and lymph

A study of drug binding in blood and lymph was performed *in vitro* using the three compartment equilibrium dialysis method described by La Rosa et al., (1984b).

Collection of blood and lymph samples

These samples were collected from chronically catheterised sheep that had not received any drug for at least 3 days. Arterial blood (20 ml) was collected from the catheter placed in the abdominal aorta using a preheparinised 20 ml syringe (9) and allocated into two 10 ml polypropylene tubes (8) containing lithium heparin. Sodium fluoride (20 mg, (10)) was added to each 20 ml of blood to stop metabolism in red blood cells. 20 ml of hindquarter lymph was collected into the same type of polypropylene tubes.

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9 Terumo, Melbourne, VIC, AUS

10 AJAX Chemicals Ltd., Sydney and Melbourne, AUS

The pH of the blood and lymph was adjusted to 7.4 with 0.1% HCl or 0.1% NaOH immediately before dialysis.

Buffer preparation

Sorensen's 0.1 M phosphate buffer (pH 7.4) was prepared by diluting 1.79 g KH_2PO_4 and 7.6 g Na_2HPO_4 in 1 L of filtered distiled water. 100 ml of this buffer was put into six different glass containers. Small volumes of lignocaine, procainamide or pethidine solutions were then added to achieve final drug concentrations of 5 and 15 μg/ml for each drug.

Equilibrium dialysis and calculation of drug binding

The three cell dialysis chambers were assembled (La Rosa et al., 1984b) with prewashed dialysis membranes (11). Buffer containing drug (2 ml) was placed into the central cell using 2 ml syringes (10), and blood and lymph were placed in the cells on each side of the central cell. Dialysis was performed at 37°C for 6 hr in an oscillating water bath. It has been shown that this equilibration time is appropriate for studies of pethidine and lignocaine binding in blood and plasma (La Rosa et al., 1984a; 1984b; Nancarrow, 1986). This dialysis time was also adopted for procainamide in order to compare binding under the same conditions. After dialysis, 0.5 ml of blood, buffer and lymph were collected from the cells using 1 ml syringes (10) and transferred into 1.5 ml Eppendorf microtubes (7) or 10 ml glass tubes, and stored for drug assay (see 2.5). These studies of drug binding were repeated 3 times for each drug.

The proportions of drug bound in blood or lymph were calculated using the following equation:

$$\text{Percentage of drug bound} = \frac{C_{bl} - C_{buf}}{C_{bl}} \times 100\% \qquad \ldots(4.1)$$

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11 Union Carbide Corporation, Films - Packaging Division, Chicago, Illinois, USA

Where C_{bl} is drug concentration in either blood or lymph and C_{buf} is drug concentration in buffer.

4.3 Data analysis

The arterial and IVC blood and the lymph drug concentrations during the last 100 min of the infusions were used for statistical analysis. Paired t - tests were used to compare simultaneously measured arterial, venous and lymph drug concentrations either in anaesthetised or in conscious, unrestrained sheep. Two sample t - tests were used to compare the results between studies in anaesthetised and conscious, unrestrained sheep. $P < 0.05$ was considered statistically significant.

4.4 Results

4.4.1 Lymph flow rate and protein content

The mean ± SD, (n = 9) of the hindquarter lymph flow rate in the anaesthetised sheep was 11.4 ± 5.6 ml/min. This was considerably lower than the value of 31.0 ± 12.2 ml/min in the conscious, unrestrained sheep. The total protein and albumin concentrations in lymph were approximately 50 and 70%, respectively, of those in plasma (Fig. 4.2). There were no significant differences in the concentrations of protein in blood or lymph between anaesthetised and conscious, unrestrained animals (Fig. 4.2).

4.4.2 Blood and lymph drug concentrations

Examples of the time - courses of lignocaine, procainamide and pethidine concentrations in the arterial and IVC blood and hindquarter lymph of both anaesthetised and conscious, unrestrained sheep are shown in Fig. 4.3. The mean ± SD (n = 15; Five samples of arterial, venous blood and lymph, respectively, were collected in each study during the last 100 min of drug infusions, therefore, generating 15 samples for each of them in three studies) of lignocaine, procainamide and pethidine concentrations in arterial, IVC blood and lymph during the last 100 min of the infusions are shown in Fig. 4.4. During this period in the anaesthetised sheep the mean ± SD of lignocaine, procainamide and pethidine concentrations in IVC blood were, respectively, 32 ± 17, 30 ± 19 and 18 ±

8% higher than in the lymph ($P < 0.05$). In the conscious, unrestrained sheep, the mean concentration in IVC blood was 11 ± 17% higher than in lymph for lignocaine ($P < 0.05$), the mean concentration of procainamide in IVC blood was not statistically different to that in lymph, and the mean pethidine concentration in IVC blood was 23 ± 15% lower than that in lymph ($P < 0.05$).

In anaesthetised sheep, the mean arterial and IVC blood and the lymph concentrations of lignocaine were, respectively, 69, 73, 49% higher than those in conscious, unrestrained sheep. The corresponding values for procainamide were 179, 155 and 104%. Such a comparison was not possible for pethidine because a higher infusion rate was used in the conscious studies than in the anaesthetised studies (see 4.2).

4.4.3 The rate of drug transport by lymph

The products of the mean lymph drug concentrations during the last 100 min of the infusions and the mean lymph flow rates were used to calculate the mean rates at which the drugs were transported from the hindquarters by lymph (Table 4.1). These rates were then expressed as percentages of the arterial drug influx into the hindquarters (i.e. the products of mean arterial blood drug concentrations and hindquarter blood flows) and venous drug efflux from the hindquarters (Table 4.1). It is apparent from this table that the rates at which lignocaine, procainamide and pethidine were transported by lymph were less than 0.1% of the drug influx or efflux in the arterial or venous blood.

4.4.4 Effect of anaesthesia on drug concentrations

Anaesthesia significantly decreased lymph/arterial blood and lymph/venous blood drug concentration ratios during the last 100 min of the infusions of the three drugs when compared to those ratios in conscious, unrestrained animals (Fig. 4.5).

4.4.5 Drug binding

The binding of lignocaine, procainamide and pethidine in lymph (Fig. 4.6) was not significantly different to that in blood for both initial buffer concentrations, with

the exception of lignocaine at an initial buffer concentration of 5 μg/ml. In this case, the binding in lymph was 12% higher than that in blood ($P < 0.05$).

4.5 Discussion

The method of collecting lymph was based on the anatomy of the lymph system from the hindquarters of sheep (May, 1964) and that used by Walsh (1968) for studying the concentrations of vasoactive substances in the hindquarters of sheep and was relatively easy and reliable. It proved suitable for long term studies if the lymph was collected aseptically and reinfused to prevent fluid, electrolyte and protein loss.

4.5.1 Validation of mass balance principles

The assumption that the rate of drug transport from a region by lymph drainage is negligible compared to that transported by the blood (Upton et al., 1988a) was experimentally tested. The results of this study showed that the rate of drug transported in lymph accounted for only 0.02 to 0.07% of the rate of transport by either arterial or venous blood (Table 4.1). The validation of this assumption adds weight to the proposed usefulness of the application of mass balance principles to pharmacokinetics (Upton et al., 1988a).

4.5.2 Lymph drug concentrations as an insight into interstitial drug disposition

The use of the hindquarters of the sheep for studying the disposition of drugs in a representative "extra-visceral" region of the body (i.e. muscle, fat, skin and bone) has been demonstrated (Upton et al., 1988b). These "non - visceral" regions are quantitatively important in determining the rate and extent of drug distribution in the body.

Several studies have shown that the lymph draining from a region is representative of the interstitial fluid of that region. For example, it was concluded from studies of the biochemical composition and the anatomical structure of lymph vessels that lymph and tissue fluid were identical (Taylor and Gibson, 1975), and that lymph represented a cross-section of the tissue fluid contained in the area concerned (Drinker and Field, 1931). It was also concluded from the study of the transport

of large molecules from plasma to interstitial fluid and lymph that the barrier to blood - lymph transport is at the blood capillary wall, not at the lymphatic capillary or within the interstitial space (Garlick and Renkin, 1970). A study of the anatomical structure of connective tissue and lymphatics showed that the interstitial space is divided into two parts by a connective tissue capsule (the epimysial capsule) - the intracapsular part surrounding the parenchymal cells of the tissue and the adjacent extracapsular part containing the lymph vessels (Rodbard, 1975). Therefore, lymph is at least representative of the extracapsular interstitial fluid, and should closely resemble the intracapsular interstitial fluid. It is therefore reasonable to view the lymph drug concentrations as representative of the drug concentrations in the interstitial fluid around the parenchymal cells of the tissue.

It has been suggested that a preparation for measuring drug concentrations in lymph draining different tissues would be an almost ideal model for the study of drug distribution in the interstitial space (Holm, 1978). Despite reports of the cannulation of lymph vessels in physiological studies (Bell et al., 1983), there have been few reports of measurements of lymph drug concentrations. Most studies of this nature have concerned the extravascular disposition of antibiotics in the lungs. The interpretation of the relationships between blood and lymph drug concentrations in many of these studies is complicated by the fact that the blood sampling sites were not related, pharmacokinetically, to the lymph vessels cannulated (Verwey and Williams, 1962; Brown, 1964; May et al., 1987). For example, it is difficult to interpret the relationship between blood and interstitial drug concentrations in rats when lymph was collected from the thoracic duct (which is the mixed collection of lymph from visceral and extra - visceral organs and tissues) and blood was collected from a tail vein, or in dogs, when lymph was collected subcutaneously and blood was collected from a femoral vein.

Some other methods for studying interstitial drug concentrations involve the collection of interstitial fluid formed under pathological conditions. For example,

collecting interstitial fluid from the inside of a multi - micropore capsule implanted in the tissue (Jain et al., 1979) can result in extraordinarily high protein and cell contents in the collected fluid due to pathological inflammatory processes and increased capillary permeability related to surgery and tissue damage (Szabo et al., 1976). These influences were still evident six weeks after the implantation of the capsule. Skin blisters have been used, but it has been shown that the half - life of flucloxacillin concentrations in skin blister fluid was 5 and 6 times longer than that in blood and lymph, respectively. Presumably, the fluid in the blister was in a state of stagnation and was therefore not in effective contact with either the capillaries or cells of the tissue (Bergan et al., 1986). Methods of collecting interstitial fluid from a skin window, skin chamber or the assay of drug concentrations in threads inserted subcutaneously would be subject to similar problems of increased protein content in the fluid, and of fluid stagnation (Frongillo et al., 1981; Raeburn, 1971; Ryan et al., 1982).

There is presumably a time lag between the interstitial and lymph drug concentrations due to the transit of lymph along the lymph vessel and through the sampling cannulae. However, in our study it was shown that Patent Blue dye injected intramuscularly into a hindquarter appeared in the collected lymph in less than 5 min. This time lag has been observed in other studies using lymph cannulation (Bergan et al., 1986; Cohen et al., 1987), and was also observed to be minimal in these studies. In our studies, this small time lag would not have made significant contributions to the ratio of the lymph to blood drug concentrations, particularly during the last 100 min of the drug infusions when the arterial blood concentrations changed relatively slowly, or were at steady-state. However, this time lag may become significant for pharmacokinetic studies after rapid intravenous drug administration.

It has been proposed that the study of lymph could have clinical and pharmacokinetic implications (Whitwam et al., 1984). In general terms, in this study the lymph drug concentrations were approximately proportional to the blood

concentrations. This has an important pharmacodynamic implication. Many drugs act by interacting with receptors on the surfaces of parenchymal cells (Goldstein et al., 1974), and these "surface" receptors are surrounded by interstitial fluid. Thus it would be expected that the interstitial concentrations of these drugs in the organs in which they have their effects may be a major determinant of the magnitude of their effect. Further understanding of the relationship between the blood and interstitial concentrations of drugs, and their effects, could well be used to improve the predictability, safety and efficacy of drugs.

However, despite this general relationship between lymph and blood drug concentrations, there were small significant differences between the concentrations of lignocaine and pethidine (Fig. 4.4) in conscious, unrestrained animals. To discuss this, it is worthwhile considering the mechanisms by which drugs can move between blood and lymph. Most capillary walls have pores that are large enough to allow direct passage of plasma water and its solutes, including many proteins, into the interstitial space. Drugs can enter the interstitial space by direct diffusion through these pores, by binding to proteins that can diffuse through the pores, or, in the case of lipophilic drugs, by direct diffusion though the walls of capillary cells. It has been reported that under normal conditions, the rate of lymph flow does not limit the filtration of protein in the interstitial space (Pilati, 1990), and presumably does not influence the ratio of the lymph and blood drug concentrations. Furthermore, it is clear from this study and those of others that the binding of lignocaine, procainamide and pethidine in lymph *in vitro* is at least as high as that in blood (Fig. 4.6; Tucker and Mather, 1979; La Rosa et al., 1984a; Karlsson, 1978). Thus, the observed differences between the blood and lymph concentrations are likely to be due to differences in the extent of drug binding between blood and lymph not detected in the *in vitro* binding study. Principally, the concentration gradients of carbon dioxide, and lactic and other acids, would produce a pH gradient between blood and lymph which could alter drug binding, because the protein binding of both pethidine (La Rosa et al.,

1984a) and lignocaine (Nancarrow et al., 1987) has been shown to be pH dependent. Note that any "ion trapping" of these drugs in the interstitial space due to these pH gradients is unlikely because the pores of the capillary and lymph vessel walls are permeable to both the charged and uncharged forms of the drugs. Similarly, there should be minimal effects of the pKa of a drug on its interstitial concentrations, nor should the lipophilicity of a drug influence its passage through capillary pores.

4.5.3 The effect of general anaesthesia on drug distribution in lymph

In anaesthetised sheep, the mean concentrations of lignocaine, procainamide and pethidine in the lymph were all significantly lower than the simultaneous IVC blood drug concentrations during the last 100 min of the infusions (Fig. 4.4). This is more evident when hindquarter lymph/arterial blood and lymph/venous blood drug concentration ratios in anaesthetised animals are compared to those in conscious, unrestrained animals (Fig. 4.5). Anaesthesia significantly decreased these ratios for all three drugs. Halothane anaesthesia decreased lymph flow from the hindquarters by 63% compared to values in conscious, unrestrained animals. Similar effects due to halothane anaesthesia have been reported in studies which showed lymph flow in the hindquarters of sheep decreased by 50 - 69% (Quin and Shannon, 1975) and thoracic duct lymph flow in dogs decreased by 59% (Whitwam et al., 1984). However, it is unlikely that reductions in lymph flow account for changes in the lymph concentration of drugs under anaesthesia because these lymph drug concentrations were studied under steady - state arterial drug concentration conditions and it was reported that lymph filtration rate does not limit the transportation of proteins (and presumably drugs) from the interstitial space (Piliati, 1990). Thus, the changes due to anaesthesia are more likely to be due to changes in the extent of drug binding in lymph or blood. In this study, the mean plasma and lymph total protein concentrations were not significantly different between anaesthetised and conscious, unrestrained sheep (Fig. 4.2). However, binding changes could be produced by a direct effect of halothane on the binding of drugs studied *in vivo*. In support of this, the free

fraction of propranolol in blood increased from 15.6 to 18.1% under halothane anaesthesia (Gordon et al., 1987), but it would be expected that halothane would have the same effect on drug binding in both blood and lymph. Local pH changes under halothane anaesthesia could also affect drug protein binding in the manner discussed previously. Although it is possible that reduced hindquarter activity under halothane anaesthesia changed local pH, this factor can not be determined in the present study because lymph pH was not measured. Alternatively, halothane anaesthesia decreases total perfusion of many organs, including the hindquarters (Runciman and Mather, 1986), increases arterio - venous shunting, and reduces the number of capillaries perfused at a given time. It could be speculated that these changes in the microcirculation might reduce the rate of equilibration between blood and the interstitial fluid by increasing diffusion distances (Milnor, 1980). From these results it is clear that studies of drug kinetics in the interstitial space should be performed in conscious, unrestrained animals, as general anaesthesia with halothane significantly decreased lymph flow and lymph/arterial blood and lymph/venous blood drug concentration ratios.

Table 4.1 The mean rates during the last 100 min of the infusions at which lignocaine, procainamide and pethidine were transported from the hindquarters by lymph, and the mean rates expressed as percentages of the arterial influx* (L/A) and IVC efflux* (L/IVC) in both anaesthetised and conscious, unrestrained sheep.

	Anaesthetised			Conscious, unrestrained		
	Rate (μg/min)	L/A (%)	L/IVC (%)	Rate (μg/min)	L/A (%)	L/IVC (%)
Lignocaine	0.28	0.02	0.02	0.47	0.03	0.04
Procainamide	1.23	0.02	0.03	1.63	0.05	0.05
Pethidine	0.17	0.02	0.03	1.05	0.05	0.07

* Based upon typical values of hindquarter blood flow of 1.0 and 0.6 L/min in conscious, unrestrained and in anaesthetised sheep, respectively, measured using an indicator dilution method (R.N. Upton, unpublished data).

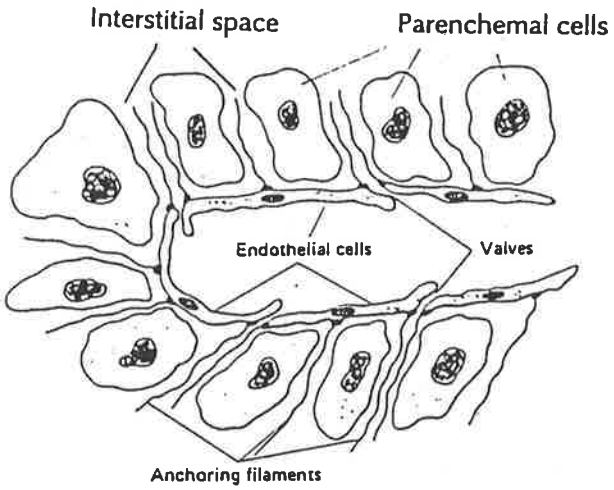


Figure 4.1

An illustration of the structure of lymphatic capillaries in relation to the parenchemal cells in the interstitial space (From Guyton, 1986a)

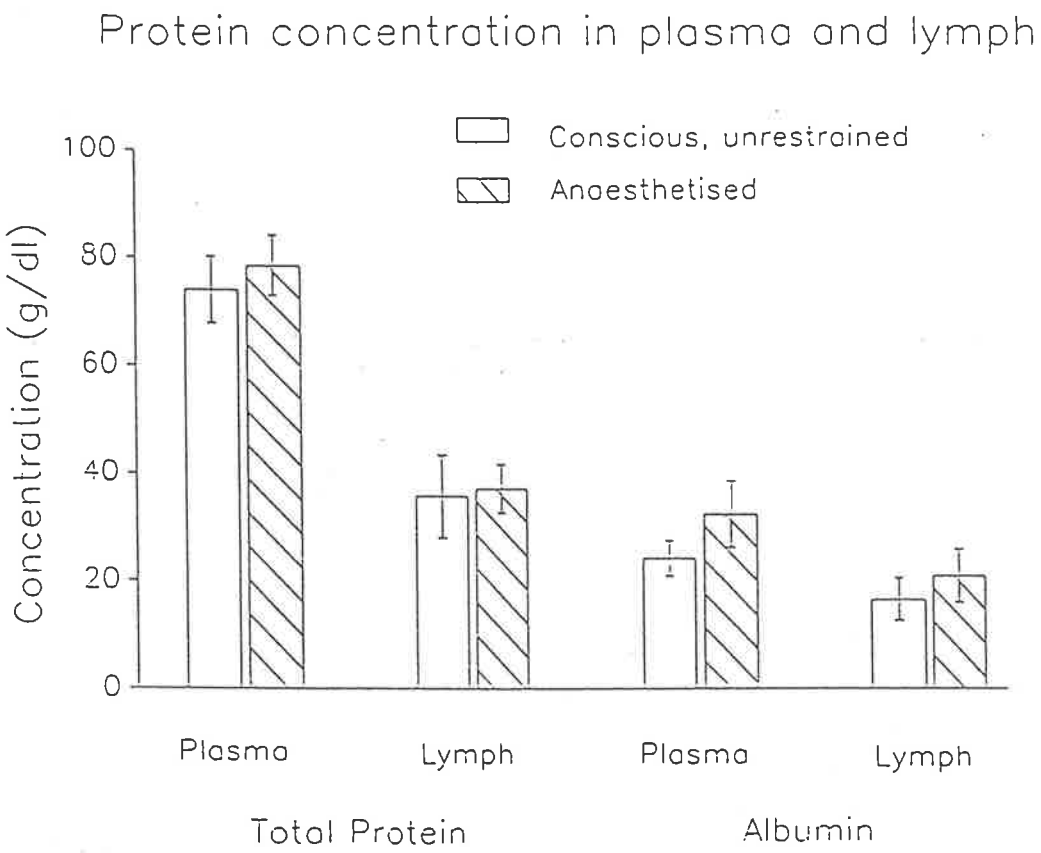


Figure 4.2

Total protein and albumin concentrations in plasma and lymph of conscious, unrestrained sheep and anaesthetised sheep. The values (Mean ± S.D., n = 9) for anaesthetised and conscious, unrestrained sheep were not significantly different (Two Sample t- test).

Figure 4.3

Typical time - courses of blood and lymph drug concentrations. (A) The time - courses of lignocaine in arterial (open circles) and IVC (filled circles) blood and hindquarter lymph (open triangles) in an anaesthetised sheep during a constant rate intravenous infusion. The infusion was stopped at the time indicated by the arrow. (D) The equivalent data during and after a constant rate infusion of lignocaine in a conscious, unrestrained animal. (B) The equivalent data during and after a constant rate infusion of procainamide in an anaesthetised animal. (E) The equivalent data during and after a constant rate infusion of procainamide in a conscious, unrestrained animal. (C) The equivalent data during and after a constant rate infusion of pethidine in an anaesthetised animal. (F) The equivalent data during and after a constant rate infusion of pethidine in a conscious, unrestrained animal.

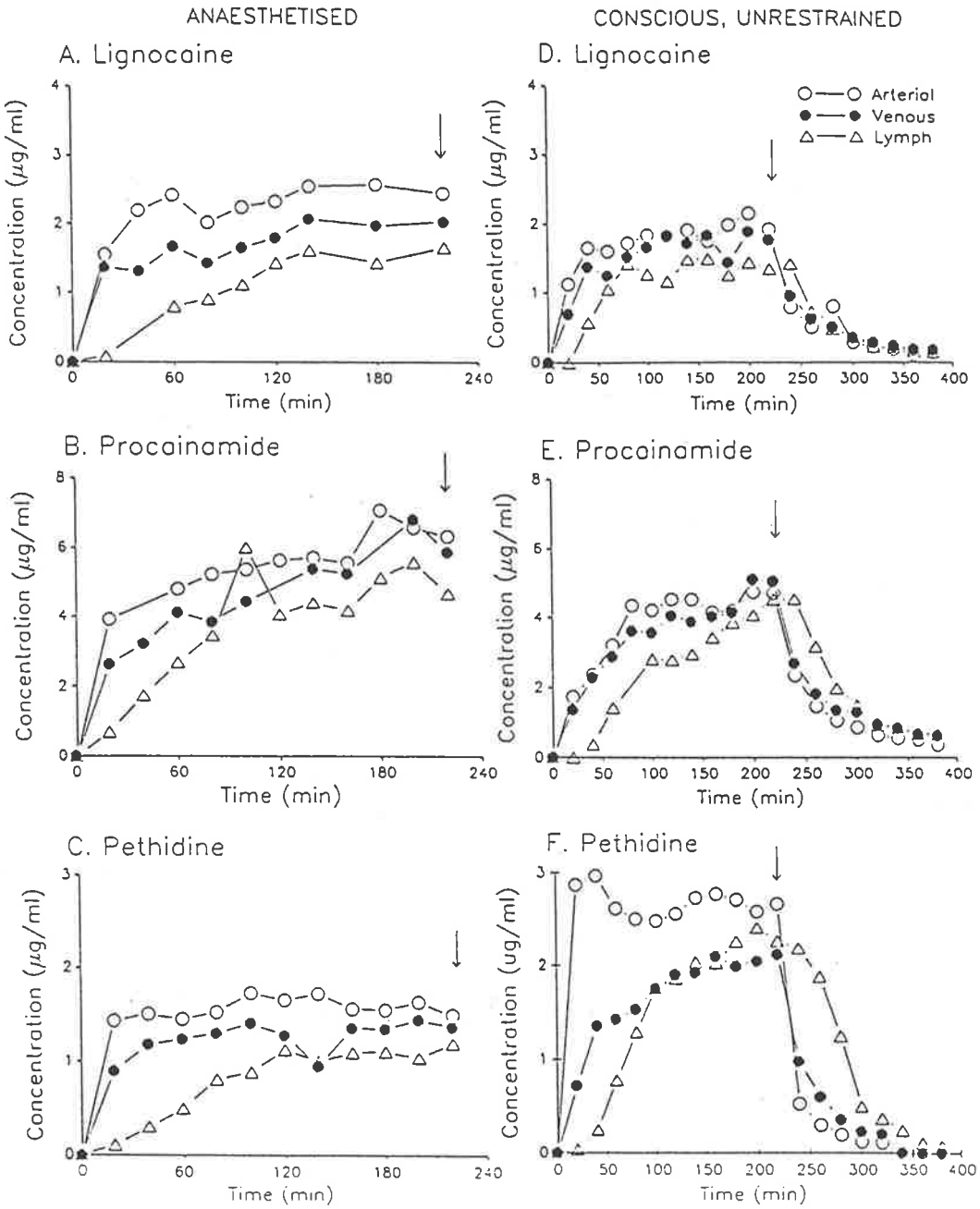
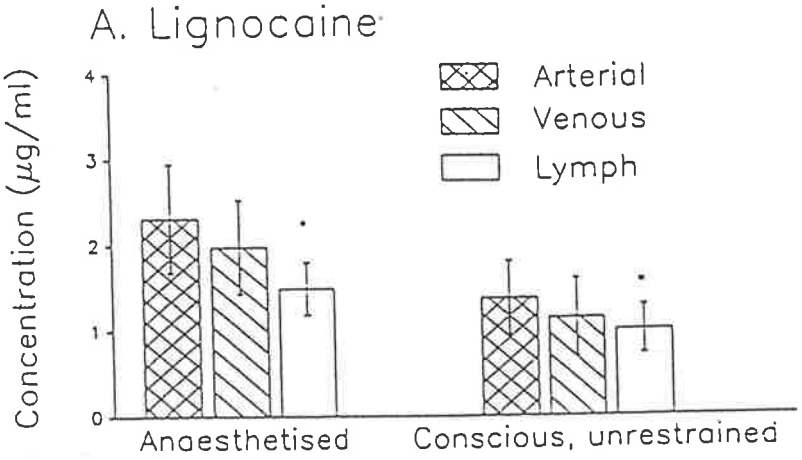
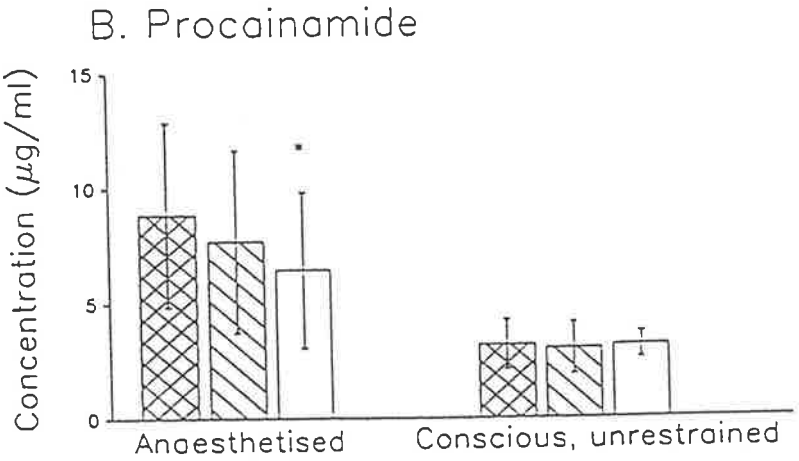

ANAESTHETISED
CONSCIOUS, UNRESTRAINED
A. Lignocaine
D. Lignocaine
B. Procainamide
E. Procainamide
C. Pethidine
F. Pethidine
Arterial
Venous
Lymph
Concentration (μg/ml)
Concentration (ug/ml)
Time (min)

Figure 4.4

Drug concentrations during the last 100 min of the infusions. (A) Left: the means (± S.D., n = 15) of the lignocaine concentrations in arterial and IVC blood and hindquarter lymph of anaesthetised sheep. Right: The equivalent data for conscious, unrestrained animals. (B) The equivalent data to A but for procainamide. (C) The equivalent data to A but for pethidine. Asterisks indicate lymph drug concentrations were statistically different from the corresponding venous drug concentrations (Paired t - test).


A. Lignocaine
Arterial
Venous
Lymph
Concentration (μg/ml)
0
1
2
3
4
Anaesthetised
Conscious, unrestrained


B. Procainamide
Concentration (μg/ml)
0
5
10
15
Anaesthetised
Conscious, unrestrained

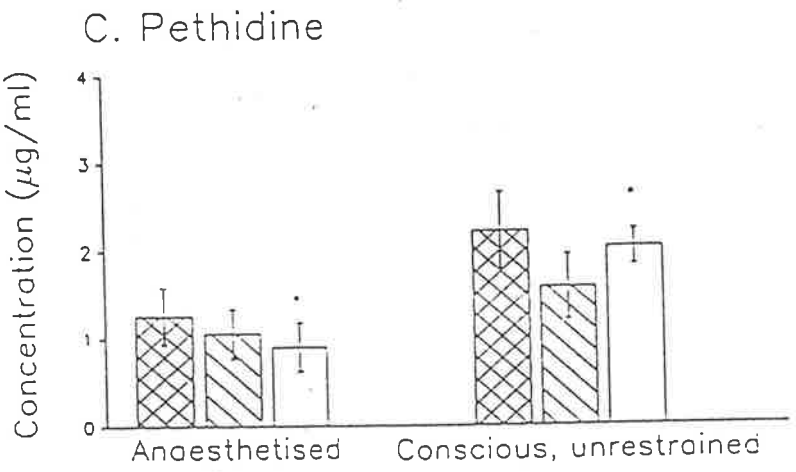
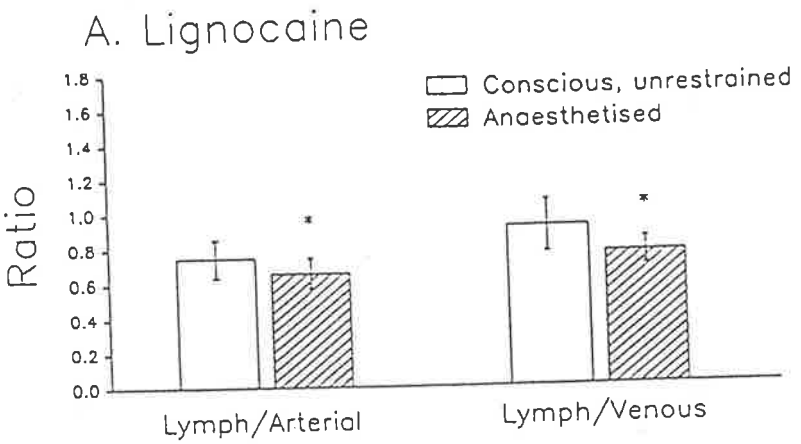
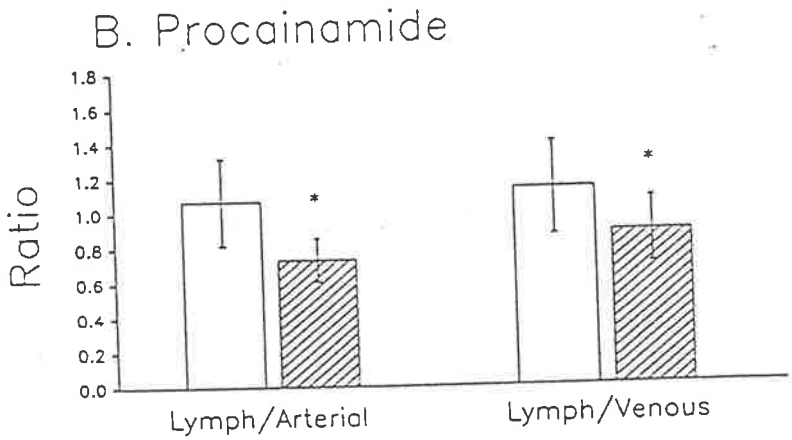

C. Pethidine
Concentration (μg/ml)
0
1
2
3
4
Anaesthetised
Conscious, unrestrained

Figure 4.5

Lymph/arterial blood and lymph/venous blood drug concentration ratios (Mean ± S.D., n = 15) for lignocaine (A), procainamide (B) and pethidine (C) during the last 100 min of the infusion periods in conscious, unrestrained sheep and anaesthetised sheep. Asterisks indicate the ratios in anaesthetised sheep which were significantly less than those in conscious, unrestrained sheep (Two Sample t - test).


A. Lignocaine
Conscious, unrestrained
Anaesthetised
Ratio
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Lymph/Arterial
Lymph/Venous


B. Procainamide
Ratio
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Lymph/Arterial
Lymph/Venous

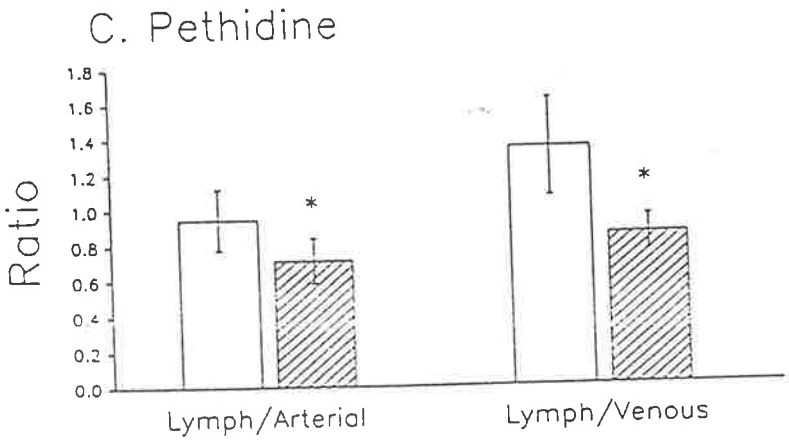
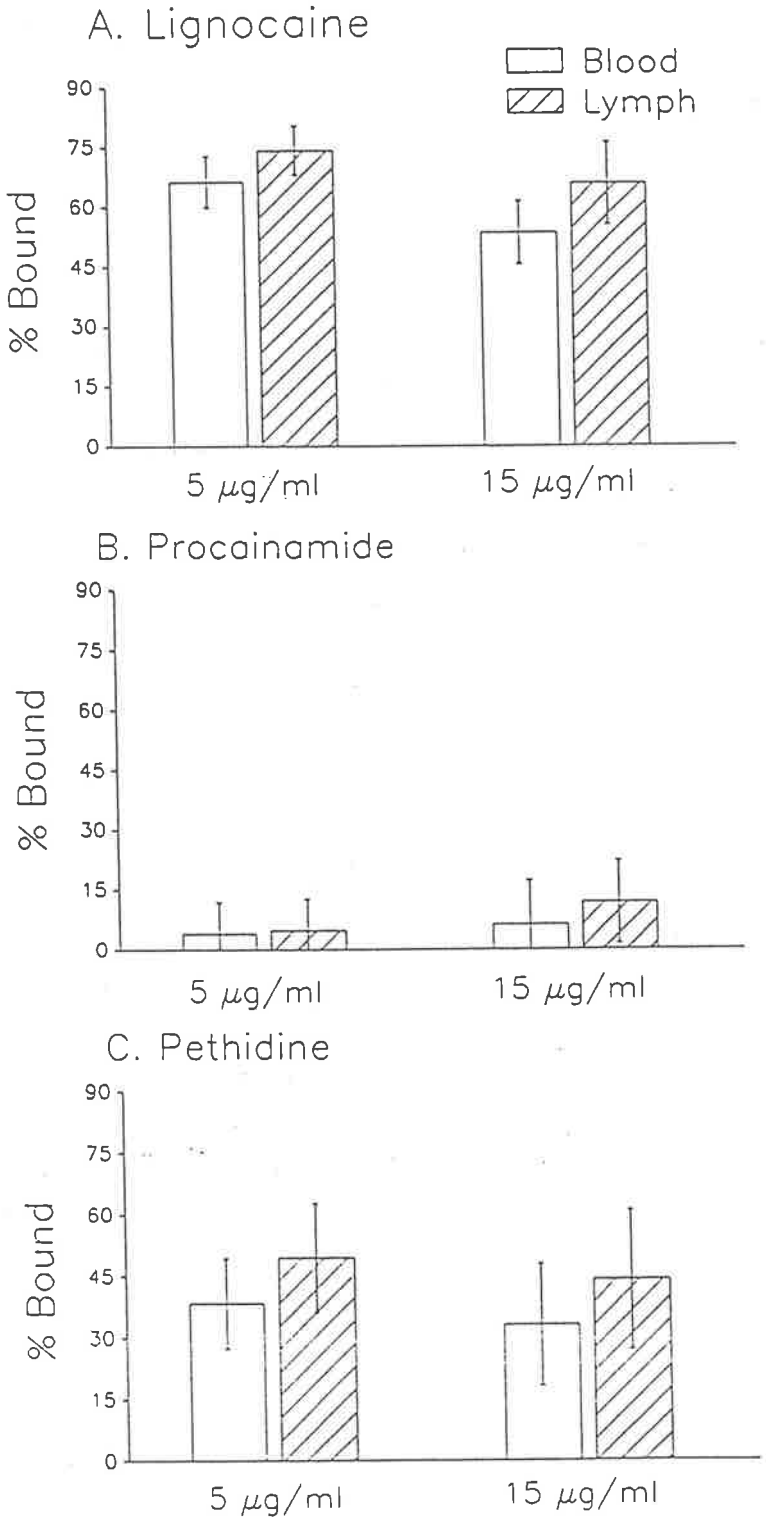

C. Pethidine
Ratio
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Lymph/Arterial
Lymph/Venous

Figure 4.6

The mean (± S.D., n = 3) of drug binding in blood and lymph studied *in vitro*. The percentage of bound lignocaine (A), procainamide (B) and pethidine (C) were determined in blood and lymph by equilibrium dialysis at initial buffer concentrations of 5 μg/ml (left) and 15 μg/ml (right). There were no statistically significant differences in drug binding between the two concentrations of each drug, or between drug binding in blood and lymph, with one exception in that lignocaine, at an initial buffer concentration of 5 μg/ml binding in lymph was 12% higher than that in blood (Paired t - test).


A. Lignocaine
Blood
Lymph
% Bound
90
75
60
45
30
15
0
5 μg/ml
15 μg/ml
B. Procainamide
% Bound
90
75
60
45
30
15
0
5 μg/ml
15 μg/ml
C. Pethidine
% Bound
90
75
60
45
30
15
0
5 μg/ml
15 μg/ml

CHAPTER 5: DEVELOPMENT OF A BLOOD SAMPLING METHOD TO CHARACTERISE RAPIDLY CHANGING BLOOD DRUG CONCENTRATIONS.

5.1 Introduction

It is known that in the first few minutes after an intravenous bolus injection of a drug, there are often rapid increases and decreases in blood drug concentrations. This period of rapid uptake and elution in organs such as the lungs, heart and brain may be associated with the most profound effects of many drugs, such as cardioactive (Horowitz and Powell, 1986) and anaesthetic agents. In conventional "systemic" pharmacokinetics (Upton, 1990) this "initial mixing" is usually ignored, and the blood concentrations in this period are extrapolated from blood concentration data obtained at later times (Chiou, 1979). However, in order to study the pharmacokinetics and pharmacodynamics of drugs immediately after an intravenous bolus injection using mass balance principles (Upton et al., 1988a), it is necessary to characterise the time - courses of the rapidly changing drug concentrations in the blood entering and leaving the various organs of the body. An inadequate frequency of blood sampling in this period can result in errors in calculated pharmacokinetic parameters (Upton et al., 1988a; Chiou, 1980; 1989a; 1989b).

A number of blood sampling methods have been used in order to achieve rates of blood sampling of the order of one sample every few seconds. Of these, many involve blood being continuously withdrawn through a blood sampling catheter during the study period, i.e. the fraction collection principle (Bertler et al., 1978; Jorfeldt, 1979; Roerig et al., 1989). However, it is known from early studies of dye dilution for blood flow measurement that blood continuously drawn through a sampling catheter can distort the dye dilution curves emerging from the catheter due to dispersion of the blood resulting from laminar and/or turbulent flow within the catheter (Lancy et al., 1957; Sherman et al., 1958; Sheppard et al., 1959). It can be postulated that the same effects would occur for studies of rapidly changing drug concentrations in blood that utilise the fraction collection principle. Therefore, the overall aim of the present study was to examine if these effects occur with several commonly used rapid blood sampling methods. The specific aims were firstly to

examine *in vitro* the influence of catheter size and length on the responses of the fraction collection blood sampling methods to a "step" change in blood drug concentrations, and secondly to compare *in vivo* how different rapid blood sampling methods characterised drug concentration - time curves in the first few minutes after an intravenous bolus drug injection.

5.2 Methods

5.2.1 Blood sampling methods

Two methods utilising the fraction collection principle were examined. In the first, blood was continuously withdrawn out of the blood sampling catheter using a roller pump, and the effluent was collected in discrete sample tubes (designated the "roller pump" method). The second differed in that discrete samples were intermittently withdrawn from the catheter using hand - held syringes with no flushing of the catheter between samples (designated the "intermittent withdrawal" method).

5.2.2 Catheter length and diameter

The following sampling systems were studied for each blood sampling method: System A - a 7 French (F) angiographic catheter (1) connected to a 91 cm (1.7 mm internal diameter) extension tube (2) System B - an 8 F angiographic catheter (1) connected to a 91 cm (1.7 mm internal diameter) extension tube (2); System C - a 9 F polyethylene catheter (3) connected to a 91 cm (1.7 mm internal diameter) extension tube (2); System D - a 9 F polyethylene catheter (3) with no extension tube. The size, length and dead - space of the sampling systems are summarised in Table 5.1. Note that in systems A, B and C, the catheters were connected to extension tubes to simulate *in vivo* blood sampling conditions where the blood sample may be taken at a site distant from the site at which the catheter was inserted.

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1 Cordis Ducor Angiographic Products, Cordis Corporation, Miami, Florida, USA

2 Sorenson Research, Division of Abbott Laboratories, Salt Lake City, Utah, USA

3 William A. Cook Australia Pty. Ltd., Eight Mile Plains, Queensland, AUS

For studies using the "roller pump" method and sampling systems A, B and C, the extension tube was replaced with an 82 cm length of Silastic tubing (4) with 1.8 mm interior diameter that had the same dead - space volume as the extension tube.

5.2.3 Generation of a "step" change in drug concentration

To generate a "step" change of blood drug concentration (Fig. 5.1), 200 ml of venous blood was collected from the IVC catheter of a sheep which had not received any drug for at least 2 days with syringes (5) pre - wetted with heparin (1000 i.u./1 ml) and was allocated into two beakers. The first contained 100 ml of lignocaine free ("blank") blood and the second 100 ml of blood to which lignocaine HCl solution (250 μg in 25 μl (6)) was added and thoroughly mixed to achieve a blood lignocaine concentration of 2.5 μg/ml ("spiked" blood). Lignocaine was chosen as a representative drug because of its ease of assay (see 2.5).

At the start of each study, the sampling system was filled with blank blood and the catheter tip was then transferred to the spiked blood and sample collection through the catheter was initiated immediately using one of the following sampling methods. If no dispersion of drug in the catheters occurs, the measured concentrations should reproduce the "step" change in concentration at the tip of the catheter (Fig. 5.1).

5.2.4 Study design

The sampling regimens for each method were as follows:

5.2.4.1 "Intermittent withdrawal" method

The responses of sampling systems A, B, C and D to "step" changes of lignocaine blood concentration were studied. Consecutive samples (0.5 ml)

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4 Silastic Medical - Grade Tubing, Dow Corning Corporation, Medical Products, Michigan, USA

5 Terumo, Melbourne, AUS

6 ASTRA Pharmaceuticals Pty. Ltd., North Ryde, NSW, AUS

were taken at 3 sec intervals using a series of 1 ml syringes, and were placed into 1.5 ml Eppendorf microtubes (7) for lignocaine assay using the method described in Chapter 2 (see 2.5). The study was repeated in triplicate. From the ratio of the dead - space volumes of the sampling systems to the volumes of each sample, it was possible to predict the time of the first sample which should have contained the expected spiked concentration of lignocaine if no dispersion occurred. The time at which this sample was taken was designated "time zero". Thus, in the absence of dispersion, blood samples taken prior to this time (i.e. negative times) should have contained no drug, while those taken after this time (i.e. positive times) should have contained lignocaine at the spiked concentration (Fig. 5.1).

5.2.4.2 "Roller pump" method

Sampling systems A, B and C were connected via their extension tubes to a "roller pump" (8). Sampling system D could not be used because the roller pump could not be used on the catheter alone. The pump speed was set to produce a sampling rate of 0.5 ml every 3 sec and samples were collected into 1.5 ml vials (7) for lignocaine assay using the method described in Chapter 2. The study was repeated in triplicate. As for the "intermittent withdrawal" studies, time zero was designated as the calculated time at which spiked blood should have first emerged in the absence of dispersion.

For each sampling method and catheter system, the shape of the concentration - time curve obtained in response to the "step" concentration change was described by the mean time at which drug first appeared in the sample, and the mean times at which 50% and 95% of the spiked concentration were first reached or exceeded.

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7 Micro Test Tubes 3180, Eppendorf - Netheler - Hinz GmbH, FRG

8 MHRE 100, Watson - Marlow Ltd., Falmouth, Cornwall, England

5.2.5 Development of an alternative rapid blood sampling method

For the *in vivo* studies, a method was developed in which minimal dispersion effects would be expected. In this method, a series of 5 ml syringes (5) were "preloaded" with a volume of 0.9% saline 2 times greater than the dead - space of the sampling system. For each sample, the pre - loaded syringe was connected to the sampling system, and the saline rapidly injected to clear the sampling system of any blood or saline remaining from the previous sample. Blood was then rapidly withdrawn out of the catheter until twice the volume of the sampling system dead - space was sampled. The sampling syringe therefore contained a volume of saline corresponding to the deadspace of the sampling system, and a known volume of blood. Measured drug concentrations in the sample were corrected for this dilution using Equation 5.1.

$$C_{corrected} = C_{assay} \times \frac{V_{sample}}{V_{sample} - V_{deadspace}} \qquad ...(5.1)$$

where $C_{corrected}$ is the corrected drug concentration, C_{assay} is the assayed drug concentration, V_{sample} is the total volume sampled and $V_{deadspace}$ is the dead - space volume of the sampling system. Sample volumes were determined by net differences in weight of the syringes before and after sampling. This method was designated the "flush and withdrawal" method.

5.2.5.1 Theoretical basis of the "flush and withdrawal" method

This method was based on different principles to the fraction collection methods, and could not be validated using the experimental design used for testing those methods. However, there are two steps that could potentially limit the accuracy of the "flush and withdrawal" method. Firstly, it is important that all the drug remaining in the catheter from the previous sample be completely washed out of the catheter with the initial "flush" of saline. Secondly, it is important that the "withdrawal" step results in all of the saline in the deadspace of the sampling system (resulting from the flush step) being

collected in the sample. Problems with either of these steps will result in errors during the subsequent correction for the dilution of the sampled blood with 0.9% saline (Equation 5.1). Therefore, two tests were carried out to assess the completeness of these steps.

5.2.5.2 Testing the "flush and withdrawal" method *in vitro*

To test the first step, a 7F catheter was connected to a threeway stopcock (9) with a dead - space of 1.5 ml and filled with blood previously "spiked" to a lignocaine concentration 5 μg/ml. Three ml of saline was flushed down the catheter and saline remaining in the catheter was collected and assayed for lignocaine. To test the second step, the catheter was filled with 0.9% saline (1.5 ml), the tip was put into blood containing 5 μg/ml of lignocaine and a 3 ml sample was aspirated from the catheter. The samples were assayed for lignocaine. The measured concentrations were corrected for the anticipated dilution and compared with the concentrations obtained from the direct assay of the spiked blood. These studies were performed 9 and 10 times, respectively.

5.2.6 An assessment of rapid blood sampling methods in vivo

Adult female Merino sheep were prepared using the method described in Chapter 2 (see 2.2) with two identical 7F angiographic catheters in the aortic arch placed via the right carotid artery. Another 7F catheter was placed in the inferior vena cava (IVC) via the right jugular vein. Experiments were performed in conscious, unrestrained sheep after 3 - 4 days were allowed for them to recover from the surgery. In a given study, 50 mg of lignocaine HCl (in 10 ml of 0.9% saline) was injected into the IVC over 2 sec and samples were collected from the two arterial catheters using either the "intermittent withdrawal" and "flush and withdrawal", or the "roller pump" and "flush and withdrawal", methods.

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9 Cobe Laboratories, Lakewood, CO, USA

Samples were taken using the "intermittent withdrawal" method described previously except that the samples were collected via a three - way stopcock connected to the end of the 7F catheter (to reduce the deadspace volume of the system). The deadspace volume was therefore 1.5 ml and samples were taken at a rate of 0.5 ml every 4 sec. Samples were taken using the "roller pump" method described previously, with the silastic tube attached to the end of the other 7F arterial catheter. The deadspace was 3.0 ml and the sampling rate was 0.5 ml every 3 sec. In each of the studies, samples were also collected using the "flush and withdrawal" method at a rate of 3 ml every 5.3 sec, simultaneously with either the "intermittent withdrawal" method or the "roller pump" method as described previously. Each experiment was repeated four times.

5.2.7 Lignocaine analysis

In all studies the amount of blood collected in each sample was determined by weight and the measured lignocaine concentration was corrected for the actual amount of blood sampled. Sample storage and assay methods were described in Chapter 2. (see 2.4 and 2.5).

5.2.8 Statistical analysis

The areas under the concentration time - curves were calculated using the trapezoidal method. Statistical analysis of results was performed using Paired t - tests for comparisons of the *in vivo* peak arterial blood drug concentrations in samples simultaneously obtained by the "intermittent withdrawal" or the "roller pump" method and the "flush and withdrawal" method and the AUC of these curves, and One Way Analysis of Variance and Two Sample t - tests were used for the comparison of the data from the *in vitro* studies using different systems and methods (see the corresponding tables of this chapter). $P < 0.05$ was considered statistically significant.

5.3 Results

5.3.1 Catheter length and diameter and the accuracy of fraction collection methods

Fig. 5.2 shows the response of the various sampling systems *in vitro* to the "step" lignocaine concentration change for the "intermittent withdrawal" sampling method and "roller pump" sampling method. It is apparent from these figures that the dead - space samples contained drug, and that the drug concentrations increased gradually to reach the spiked concentration (Fig. 5.1). Table 5.2 shows the times at which drug first appeared in the samples, and the times required for the sampled drug concentrations to reach 50% and 95% of the spiked drug concentrations for the "intermittent withdrawal" and "roller pump" sampling methods. Changing the internal diameter of the catheters from 7F to 9F did not change the responses to the "step" drug concentration change. Likewise, reducing the length of the sampling system from 171 cm to 80 cm did not significantly alter the response to the "step" drug concentration change (Fig. 5.2 C).

The times required to reach 50% and 95% of the spiked concentration in response to the "step change" in drug concentration were not statistically different for the "roller pump" and "intermittent withdrawal" methods ($P > 0.05$). Table 5.2 also shows that for both the "intermittent withdrawal" and "roller pump" sampling methods, and for all the catheter sizes and lengths used, drug always appeared in the samples which were expected to contain only blank blood from the dead - space volume (also indicated by the drug concentrations measured at negative times in Fig. 5.2). Furthermore, only 50% of the spiked drug concentration was achieved at around time 0 (Table 5.2), and 95% of the spiked concentrations were reached at times significantly later than expected (i.e at time 0). Statistical analysis showed no significant differences in the times when drug first appeared between catheters of different sizes and lengths, when using either the "intermittent withdrawal" or "roller pump" methods. There were no significant differences between the times at which the measured drug concentrations reached 50% and 95% of the spiked concentration using the different sampling systems for the "intermittent withdrawal" sampling method. The times at which 95% of the

spiked concentration were reached were significantly longer for the 7F catheters compared to both the 9F and 8F catheters when using the "roller pump" sampling method (Table 5.2).

It is also apparent that the measured drug concentrations varied most dramatically, as shown by large variations from the mean values in Fig. 5.2, in the samples which were on the up - slope of the concentration - time curves (i.e. before 95% of the spiked concentration was achieved). During this time period for all the studies, the coefficient of variations of the blood concentrations ranged from 3 - 173% and 2 - 113% for the "intermittent withdrawal" and "roller pump" methods, respectively. The corresponding values after this time for the "intermittent withdrawal" and "roller pump" methods decreased to 1 - 7% and 0.4 - 38%, respectively.

5.3.2 Testing the "flush and withdrawal" method in vitro

When the 7F catheters filled with blood at lignocaine concentration of 5 μg/ml were flushed with 3 ml of saline, the drug concentration in the saline remaining in the catheter was 0.9 ± 0.4% (n = 9) of the original blood concentration. The mean calculated concentration in samples corrected for dilution using Equation 5.1 was 4.7 ± 0.6 μg/ml (n = 10) which was not significantly different (Two Sample t - test, $P > 0.05$) to the concentration determined by the direct assay of the spiked blood (5.0 ± 0.3 μg/ml, n = 10).

5.3.3 Rapid blood sampling methods in vivo

Examples of the time - courses of arterial blood lignocaine concentrations *in vivo* determined simultaneously using the "intermittent withdrawal" and "flush and withdrawal" methods are shown in Fig. 5.3 A, and the "roller pump" and "flush and withdrawal" methods are shown in Fig 5.3 B. It is apparent that following an intravenous bolus injection of lignocaine, the drug appeared in arterial blood samples earlier when they were collected by either the "intermittent withdrawal" or "roller pump" methods than the "flush and withdrawal" method. Peak lignocaine concentrations measured by the "intermittent withdrawal" and "roller

pump" methods were significantly lower (20% and 22%, respectively) than those measured by the "flush and withdrawal" method ($P < 0.05$). There were no differences in the times at which the peak concentration occurred, in the area under concentration - time curves (AUC) measured until the time of the last paired blood samples, or the time required to reach 95% of the AUC between the "intermittent withdrawal" and "flush and withdrawal", "roller pump" and "flush and withdrawal" methods (Table 5.3).

5.4 Discussion

These data, and the reports of others (Roerig et al., 1989) show that the "roller pump" and "intermittent withdrawal" blood sampling methods allow blood samples to be taken at up to 0.5 - 1 sec intervals. However, these methods are subject to dispersion of drug concentration peaks in the blood sampling systems analogous to that which occurs in studies of dye curves (Lancy et al., 1957). These data show that this dispersion is of a magnitude sufficient to cause significant errors in measured drug concentrations after intravenous bolus administration.

The *in vitro* studies showed the failure of the "intermittent withdrawal" and "roller pump" sampling methods to describe accurately sudden changes in drug concentrations. "Step" changes in drug concentration were invariably recorded as sigmoid shaped concentration - time curves: drug appeared about 5 - 9 sec earlier, and reached 95% of the spiked concentration about 9 - 15 sec later, than expected if no dispersion occurred.

There are two possible mechanisms of dispersion. Firstly, if the fluid flow through the catheter is Newtonian (non - turbulent), it would be expected that the velocity of each fluid element would be inversely proportional to the square of its distance from the axis of the tube, and would be zero at the lumen wall (Dryden, 1956). Thus, the blood in the centre of the lumen will travel up the catheter faster than that at the lumen wall. Theoretically, the interface between blank and spiked blood within the catheter following a "step" change in drug concentration will change from a vertical straight line at the tip of the catheter to a parabolic shape at the head of the catheter

(Fig. 5.4 and Guyton, 1986b). Consequently, blood containing drug will appear in the anticipated dead - space samples.

Secondly, dispersion could be the result of turbulent flow within the catheter. This would be exacerbated by the inconstant rate of withdrawal of blood from the catheter such as that which occurs with the "intermittent withdrawal" method, or by the cyclic nature of flow produced by the "roller pump". It would be anticipated that such turbulent flow would produce mixing between spiked and blank blood within the catheter, particularly at the junctions of catheters, taps and extension tubes. Indeed, this may explain the large variation in drug concentrations in the samples during the upslope of the concentration - time curves seen in Fig. 5.2. It has been noted that in the presence of turbulent flow it is difficult to establish a mathematical correction for the dispersion incurred by these types of sampling methods (Lancy et al., 1957).

It is anticipated that both of these mechanisms of dispersion would not be influenced by the physicochemical properties of drugs under study. Thus, in addition to lignocaine, most drugs would be subject to these effects in certain blood sampling systems.

In contrast with an earlier report on the effect of blood sampling methods on the shape of indicator dilution curves (Lancy et al., 1957), these data showed that catheter length, diameter and the blood sampling rate did not notably influence the response of the sampling system to a "step" drug concentration change. Furthermore, in contrast to the suggestion of Sherman et al., (1958), the times at which drug first appeared in the samples were not notably influenced by the catheter volume and blood withdrawal rate. These findings suggest that the dispersion in the sampling systems tested in this chapter may have been due to turbulent rather than laminar flow.

It would be anticipated that a reduction of catheter length and/or internal diameter such that the catheter deadspace volume was much less than the sample volume

would greatly reduce dispersion in the catheter. Such short sampling catheters would be practical for sampling blood from peripheral blood vessels. However, the use of longer catheters is necessary to sample blood from vessels such as the coronary sinus and pulmonary artery in order to study drug uptake and elution in the heart and lungs, respectively, using mass balance principles (Upton et al., 1988a).

The "flush and withdrawal" method was investigated with the intention of developing a rapid blood sampling method that would not be influenced significantly by dispersion. The *in vitro* studies showed that these aims were achieved, but with a maximum sampling frequency of approximately 5.3 sec which was not as rapid as the "intermittent withdrawal" and "roller pump" methods. This method was therefore used as the "control" sampling method for accurately defining rapidly changing blood drug concentrations *in vivo*. This method was preferred to the use of a "free flowing" catheter as a control (presumably under arterial blood pressure (Lancy et al., 1957)), because it is highly probable that dispersion will also occur in the "free flowing" catheter. Note that the equal AUC measurements obtained by the different sampling methods (Table 5.3) demonstrates that the rapid administration of saline associated with the "flush and withdrawal" method did not significantly dilute the blood in the aorta and reduce the drug concentrations sampled through the other catheter using either the "intermittent withdrawal" or "roller pump" methods.

As anticipated from the results of the *in vitro* studies, the *in vivo* studies showed significant inaccuracies in the measured time - courses of drug concentrations after bolus administration when blood was sampled by the "intermittent withdrawal" and "roller pump" methods. Intuitively, it would be expected that the dispersion observed in response to the "step" drug concentration changes *in vitro* would flatten and broaden drug concentration - time curves *in vivo*. This phenomenon was also observed in a study of the effects of blood sampling methods on dye curves (Dow, 1958). However, as expected, the areas under the concentration - time curves did not differ between the blood sampling methods (Huang and Oie, 1982a; Vofelstein et al., 1977).

The importance of dispersion in blood sampling catheters depends on the frequency at which blood samples need to be taken in order to characterise the blood drug concentration - time curve. For example, when mass balance principles are used for studying tissue drug uptake after an intravenous bolus drug administration (Upton et al., 1988a), a sampling frequency of 5 sec is adequate for defining the time - course of arterial and regional venous, but not pulmonary arterial, blood drug concentrations. This frequency can be adequately achieved using the "flush and withdrawal" method. However, in order to sample at frequencies of less than 5 sec, other sampling methods must be developed, or two catheters must be placed at the same site, with two people sampling. Alternatively, the process of deconvolution (used in other pharmacokinetic applications) could be used to correct for dispersion in catheters provided the dispersion is both linear and reproducible.

Table 5.1 The diameters, lengths and dead - space volumes of the catheter systems used in the *in vitro* fraction collection experiments.

Catheter system	Diameter	Length (cm)	Extension	Deadspace (ml)
A	7 F	100	yes	3.0
B	8 F	100	yes	3.5
C	9 F	100	yes	4.5
D	9 F	80	no	2.5

Table 5.2 The times at which lignocaine first appeared in the samples, and the times at which the measured concentrations achieved 50 and 95% of the spiked concentration in the *in vitro* fraction collection experiments (mean ± S.D., n = 3).

"Intermittent withdrawal" method

Sampling system	First appearance (sec)	Time at 50% (sec)	Time at 95% (sec)
A	-5 ± 2	1 ± 1.7	11 ± 5
B	-6 ± 0	0 ± 0	11 ± 2
C	-5 ± 5	2 ± 3.5	14 ± 3
D	-6 ± 3	-1 ± 3.5	9 ± 3

"Roller pump" method

Sampling system	First appearance (sec)	Time at 50% (sec)	Time at 95% (sec)
A	-9 ± 0	1 ± 1.7	16 ± 2[a]
B	-9 ± 0	1 ± 1.7	10 ± 2
C	-9 ± 0	0 ± 0	11 ± 2

[a]Significantly longer than B and C (One Way Analysis of Variance).

Table 5.3 A comparison of the times and magnitudes of the peak lignocaine concentrations, and the areas under the concentration time - curves (AUC) when the "intermittent withdrawal" and "flush and withdrawal", or "roller pump" and "flush and withdrawal" methods were compared simultaneously following an intravenous bolus injection of lignocaine *in vivo* (mean S.D., n = 4).

	Peak concentration (μg/ml)	**Peak time** (sec)	**AUC** ((μg/ml) x sec)
Intermittent withdrawal	20 ± 8[a]	15 ± 7	464 ± 176
Flush and withdrawal	25 ± 6	16 ± 6	462 ± 142
Roller pump	18 ± 7[a]	12 ± 4	518 ± 82
Flush and withdrawal	23 ± 5	15 ± 5	497 ± 44

[a]Significantly lower than the "flush and withdrawal" method (Paired t - test).

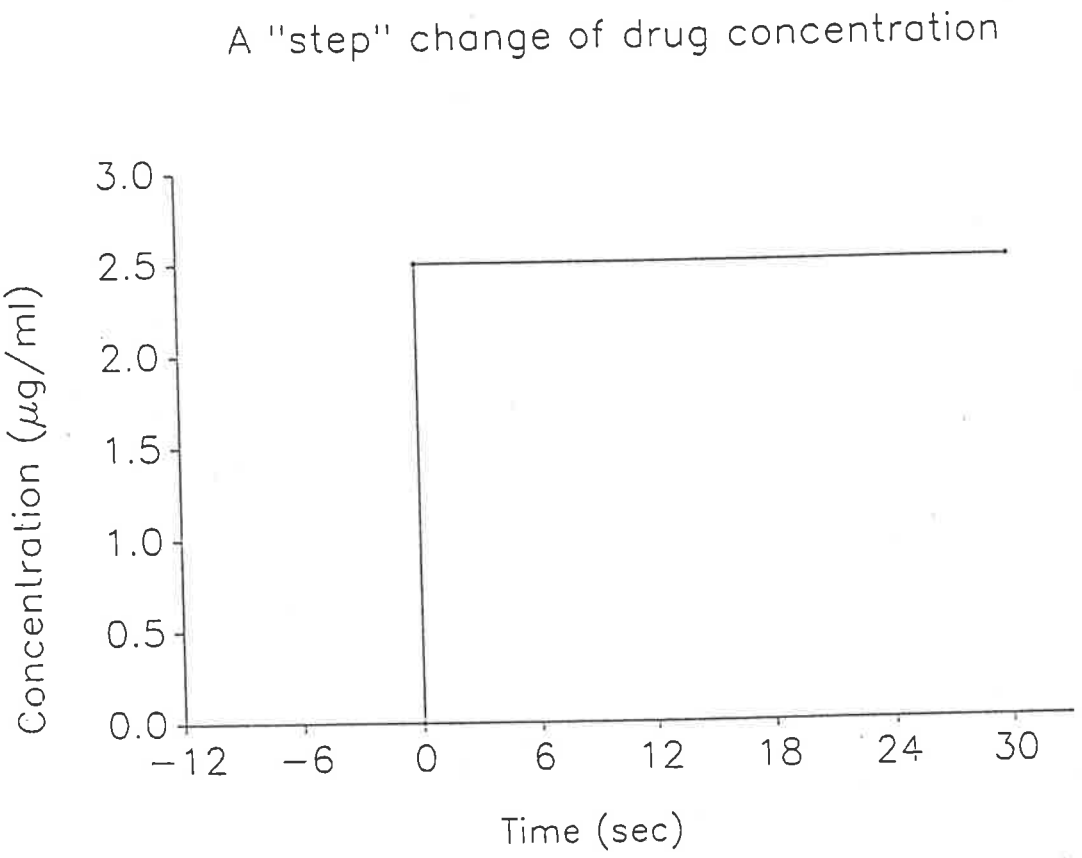


Figure 5.1

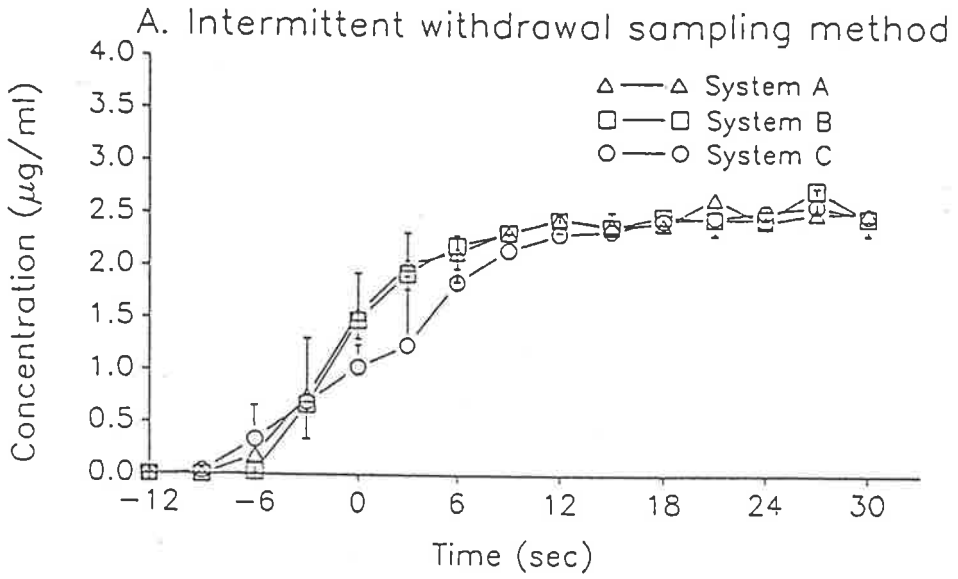
A theoretical example of a "step" drug concentration change measured by a sampling system in which no dispersion occurs.

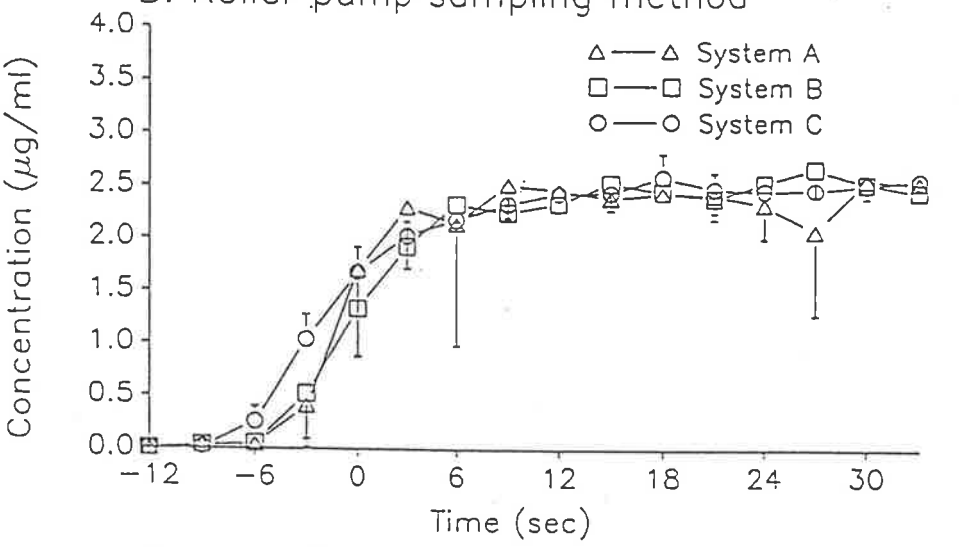
Figure 5.2

(A) Lignocaine concentrations (mean ± S.D., n = 3) obtained using the "intermittent withdrawal" sampling method to characterise an *in vitro* "step" concentration change using blood sampling systems A (7F, open triangles), B (8F, open squares) and C (9F, open circles).

(B) Lignocaine concentrations (mean ± S.D., n = 3) obtained using the "roller pump" sampling method to characterise an *in vitro* "step" concentration change using blood sampling systems A (7F, open triangles), B (8F, open squares) and C (9F, open circles).

(C) Lignocaine concentrations (mean ± S.D., n = 3) obtained using the "intermittent withdrawal" sampling method to characterise an *in vitro* "step" concentration change using blood sampling systems C (171 cm, 9F, open circles) and D (80 cm, 9F, open diamonds) that are of different lengths.


A. Intermittent withdrawal sampling method
Concentration (μg/ml)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
−12
−6
0
6
12
18
24
30
Time (sec)
System A
System B
System C


B. Roller pump sampling method
Concentration (μg/ml)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
−12
−6
0
6
12
18
24
30
Time (sec)
System A
System B
System C

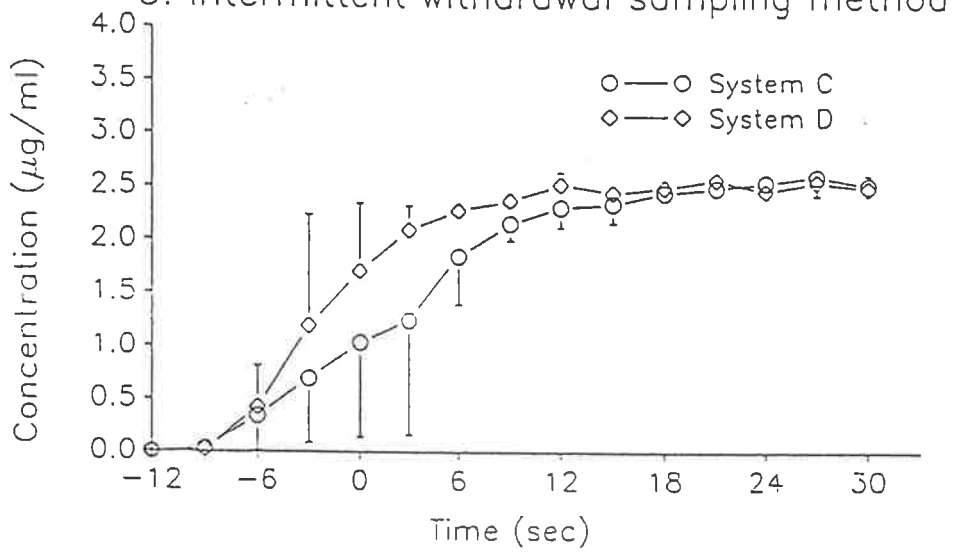
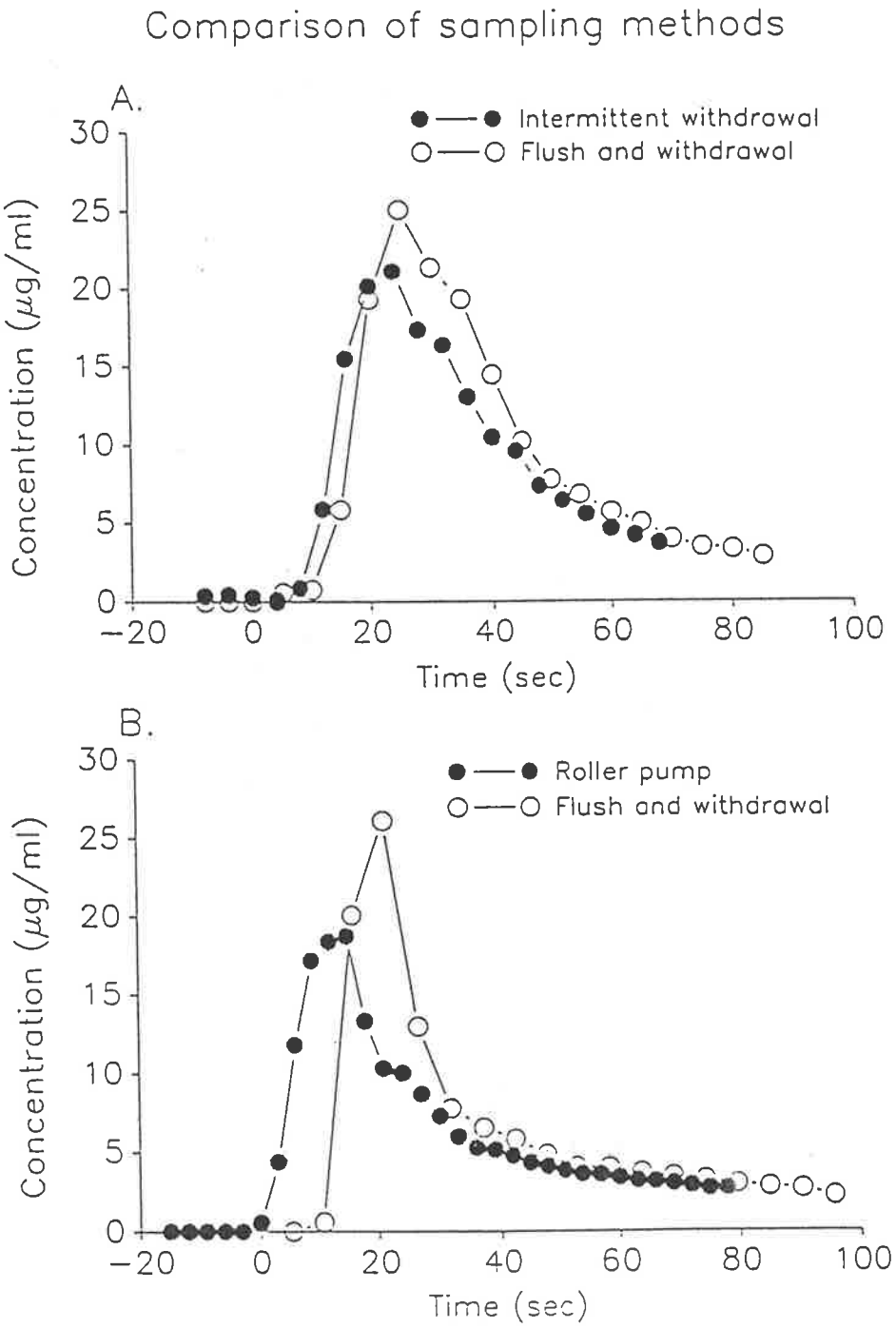

C. Intermittent withdrawal sampling method
Concentration (μg/ml)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
−12
−6
0
6
12
18
24
30
Time (sec)
System C
System D

Figure 5.3

An example of a comparison of the measured time - courses of the arterial blood lignocaine concentrations *in vivo* following an intravenous bolus. Blood samples were collected simultaneously using: (A) the "intermittent withdrawal" (solid circles) and "flush and withdrawal" methods (open circles) and (B) the "roller pump" (solid circles) and "flush and withdrawal" methods (open circles).


Comparison of sampling methods
A.
Intermittent withdrawal
Flush and withdrawal
Concentration (μg/ml)
30
25
20
15
10
5
0
−20
0
20
40
60
80
100
Time (sec)
B.
Roller pump
Flush and withdrawal
Concentration (μg/ml)
30
25
20
15
10
5
0
−20
0
20
40
60
80
100
Time (sec)

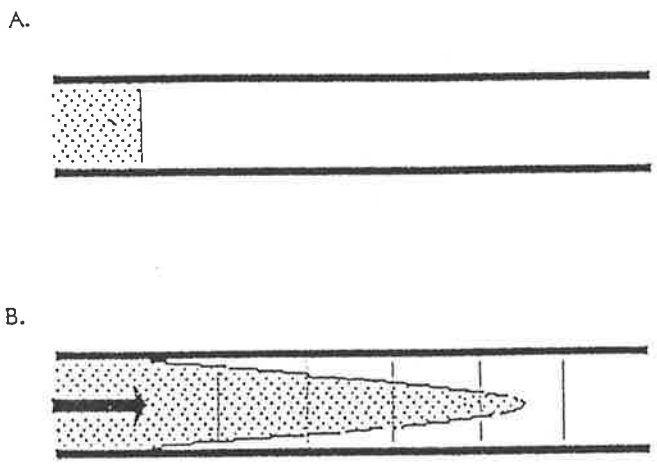


Figure 5.4

Laminar flow shown in a hypothetical longitudinal section of a catheter.

(A) Initially with no flow in the catheter, the interface between drug free (white) and spiked (shaded) blood is a vertical line

(B) Once flow has started, the interface becomes parabolic in shape with higher velocities at the centre of the catheter. When the sample volume (indicated by the areas between the vertical lines) is less than the deadspace volume of the catheter, it is possible for some blood samples to contain a mixture of blank and spiked blood.

SECTION III: STUDIES OF MYOCARDIAL PHARMACOKINETICS AND PHARMACODYNAMICS IN THE SHEEP.

Introduction

The applications of mass balance principles for the study of regional pharmacokinetics have been discussed in Chapter 1. The experiments in Section II validated a number of assumptions previously made for the use of mass balance principles (Upton et al., 1988a). Firstly, the experiments in Chapter 3 showed that the rate of drug transfer from the surface of organs (heart and kidney) by direct diffusion was negligible. Secondly, the experiments in Chapter 4 showed that the rate of drug removal from organs (the hindquarters was used as an example) by lymph was also negligible with respect to mass balance calculations. Thirdly, the experiments in Chapter 5 showed the inability of some widely used rapid blood sampling methods to adequately characterise rapidly changing blood drug concentrations, and validated a new blood sampling method.

In this section, this preliminary work is utilised in studies of the myocardial pharmacokinetics and pharmacodynamics of lignocaine and pethidine using mass balance principles in chronically prepared sheep with blood sampling catheters in the aortic arch and coronary sinus, Doppler probes to measure left coronary artery blood flow and cardiac output, and additional instrumentation to measure other haemodynamic parameters. In Chapter 6, the general methods used for the preparation of these sheep over and above those described in Chapter 2, are presented. Studies to select a suitable vehicle for the rapid intravenous administration of drugs in small volumes of solutions are presented in Chapter 7. Chapters 8 and 9 report studies of the intravenous administration of lignocaine and pethidine, and describe the haemodynamics effects and the myocardial pharmacokinetics of the drugs, respectively. The relationships between the myocardial pharmacokinetics and the haemodynamic effects of the drugs are examined in Chapter 10.

CHAPTER 6: GENERAL METHODS FOR STUDIES OF MYOCARDIAL PHARMACOKINETICS AND PHARMACODYNAMICS IN CONSCIOUS, UNRESTRAINED SHEEP.

6.1 Introduction

The use of mass balance principles requires the simultaneous measurement of the arterio - venous blood drug concentration gradients across, and blood flow to, the organ or tissue under study (see 1.5). Thus, in the study of myocardial pharmacokinetics using mass balance principles it is important to accurately define the region of the myocardium that is bounded by the chosen blood sampling sites and the region for which blood flow is measured. This will be addressed in the first part of this chapter. Subsequently, the general methods for the measurement of myocardial blood flow are developed and verified and the methods used for the catheterisation of the specific blood vessels are presented. Finally, the methods employed for the measurement of the haemodynamic parameters used as indices of drug effect are presented.

6.2 Defining the area of the myocardium under study

Like most mammals, the left and right coronary arteries of the sheep supply arterial blood to the myocardium, and the coronary sinus is the major venous vessel of the heart (May, 1964). Sheep differ from man in that the coronary sinus also receives blood from the hemiazygous vein draining the chest wall on the left side. However, by tying off the hemiazygous vein it is possible to collect pure myocardial effluent from the coronary sinus (May, 1964; Nancarrow, 1986). In a study of the anatomy of the coronary sinus in four lamb hearts, Downing et al., (1973) found that after ligation of the haemiazygous vein outside the pericardium, the blood from the coronary sinus of sheep was derived principally from the left ventricle and ventricular septum (85%) with a small proportion from the left atrium (8%) and the right ventricle (7%). Thus the region defined by the effluent venous blood of the coronary sinus, with the haemiazygous vein ligated consists predominantly (93%) of the left heart. A series of studies, described below, was conducted to further investigate the

distribution of arterial blood to this region and define the area of myocardium under study in the sheep.

6.2.1 Defining the region of myocardium perfused by the left coronary artery in sheep

This was investigated *in vitro* using the method of Bond et al., (1973) in which perfusion of the left decending coronary artery with indian ink was used to define the region of myocardium perfused by branches of the left coronary artery in dogs. Ten normal sheep hearts were obtained from sheep used for experiments not involving the heart. To do this, the sheep were systemically heparinised (200 i.u. of heparin sodium/Kg, I.V. injection) and then sacrificed with a barbiturate overdose. The hearts were examined and none showed any signs of previous myocardial infarction or other obvious abnormalities. In each heart, cannulae (extension tubing 75 cm long (1)) were secured in the ostia of the left and right coronary arteries, with their tips no further than 3 mm into the vessels. Each cannula was connected to a modified 20 ml syringe (2) by a three - way stopcock (Fig. 6.1). One of the syringes was filled with indian ink (3) and the other with 0.9% saline. The other ends of the syringes were connected via Y - connectors and silastic tubes to a pneumatic bulb and a sphygmomanometer. The heart was suspended in a large container with the base of the heart down to prevent the perfused indian ink emerging from the coronary sinus flowing into the right ventricular cavity. A pressure of 100 mmHg (to mimic the *in vivo* mean arterial blood pressure of sheep) was simultaneously applied to both of the syringes and the myocardium was perfused by the ink through either the left or right coronary artery and the other artery was perfused by 0.9% saline simultaneously under the same perfusion pressure.

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1 Lane Cove, NSW, AUS

2 Terumo, Melbourne, AUS

3 Penguin Indian ink, Melbourne, AUS

After perfusion, the stained tissue was dissected from the unstained tissue (Fig. 6.2). It was found that in all 10 hearts the left ventricular free wall, left atrium and most of the ventricular septum were supplied by the left coronary artery. The right ventricular free wall, the right atrium and a small part of the ventricular septum (near the base of the heart) were supplied by the right coronary artery. After the dissection, the epicardial fat and the large blood vessels were removed and the stained and unstained tissues were blotted dry and weighed separately. The percentages of the myocardium perfused by the left and right coronary arteries are presented in Table 6.1. These findings are in general agreement with findings in other domestic ruminants (Swenson, 1984). From this study and the study of Downing et al., (1973) it can be deduced that, in the sheep, a region of myocardium comprising left ventricular free wall, ventricular septum and left atrium is perfused by arterial blood from the left coronary artery and is "drained" by the coronary sinus, provided the haemiazygous vein has been ligated. This was the region of the myocardium chosen for the study of myocardial pharmacokinetics using mass balance principles in the following chapters.

6.3 Other aspects concerning the application of mass balance principles to the "myocardium" studied

6.3.1 Collecting blood for measuring left coronary artery blood drug concentrations

It is generally accepted that arterial blood is homogeneous in drug concentration due to the mixing of blood that occurs in the chambers of the heart, but there is not a large body of experimental work to confirm this assumption. In one study, Horowitz et al., (1986) found no differences in the time - courses of lignocaine concentrations in the aorta and femoral artery following intravenous bolus administration. It was therefore assumed that blood drug concentrations in the ascending aorta and at the entrance of the left coronary artery are essentially identical. Thus, arterial blood sampled from the ascending aorta was considered to be representative of the blood supplying the myocardial tissue via the left coronary artery.

It is also recognised that the intravascular transit time of drug molecules in the heart can contribute to the calculated net myocardial drug uptake using mass balance principles. The contribution of the transit time across the myocardium to the calculated myocardial drug uptake and elution is difficult to quantitate, because of the skewed nature of the distribution of these transit times (Coulam et al., 1966). However, in previous experiments it was found that the mean intravascular transit time of the heart in dog and sheep was less than 5 sec (Selden and Neill, 1975; Upton et al., 1988a). In most pharmacokinetic studies, the times needed to reach equal arterial and venous blood drug concentrations are much longer than this intravascular transit time, which makes the artifact resulting from intravascular transit negligible (Upton et al., 1988a; see 1.5).

6.3.2 Methods to measure left coronary artery blood flow in conscious, unrestrained sheep

There are a number of methods which have been used to determine coronary artery blood flow. These include thermodilution (Horowitz et al., 1986), radioactive microsphere distribution (Consigny et al., 1982), methods based on the nitrous oxide method of Kety and Schmidt (Kety and Schmidt, 1945; Krasnow et al., 1963) and methods using electromagnetic (Khouri et al., 1968; Skarvan and Priebe, 1988) and Doppler flowmeters (Vatner et al., 1985). However, the use of mass balance principles to quantify myocardial drug uptake and elution in conscious, unrestrained animals requires continuous measurement of the coronary blood flow using a method that does not interfere with the measurement of blood drug concentrations or the haemodynamic status of the sheep.

The specific features of the coronary anatomy of the sheep (see 6.4) and the apparent reliability of chronically implanted Doppler flow probes made these the method of choice in these studies. To measure total left coronary artery blood flow, the Doppler flow probe must be placed on the mainstem of the left coronary artery. Although this method has been used successfully in both anaesthetised (Wangler et al., 1981) and conscious experimental animal preparations (Vatner et

al., 1971; Bernard et al., 1990), most of these studies only measured blood flow in the left descending or circumflex coronary arteries. This method was further developed for the measurement of the left main coronary artery blood flow of sheep, as described below.

The Doppler flow probe method also had the advantage that it could be used to provide simultaneous and continuous measurement of cardiac output (an important haemodynamic parameter, see 6.6) in conscious, unrestrained sheep by placing another probe on the pulmonary artery.

6.4 Sheep preparation

Six adult, female Merino sheep of haemoglobin type A (see 2.2), weighing between 37 and 52 kg were used for the myocardial pharmacokinetic and pharmacodynamic studies. These sheep were chronically instrumented using sterile techniques in two stages. Two weeks before the studies, pulsatile Doppler flow probes were placed on the pulmonary and left main coronary arteries. Approximately 10 days later, after the probes had "grown in" and were giving stable signals, the sheep were chronically catheterised (see 6.4.2). Pharmacokinetic and pharmacodynamic studies were performed from approximately 3 - 4 days after the catheterisation.

6.4.1 Surgical procedures for placing Doppler flow probes on the left coronary artery and pulmonary artery

Sheep were acclimatized to their metabolic crates for 1 - 2 days. The night before surgery they were fasted. On the day of surgery, they were anaesthetised using the methods described in Chapter 2. Using sterile technique, a left lateral thoracotomy was made at the 4th intercostal space and the heart was exposed through a pericardiotomy. Care was taken to protect the left phrenic nerve. The main left coronary artery of sheep lies between the left atrium and the pulmonary artery and is covered entirely by epicardial fat. The artery branches into the left descending and the circumflex coronary arteries at distances approximately 1.2 to 1.5 cm from the aorta. Just proximal to this bifurcation, the vessel becomes gradually embedded in the myocardium of the left ventricle.

To expose the vessel, the left atrial appendage and pulmonary artery were gently retracted to the right and left sides, respectively. The left coronary artery was dissected carefully from the epicardial fat and the surrounding tissues, and a modified silastic cuff - type pulsatile Doppler flow probe (4) was placed on the artery. The probes were modified because in initial investigations it was found that the standard probes were too thick and wide to fit around the exposed left coronary artery. The cuff was trimmed so that approximately 0.5 cm by 0.5 cm of silastic remained around the Doppler crystal (Fig. 6.3 A). A cotton tape (0.5 cm wide and 5 cm long) was mounted onto the probe so that it covered the back of the remaining silastic. To place the probe, one end of the tape was passed under the coronary artery so that the Doppler crystal was mounted on the top of the artery, and the tape was then carefully tightened and cut. The two ends of the cotton tape were sutured together to secure the probe onto the artery.

To place the pulmonary artery probe for cardiac output measurement, the main pulmonary artery was dissected free from the adjacent ascending aorta. A "suture - down" Doppler flow probe (4) modified (Fig. 6.3 B) by gluing the Acrylic pad of the probe onto cotton tape (1.5 cm wide and 10 cm long). The probe was secured around the artery using the cotton tape, and was tightly sutured in place.

After placement of the Doppler flow probes, both the left coronary and pulmonary arteries were always examined to ensure that they had not been constricted. The pericardiotomy was then repaired with a silk suture (5). At this stage, the haemiazygous vein was double ligated outside the pericardium using silk ligatures (5) to ensure the coronary sinus contained pure myocardial effluent (see 6.2).

The chest was closed with coated vicryl sutures (6) and the pneumothorax was evacuated by inflating the lungs prior to tying the last suture. The wires of both

4 Titronics Medical Instruments, Iowa City, Iowa, USA

5 American Cyanamid Company, New York, USA

6 5 metric, Ethicon, USA

the Doppler flow probes were protected by silastic tubing (to minimise damage to the wires due to the constant movement of the heart) and were exteriorised through the chest incision. They were passed through a subdermal tunnel and secured to the skin on the left - side of the back of the sheep. After recovery from anaesthesia, the sheep were returned to their metabolic crates (see 2.2).

6.4.2 Catheterisation of blood vessels

For the second stage of surgery, the sheep were again anaesthetised and were prepared with intravascular catheters using the methods described in Chapter 2. The coronary sinus and ascending aorta were cannulated with 7F catheters (7)(8), in order to sample afferent and efferent blood, respectively, from the region of myocardium defined for study (see 6.2). Each sheep was also prepared with a 7F catheter (8) in the inferior vena cava (IVC) for drug administration; a quadrapolar intracardiac electrocardiographic (ECG) wire (9) in the coronary sinus for electrocardiography; 7F (8) and 9F (10) catheters in the ascending aorta for the measurement of arterial blood pressure and for the introduction of a pressure transducer tipped catheter into the left ventricle (see 6.6.1), respectively. A 7F Swan - Ganz catheter was placed in the pulmonary artery for measurement of blood temperature and for the measurement of cardiac output by the thermodilution method (for calibration of the pulmonary artery Doppler flow probe, see 6.5.2.1). The post - operative care, maintenance and handling of the chronically instrumented sheep has been described in Chapter 2 (see 2.2).

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7 Multi - purpose B1 catheter, Cordis Corporation, Miami, FL, USA

8 Multi - purpose A1 catheter, Cordis Corporation, Miami, FL, USA

9 USCI Ltd., Billerica, MA, USA

10 Willian A. Cook, Sydney, NSW, AUS

6.5 Measurement of left coronary artery blood flow and cardiac output

6.5.1 Blood flow rate in the left coronary artery and pulmonary artery

6.5.1.1 Background

The Doppler flow probe and the flowmeter measure the velocity of blood using the Doppler principle. This principle states that sound reflected from a moving target will be shifted in frequency by an amount proportional to the target velocity, and is described by the following equation:

$$\text{Doppler frequency shift} = \frac{2F_t \times V \times \text{Cos } \alpha}{C} \qquad ...(6.1)$$

In the case of blood in a vessel, F_t is the incident sound frequency from the Doppler flowmeter, V is erythrocyte velocity (i.e. blood flow velocity being measured), α is the angle between the sound beam and the direction of blood flow, and C is the speed of sound in blood (Fig. 6.4).

A 545C - 4 directional pulsed Doppler flowmeter (11) was used for the measurement of the velocity and direction of blood flow in the left coronary and pulmonary arteries. Fig. 6.4. also shows the principle by which the pulsed Doppler flowmeter and flow probe work. The Doppler flow probe (A) consists of a 1 mm diameter 20 kHz piezoelectric transducer mounted on a silastic cuff. The transducer is placed in such a position in the cuff that when the probe is placed on the blood vessel, ideally, the angle between the direction of blood flow and the ultrasonic signal is 45°. The Doppler flowmeter and the probe send bursts of ultrasound of 4 μsec duration at a pulse repetition frequency of 62.5 kHz into the blood. The resulting echoes from the vessels walls and blood cells are received by the same transducer and amplified by the Doppler flowmeter. Because the emitted ultrasound is pulsed, the returning echoes are separated in time according to the distances travelled. Therefore, the portions

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11 Bioengineering, The University of Iowa, 56 M.R.F. Iowa City, Iowa, USA

of echoes generated from different distances in the blood vessel can be selectively sampled by the "time gating", which can be controlled by the "Range Gate" of the Doppler flowmeter. Functionally, the sampling point of the ultrasound echoes can be controlled to be anywhere across the diameter of the blood vessel. The sampled echoes are compared in frequency to the signal from the transmitting oscillator and the Doppler frequency shifts are recorded. Because the speed of sound in blood (C) is a constant (1,500 m/sec), blood flow velocity in the relevant blood vessel can be calculated from equation 6.1.

It has been shown that the flow velocities recorded by Doppler flowmeters chronically implanted in experimental animals can be converted to blood flow rates in several ways if the Doppler flow probe has been implanted around the vessels for more than two weeks (Vatner et al., 1973; White et al., 1974a). This time is required for fibrous scar tissue to form around the Doppler probe and the blood vessel, which minimises changes in the angle between the ultrasonic beam and the direction of blood flow (α) and minimises any movement between the Doppler probe and the blood vessel (i.e. to ensure good acoustical coupling between transducer and blood vessel). The scar tissue also minimizes changes in vessel diameter due to changes in perfusion pressure, reaction to vasoactive substances or drug effects which may be encountered during experiments.

Theoretically, blood flow rate is the product of the blood flow velocity and the diameter of the blood vessel. This method was not commonly used because of the difficulty of accurately measuring the inner diameter of the vessels and because blood flow is usually laminar (i.e. there are substantial velocity gradients over any cross section of a vessel). Small errors in the measured blood vessel diameter will produce relatively large errors in the calculated blood flow rate. Most commonly, the Doppler flow probes are calibrated *in vivo* or *in vitro* to convert blood flow velocity to blood flow rate. *In vivo*, good correlations between blood flow rates simultaneously measured by Doppler

flowmeter and electromagnetic flowmeter, and Doppler flowmeter and the thermodilution method have been reported (White et al., 1974b). Using the beaker and stop - watch method, a linear relationship between blood flow rates and blood flow velocities measured by Doppler flowmeter was also found when the Doppler flow probes were calibrated *in vitro* (Vatner et al., 1971; 1973). The methods for calibrating the Doppler probes used in the studies of this thesis will be described below.

6.5.1.2 Pre - experimental calibration and recording of Doppler frequency shifts during experiments

Two weeks after the surgery for placing the Doppler probes, the probes were connected to two separate channels of the four channel directional pulsed Doppler flowmeter (12) operated at 20 kHz. The outputs of mean and phasic blood flow velocities (Doppler frequency shift) from each channel were fed to a high frequency - response ink - jet chart recorder ((13), subsequently called the Mingograf82 chart recorder). Before each experiment, the 0 to 4 kHz ultrasound frequency shift was calibrated on the chart as 0 to 20 and 0 to 40 mm pen shifts for the left coronary and pulmonary arteries respectively (Fig. 6.5, "Calibration").

As outlined previously, the sample point within the vessel at which blood velocity is measured can be adjusted by changing the "Range gate" on the Doppler flowmeter. Because blood flow in blood vessels is laminar, the blood velocity profile across the radius of a blood vessel is parabolic in shape with the highest velocities in the centre of the vessel (Guyton, 1986b). To determine the optimal sample point, the flow velocity profiles (Nerem, 1985) in both the left coronary and pulmonary arteries of each sheep were determined 13 to 14 days after the implantation of the Doppler flow probes when the sheep were standing quietly in the metabolic crates. This was done by recording the blood

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12 Bioengineering, The University of Iowa, 56 M.R.F. Iowa City, Iowa, USA

13 Mingograf82, Siemens - Elema, Solna, Sweden

flow velocities across the radius of the vessels at points 0.2 mm apart. Examples of blood velocity profiles in the left coronary and pulmonary arteries measured in one of the sheep are shown in Fig. 6.6. These velocity profiles are in agreement with theoretical and measured profiles reported previously (Nerem, 1985). The influence of heart rate on the velocity profiles was also examined. It was found that the velocity profiles did not change significantly between heart rates of 80 beats/min and 140 beats/min (Fig. 6.6) altered using cardiac pacing via the intracardiac ECG wire and a cardiac pace - maker (14). This range of heart rates spanned the heart rate changes observed in subsequent pharmacokinetic and pharmacodynamic studies.

For the pharmacokinetic and pharmacodynamic studies, the sampling point was always chosen as the point with the highest flow velocity. This sampling point was always used for studies in the same sheep and at the time of Doppler flow probe calibration.

6.5.2 Calibration of Doppler flow probes

An *in vitro* method of calibrating the Doppler flow probe on the left coronary artery was chosen because of the limited number of methods available for continuously and reliably measuring coronary artery blood flow *in vivo*. Unlike other species (White et al., 1974a; 1974b), the left main coronary artery of the sheep is too short to accommodate an electromagnetic flow probe and a Doppler flow probe simultaneously.

For calibrating the pulmonary artery Doppler flow probe, an *in vitro* method was ruled out when initial studies showed that the wall of the pulmonary artery of sheep is so thin that the vessel grossly distended when pressure was exerted to drive blood flow through the pulmonary artery preparation, thereby making accurate *in vitro* calibration impossible. The thermodilution cardiac output method was therefore used to calibrate the pulmonary artery Doppler flow probe

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14 A - V sequential demand pulse generator, Medtronic Inc., 5330, MN, USA

in vivo. This is a standard method for measuring cardiac output and has been used routinely in our laboratory (Runciman et al., 1981a; 1981b). Good linear correlations between cardiac outputs measured by the thermodilution method and a Doppler flowmeter in experimental rabbits have previously been reported (White et al., 1974b).

6.5.2.1 *In vivo* calibration of the Doppler flow probe on the pulmonary artery

For accurate *in vivo* calibration of the Doppler probe on the pulmonary artery it was essential that the cardiac output of sheep could be varied to cover the range of cardiac outputs encountered in the pharmacokinetic and pharmacodynamic experiments described in the following chapters. It is known that halothane can depress myocardial function (Warren and Stoelting, 1986). In our previous studies (Upton et al., 1990b) in anaesthetised sheep, it was found that this decrease in cardiac output can be readily regulated by varying the inhaled halothane concentrations from 1% to 5%. When sheep were recovering from halothane anaesthesia, there was a period in which cardiac output exceeded normal values. Therefore, the *in vivo* calibration of pulmonary artery Doppler probes was carried out in sheep during and after halothane anaesthesia to achieve this range of cardiac outputs.

When the pharmacokinetic and pharmacodynamic studies were completed in a sheep, it was anaesthetised using the method described in Chapter 2. Cardiac output was measured using a thermodilution method previously described in detail by Runciman et al., (1984a). Briefly, the chronically placed 7F Swan - Ganz catheter in the pulmonary artery (see 6.4.2) was connected to a cardiac output computer (15) and cardiac output was determined following the injection of 10 ml of 0.9% saline at a temperature of $0^{\circ}C$ via a chronically placed 7F polyethelene catheter in the right atrium (see 6.4.2). The cardiac output was determined as the average of three such injections made at 15 to 20 sec intervals.

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15 Model 9520A, Edwards Laboratories Inc., Irvine, CA, USA

As the cardiac output was manipulated, the pulmonary artery blood flow velocities (mean and phasic Doppler frequency shifts) were measured using the Doppler flowmeter and were recorded using the Mingograf82 chart recorder at the times of each injection for thermodilution cardiac output measurement. The Doppler apparatus was pre - calibrated as described previously (see 6.5.1). A linear regression of the thermodilution cardiac outputs (L/min) and the pen shift (mm) of the Mingograf82 chart recorder representing the mean Doppler frequency shift (blood flow velocity in the pulmonary artery) was constructed. These regressions for the six sheep studied are shown in Fig. 6.7. The coefficients of correlation of these linear regressions (r) ranged from 0.97 to 0.99. These correlations were later used to convert the recorded mean pulmonary artery Doppler frequency shifts (blood flow velocities) to cardiac output in volume terms (L/min) using the formula:

$$CO = a + b\,X \qquad \ldots(6.2)$$

Where CO is cardiac output (L/min), a and b represent the intercept and slope of the regression line and X is the pen shift (mm) for the pulmonary artery Doppler frequency shifts.

It would be expected that the angle between the Doppler transducer and the direction of blood flow in the vessel (α in Equation 6.1 and Fig. 6.4) would vary between sheep because of variations in the growth of tissue between the cuff of the Doppler probe and the vessel, and the shape and curvature of the vessels. Consequently, the measured Doppler frequency shifts would be different between sheep, even if the blood flow velocities in the blood vessels of different sheep were the same (Equation 6.1). This explains the need for calibration of the Doppler probes in each sheep, and may also explain the different slopes of the calibrations of the Doppler probes in the six sheep (Fig. 6.7)

6.5.2.2 *In vitro* calibration of Doppler flow probes on the left coronary artery

After the *in vivo* calibration of the Doppler flow probe on the pulmonary artery, sheep were systemically heparinised (heparin sodium 200 i.u./kg (16)) and sacrificed with an intravenous overdose of barbiturate. Approximately 2 L of blood was collected via the intravascular catheters into a large air - tight container and an autopsy was performed. In each of the six sheep studied, there were adhesions in the left thoracic cavity as the results of previous surgery. No pneumothorax, collapsed lung tissue or visual evidence of myocardial infarction was found. Pericardial adhesion was found in one of the sheep.

The heart complete with the left coronary artery Doppler flow probe on the vessel was excised. The ascending aorta, approximately 5 cm above the aortic valves, was cannulated with a plastic cannula of 1.5 cm diameter (Fig. 6.8). The left descending and the circumflex coronary arteries were retrogradely cannulated with modified 2.5 cm long 9F polyethelene catheters (10). These catheter tips were placed inside the left descending and circumflex coronary arteries, approximately 1 cm from the bifurcation of the left main coronary artery. The vessels were tied off around the cannulae and secured with stay - sutures to the myocardium. The orifices of the pulmonary veins on the left atrium were also tied.

The cannula in the aorta was connected by a 1.5 cm diameter silastic tube to the outlet of the air - tight container containing the heparinised sheep blood. The pressure inside the blood container was controlled by pumping air into the container using a pneumatic bulb (Fig. 6.8), and the pressure was monitored using a sphygmomanometer (17). The blood container was pressurised at various pressures so that the blood flowed through the left coronary artery at different rates. After passing the probe, the blood was collected via the

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16 David Bull Laboratories Pty. Ltd., Mulgrave VIC, AUS

17 Focus, Japan

cannulae in the left descending and circumflex coronary arteries, and was directed using extension tubes (Fig. 6.8) into a 2 L plastic bag. The weight of this plastic bag was measured continuously by a force displacement transducer (18) and was recorded using a Mingograf82 chart recorder at a chart speed of 25 mm/sec. The chart recorder was precalibrated so that a 0 to 50 mm pen shift was produced by a weight change of 25 g in the blood collection bag. The rate of weight increase of the plastic bag represented the blood flow rate in the left coronary artery.

Prior to calibration, the Doppler probe was pre - calibrated as described previously for the *in vivo* studies (see 6.5.1). Flow through the probe and vessel was initiated, and the Mingograf82 chart recorder was used to simultaneously record the Doppler frequency shift and the rate of weight gain of the blood collection bag. The pressure inside the blood container was then increased in increments up to 160 mmHg. Each pressure was kept constant long enough to achieve constant flow and Doppler frequency shifts (Fig. 6.9). The blood flow rates and measured Doppler frequency shifts (pen shift in mm) were then correlated by linear regression. The coefficients of correlation of these regressions (r) for the six calibrations ranged from 0.98 to 0.99 and are shown in Fig. 6.10. These correlations were used to convert the recorded mean coronary artery Doppler frequencies (blood flow velocities) into left coronary blood flows (ml/min) using the formula:

$$LCBF = a + b\ X \qquad \ldots(6.3)$$

Where LCBF is the left coronary artery blood flow rate (ml/min); a and b represent the intercept and slope of the regression line, and X is the pen shift (mm) representing blood flow velocities in the left coronary artery. For similar reasons to those described in the calibration of pulmonary artery Doppler

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18 Grass Instrument Co., Quincy, MA, USA

probes, the observed slopes of the regression lines of the calibrations differed between sheep.

A possible source of error for this type of *in vitro* calibration of Doppler flow probes is turbulent flow in the vessel *in vitro*. To test this, at the time of any *in vitro* calibration of the coronary artery Doppler probe, the flow profiles inside the vessel were also recorded at a perfusion pressure of 100 mmHg, using the same method as used *in vivo* (see 6.5.1). An example of such an *in vitro* flow velocity profile in the left coronary artery is shown in Fig. 6.11. It is apparent that the *in vitro* blood flow velocity profile was essentially similar to that measured *in vivo*, and that the flow was therefore not turbulent. The sampling point was chosen as the point of maximum velocity, as for the *in vivo* studies.

After *in vitro* calibration of the Doppler flow probes, the epicardial fat of the sheep hearts was stripped off, the large blood vessels were removed and the hearts were weighed to obtain the lean tissue mass (Table 6.2).

6.6 Measurement of haemodynamic parameters used as an index of drug effect

Although coronary artery blood flow measurement is necessary to apply mass balance principles to the myocardium, it is also an important index of the haemodynamic state. This, together with cardiac output measured using the pulmonary artery Doppler probe, was also used as a haemodynamic parameter to provide an index of drug effect.

In addition, the following haemodynamic parameters were measured in each sheep: left ventricular pressure (LVP) and the maximum speed of left ventricular pressure rise (LV dp/dt_{max}), mean arterial blood pressure (MAP) and intracardiac electrocardiogram (ECG). All the haemodynamic parameters were amplified and recorded using the Mingograf82 chart recorder at the chart speed of 25 mm/sec (Fig. 6.5, "Control recording").

6.6.1 Measurement of LVP and LV dp/dt$_{max}$

The methods for measuring LVP and LV dp/dt$_{max}$ in chronically catheterised sheep had been developed and validated during previous studies of local anaesthetic toxicity (Nancarrow, 1986), and these methods were used for the experiments presented in this thesis. Briefly, on an experimental day the sheep was brought into laboratory and supported on a sling in the metabolic crate (see Chapter 2). Just before the experiment, a 5F Millar Mikro - Tip pressure transducer catheter (the "Millar catheter", (19)) was introduced into the left ventricle using sterile technique via a Touhy - Borst adaptor (20) and the 9F catheter placed in the aortic arch (the "introducer catheter") described previously (see 6.4.2). The Touhy - Borst adapter and the introducer catheter were connected to the catheter flushing system (see Chapter 2) and the system was flushed continuously at a pressure of 300 mmHg while the Millar catheter was inserted. This reduced blood loss to a minimum and prevented blood clot formation inside the introducer catheter.

The signal from the Millar catheter was recorded by the Mingograf82 chart recorder via an interface unit (21) and a switch unit connected between the Millar catheter and the chart recorder. At the level of the switch, left ventricular pressure signals were also sent to an analogue differentiator (22) which was pre - calibrated to generate a 0 to 100 mmHg pressure change in 0.1 sec (i.e. 0 to 1000 mmHg in 1 sec) in response to an equivalent 100 mmHg electric sawtooth waveform generated by a signal generator (23). The response of the differentiator was linear between 0 and 4000 mmHg/sec (Nancarrow, 1986) - this spanned the rates of left ventricular pressure rise observed in the experiments described in Chapters 7 and 9. The differentiated LVP signal - LV dp/dt was also recorded on

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19 Millar Instruments.Inc., Houston, Texas, USA

20 William A. Cook, Sydney, AUS

21 TC510 Transducer Control Unit, Houston, TA, USA

22 Model 3640, Devices Pty. Ltd., Sydney, AUS

23 Model 160A, BWD Electronics, Melbourne, AUS

the chart recorder. The peak value of each recorded LV dp/dt represents the individual LV dp/dt_{max}.

The chart recorder and the Millar catheter were statically calibrated from 0 mmHg (with the catheter tip just under the surface of water) to 100 mmHg (generated by an oil - filled glass manometer marked in mmHg equivalents (24)) before each experiment. The transducer was sterilised by immersion in the glutaraldehyde (25) for 20 min before use. Correct positioning of the tip of Millar catheter in the left ventricle was confirmed by the presence of the characteristic left ventricular pressure trace (Fig. 6.5, "Control recording").

It is generally accepted that the accuracy of LV dp/dt_{max} as the measurement of myocardial contractility is affected when there are simultaneous changes in LV end diastolic pressure and heart rate (Mason, 1969). In our pilot studies in sheep, the relationship between the heart rate and the LV dp/dt_{max} changes was examined. It was found that when heart rates were increased from 100 to 190 by intracardiac pacing, the LV dp/dt_{max} changes were less than 8% of the control values. The heart rate variations during the subsequent studies were within this range and therefore were considered not to be a significant factor influencing LV dp/dt_{max} as an index of myocardial contractility in these experiments in the sheep.

6.6.2 Measurement of mean arterial blood pressure (MAP)

MAP was measured by a pressure transducer (26) connected via polyethylene tubing (27) to the arterial catheter in the ascending aorta. The pre - experimental calibrations of the chart recorder and the transducers were the same as described for the Millar pressure transducer catheter. An example of the MAP recording is shown in Fig. 6.5.

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24 Dwyer Instruments

25 Aldecyde, ICI Australia Operations Pty. Ltd., VIC, AUS

26 Model 4 - 327 - I, Bell and Howell Inc., Pasadena, CA, USA

27 PT 36, Sorenson Research Co., Salt Lake City, UT, USA

6.6.3 Measurement of intracardiac ECG

The intracardiac ECG was measured using the intracardiac ECG wire described previously (see 6.4), and was amplified and recorded using the Mingograf82 chart recorder (Fig. 6.5 "Control recording"). The heart rate (HR) was determined from the frequency of the QRS complex of the ECG recordings.

6.7 Calculated haemodynamic parameters

Systemic vascular resistance (SVR), stroke volume (SV), left ventricular stroke work (LVSW), left ventricular minute work (LVMW), left coronary vascular resistance (CVRl) and double product (DP, which is an indicator of myocardial oxygen consumption) were calculated according to standard formulae (Bonica et al., 1971; Skarvan and Priebe, 1988):

$$SVR = \frac{MAP - RVEDP}{CO} \times 80 \ (dynes/sec/cm^{-5}) \qquad ...(6.4)$$

$$SV = \frac{CO}{HR} \ (ml) \qquad ...(6.5)$$

$$LVMW = CO \times MAP \times 0.0144 \ \ (kg/m/min) \qquad ...(6.6)$$

$$LVSW = SV \times MAP \times 0.0135 \ \ (g \cdot meters) \qquad ...(6.7)$$

$$CVRl = \frac{AoPd^{*} - LVEDP}{CBFl} \times 80 \ (dynes/sec/cm^{-5}) \qquad ...(6.8)$$

$$DP = HR \times AoPs. \qquad ...(6.9)$$

*It is known that left ventricular systolic pressure (LVSP) is equal to the aortic systolic pressure and mean arterial blood pressure can be approximated using the following equation:

$$MAP = \frac{2 * AoPd + AoPs}{3} \qquad ...(6.10)$$

where AoPs is the aortic systolic blood pressure. Therefore, the diastolic aortic blood pressure (AoPd) was calculated using the following equation:

$$AoPd = \frac{3 \text{ X } MAP - LVSP}{2} \text{ (mmHg)} \qquad ...(6.11)$$

6.8 Data handling for blood flows and haemodynamic parameters

The means of left ventricular systolic and diastolic pressures and LV dp/dt_{max} were obtained by averaging the individual readings recorded during the 5 sec before the scheduled data sampling times. The mean pulmonary artery and left coronary artery Doppler frequencies were also read at these times. Heart rate (beats/min) was obtained by counting the QRS waves of the intracardiac ECG for the 6 sec prior to the scheduled data sampling times and multiplying by 10.

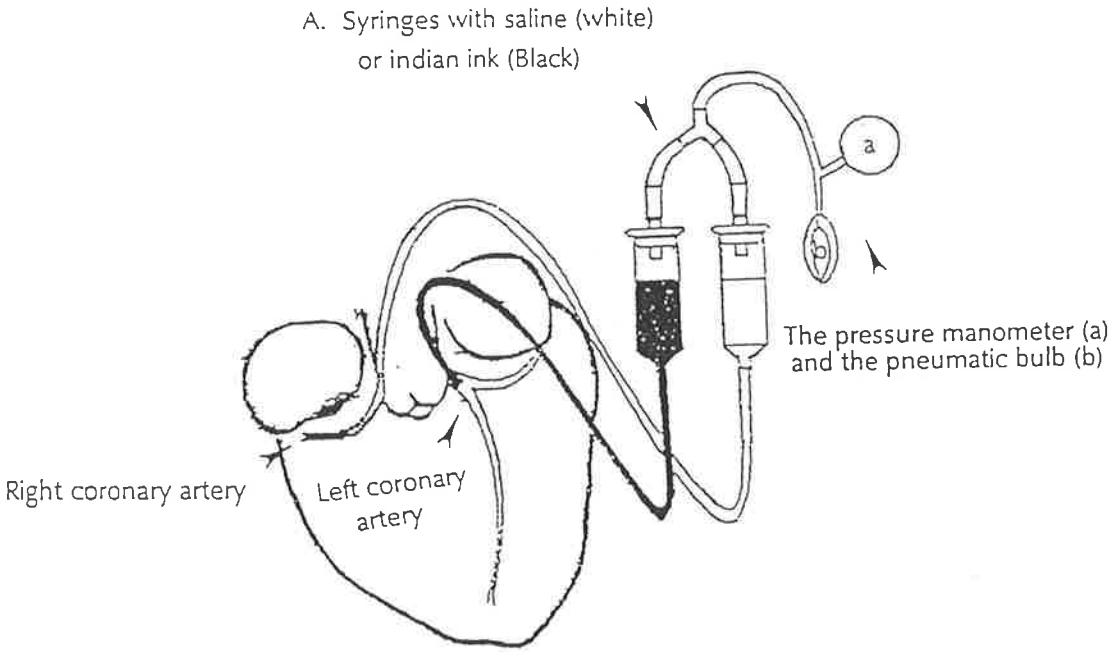
These data were entered into a spreadsheet program run on an IBM(R) compatible personal computer. Details of these and the methods of data analysis and graphing are discussed in Chapter 2.

Table 6.1 The proportions of the myocardial tissue perfused by the left and right coronary arteries as determined by *in vitro* tissue staining with indian ink (mean S.D., n = 10).

Tissue perfused by the right coronary artery (n = 4 hearts)		**Tissue perfused by the left coronary artery (n = 6 hearts)**	
Weight (g)	*Percent of total*	*Weight (g)*	*Percent of total*
63 ± 10	23 ± 2	216 ± 37	77 ± 2

Table 6.2 The lean tissue weight of the hearts of the six sheep used for studies of myocardial pharmacokinetics and pharmacodynamics.

Sheep number	Lean tissue weight (g)
309	237
312	243
315	243
316	242
318	226
320	218



Heart of sheep with cannulae in the right and left coronary arteries, respectively

Figure 6.1

In vitro experimental set - up for the study of the proportion of myocardial tissue perfused by the left coronary artery in the sheep. The left and right coronary arteries are perfused by indian ink and saline respectively under a perfusion pressure of 100 mmHg.

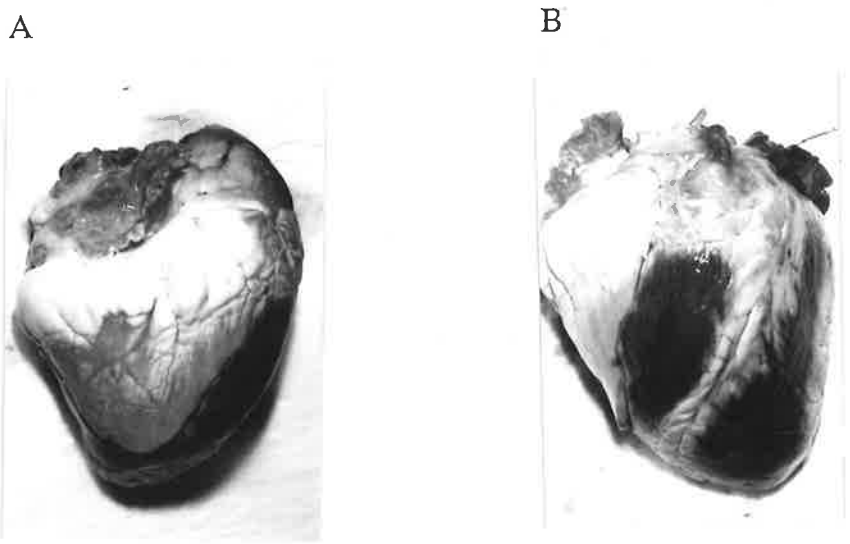


Figure 6.2

The area of myocardial tissue perfused by the left coronary artery (stained black). (A) view from the right side of the heart - the right ventricular free wall was not stained, and (B) view from the left side of the heart - the left ventricular wall and left atrium were stained.

Figure 6.3

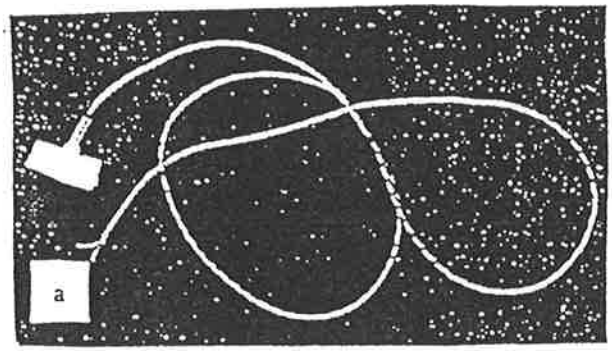
(A) a. The "cuffed" Doppler flow probe with leads;

b. The position of the piezoelectric transducer (crystal) on the probe.

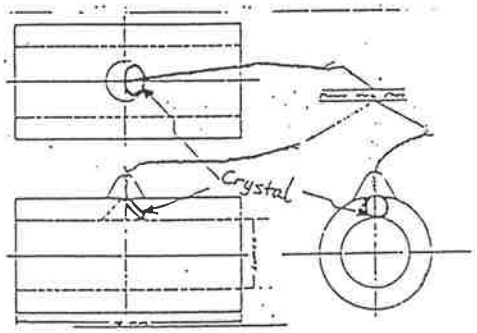
(B) a. The "suture - down" Doppler flow probe with leads;

b. The position of the piezoelectric transducer (crystal) on the probe.

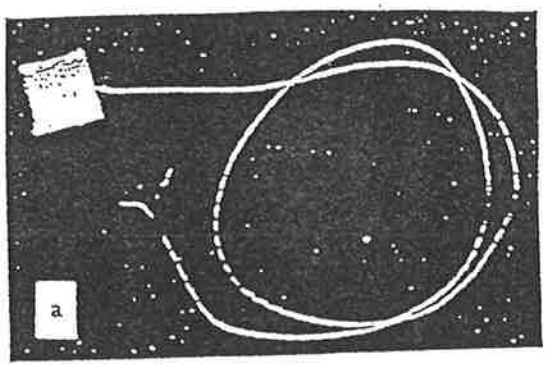
A



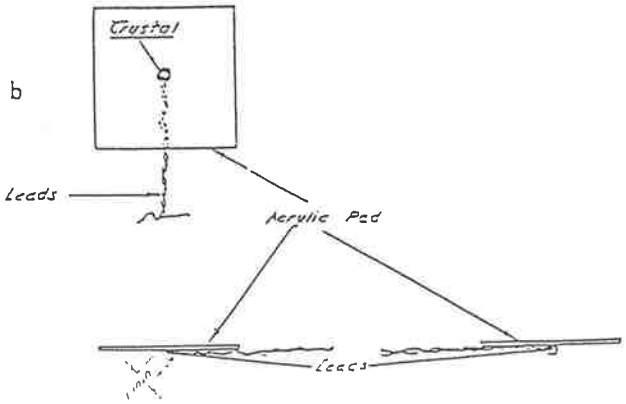
b



B



b



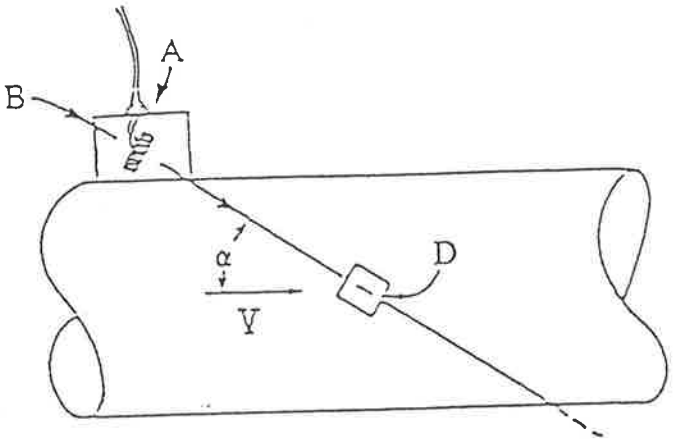


Figure 6.4

Principles of the Doppler method for measuring blood flow velocity in a blood vessel. (A) is the Doppler flow probe mounted on a blood vessel, (B) the direction of the ultrasound generated by the Doppler flowmeter and probe, α the angle between the direction of blood flow (V) and the ultrasound, and (D) the sample point.

Figure 6.5

Calibration of the Mingograf82 chart recorder (left) and examples of the control recordings of the measured haemodynamic parameters (right). Abbreviations used are:

CA: coronary artery

CABF: coronary artery blood flow

ECG: electrocardiogram

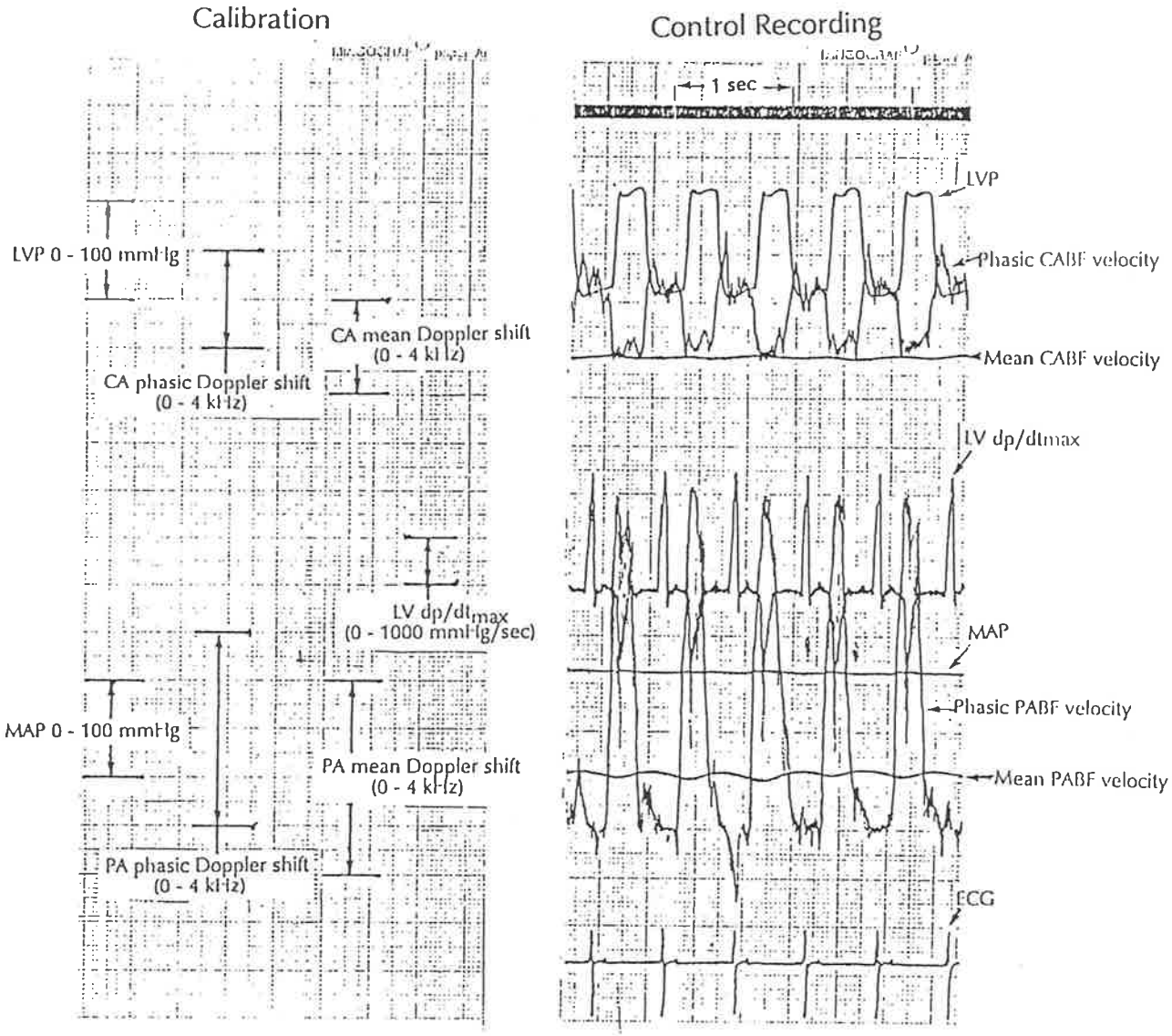
LV dp/dt_{max}: maximum rate of left ventricular pressure rise

LVP: left ventricular pressure

MAP: mean arterial blood pressure

PA: pulmonary artery

PABF: pulmonary artery blood flow


Calibration
LVP 0 - 100 mmHg
CA phasic Doppler shift
(0 - 4 kHz)
CA mean Doppler shift
(0 - 4 kHz)
LV dp/dtmax
(0 - 1000 mmHg/sec)
MAP 0 - 100 mmHg
PA mean Doppler shift
(0 - 4 kHz)
PA phasic Doppler shift
(0 - 4 kHz)
Control Recording
1 sec
LVP
Phasic CABF velocity
Mean CABF velocity
LV dp/dtmax
MAP
Phasic PABF velocity
Mean PABF velocity
ECG

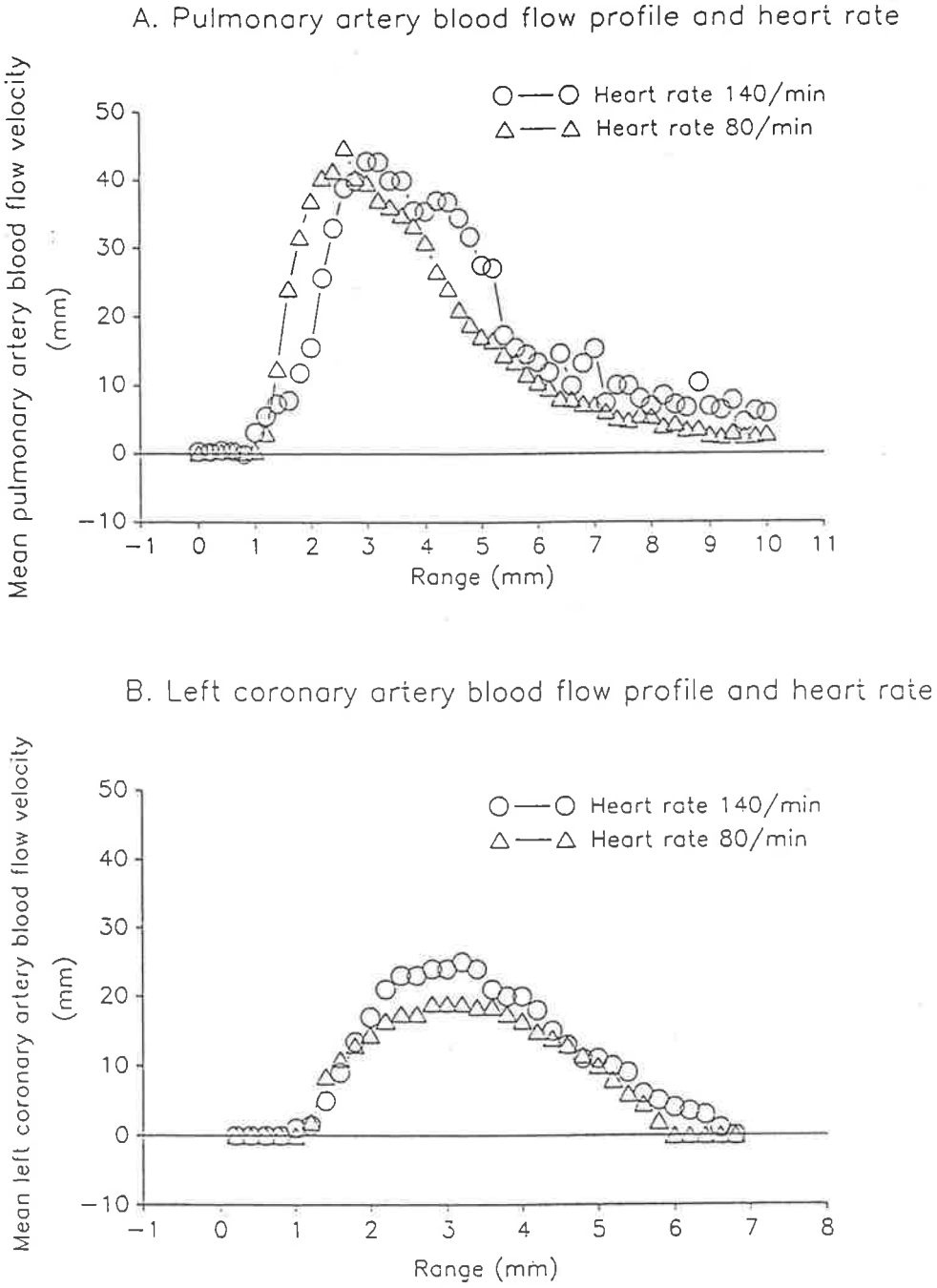


Figure 6.6

Blood flow profiles in the pulmonary artery (A) and the left coronary artery (B). The horizontal axis shows the distance between the sampling point and the Doppler transducer, i.e. the "Range". The vertical axis is the Doppler frequency shift in millimeters (equivalent to blood flow velocity). Open triangles are blood flow profiles at a heart rate of 80 beats/min and open circles are blood flow profiles at a heart rate of 140 beats/min.

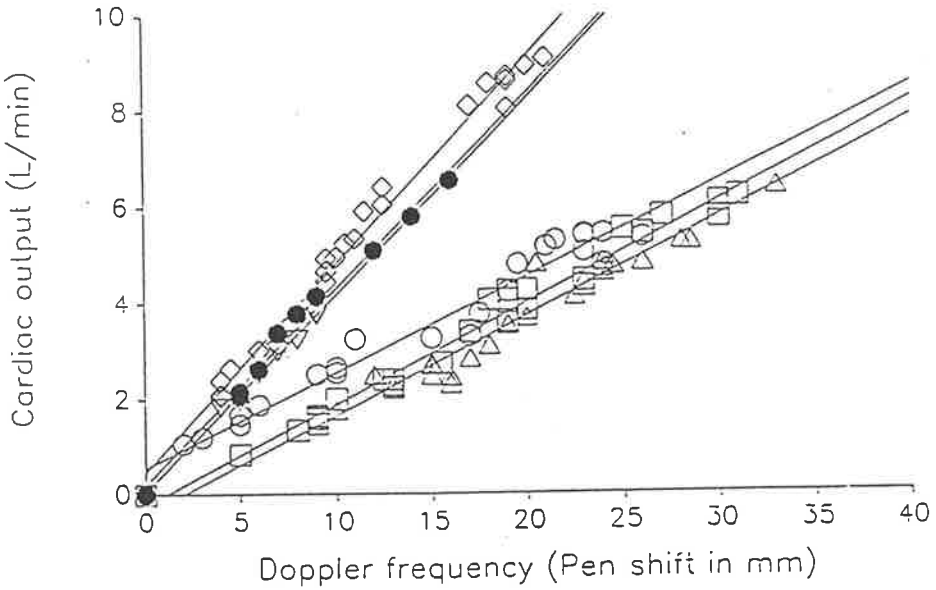
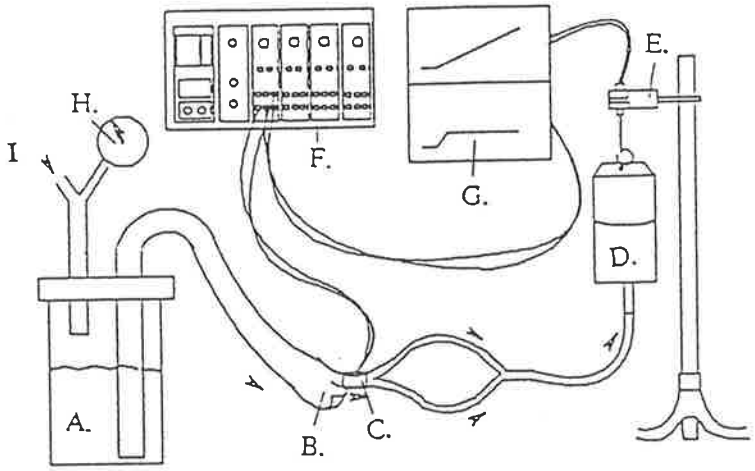


Figure 6.7

Linear regression of the *in vivo* Doppler frequency shift in pulmonary artery and cardiac output simultaneously detected by the thermolution method (six sheep, and each regression line represent one sheep over a range of cardiac outputs).



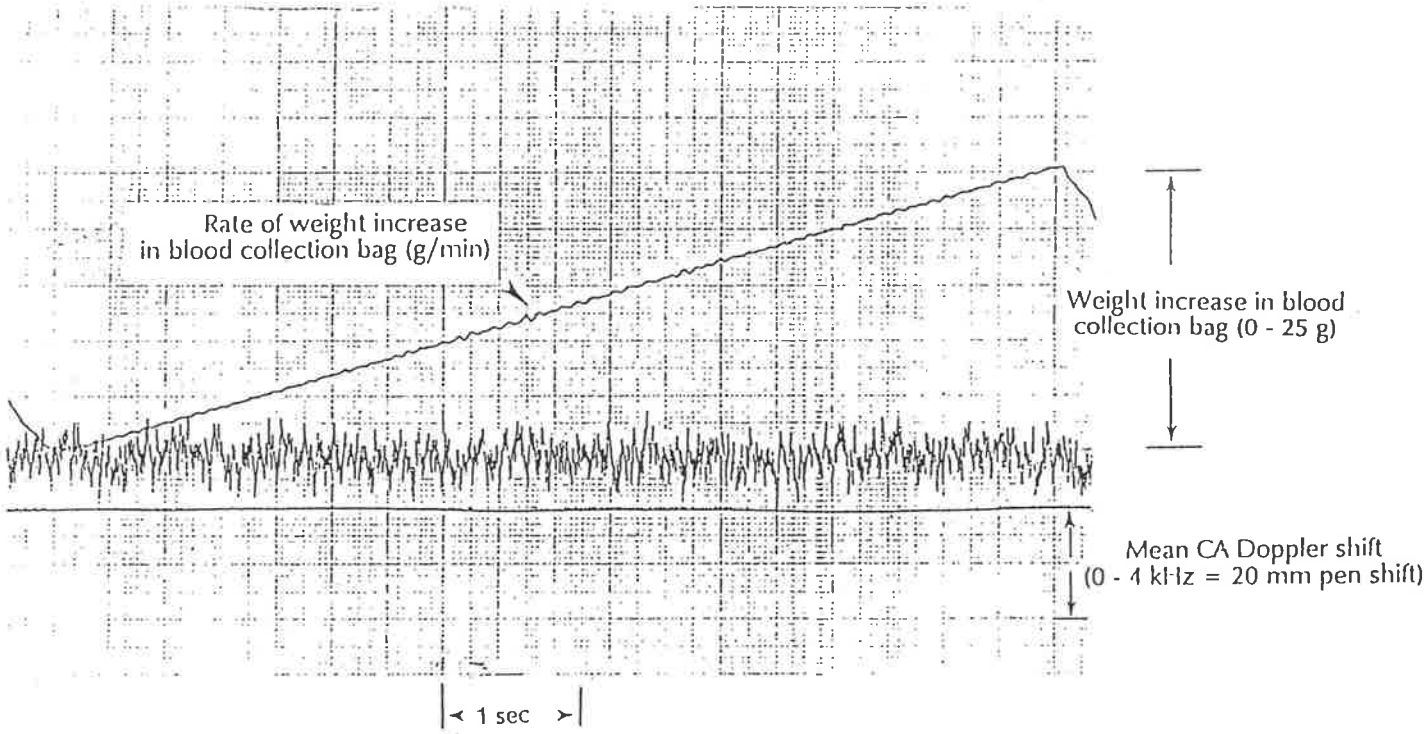
A. Bucket containing blood; B. Cannulated aorta; C. Doppler flow probe on left coronary artery; D. Blood collection bag; E. Force replacement transducer; F. Doppler flowmeter; G. Mingograf82 chart recorder; H. Sphygmonanometer; I. Connection to the pneumatic bulb.

Figure 6.8

Experimental set - up for the *in vitro* calibration of Doppler flow probes on the left coronary artery.

Figure 6.9

An example of the chart recording of the *in vitro* calibration of the Doppler flow probe on the left coronary artery. The constant blood flow and mean Doppler frequency shift are apparent.


Rate of weight increase
in blood collection bag (g/min)
Weight increase in blood
collection bag (0 - 25 g)
Mean CA Doppler shift
(0 - 4 kHz = 20 mm pen shift)
1 sec

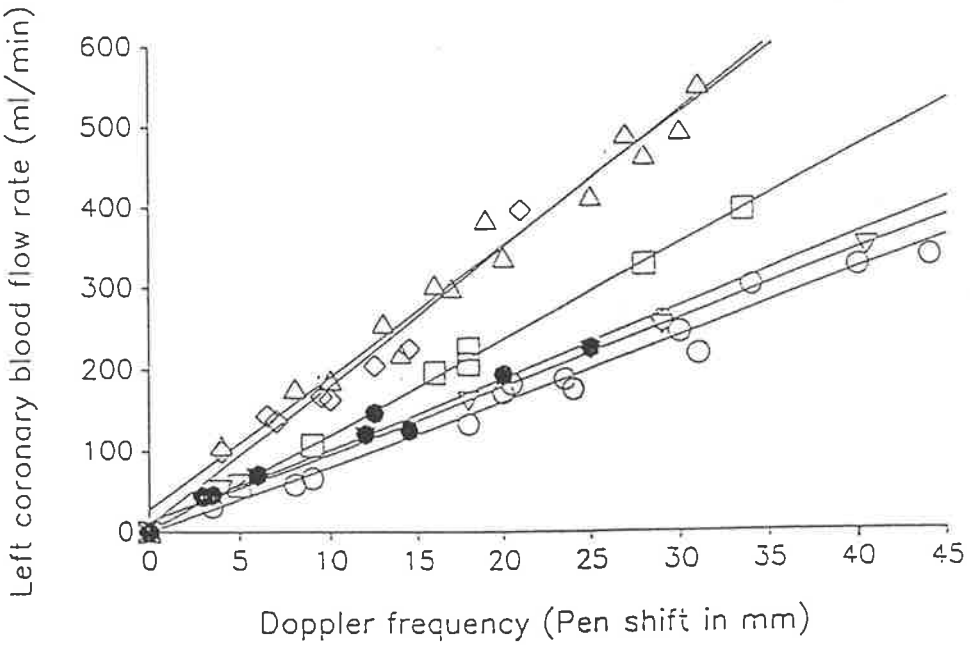


Figure 6.10

Linear regression of the *in vitro* Doppler frequency shift and blood flow rate in the left coronary artery of six sheep. Each regression line represents one calibration over a range of left coronary artery blood flow.

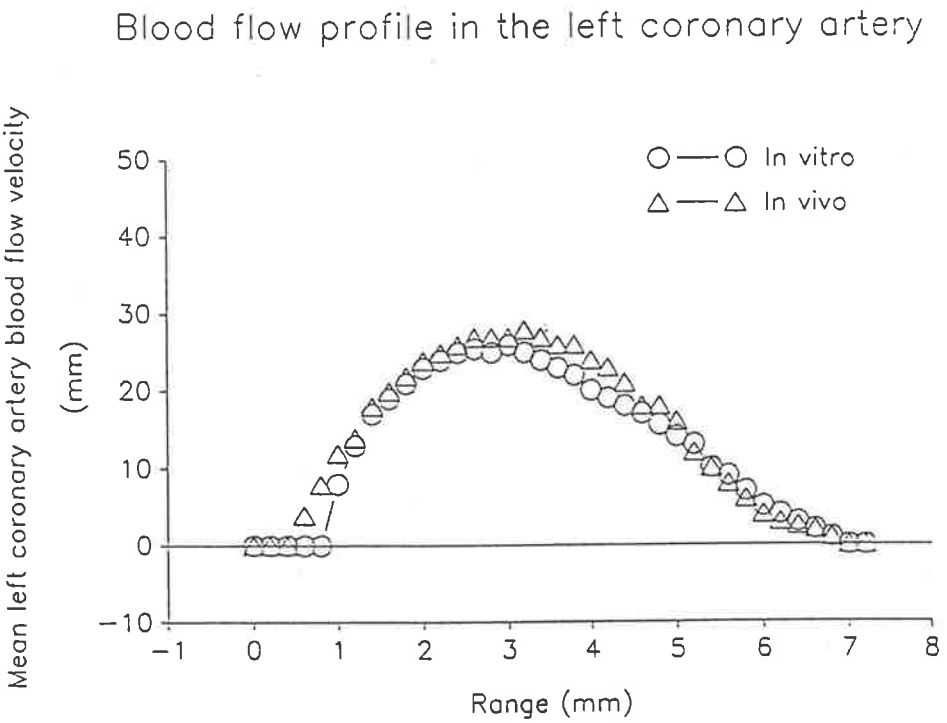


Figure 6.11

In vivo (open triangles) and *in vitro* (open circles) blood flow profiles measured for a Doppler flow probe on the left coronary artery.

CHAPTER 7: HAEMODYNAMIC EFFECTS OF SMALL VOLUMES OF HYPOTONIC SOLUTIONS AFTER INTRAVENOUS ADMINISTRATION TO CONSCIOUS, UNRESTRAINED SHEEP.

7.1 Introduction

The side - effects of the intravenous administration of relatively large volumes of hyper - and hypo - tonic solutions are well known. These include post - infusion phlebitis, intravaslucar haemolysis (Tomas et al., 1970; Ponder, 1971), and the disturbance of fluid balance in paediatric and fluid - restricted patients (Robinson et al., 1987; Santeiro et al., 1990). Although it has been reported that the rapid intravenous injection of small volumes of high osmolality solutions caused significant haemodynamic changes (Huseby and Gumprecht, 1981), little is known about the haemodynamic effects of the intravenous injection of small volumes (e.g. less than 20 ml) of hypotonic solutions, such as those which occur when some drugs formulated as a solution in water are administered as an intravenous bolus. Indeed, the British Pharmacopoeia, (1988) and the United States Pharmacopoeia (1989) make no recommendations regarding the osmolarity of solutions for intravenous bolus administration that have volumes of less than 100 ml. The conventional wisdom on this matter is that after intravenous administration the rapid mixing of such small volumes of hypotonic solutions, and water itself, with venous blood disperses the hypotonic solution so rapidly that no adverse effects are apparent.

In preparation for the series of studies of myocardial pharmacokinetics and pharmacodynamics, it was considered important to confirm that there were no haemodynamic effects due to the i.v. injection of the solutions used to dilute the drugs under study (either Water for Injection B.P or 0.9% Sodium Chloride Injection B.P.). These studies would therefore act as control studies for the drug studies described later (Chapters 8 and 9). However, further studies were conducted when it was found that while the injection of 0.9% Sodium Chloride Injection B.P. caused no haemodynamic effects, the injection of Water for Injection B.P. caused severe, transient haemodynamic depression.

The aims of the studies were as follows. 1. To determine the haemodynamic effects of the intravenous injection of small volumes of Water for Injection B.P. in conscious, unrestrained sheep, and to compare them with those of small volumes of 0.9% Sodium Chloride Injection B.P. 2. To determine the relationship between the haemodynamic effects and the osmolalities of small volumes of solutions injected intravenously, and of the rate and site of injection,. 3. To make preliminary investigations into the mechanisms of the observed haemodynamic effects.

7.2 Methods

7.2.1 Animal preparation and haemodynamic measurements

Five adult female Merino sheep weighing between 37 and 52 kg were prepared with the chronic instrumentation and blood sampling catheters described in Chapter 6. The haemodynamic parameters were measured and recorded using the methods described in Chapter 6.

7.2.2 Study design

On an experimental day, each sheep was supported in a comfortable sling inside its metabolic crate to minimize movement that would influence the haemodynamic measurements. All injections were made using an angiographic injector (1) that enabled accurate control of injection rates and volumes. Immediately before each injection of a solution, a 30 sec period of control haemodynamic parameters and left coronary artery blood flow was recorded. The solution under study was then injected, and the parameters were recorded for a further 2 min. Subsequent injections were not made until haemodynamic parameters and left coronary blood flow had returned to stable control values.

The injection solutions, injection sites and injection rates studied were as follows; the order in which they were studied was selected randomly.

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1 Angiomat 3000, Viamonte HOBBS, Barber - Colman Company, Rockford, Illinois, USA

7.2.2.1 Solutions for injection

1. 10 ml of Water for Injection B.P. (2)

2. 10 ml of 0.9% Sodium Chloride Injection B.P. (3). In addition, 10 ml of 0.225%, 0.45%, 0.6% and, 0.675% solutions of sodium chloride were studied. These solutions were prepared by diluting 0.9% Sodium Chloride Injection B.P. with Water for Injection B.P. The osmolalities of the resulting solutions were determined using an osmometer (4) calibrated over the range 100 - 900 mOsm/kg.

3. 10 ml of blood taken from the arterial catheter of the same sheep with a pre - heparinised syringe immediately before injection.

4. 10 ml of haemolysed blood prepared by adding 5 ml of Water for Injection B.P. to 5 ml of blood taken from the arterial catheter of the same sheep with a heparinised syringe immediately before injection. 0.23 ml of 20% NaCl was added to the haemolysed blood solution to increase its osmolarity to over 310 mOsm/kg (greater than isotonic).

7.2.2.2 Injection sites

Injections were made into the inferior vena cava (IVC). Water for Injection B.P. was also injected into the pulmonary artery and retrogradely into the ascending aorta.

7.2.2.3 Injection rates

The 10 ml volume of the solutions was injected over 1 sec. In addition, 10 ml of Water for Injection B.P. was injected into the IVC over 2, 4 and 10 sec.

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2 Abbot Australia Pty. Ltd., NSW, AUS, or Astra Pharmaceuticals, North Ryde, NSW, AUS

3 Travenol Laboratories Pty. Ltd., Oakes Road, Old Toongabbie, NSW, AUS

4 Advanced DigiMatic Osmometer, Model 3DL, Needham Heights, MA, USA

7.2.3 Data and statistical analysis

The haemodynamic parameters were averaged and calculated for the following intervals. The 30 sec immediately before injection (the control period), for 5 sec intervals for the first 30 sec following injection, for 10 sec intervals for the next 30 sec, and for 20 sec intervals for the following 60 sec.

Each solution for a given injection rate or site was injected once in each of the 5 sheep.

Paired t - test was used for statistical analysis of the differences of haemodynamic parameters measured during the control periods and those measured after the intravenous injection of different solutions. Linear regressions were used for correlating injection rates or injectate concentrations with haemodynamic effects. $P < 0.05$ was considered statistically significant.

7.3 Results

7.3.1 Water for Injection B.P. and 0.9% Sodium Chloride Injection B.P.

In conscious, unrestrained sheep the injection of Water for Injection B.P. into the IVC over 1 sec caused significant depression of haemodynamic status and a decrease in left coronary artery blood flow, while 0.9% Sodium Chloride Injection B.P. had no such effects (Table 7.1). The haemodynamic depressant effects of Water for Injection B.P. started approximately 12 sec after injection, and lasted between 10 and 20 sec. After this period of depression, there was a short period of "rebound" during which many parameters exceeded their control values. An example of the time - course of these effects is shown in Fig. 7.1.

During the period of depression produced by the intravenous injection of Water for Injection B.P., left coronary artery blood flow was significantly decreased and was also subject to rebound (Table 7.1 and Fig. 7.1). Heart rate significantly increased during the first 30 sec following injection and then usually slowed down for the next 30 to 60 sec during which bradycardia and atrio - ventricular conduction block were often apparent on the intracardiac ECG (Fig. 7.2).

7.3.2 Different concentrations of NaCl

The measured osmolalities of 0.225%, 0.45%, 0.6%, 0.675% and 0.9% Sodium Chloride Injection B.P. solutions were 81, 164, 208, 264 and 335 mOsm/kg, respectively. It is apparent from Table 7.2 that of the four concentrations of NaCl solutions used, the intravenous injection of 10 ml of 0.6% and 0.67% NaCl over 1 sec into the IVC did not cause any significant perturbation of the measured haemodynamic parameters or left coronary blood flow. The injection of 0.45% NaCl decreased the mean arterial blood pressure and increased the heart rate. However, the injection of 0.225% NaCl resulted in significant decreases in the measured haemodynamic parameters and left coronary artery blood flow, and increases of heart rate in a manner comparable to the effects of the injection of Water for Injection B.P.

7.3.3 Heparinised blood

The injection of 10 ml of heparinised blood into the IVC over 1 sec had no statistically significant effect on the haemodynamic parameters, left coronary artery blood flow or the intracardiac ECG (Table 7.3).

7.3.4 Haemolysed blood

The injection of 10 ml of haemolysed blood solution into the IVC over 1 sec induced changes in the haemodynamic parameters, left coronary artery blood flow and the intracardiac ECG similar to those produced by the injection of Water for Injection B.P. and 0.225% NaCl (Table 7.3 and Fig. 7.3).

7.3.5 Injection rates

It is apparent from Table 7.4 that, apart from statistically significant decreases in left ventricular systolic and diastolic pressures and the mean arterial pressure, the injection of 10 ml of Water for Injection B.P. over 10 sec into the IVC did not cause great changes in other haemodynamic parameters. In contrast, injections of 10 ml over 2 and 4 sec had adverse effects on the haemodynamic parameters, decreased left coronary artery blood flow and produced intracardiac ECG changes similar to those induced by injection over 1 sec.

7.3.6 Injection sites

The injection of 10 ml of Water for Injection B.P. into the pulmonary artery over 1 sec had no significant effects on haemodynamic status or left coronary artery blood flow (Table 7.5). The injection of 10 ml of Water for Injection B.P. into the ascending aorta over 1 sec had no depressant effects but did cause some slight changes to the myocardial function and haemodynamic status, characterised by increases in LV and mean arterial blood pressures, LV stroke work, LV minute work and left coronary artery blood flow (Table 7.5).

7.4 Discussion

As discussed in Chapter 6, the accuracy of LV dp/dt_{max} as a measurement of myocardial contractility is affected by simultaneous changes in LV end diastolic pressure, aortic diastolic pressure and heart rate (Mason, 1969). However, heart rate variations did not induce significant changes in LV dp/dt_{max} in the sheep (see 6.6). The slight decreases of left ventricular end diastolic pressure and mean arterial pressure can not account for the observed greater than 50% reduction of LV dp/dt_{max} in response to intravenously injected Water for Injection B.P. (Table 7.1 and 7.4), and this must be attributed to compromised myocardial contractility. This is supported by the other indicators of cardiac performance such as cardiac output, stroke volume, LV pressures, LV stroke work and LV minute work, all of which showed depression of myocardial function (Table 7.1 and Fig. 7.1). Together with the significantly decreased mean arterial blood pressure and increased heart rate and systemic vascular resistance, an overall picture of a temporary deterioration in myocardial function and haemodynamic status shortly after the injection of 10 ml of Water for Injection B.P. into the IVC of the conscious, unrestrained sheep is apparent. This phenomenon appears not to have been reported previously.

In contrast to the work of Mason (1968), it is unlikely that these unexpected effects were the result of an autonomic nervous system reaction due to the rapid injection stimulating sensors on the vessel wall of IVC, because similar injections of 0.9% Sodium Chloride Injection B.P. did not induce any changes in haemodynamic status,

left coronary artery blood flow or intracardiac ECG (Table 7.1). However, it is apparent that the depressant effects were related to the concentrations (and therefore osmolalities) of the NaCl injection solutions (Table 7.2). The injection of NaCl solutions with concentrations above 0.45% (164 mOsm/kg) did not cause depressant effects, apart from slight increases in left coronary blood flow. The injection of 0.45% NaCl had some slight effects, characterised by a decrease in mean arterial blood pressure and an increase in heart rate. The injection of 0.225% NaCl (81 mOsm/kg) produced similar myocardial and haemodynamic effects to the injection of Water for Injection B.P. Thus, it seems that an osmolality of approximately 164 mOsm/kg is the threshold, below which significant depressant effects were found for injections over 1 sec. Linear regression showed inverse linear correlations between the haemodynamic effects and the concentrations of NaCl used (Fig. 7.4). The coefficients of these correlations were all greater than 0.9.

Furthermore, it appears that the depression was a result of the action of these hypotonic solutions on blood. The injection of heparinised blood had no effect, yet the injection of blood which had been haemolysed with Water for Injection B.P *and then had its osmolarity restored to slightly greater than isotonic* had profound depressant effects (Table 7.3). It appears therefore that the intravenous injection of hypotonic solutions such as Water for Injection B.P. and 0.225% NaCl caused haemolysis or other unknown biochemical changes in blood, and these caused the observed effects on haemodynamic status and left coronary artery blood flow. This mechanism was therefore different to that causing depression after the intravenous injection of hypertonic solutions which was thought to be the result of direct peripheral vasodilatation or the release of histamine (Huseby and Gumprecht, 1981; Findlay et al., 1981).

It has also been recognised that non - ionic solutions can cause decreases in the concentration of ionised calcium in the myocardium and subsequently cause haemodynamic changes (Fischer and Morris, 1980). However, in the present study, the lack of ions in the solutions is unlikely to be the only cause of the observed

haemodynamic depression and decrease of left coronary blood flow because depression was not produced when Water for Injection B.P. was injected into the pulmonary artery and the ascending aorta (Table 7.5). Alternatively, solutions low in ions exert their effects only on the endocardium of the right side of the heart. However, this argument is not supported by the observation that in all studies a time lag of approximately 12 sec occurred before the onset of the depressant effects. Our previous studies have shown that a solution injected into the IVC of a sheep requires only 5 to 6 sec to reach the right side of the heart (Upton et al., 1990b).

Further evidence that the primary site of depressant action was not on the peripheral vascular system was also provided by the fact that the injection of Water for Injection B.P. into the pulmonary artery and ascending aorta did not have depressant effects (Table 7.5). The hypothesis that the depressant effect of these hypotonic solutions was related to the effects of haemolysed blood or other unknown materials on the myocardium is consistent with the studies of the influence of injection rate and concentrations of the injection solutions. Linear relationships were found between the percentage changes of the haemodynamic parameters and the injection rates, and inverse linear relationships between the concentrations of the injection solutions and the haemodynamic changes (Fig. 7.4). It would be expected that at slower injection rates the extent of haemolysis as the water entered the blood stream would be less than that occurring for faster injection rates, and more severe haemolysis will occur with the use of lower concentrations of NaCl. Thus, the injection of Water for Injection B.P. into the IVC over 10 sec and higher concentrations of NaCl had less or no effects on the myocardium (Table 7.4).

In view of the depressant effects of hypotonic solutions, it would be expected that the intravenous injection of drug solutions with osmolalities lower than that of 0.45% NaCl (164 mOsm/kg) would depress myocardial function, haemodynamic status and coronary artery blood flow due to their hypotonicity alone. This depression of the haemodynamic status would compound the myocardial depressant effects caused by drugs such as some anti - arrhythmics and calcium antagonists. A pilot study in our

laboratory showed a dramatic difference between the myocardial effects of procainamide diluted in Water for Injection B.P. or in 0.9% Sodium Chloride Injection B.P. (Fig. 7.5). Although this dose of procainamide diluted in 0.9% Sodium Chloride Injection B.P. did not have depressant effects on the myocardium, when administered as a hypotonic solution in water, it had greater depressant effects than Water for Injection B.P. alone (compare Fig. 7.1 and 7.5).

If these findings are also found in man, the data have several important clinical implications. Many commercial drug products such as antiarrhythmics, analgesics and anaesthetics are dissolved in Water for Injection B.P. and are approved for intravenous bolus injection. Their osmolalities are not often stated on the label, although this is now recommended by the United States Pharmacopoeia. It could be argued that in clinical practice drugs are rarely administered as an intravenous bolus over 1 - 10 sec. However, in practice there is great variability in the rate that drugs are administered as bolus injections. The potential effects are transient but severe and could be more severe in patients whose circulatory function is already compromised by age or disease. Therefore, controlling the osmolality and injection rate of drugs administered as a bolus may be clinically important, particularly with critically ill patients; further work in this area is warranted.

Table 7.1 The effects of the intravenous injection of 10 ml of Water for Injection B.P. or Sodium Chloride Injection B.P. on the haemodynamic parameters and left coronary artery blood flow of conscious, unrestrained sheep (mean ± S.D., n = 5).

	Water for Injection B.P.		**0.9% Sodium Chloride Injection B.P.**	
	Control	*Maximum Effect*	*Control*	*Maximum Effect*
Cardiac output (L/min)	6.2 ± 2	2.7 ± 2 [b]	5.2 ± 2.5	5.4 ± 2.3
LV systolic pressure (mmHg)	114 ± 15	68.5 ± 22 [b]	112 ± 7	114 ± 7
LV end diastolic pressue (mmHg)	11 ± 3	4 ± 2 [b]	11 ± 3	10 ± 3
LV dP/dt_{max} (mmHg/sec)	2125 ± 263	1280 ± 639 [b]	2240 ± 295	2235 ± 307
Mean arterial blood pressure (mmHg)	102 ± 13	68 ± 16 [b]	102 ± 10	102 ± 5
Heart rate (beats/min)	108 ± 21	148 ± 33 [b]	105 ± 27	108 ± 28
Stroke volume (ml/beat)	58 ± 23	29 ± 26 [b]	54 ± 28	54 ± 28
LV Stroke work (g·meters)	84 ± 46	33 ± 35 [b]	76 ± 44	79 ± 47
LV Minute work (kg/m/min)	9.4 ± 4.8	4.7 ± 5.2 [a]	7.8 ± 4.2	8.3 ± 4.4
Systemic vascular resistance (dynes/sec/cm^{-5})	1407 ± 282	2665 ± 1498 [a]	1784 ± 691	1687 ± 541
Double product (rate - pressure $^{-3}$)	11.4 ± 4.9	12 ± 5.8	12.3 ± 2.1	12.8 ± 2.5
Left coronary artery blood flow (ml/min)	132 ± 58	51 ± 38 [b]	143 ± 40	145 ± 41
Coronary vascular resistance (dynes/sec/cm^{-5})	64 ± 28	552 ± 1002	50 ± 21	51 ± 22

[a]($P < 0.05$) and [b]($P < 0.01$): differences to controls values are statistically significant (Paired t - test).

Table 7.2 Maximum changes in the haemodynamic parameters and left coronary artery blood flow as percentages of their control values after the i.v. injection of 10 ml solutions of different concentrations of NaCl (mean ± S.D., n = 5).

	Percentage of Control Values			
	0.225% NaCl	*0.45% NaCl*	*0.6% NaCl*	*0.67% NaCl*
Cardiac output	70 ± 53 [a]	96 ± 25	107 ± 22	104 ± 4 [a]
LV systolic pressure	66 ± 16 [b]	93 ± 10	101 ± 2	100 ± 1
LV diastolic pressure	26 ± 26 [b]	85 ± 49	103 ± 6	97 ± 10
LV dP/dt_{max}	64 ± 19 [b]	92 ± 14	100 ± 1	101 ± 3
Mean arterial blood pressure	74 ± 8 [b]	91 ± 8 [a]	100 ± 1	100 ± 3
Heart rate	142 ± 12 [b]	113 ± 18 [a]	99 ± 1	101 ± 4
Stroke volume	40 ± 25 [b]	94 ± 29	105 ± 19	104 ± 5
LV Stroke work	64 ± 56 [a]	89 ± 35	106 ± 18	1.05 ± 5
LV Minute work	94 ± 81	94 ± 24	105 ± 19	105 ± 4
Systemic vascular resistance	103 ± 5	101 ± 21	99 ± 16	97 ± 4
Double product	124 ± 37	113 ± 2	100 ± 3	101 ± 4
Left coronary artery blood flow	58 ± 25 [b]	84 ± 23	106 ± 4 [a]	108 ± 8 [a]
Coronary vascular resistance	117 ± 1	111 ± 40	95 ± 6 [a]	95 ± 5 [a]

[a]($P < 0.05$) and [b]($P < 0.01$): differences to controls values are statistically significant (Paired t - test).

Table 7.3 The effects of the i.v. injection of 10 ml of heparinised and haemolysed blood on haemodynamic parameters and on left coronary artery blood flow (mean ± S.D., n = 5).

	Haemolysed blood		**Heparinised blood**	
	Control	*Maximum effect*	*Control*	*Maximum effect*
Cardiac output (L/min)	5.6 ± 2.7	3.9 ± 2.1 [b]	6.1 ± 2.4	6.5 ± 2.8
LV systolic pressure (mmHg)	122 ± 12	78 ± 17 [b]	124 ± 11	121 ± 13
LV end diastolic pressue (mmHg)	11 ± 3	4 ± 2 [a]	12 ± 4	11 ± 4
LV dP/dt_{max} (mmHg/sec)	2160 ± 287	1440 ± 469 [b]	2281 ± 267	2337 ± 260
Mean arterial blood pressure (mmHg)	111 ± 13	78 ± 16 [b]	111 ± 17	109 ± 18
Heart rate (beats/min)	106 ± 23	144 ± 34 [b]	105 ± 20	103 ± 20
Stroke volume (ml/beat)	56 ± 26	45 ± 27	62 ± 28	64 ± 32
LV Stroke work (g·meters)	87 ± 51	54 ± 42 [b]	99 ± 55	99 ± 59
LV Minute work (kg/m/min)	9.3 ± 5.4	8.4 ± 7	10.3 ± 5.7	10.5 ± 5.9
Systemic vascular resistance (dynes/sec/cm^{-5})	1741 ± 450	1399 ± 763	1576 ± 363	1577 ± 567
Double product (rate - pressure $^{-3}$)	12.9 ± 3.5	15 ± 6.3	12.9 ± 2.5	13.2 ± 3.1
Left coronary artery blood flow (ml/min)	137 ± 62	94 ± 41 [b]	153 ± 55	157 ± 54
Coronary vascular resistance (dynes/sec/cm^{-5})	65 ± 24	46 ± 25 [a]	57 ± 28	55 ± 26

[a]($P < 0.05$) and [b]($P < 0.01$): differences to controls values are statistically significant (Paired t - test).

Table 7.4 Maximum changes in the haemodynamic parameters and left coronary artery blood flow as percentages of their control values after the i.v. injection of Water for Injection B.P. at injection rates of 10 ml in 1, 2, 4 or 10 sec (mean ± S.D., n = 5).

	Percentage of Control Values			
	10 ml/1 sec	*10 ml/2 sec*	*10 ml/4 sec*	*10 ml/10 sec*
Cardiac output	45 ± 28 [b]	39 ± 33 [b]	62.4 ± 42.7 [a]	97.3 ± 17
LV systolic pressure	60 ± 18 [b]	61 ± 21 [b]	71 ± 21 [b]	89 ± 11 [a]
LV end diastolic pressue	36 ± 22 [b]	31 ± 23 [b]	50 ± 23	80 ± 20 [a]
LV dP/dt_{max}	53 ± 10 [b]	61 ± 29 [b]	69 ± 22 [b]	90 ± 15
Mean arterial blood pressure	67 ± 9 [b]	67 ± 9 [b]	79 ± 12 [b]	94 ± 5 [a]
Heart rate	138 ± 26 [b]	143 ± 27 [b]	128 ± 20 [b]	112 ± 18
Stroke volume	45 ± 39 [b]	49 ± 41 [b]	62 ± 43 [a]	89 ± 33
LV Stroke work	35 ± 41 [b]	41 ± 41 [b]	59 ± 47 [a]	83 ± 34
LV Minute work	48 ± 61 [a]	61 ± 70	66 ± 46 [a]	89 ± 30
Systemic vascular resistance	185 ± 98 [b]	265 ± 289	145 ± 85	100 ± 28
Double product	112 ± 47	110 ± 67	95 ± 22	101 ± 8
Left coronary artery blood flow	43 ± 39 [b]	49 ± 28 [b]	75 ± 18 [b]	98 ± 4
Coronary vascular resistance	683 ± 1184	682 ± 1303	120 ± 50	97 ± 7

[a]($P < 0.05$) and [b]($P < 0.01$): differences to controls values are statistically significant (Paired t - test).

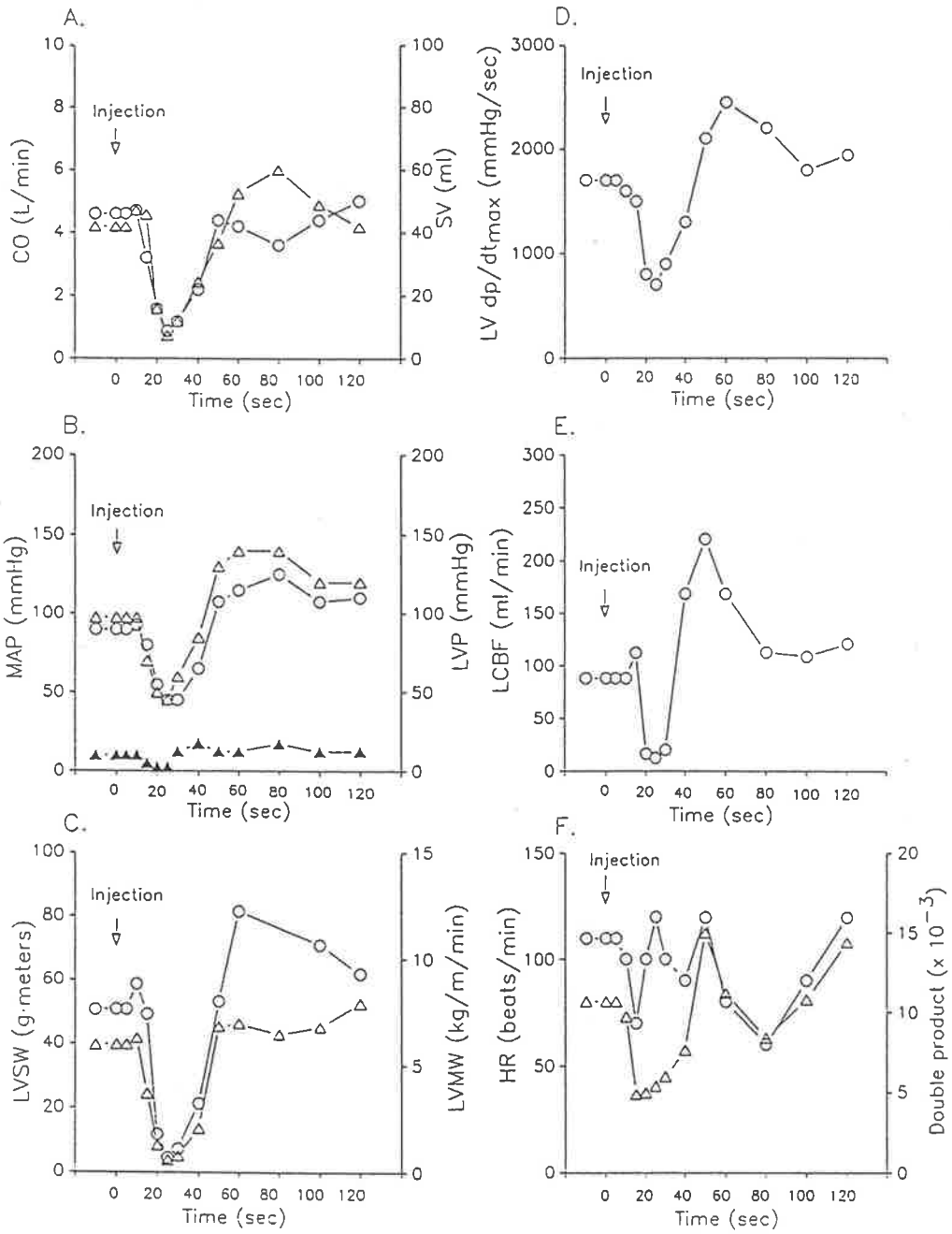
Figure 7.1

Effects of 10 ml of Water for Injection B.P. injected into the IVC over 1 sec. Scales for the open and closed circles are shown by the vertical axis on the left, and those for open and closed triangles are shown by the vertical axis on the right side of the graphs. (A) CO = Cardiac output, SV = Stroke volume, (B) MAP = Mean arterial pressure, LVP = Left ventricular pressure, (C) LVSW = Left ventricular stroke work, LVMW = Left ventricular minute work, (D) LV dP/dt_{max} = Maximum rate of change of left ventricular pressure, (E) LCBF = Left coronary artery blood flow, and (F) HR = Heart rate.

Table 7.5 Haemodynamic parameters and left coronary artery blood flow after the injection of 10 ml of Water for Injection B.P. into the pulmonary artery and the ascending aorta (mean ± S.D., n = 5).

	Pulmonary artery		**Ascending aorta**	
	Control	*Maximum Effect*	*Control*	*Maximum effect*
Cardiac output (L/min)	5.5 ± 2.5	5.6 ± 2.3	5.5 ± 2.6	6 ± 2.8
LV systolic pressure (mmHg)	117 ± 13	117 ± 17	116 ± 13	125 ± 13 [a]
LV end diastolic pressue (mmHg)	10 ± 3	11 ± 5	10 ± 3	12 ± 3 [a]
LV dP/dt_{max} (mmHg/sec)	2185 ± 193	2150 ± 338	2185 ± 272	2265 ± 387
Mean arterial blood pressure (mmHg)	105 ± 13	102 ± 20	103 ± 13	109 ± 14 [b]
Heart rate (beats/min)	104 ± 23	109 ± 21	107 ± 25	113 ± 21
Stroke volume (ml/beat)	56 ± 28	54 ± 28 [a]	55 ± 29	56 ± 27
LV Stroke work (g.meters)	84 ± 49	82 ± 54	80 ± 49	89 ± 52 [a]
LV Minute work (kg/m/min)	8.7 ± 5.1	8.9 ± 5.5	8.5 ± 5.1	9.7 ± 5.3 [b]
Systemic vascular resistance (dynes/sec/cm^{-5})	1728 ± 550	1751 ± 527	1752 ± 664	1772 ± 670
Double product (rate - pressure $^{-3}$)	12.1 ± 2.8	13.1 ± 2.9	12.4 ± 3.2	13.6 ± 2.3
Left coronary artery blood flow (ml/min)	129 ± 47	128 ± 54	131 ± 42	146 ± 39 [a]
Coronary vascular resistance (dynes/sec/cm^{-5})	63 ± 25	60 ± 26	58 ± 20	58 ± 22

[a]($P < 0.05$) and [b]($P < 0.01$): differences to controls values are statistically significant (Paired t - test).


A.
CO (L/min)
SV (ml)
Injection
Time (sec)
B.
MAP (mmHg)
LVP (mmHg)
Injection
Time (sec)
C.
LVSW (g·meters)
LVMW (kg/m/min)
Injection
Time (sec)
D.
LV dp/dtmax (mmHg/sec)
Injection
Time (sec)
E.
LCBF (ml/min)
Injection
Time (sec)
F.
HR (beats/min)
Double product (x 10⁻³)
Injection
Time (sec)

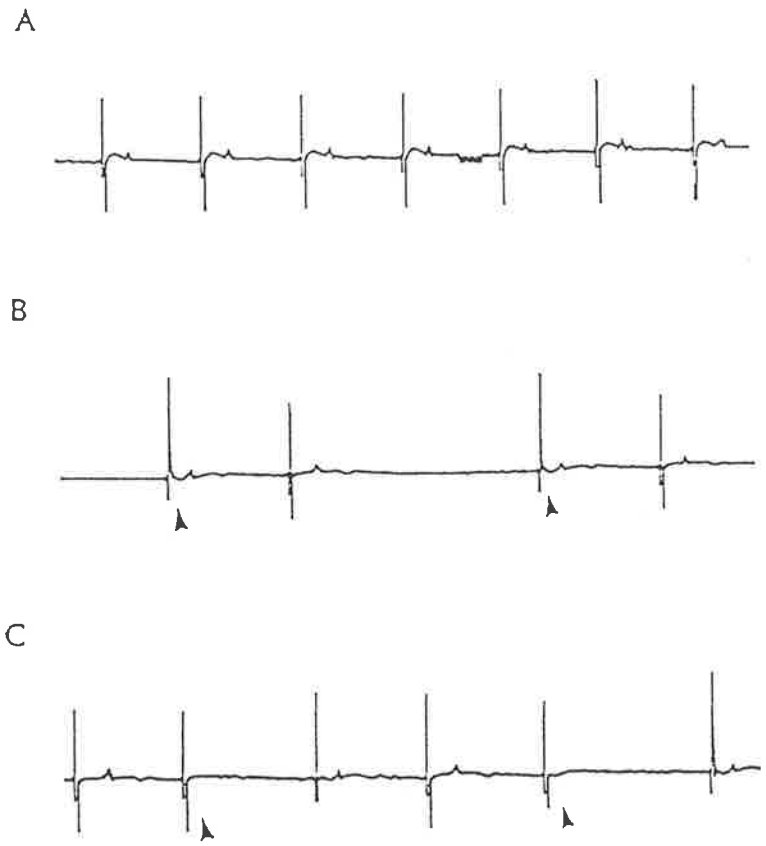


Figure 7.2

(A) An example of an intracardiac ECG trace recorded during the control period (heart rate = 120/min)

(B) and (C) Intracardiac ECG traces recorded approximately 60 sec after the injection of Water for Injection B.P. into the IVC. Supraventricular bradycardia ((B) heart rate = 60/min) and atrio - ventricular conduction block were detected ((C) as shown by the missing QRS indicated in the Figures by the arrows).

Figure 7.3

The Effects of 5 ml of blood haemolysed with 5 ml of Water for Injection B.P. and made greater than isotonic with 20% NaCl injected into the IVC over 1 second. Scales for the open circles are shown by the vertical axis on the left and those for open and solid triangles are shown by the vertical axis on the right side of the graphs. (A) CO = Cardiac output, SV = Stroke volume, (B) MAP = Mean arterial pressure, LVP = Left ventricular pressure, (C) LVSW = Left ventricular stroke work, LVMW = left ventricular minute work, (D) LV dP/dt_{max} = Maximum rate of change of left ventricular pressure, (E) LCBF = Left coronary artery blood flow, and (F) HR = Heart rate.

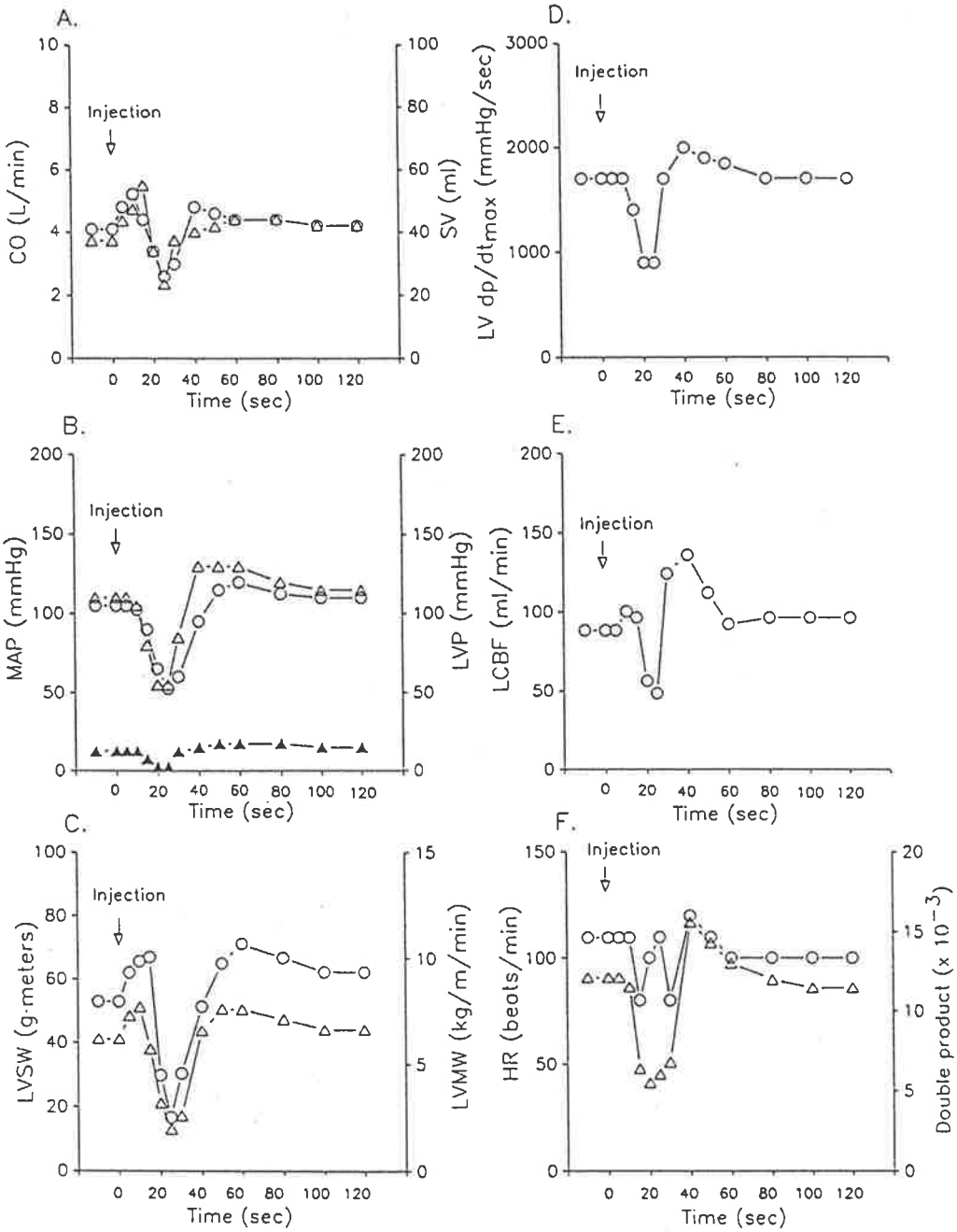

A.
Injection
CO (L/min)
SV (ml)
Time (sec)
D.
Injection
LV dp/dtmax (mmHg/sec)
Time (sec)
B.
Injection
MAP (mmHg)
LVP (mmHg)
Time (sec)
E.
Injection
LCBF (ml/min)
Time (sec)
C.
Injection
LVSW (g·meters)
LVMW (kg/m/min)
Time (sec)
F.
Injection
HR (beats/min)
Double product (x 10⁻³)
Time (sec)

Figure 7.4

(A) The linear relationships bewteen the percentage change of the haemodynamic parameters and the injection rates used. The correlation coefficients of these were all greater than 0.9.

(B) The inverse linear relationships between the percentage change of the haemodynamic parameters and the concentrations of NaCl injected. The correlation coefficients of these were all greater than 0.9.

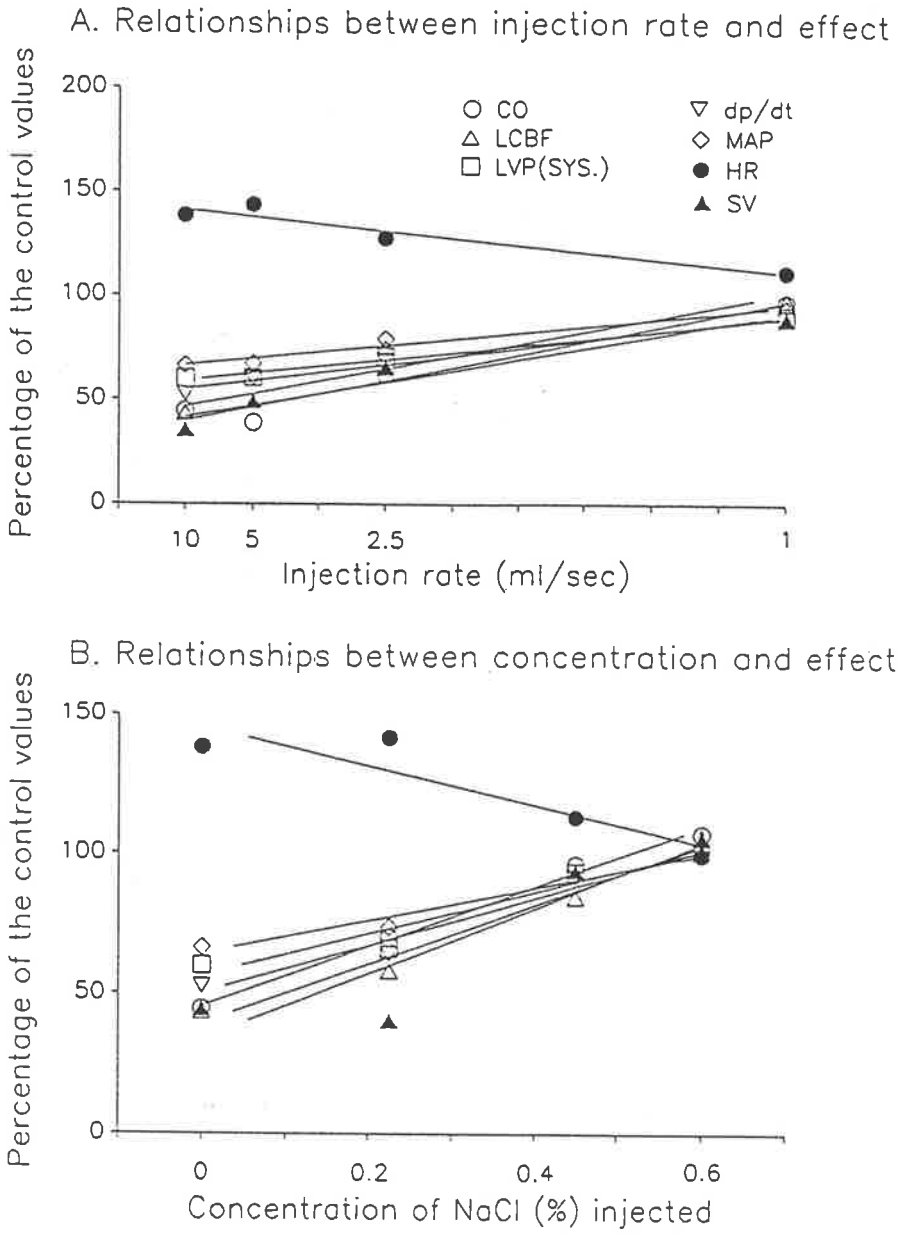
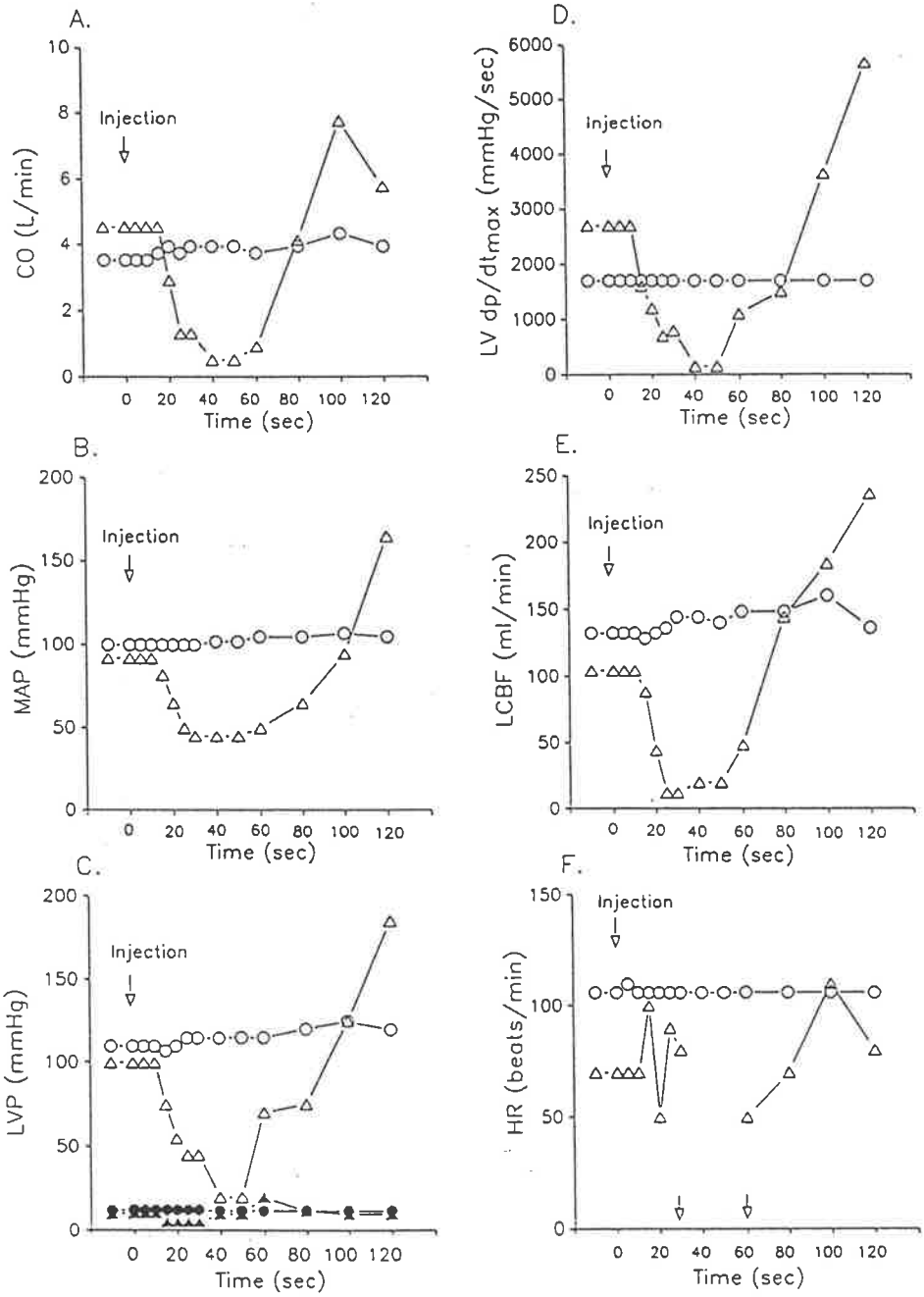

A. Relationships between injection rate and effect
Percentage of the control values
200
150
100
50
0
CO
LCBF
LVP(SYS.)
dp/dt
MAP
HR
SV
10
5
2.5
1
Injection rate (ml/sec)
B. Relationships between concentration and effect
Percentage of the control values
150
100
50
0
0
0.2
0.4
0.6
Concentration of NaCl (%) injected

Figure 7.5

Examples of the time - courses of the effects on myocardial and haemodynamic function following IVC injection of either 25 mg of procainamide diluted in 10 ml of water (osmolality 18 mOsm/kg, open and solid triangles) or 25 mg of procainamide diluted in 10 ml of Sodium Chloride Injection B.P. (osmolality over 300 mOsm/kg, open and solid circles). Both solutions were injected over 1 sec. (A) CO = Cardiac output, (B) MAP = Mean arterial pressure, (C) LVP = Left ventricular pressure, (D) LV dP/dt_{max} = Maximum rate of change of left ventricular pressure, (E) LCBF = Left coronary blood flow and (F) HR = Heart rate. In (F) the heart beat of the sheep stopped between the times indicated by the two arrows on the vertical axis.


A.
CO (L/min)
Injection
Time (sec)
B.
MAP (mmHg)
Injection
Time (sec)
C.
LVP (mmHg)
Injection
Time (sec)
D.
LV dp/dtmax (mmHg/sec)
Injection
Time (sec)
E.
LCBF (ml/min)
Injection
Time (sec)
F.
HR (beats/min)
Injection
Time (sec)

CHAPTER 8: HAEMODYNAMIC EFFECTS OF LIGNOCAINE AND PETHIDINE AFTER INTRAVENOUS BOLUS ADMINISTRATION TO CONSCIOUS, UNRESTRAINED SHEEP.

8.1 Introduction

Lignocaine is a local anaesthetic and also a Class Ib antiarrhythmic drug with a narrow margin of safety when used intravenously (Lucchesi and Patterson, 1982; Scott, 1984; Reiz and Nath, 1986; Vogt et al., 1988). The accidental intravenous injection of local anaesthetics is associated with severe central nervous and cardiovascular system toxicity (Albright, 1979; Jonville et al., 1990). Although there are numerous reports concerning the cardiovascular toxicity of lignocaine, several points are still not clear. Firstly, there is wide variation in the doses of lignocaine at which toxicity has been reported. Contributing factors are likely to be the differences in the animal species, experimental preparations and intravenous injection rates used in these investigations. It is known that slower i.v. injection rates are associated with higher doses and blood drug concentrations at the onset of toxicity (Malagodi et al., 1977; Morishima et al., 1981; Rutten et al., 1989), presumably due to the greater time available for the redistribution of the drug. Secondly, it has been reported that higher lignocaine blood drug concentrations were needed for the onset of toxic effects to the cardiovascular system than to the central nervous system (Munson et al., 1975; Malagodi et al., 1977; Liu et al., 1983). The latter was manifested by drowsiness, seizure and convulsion of the experimental animals (Munson et al., 1972; 1975; Liu et al., 1983; Rutten et al., 1989). It is known that these central nervous system effects can stimulate the sympathetic nervous system which can "offset" the toxic effects on the cardiovascular system (Doba et al., 1975; Bayne and Simon, 1981; Rutten et al., 1989). However, animal studies have also suggested that toxic blood concentrations can be achieved following i.v. bolus doses of lignocaine recommended for the treatment of cardiac arrhythmia (Vogt et al., 1988). Therefore in the studies described in this chapter, intravenous bolus doses of lignocaine between those

suggested for the treatment of cardiac arrhythmias and those that are known to cause central nervous system toxicity were used to investigate the effects of lignocaine on the cardiovascular system in conscious, unrestrained sheep.

Despite the fact that pethidine has been widely used as an analgesic following myocardial infarction and cardiac surgery (Lee et al., 1976; Helgesin and Refsum, 1987), the studies investigating its effects on the myocardium have not reached a consensus as to whether pethidine is a positive or negative ionotrope (Grundy, 1971; Helgesin and Refsum, 1987; 1990; Helgesin et al., 1990). Most of these studies used isolated myocardial tissues or Langendorff perfused heart preparations (Grundy, 1971; Strauer, 1972; Grundy and Tritthart, 1972; Rendig et al., 1980); the remainder were *in vivo* studies in anaesthetised animal preparations (Grundy, 1971). It is probable that this lack of consensus is due in part to differences in the type of *in vitro* preparations used in these studies. It is also known that general anaesthesia causes significant physiological changes to the central nervous system and the cardiovascular system (Warren and Stoelting, 1986) and that conclusions drawn from *in vivo* studies in anaesthetised animals can be different from studies of conscious, unrestrained animals. Therefore, the effects of intravenously administered pethidine on left coronary artery blood flow, myocardial function and haemodynamics in conscious, unrestrained sheep were investigated.

This chapter reports the haemodynamic data recorded following the intravenous injections of lignocaine and pethidine as part of the studies which will also be described in Chapter 9. In Chapter 10, the drug concentration data of Chapter 9 will be correlated with the haemodynamic data reported in this chapter.

Pilot studies of intravenous injections of procainamide diluted in 0.9% saline up to doses of 200 mg injected over 1 sec showed no significant effects on the haemodynamic parameters measured in conscious, unrestrained sheep. It was found that the predominant effect of procainamide on myocardial conduction could not be

measured using the intracardiac ECG recording method used. Time constraints prevented the development of a more precise method for measuring ECG changes in conscious, unrestrained animals. Therefore, the pharmacokinetic and pharmacodynamic data from procainamide studies were not included in this thesis.

8.2 Materials and methods

8.2.1 Animal preparation and haemodynamic measurements

The animals used in these experiments were those described in Chapter 6. The Doppler flow probes and other instruments for measuring and recording left coronary artery and pulmonary artery blood flow velocities and other haemodynamic parameters were calibrated as described in Chapter 6.

8.2.2 Drugs and drug administration

Lignocaine hydrochloride at doses of 50, 75, or 100 mg and pethidine hydrochloride at doses of 100, 200 or 300 mg were used for the study. The drug solutions used were prepared just before the experiments. Double the amount of the selected dose of the drug in its stock form (2% lignocaine hydrochloride or pethidine hydrochloride 200 mg in 2 ml) was diluted by 0.9% saline in a sterile glass mixing bottle to make an injection solution of 20 ml. This was then drawn into the plastic syringe of an Angiomat 3000 Viamonte HOBBS injector (1). After 30 sec of control recording of the haemodynamic parameters (see below and 6.6), 11.5 ml of this solution was injected using the injector into the inferior vena cava (IVC) catheter (see 2.2). Because this catheter had a dead space volume of 1.5 ml, 10 ml of the drug solution containing the selected dose was injected into the IVC blood. The dose and drug used in each experiment was selected randomly and all the doses of the two drugs were studied in each of the 6 sheep. The same sheep was not used for another study until 48 h after the experiment.

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1 Barber - Colman Company, Rockford, Illinois, USA

8.2.3 Study design

After the pre - experimental calibration of the instruments, the sheep were prepared for the measurement of both the mean and phasic blood flow velocities of the pulmonary and left coronary arteries respectively, the left ventricular pressure (LVP, mmHg), the maximum rate of left ventricular pressure rise (LV dp/dt_{max}), the mean arterial blood pressure (MAP) and the intracardiac ECG as described in Chapter 6. Sheep were allowed to "settle down" for approximately 30 min after the placement of the measuring devices. Control measurements of the haemodynamic parameters were recorded on the Mingograf82 chart recorder at a paper speed of 25 mm/sec for approximately 30 sec. The selected dose of lignocaine or pethidine was injected into the IVC of the sheep over 1 sec. The haemodynamic parameters were continuously recorded for the next 10 min, and for 30 sec at 15 min after drug injection. It was known from previous studies that the cardiovascular system and central nervous system effects of these doses of drugs would no longer be discernible by 10 min after drug injection (Rutten et al., 1989).

In addition to the haemodynamic measurements, the sheep were observed during the studies and any manifestation of central nervous system toxicity, as shown by agitation, fitting or convulsion was noted.

8.2.4 Data handling and statistical analysis

All the recorded haemodynamic parameters except heart rate were averaged for the 5 sec period before the times of 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 15 min after drug injection. The control values of these parameters were the mean (± S.D.) of the recorded parameters during the 30 sec period immediately before drug injections. Heart rate (HR, beats/min) was obtained by counting the number of QRS complexes on the intracardiac ECG recording during the 6 sec period before these times and multiplying by 10. These averaged parameters were

entered into a spreadsheet program (see 2.6). After the *in vivo* calibration of the pulmonary artery Doppler flow probe, and the *in vitro* calibration of the left coronary artery Doppler flow probe (see 6.5), the cardiac output (L/min) and left coronary artery blood flow (ml/min) at these times were calculated (see 6.5). The following haemodynamic parameters were also calculated from the measured data: stroke volume, systemic vascular resistance, left ventricular minute work, left ventricular stroke work, left coronary vascular resistance and the double product. The standard formulae used for these calculations have been described in Chapter 6.

Paired t - test was used for statistical analysis of haemodynamic parameter changes between the control periods and the maximum effects. One Way Analysis of Variance was used for comparison of haemodynamic parameters between different doses, and linear regression was used to correlate doses and drug effects. $P < 0.05$ was considered statistically significant.

8.3 Results

In one of the sheep, pericardial adhesions were found at the time of autopsy (see 6.5). Inspection of the haemodynamic data showed control values of LV dp/dt_{max} and cardiac output that were significantly lower than the other sheep. Therefore, data from this sheep were considered to have been recorded under pathological conditions, and were not included in the results.

8.3.1 Intravenous bolus injections of lignocaine

8.3.1.1 Left coronary artery blood flow and cardiac output

There were no statistically significant changes in left coronary blood flow (Fig. 8.1 (A)), left coronary vascular resistance (Fig. 8.1 (B)), or cardiac output (Fig. 8.1 (C)), after the bolus intravenous injection of the three doses of lignocaine.

8.3.1.2 The maximum rate of left ventricular pressure rise (LV dp/dt_{max})

This index was significantly depressed for all the doses of lignocaine. The time - courses of the LV dp/dt_{max} , expressed as percentages of their control values after the intravenous administration of 50, 75 and 100 mg of lignocaine in conscious, unrestrained sheep are shown in Fig. 8.2.

In addition, for each sheep, the values of LV dp/dt_{max} during the period of lowest reading of the parameter were averaged for 15 sec to give the mean maximum depression of LV dp/dt_{max} produced by each lignocaine dose, and this is shown in Table 8.1. The means (± S.D.) of the times when this maximum LV dp/dt_{max} depression occurred are also shown. The durations of statistically significant LV dp/dt_{max} decreases from the mean control values after different doses of intravenously injected lignocaine are shown in Table 8.2.

8.3.1.3 Parameters influencing the LV dp/dt_{max} as an accurate index of myocardial contractility

It is apparent from Fig. 8.3 that heart rate, aortic diastolic blood pressure and left ventricular end diastolic pressure did not change significantly from their corresponding control values during the 15 min period after the bolus intravenous injection of 50, 75 and 100 mg of lignocaine.

8.3.1.4 Other haemodynamic parameters

The time - courses of the mean arterial blood pressure, stroke volume, left ventricular stroke work, left ventricular minute work, systemic vascular resistance and double product are shown in Fig. 8.4. The values of these parameters did not differ significantly from the corresponding control values.

8.3.1.5 Central nervous system effects

There were no discernible central nervous system effects after any of the lignocaine doses.

8.3.2 Intravenous bolus injections of pethidine

8.3.2.1 Left coronary blood flow and cardiac output

Left coronary artery blood flow was transiently but significantly increased by the intravenous bolus injection of all three doses of pethidine. This lasted for an average of 30 (100 and 300 mg doses) to 60 (200 mg) sec (Fig. 8.5 (A)). Left coronary vascular resistances during the initial 30 sec period after drug injection were significantly decreased after 100 mg, 200 mg ($P < 0.01$) and 300 mg ($P < 0.01$) doses of pethidine (Fig. 8.5 (B)). There was no significant change in cardiac output after the intravenous bolus injection of the three doses of pethidine (Fig. 8.5 (C)).

8.3.2.2 The maximum rate of left ventricular pressure rise (LV dp/dt_{max})

All the doses of pethidine decreased LV dp/dt_{max} significantly. The time - courses of LV dp/dt_{max} depression, expressed as percentages of control values after the intravenous bolus injection of pethidine are shown in Fig. 8.6. It is apparent that the standard deviations of the percentage depressions of LV dp/dt_{max} after i.v. injection of 300 mg pethidine were obviously larger and more variable than those after the other two doses of pethidine (Fig. 8.6).

The maximum depression of LV dp/dt_{max} after different doses of pethidine, calculated using the method described for lignocaine, and the times of the maximum LV dp/dt_{max} decrease are shown in Table 8.3. It is apparent that the 200 mg and 300 mg doses of pethidine decreased LV dp/dt_{max} more obviously than the 100 mg dose. There were no statistical differences in the times of onset of the maximal LV dp/dt_{max} depression between the doses. The durations of the statistically significant decreases in LV dp/dt_{max} from the mean control values after different doses of pethidine are shown in Table 8.2.

8.3.2.3 Parameters which can potentially influence the accuracy of LV dp/dt_{max} measurement

It is apparent from Fig. 8.7 (A) and Table 8.4 that there was a short period of significant heart rate increase after the intravenous bolus injection of 100 mg pethidine. The 200 mg and 300 mg doses of pethidine significantly increased heart rate for the prolonged periods of 9 and 15 min respectively after drug injection. Shortly after the intravenous injection of 100, 200 or 300 mg of pethidine, there were periods of significant increases in aortic diastolic blood pressure, which lasted for approximately 1 to 2 min (Fig. 8.7.(C)). There were no significant changes in left ventricular end diastolic pressure after the 100 mg dose of pethidine, whereas short periods (1 - 2 min) of significant elevation in left ventricular end diastolic pressure were observed after the 200 and 300 mg doses of pethidine (Fig. 8.7.(B)).

8.3.2.4 Other haemodynamic parameters

As shown in Fig. 8.8, there were short periods of significant increases in mean arterial blood pressure after the three doses of pethidine. Stroke volume, left ventricular minute work and left ventricular stroke work did not change significantly from control values. There were transient (approximately 60 sec) significant decreases in systemic vascular resistance after the 200 and 300 mg doses of pethidine. Significant increases in the double product were also found for all the doses of pethidine (Fig. 8.8, Table 8.5).

8.3.2.5 Central nervous system effects

During the experiments, 3 and 4 out of the 5 sheep studied appeared agitated, as shown by bleating and kicking, approximately 60 to 90 sec after the administration of 200 and 300 mg of pethidine, respectively, although in the 200 mg dose studies the agitation lasted relatively for shorter periods compared

with those of the 300 mg dose studies. This effect was not observed for the 100 mg dose studies.

8.4 Discussion

There have been no studies reported in the literature which have examined whether the major metabolites of lignocaine and pethidine have myocardial depressant effects of their own. However, it was found that the concentrations of the major metabolites of these drugs after intravenous bolus administration and when the haemodynamic changes were observed were too low to be detected by the drug assay methods used (see 9.2). Thus, it is reasonable to assume that the observed haemodynamic changes can be attributed to the effects of the parent drugs alone.

8.4.1 Haemodynamic effects of lignocaine

The widespread clinical use of lignocaine as a local anaesthetic and class Ib antiarrhythmic drug has led to detailed investigations of its haemodynamic effects since the early years of its use (Harrision et al., 1963; Nayler et al., 1969; McWhirter et al., 1973).

In agreement with the results of previous *in vitro* and *in vivo* studies (Nayler et al., 1969; Binnion et al., 1969; Feldman et al., 1982; Gee et al., 1990), the intravenous bolus injection of 50, 75 and 100 mg of lignocaine produced significant negative inotropic effects on the myocardium of conscious, unrestrained sheep as shown by the decreased LV dp/dt_{max} (Fig. 8.2). As discussed earlier, heart rate variation did not influence LV dp/dt_{max} significantly. Although left ventricular end diastolic pressure (pre - load) and aortic diastolic blood pressure (after - load) influence the accuracy of LV dp/dt_{max} as a measurement of myocardial contractility (Mason, 1969), these factors did not change significantly from their control values in the studies of this chapter (Fig. 8.3). Therefore the measured significant changes in LV dp/dt_{max} were an accurate reflection of the changes in myocardial contractility caused by the drug. Correlating the doses used to the

maximum depression of LV dp/dt_{max} (Fig. 8.9) showed that the intensity of depression of myocardial contractility produced by lignocaine was linear and dose dependent with a coefficient of correlation (r) of 0.91. This result is consistent with a previous study showing that the decreases of myocardial contractile force caused by lignocaine was dose dependent in an *in vitro* experimental preparation (Helgesin et al., 1984).

In contrast to a study of the haemodynamic effects of lignocaine in anaesthetised swine (Gee et al., 1990) in which coronary artery blood flow was transiently increased between 20 and 30 sec after the i.v. bolus injection of lignocaine, continuous recording of left coronary artery blood flow in the studies presented here did not reveal any significant changes after lignocaine administration to conscious, unrestrained sheep (Fig. 8.1), which is in agreement with another study in anaesthetised dogs (Leone et al., 1988).

The slight reductions in arterial blood pressure and heart rate observed by other workers in anaesthetised dogs (Gee et al., 1990; Friedrichs et al., 1990) were not observed for the lignocaine doses administered to the conscious, unrestrained sheep in these studies (Figs. 8.3 and 8.4). Therefore, the proposed "protective effect" of the intravenous use of lignocaine on the ischaemic myocardium (postulated to occur from a reduction in myocardial oxygen consumption as deduced from decreased values of double product (Gee et al., 1990)) was not seen in the conscious, unrestrained sheep preparation (Fig. 8.4). It is noted that the doses and injection rates of lignocaine used in these studies were similar to those used here. This proposed protective effect may occur only under anaesthesia.

The doses of lignocaine used in the studies presented here were selected to be between the "loading" dose used clinically for the treatment of arrhythmias (Aps et al., 1976; Campbell et al., 1978) and the doses which induced convulsion in conscious, unrestrained sheep (Rutten et al., 1989). In the absence of convulsions

and other observable adverse CNS effects reported in other studies of lignocaine in conscious, unrestrained sheep (Rutten et al., 1990), the deleterious effects of lignocaine on myocardial contractility were very noticeable in this study. It would be expected that this effect would be more harmful or even dangerous to patients with compromised myocardial function, such as after myocardial infarction in which lignocaine is often used for the treatment of arrhythmias. This is also important because it was shown in this study that the commonly monitored haemodynamic parameters, such as mean arterial blood pressure, heart rate and cardiac output, may not reveal the depression (Figs. 8.1 and 8.5).

In conclusion, significant depression of myocardial contractility caused by these intravenous doses of lignocaine was observed when there were no overt signs of CNS toxicity. Thus, the heart may not be more tolerant to the toxic effects of lignocaine than the CNS in conscious, unrestrained sheep, as generally believed. If these findings are applicable to man, care and close monitoring of the haemodynamic status of patients are recommended when lignocaine is administered as an intravenous bolus.

8.4.2 Haemodynamic effects of pethidine

The results of studies of the effects of pethidine on the cardiovascular system have been controversial despite the fact that the drug is often used in the management of pain after surgery and myocardial infarction (Lee et al., 1976; Helgesin and Refsum, 1987). Not long after pethidine was synthesized, a study showed that the drug caused hypotension, and animal hearts were "markedly dilated" at the time of autopsy after acute toxicity (Gruber et al., 1941). In studies using myocardial tissue preparations (Strauer, 1972; Grundy and Tritthart, 1972; Rendig et al., 1980), Langendorff preparations of animal hearts and in anaesthetised animals (Grundy, 1971), pethidine was found to decrease myocardial contractility as indicated by decreased maximum developed isometric tension, maximum rate of

isometric tension development and peak velocity of muscle shortening. The effects of pethidine were found to be more marked than those of morphine or fentanyl. Recently, pethidine was found to have positive inotropic effects on both myocardial tissue preparations and the myocardium of anaesthetised animals (Helgesin and Refsum, 1987; 1990; Helgesin et al., 1990). It was suggested that differences in the animal species and experimental preparation, in the presence or absence of anaesthetic agents or the release of histamine may be causes of the differences in results (Helgesin and Refsum, 1990).

The three doses selected in the study presented here were based on pilot studies which showed that doses lower than 100 mg of pethidine injected intravenously over 1 sec produced minimal measurable effects on the cardiovascular system of conscious, unrestrained sheep, and which showed that doses of greater than 300 mg produced overt CNS toxicity. The intravenous bolus injection of the doses of pethidine chosen produced depression of myocardial contractility in conscious, unrestrained sheep, as indicated by the significantly decreased LV dp/dt_{max} (Fig. 8.6). It is apparent that concurrently with the depression of LV dp/dt_{max}, heart rate, left ventricular pre - load (left ventricular end diastolic pressure) and after - load (aortic diastolic blood pressure) were either significantly increased or unchanged compared with their control values (Fig. 8.7, Table 8.4). It was shown in Chapter 6 (see 6.6) that heart rate changes (from 100 to 190/min, which spanned the heart rate changes observed in this study, Table 8.4) induced a less than 8% change in LV dp/dt_{max} in conscious, unrestrained sheep. Theoretically, elevated left ventricular end diastolic pressure and aortic diastolic pressure would lead to an over - estimation of the measured LV dp/dt_{max} (Mason, 1969). Thus, the reduced LV dp/dt_{max} observed can be attributed to the effect of pethidine on myocardial contractility rather than the influence of the factors mentioned above. As the dose of pethidine increased from 100 to 200 mg, greater depression of LV dp/dt_{max} was observed (Table 8.3). However, greater LV dp/dt_{max} depression

was not seen after the intravenous injection of 300 mg pethidine. This may be the result of factors which may have influenced LV dp/dt_{max}, such as the greater central sympathetic stimulating activity (Castro et al., 1979) observed at higher doses of pethidine (200 and 300 mg). Although pethidine has been reported to have membrane stabilizing effects on the myocardium (Helgesen and Refsum, 1987; 1990), the mechanism by which pethidine depresses myocardial contractility is not known.

The transient increase in coronary artery blood flow caused by pethidine has been reported previously (Patschke et al., 1977). This effect lasted for approximately 30 sec in the conscious, unrestrained sheep. It is also known that pethidine can reduce peripheral vascular resistance and increase blood flow to the limbs (Nadasdi and Zsoter, 1969). In the study presented here, the calculated left coronary vascular resistance was transiently but significantly decreased (Fig. 8.5). This rapid transient effect suggests that pethidine directly or indirectly dilates coronary blood vessels, because the increase in aortic diastolic blood pressure occurred later than the increase in coronary artery blood flow, and the flow changes did not follow the changes in left ventricular diastolic pressure or heart rate (Figs. 8.5 and 8.7), which can also influence coronary vascular resistance. Further evidence which supports a direct effect of pethidine on blood vessels is that the systemic vascular resistance decreased significantly simultaneously with the coronary vascular resistance change.

The two higher doses of pethidine used in the study presented here would have been expected to significantly increase myocardial oxygen consumption as deduced from the increase in the double products after injection compared with control values (Table 8.5). This effect was dose dependent and was principally due to the significant increase in heart rate (Fig. 8.7, Table 8.4). This change in heart rate may be partly due to CNS effects. Because the stroke volume, left

ventricular stroke work and left ventricular minute work did not change significantly (Fig. 8.8), it is unlikely that the increase in heart rate was a compensatory response to the decreased myocardial pumping function caused by pethidine.

In conclusion, the rapid intravenous bolus injection of 100, 200 or 300 mg doses of pethidine significantly decreased myocardial contractility in conscious, unrestrained sheep. This effect was dose dependent but may have been modulated by the CNS effects of pethidine for the highest dose. Transient increases in coronary artery blood flow may have been due to the vasodilatory effect of pethidine, while the significantly increased heart rate caused by pethidine may have been due to its atropine like effect or CNS effect, which substantially increased the double product, an index of myocardial oxygen consumption.

Table 8.1 The maximum depression of LV dp/dt_{max} as percentages of the control values, and the time of the maximum depression after the different doses of lignocaine (mean ± S.D., n = 5).

Lignocaine dose (mg)	Maximum depression (%)	Time of maximum (sec)
50	18 ± 3	54 ± 12
75	25 ± 4[a]	51 ± 14
100	33 ± 4[a]	37 ± 7[a]

[a]Statistically different to the corresponding values of the lower dose(s) (One Way Analysis of Variance and Paried t - test; the linear relationship between lignocaine doses and the percent depression of LV dp/dt_{max} is shown in Fig. 8.9).

Table 8.2 The durations of the periods of statistically significant depression of LV dp/dt_{max} after different doses of lignocaine or pethidine.

Lignocaine		Pethidine	
Dose (mg)	Duration (min)	Dose (mg)	Duration (min)
50	3	*100*	4
75	3	*200*	5
100	7	*300*	1

Table 8.3 The maximum depression of LV dp/dt_{max} as percentages of the control values, and the time of maximum depression after the different doses of pethidine (mean ± S.D., n = 5).

Pethidine dose (mg)	Maximum depression (%)	Time of maximum (sec)
100	30 ± 5	55 ± 13
200	39 ± 5[a]	59 ± 13
300	41 ± 9[a]	46 ± 30

[a]Statistically different from the 100 mg dose (One Way Analysis of Variance and Paired t - test).

Table 8.4 The changes in heart rates after the 100, 200 and 300 mg doses of pethidine injected intravenously at time = 0 (mean ± S.D., n = 5).

Time (min)	Heart rate (beats/min)		
	100 mg	200 mg	300 mg
Control	102 ± 16	99 ± 14	101 ± 9
0.5	108 ± 17	136 ± 12[b]	159 ± 30[b]
1	104 ± 12	121 ± 12[b]	147 ± 13[b]
2	116 ± 11[a]	127 ± 13[b]	154 ± 18[b]
3	114 ± 10	122 ± 8[b]	144 ± 16[b]
4	111 ± 13	117 ± 9[b]	133 ± 7[b]
5	110 ± 14	114 ± 8[b]	131 ± 10[b]
6	107 ± 15	115 ± 5[b]	127 ± 7[b]
7	108 ± 14	114 ± 7[b]	126 ± 13[b]
8	106 ± 14	113 ± 9[a]	123 ± 8[b]
9	106 ± 14	111 ± 9[a]	121 ± 8[b]
10	106 ± 16	110 ± 11	118 ± 11[b]
15	104 ± 16	104 ± 11	112 ± 7[b]

[a]Statistically different to control heart rates ($P < 0.05$, Paired t - test).

[b]Statistically different to control heart rates ($P < 0.01$, Paired t - test).

Table 8.5 The changes in double product after the 100, 200 and 300 mg doses of pethidine injected intravenously at time = 0 (mean ± S.D., n = 5).

Time (min)	Double product (Heart rate x systolic blood pressure x 10^{-3})		
	100 mg	200 mg	300 mg
Control	11.4 ± 1.4	10.5 ± 1.3	11.1 ± 1.7
0.5	12.9 ± 2	14.7 ± 2.2[b]	18.2 ± 6.1[b]
1	13 ± 1.5[a]	15.3 ± 2.8[b]	20.1 ± 4.8[b]
2	14.4 ± 2.7[b]	14.5 ± 2.9[b]	20.1 ± (6)[b]
3	13.9 ± 2.1[b]	13.8 ± 2[b]	19.1 ± 5.5[b]
4	13.2 ± 2.5	12.9 ± 1.6[b]	16.2 ± 2.8[b]
5	12.9 ± 1.9	12.3 ± 1.3[b]	15.7 ± 2.8[b]
6	12.4 ± 2.2	12.4 ± 1.4[b]	14.7 ± 2.2[b]
7	12.4 ± 2.7	12.2 ± 1.2[b]	14.7 ± 3.9[a]
8	11.7 ± 1.9	12.5 ± 1.8[b]	14 ± 2.4[b]
9	11.6 ± 1.9	11.9 ± 1.2 [a]	13.5 ± 2.3[a]
10	11.6 ± 2.4	11.7 ± 1.3 [a]	13.1 ± 2.3[a]
15	11.3 ± 2.3	11.0 ± 1.0	12 ± 1.5[a]

[a]Statistically different from control double products ($P < 0.05$, Paired t - test).

[b]Statistically different from control double products ($P < 0.01$, Paired t - test).

Figure 8.1

The time - courses (mean ± S.D., n = 5) of left coronary artery blood flow (A), coronary vascular resistance (B) and cardiac output (C) after 50 mg (open circles), 75 mg (open triangles) and 100 mg (open squares) doses of lignocaine. Lignocaine was injected at time 0, and the haemodynamic parameters shown at the time 0 are the mean (± S.D.) of the values recorded in the 30 sec control period immediately prior to injection. None of these parameters changed significantly.

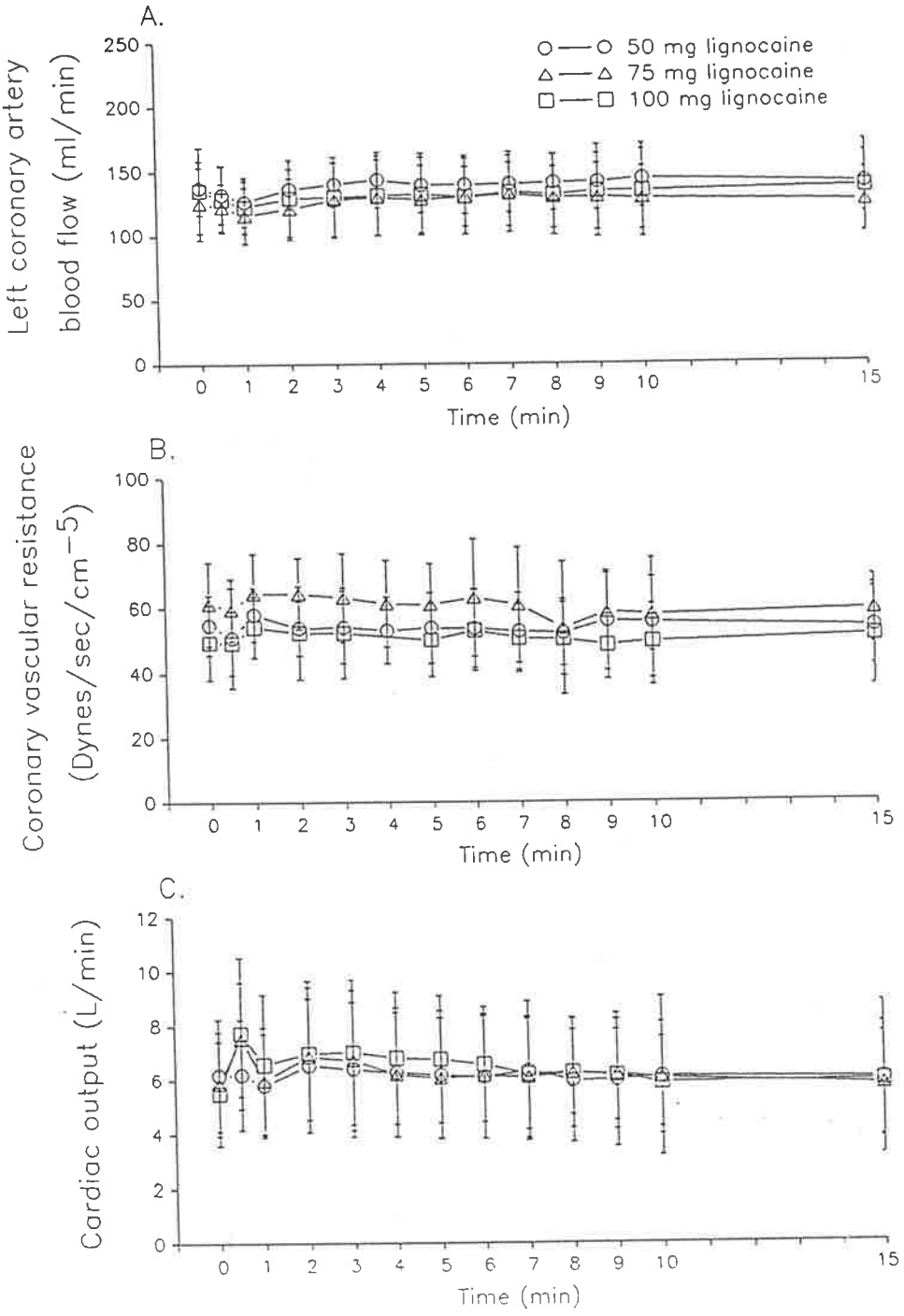

A.
Left coronary artery blood flow (ml/min)
250
200
150
100
50
0
50 mg lignocaine
75 mg lignocaine
100 mg lignocaine
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
B.
Coronary vascular resistance (Dynes/sec/cm^{-5})
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
C.
Cardiac output (L/min)
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

Figure 8.2

The time - courses of the maximum rate of left ventricular pressure rise (LV dp/dt_{max}) expressed as percentages of their control values (mean ± S.D., n = 5) after the intravenous bolus administration of 50 mg (A), 75 mg (B) and 100 mg (C) doses of lignocaine. The higher lignocaine doses caused greater depression of LV dp/dt_{max}. Lignocaine was injected at time 0, and the haemodynamic parameters shown at time 0 are the mean (± S.D.) of the values recorded in the 30 sec control period immediately prior to injection. The maximum decreases in LV dp/dt_{max}, the times of these decreases, and the duration of the statistically significant decreases of LV dp/dt_{max} are shown in Tables 8.1 and 8.2.

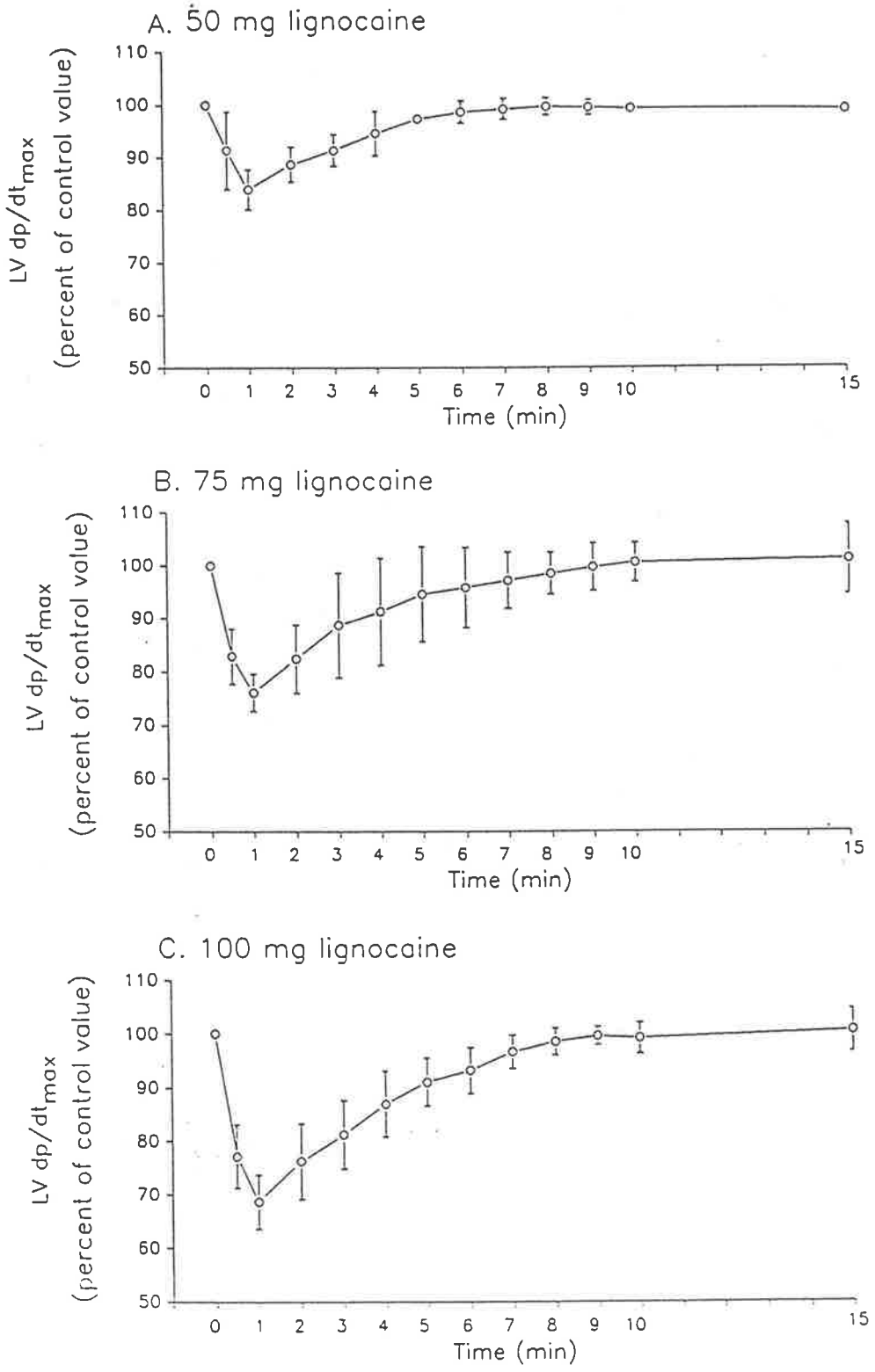

A. 50 mg lignocaine
LV dp/dtmax
(percent of control value)
110
100
90
80
70
60
50
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
B. 75 mg lignocaine
LV dp/dtmax
(percent of control value)
110
100
90
80
70
60
50
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
C. 100 mg lignocaine
LV dp/dtmax
(percent of control value)
110
100
90
80
70
60
50
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

Figure 8.3

The time - courses (mean ± S.D., n = 5) of heart rate (A), left ventricular end diastolic pressure (LVP, diastolic), (B) and aortic diastolic blood pressure (C) after 50 mg (open circles), 75 mg (open triangles) and 100 mg (open squares) doses of lignocaine. Lignocaine was injected at time 0, and the haemodynamic parameters shown at time 0 are the mean (± S.D.) of the values recorded in the 30 sec control period immediately prior to injection. None of these parameters changed significantly.

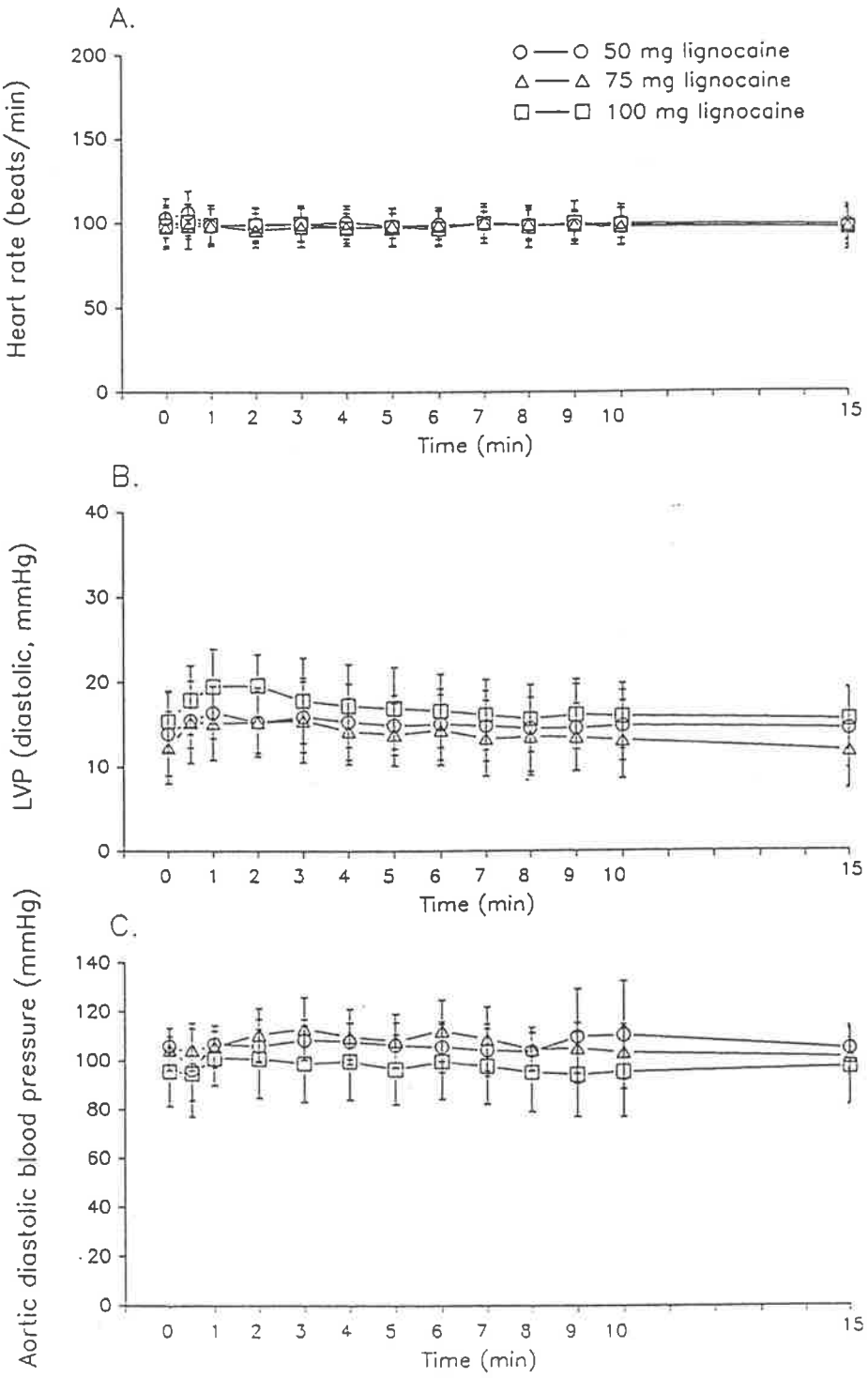

A.
50 mg lignocaine
75 mg lignocaine
100 mg lignocaine
Heart rate (beats/min)
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
B.
LVP (diastolic, mmHg)
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
C.
Aortic diastolic blood pressure (mmHg)
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

Figure 8.4

The time - courses (mean ± S.D., n = 5) of left ventricular stroke volume (A), mean arterial blood pressure (D), systemic vascular resistance (B), double product (E), left ventricular (LV) stroke work (C) and left ventricular (LV) minute work (F) after 50 mg (open circles), 75 mg (open triangles) and 100 mg (open squares) doses of lignocaine. Lignocaine was injected at time 0, and the haemodynamic parameters shown at time 0 are the mean (± S.D.) of the values recorded in the 30 sec control period immediately prior to injection. None of these parameters changed significantly.

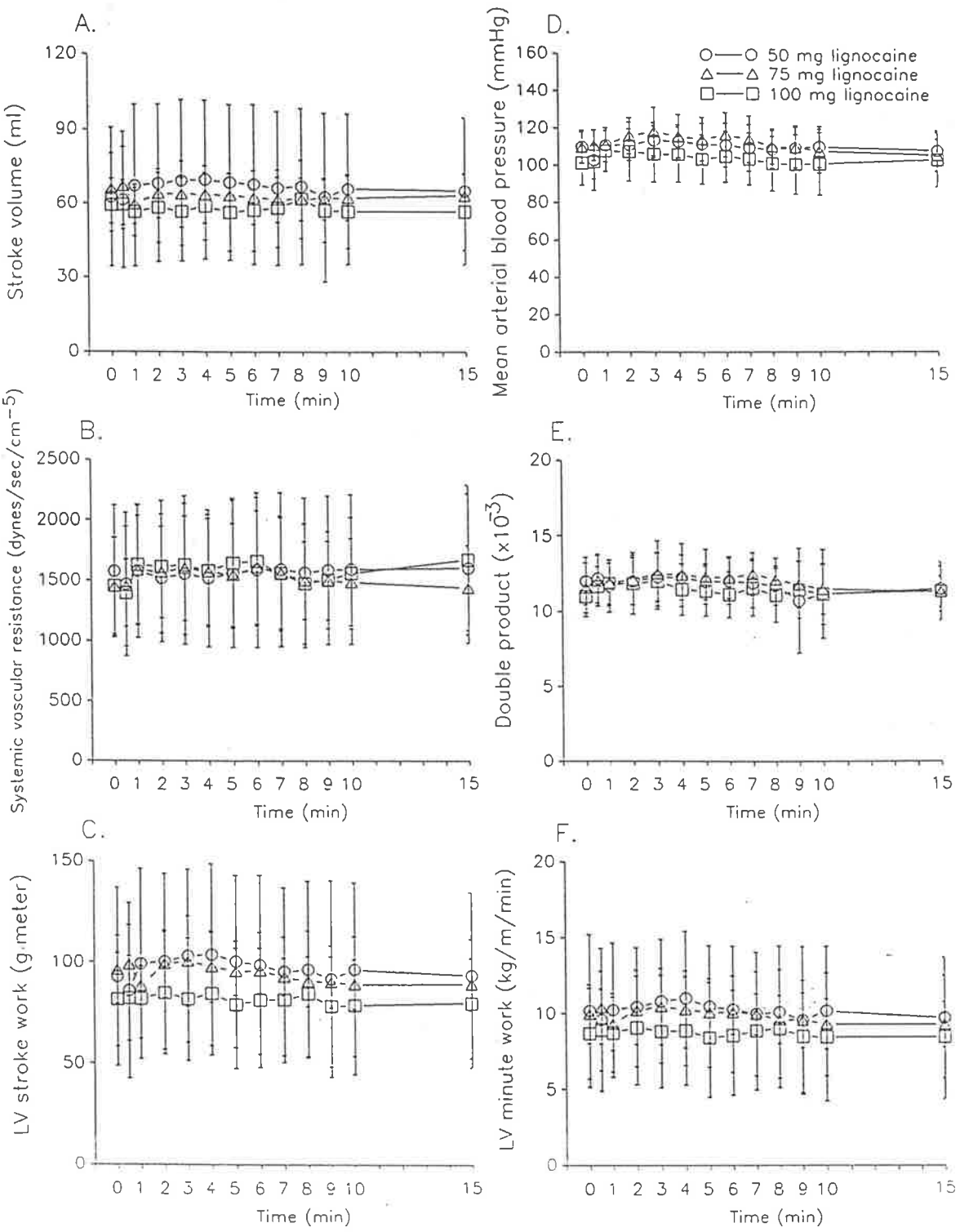

A.
Stroke volume (ml)
120
90
60
30
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
D.
Mean arterial blood pressure (mmHg)
160
140
120
100
80
60
40
20
0
50 mg lignocaine
75 mg lignocaine
100 mg lignocaine
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
B.
Systemic vascular resistance (dynes/sec/cm^{-5})
2500
2000
1500
1000
500
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
E.
Double product (x10^{-3})
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
C.
LV stroke work (g.meter)
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
F.
LV minute work (kg/m/min)
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

Figure 8.5

The time - courses (mean ± S.D., n = 5) of left coronary artery blood flow (A), coronary vascular resistance (B) and cardiac output (C) after 100 mg (open circles), 200 mg (open triangles) and 300 mg (open squares) doses of pethidine. Pethidine was injected at time 0, and the haemodynamic parameters shown at time 0 are the mean (± S.D.) of the values recorded in the 30 sec control period immediately prior to injection. After the injection of pethidine, left coronary artery blood flow increased significantly for approximately 0.5 to 1 min with a concurrent decrease in coronary vascular resistance. Cardiac output did not change significantly.

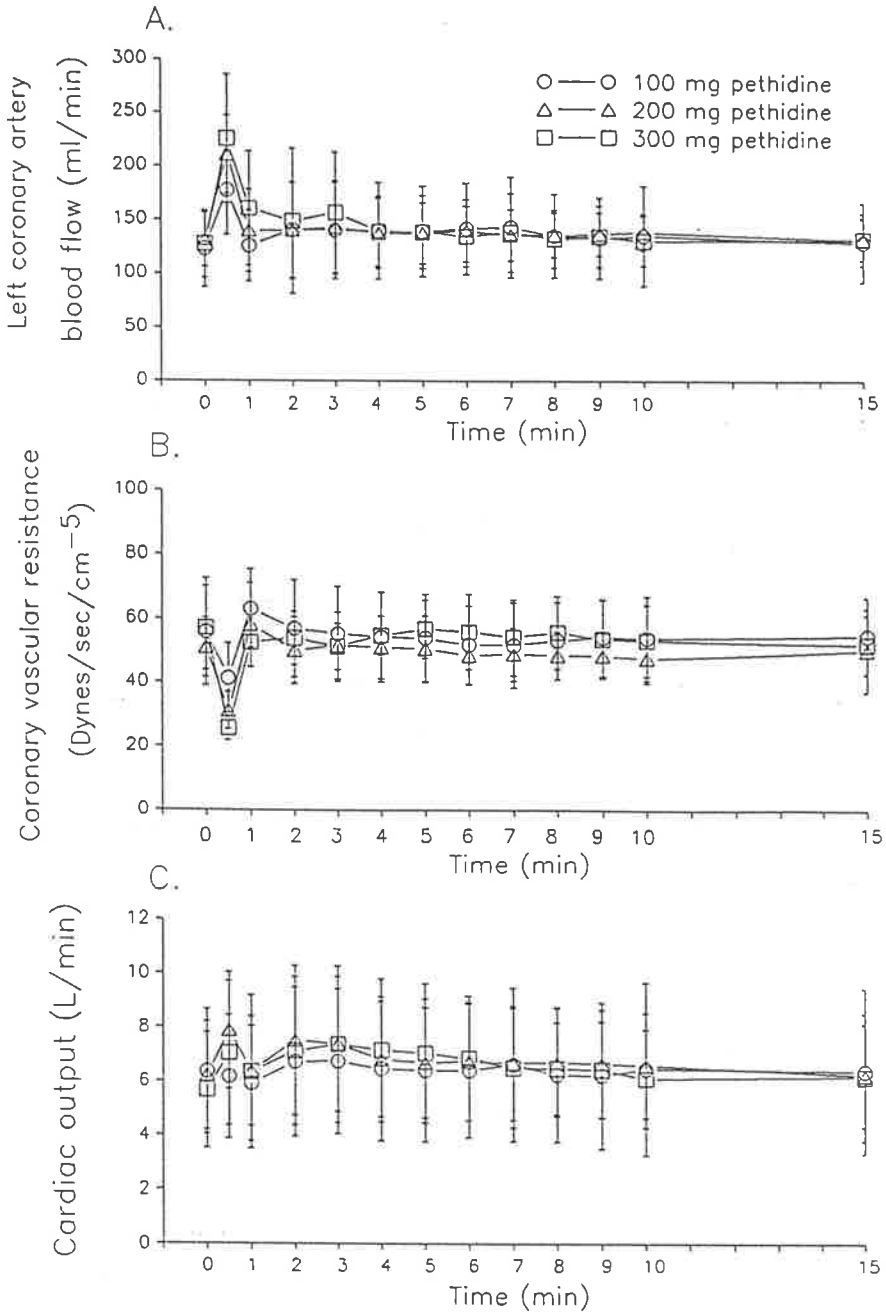

A.
Left coronary artery blood flow (ml/min)
300
250
200
150
100
50
0
100 mg pethidine
200 mg pethidine
300 mg pethidine
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
B.
Coronary vascular resistance (Dynes/sec/cm^{-5})
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
C.
Cardiac output (L/min)
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

Figure 8.6

The time - courses (mean ± S.D., n = 5) of the maximum rate of left ventricular pressure rise (LV dp/dt_{max}) expressed as percentages of their control values after the intravenous bolus administration of 100 mg (A), 200 mg (B) and 300 mg (C) doses of pethidine. Consistant dose dependent depression of LV dp/dt_{max} was not seen with the 300 mg dose of pethidine. Pethidine was injected at time 0, and the haemodynamic parameters shown at time 0 are the mean (± S.D.) of the values recorded in the 30 sec control period immediately prior to injection. The maximum decreases in LV dp/dt_{max}, the times of these decreases, and the duration of the statistically significant decreases in LV dp/dt_{max} are shown in Tables 8.3 and 8.2.

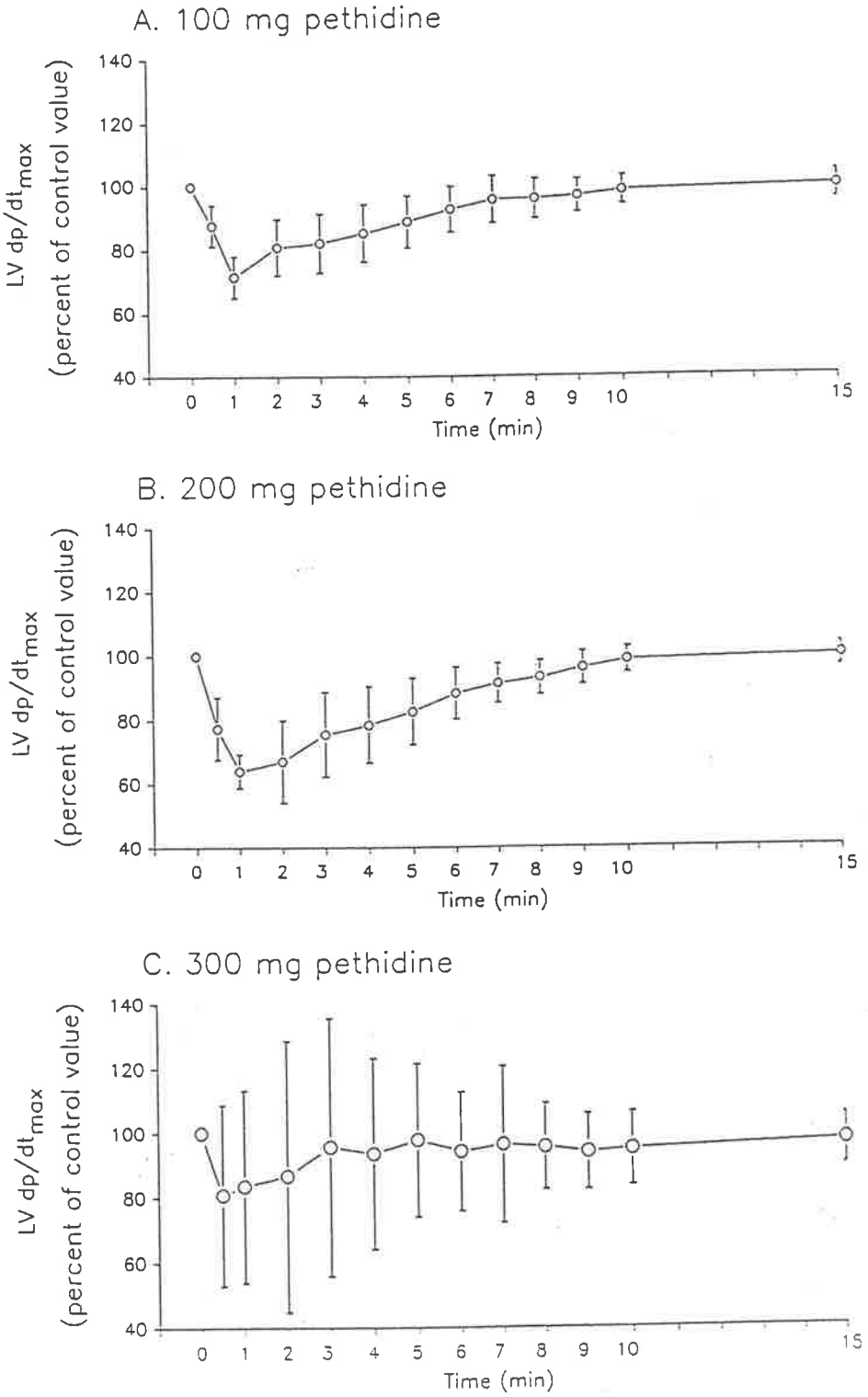

A. 100 mg pethidine
LV dp/dtmax
(percent of control value)
140
120
100
80
60
40
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
B. 200 mg pethidine
LV dp/dtmax
(percent of control value)
140
120
100
80
60
40
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
C. 300 mg pethidine
LV dp/dtmax
(percent of control value)
140
120
100
80
60
40
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

Figure 8.7

The time - courses (mean ± S.D., n = 5) of heart rate (A), left ventricular end diastolic pressure (LVP, diastolic), (B) and aortic diastolic blood pressure (C) after 100 mg (open circles), 200 mg (open triangles) and 300 mg (open squares) doses of pethidine. Pethidine was injected at time 0, and the haemodynamic parameters shown at time 0 are the mean (± S.D.) of the values recorded in the 30 sec control period immediately prior to injection. Increases in the values of these parameters were seen for different periods after different doses of pethidine (see also Table 8.4 for heart rate changes).

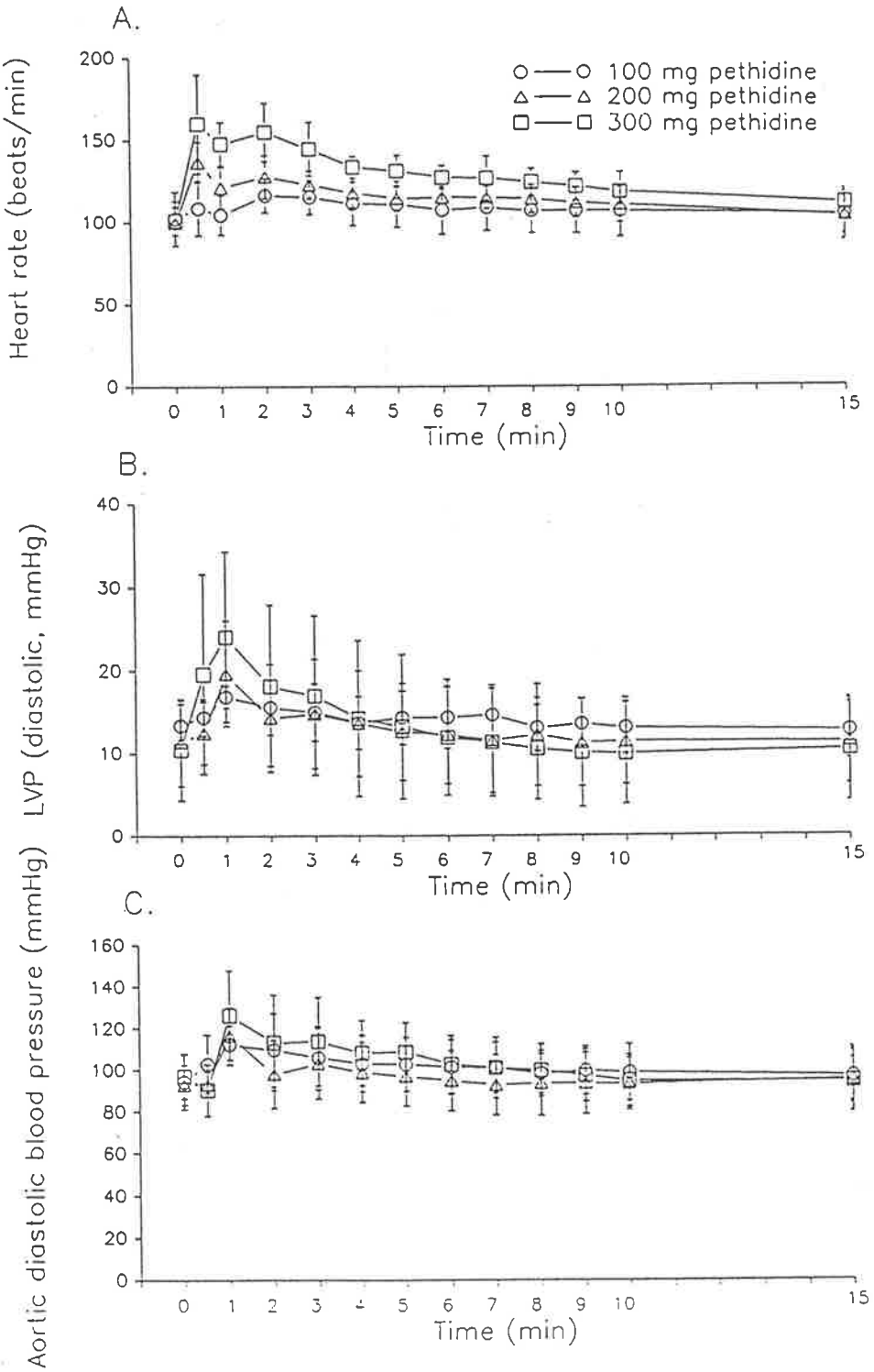

A.
Heart rate (beats/min)
200
150
100
50
0
100 mg pethidine
200 mg pethidine
300 mg pethidine
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
B.
LVP (diastolic, mmHg)
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
C.
Aortic diastolic blood pressure (mmHg)
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

Figure 8.8

The time - courses (mean ± S.D., n = 5) of left ventricular stroke volume (A), mean arterial blood pressure (D), systemic vascular resistance (B), double product (E), left ventricular (LV) stroke work (C) and left ventricular (LV) minute work (F) after 100 mg (open circles), 200 mg (open triangles) and 300 mg (open squares) doses of pethidine. Pethidine was injected at time 0, and the haemodynamic parameters shown at time 0 are the mean (± S.D.) of the values recorded in the 30 sec control period immediately prior to injection. Significant increases in double product were seen after the injection of pethidine.

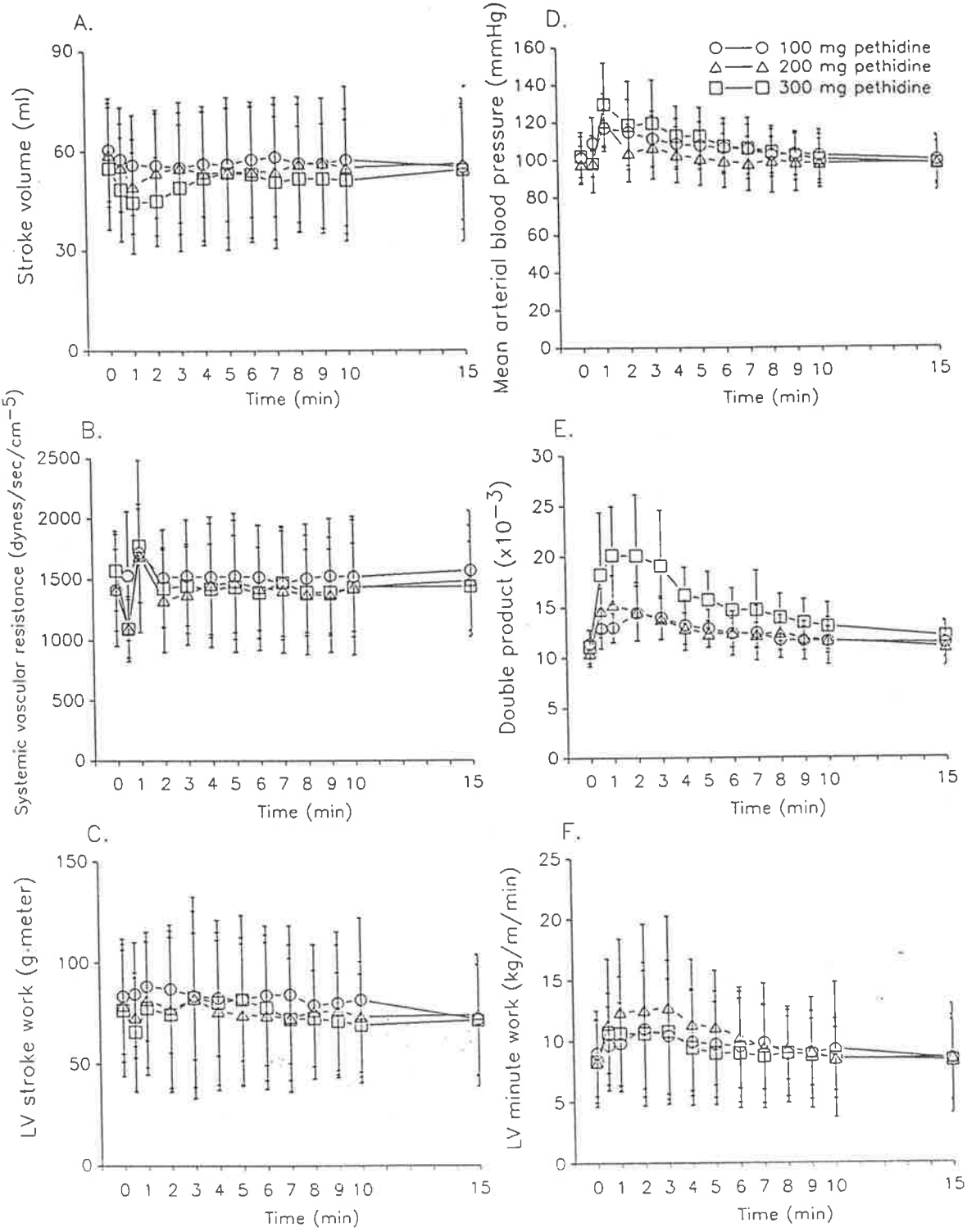
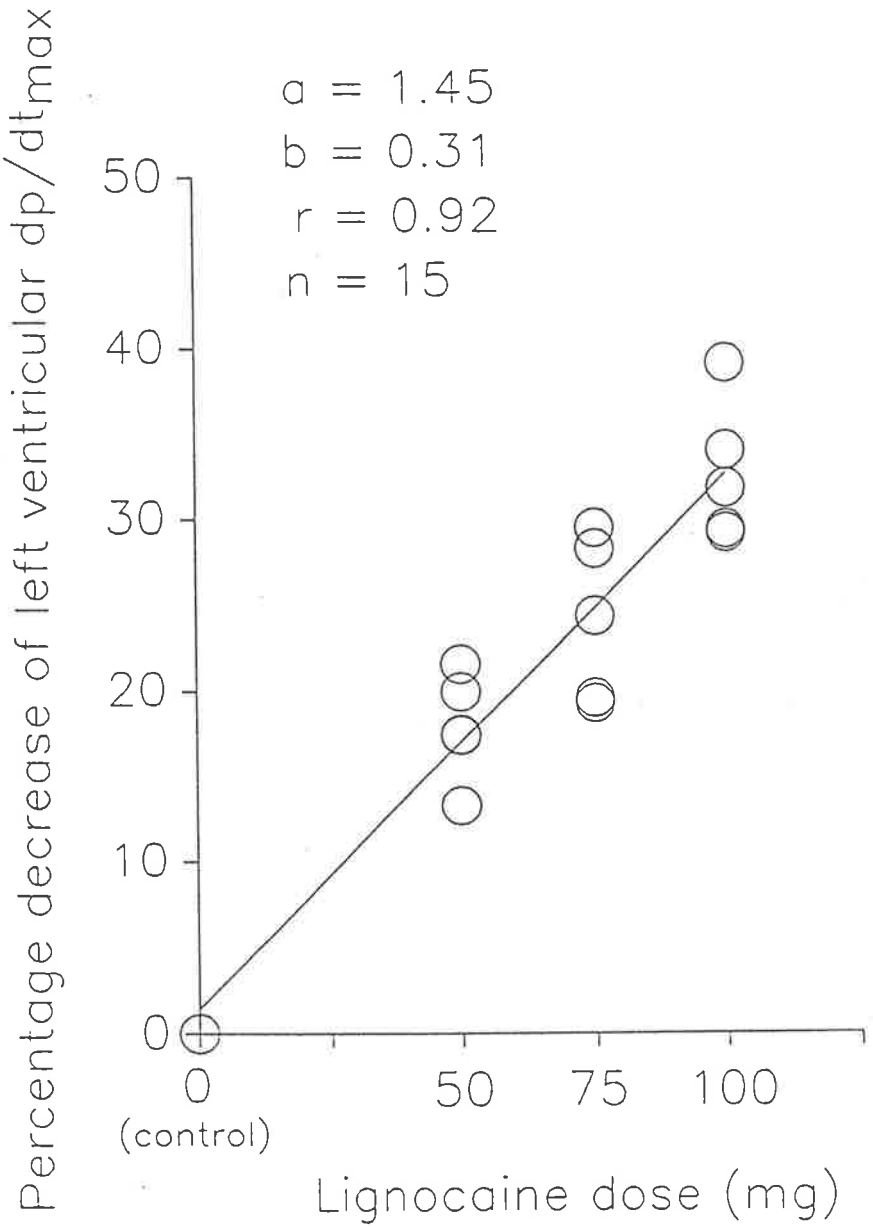

A.
Stroke volume (ml)
90
60
30
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
D.
Mean arterial blood pressure (mmHg)
160
140
120
100
80
60
40
20
0
100 mg pethidine
200 mg pethidine
300 mg pethidine
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
B.
Systemic vascular resistance (dynes/sec/cm^{-5})
2500
2000
1500
1000
500
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
E.
Double product (x10^{-3})
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
C.
LV stroke work (g.meter)
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)
F.
LV minute work (kg/m/min)
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

Figure 8.9

The relationship between the intravenous doses of lignocaine and the maximum decrease in LV dp/dt_{max} as a percent of control. The solid line indicates a line of best fit obtained by linear regression. a is the intercept of the line, b is the slope of the line, r the coefficient of correlation of the regresion and n the number of data points.


a = 1.45
b = 0.31
r = 0.92
n = 15
Percentage decrease of left ventricular dp/dtmax
50
40
30
20
10
0
0
(control)
50
75
100
Lignocaine dose (mg)

CHAPTER 9: MYOCARDIAL UPTAKE AND ELUTION OF LIGNOCAINE AND PETHIDINE AFTER INTRAVENOUS BOLUS ADMINISTRATION TO CONSCIOUS, UNRESTRAINED SHEEP.

9.1 Introduction

It is widely believed that the concentrations of a drug in its target tissues or organs, and more importantly at the sites of "receptors" in these tissues are major determinants of the quantitative therapeutic or toxic effects of the drug (Goldstein et al., 1974; Goth, 1984b). Despite this, the relationships between the blood and tissue concentrations of drugs such as antiarrhythmics, which act on the heart and are often administered as an intravenous bolus, are not well understood and have not been "properly" investigated (Horowitz et al., 1986). This lack of understanding can be attributed in part to the differences between *in vitro* and *in vivo* studies of drug distribution, and the lack of experimental methods to determine initial myocardial drug uptake shortly after intravenous bolus drug administration (Horowitz and Powell, 1986). For example, despite the fact that the maximum therapeutic and toxic effects of many drugs occur in the first few minutes after an intravenous bolus (Horowitz et al., 1986), blood drug concentrations in this period are not measured directly, but are extrapolated from blood drug concentrations measured at later times (Chiou, 1980; Runciman et al., 1990). Another common concept is that of "pseudo - equilibrium" in which tissues such as the myocardium are often thought of as part of a "well - perfused compartment" in which equilibrium between the blood and the tissues occurs so rapidly that the time - course of the tissue drug concentrations closely follows that of the blood concentrations.

These practices and assumptions have been questioned theoretically (Chiou, 1979), and it also has been shown experimentally that the concentrations of drugs in well - perfused tissues such as the myocardium can be remarkably different to those in either the arterial or venous blood (Galeazzi et al., 1976; Anderson et al., 1980; Latini et al., 1983; Horowitz et al., 1986).

Therefore, the aims of the studies reported in this chapter were as follows: 1. To examine the time - courses of the arterial and coronary sinus blood concentrations of lignocaine and pethidine after rapid intravenous bolus injection into the inferior vena cava of the conscious, unrestrained sheep. 2. To use these concentrations, and the measured time - course of myocardial blood flow, to determine the time - course of the myocardial drug concentrations using mass balance principles. 3. To examine the relationships between the calculated myocardial drug concentrations and the blood concentrations of the drugs.

9.2 Materials and methods

9.2.1 Animal preparation

The studies were performed as an additional part of the haemodynamic studies of lignocaine and pethidine performed in the six sheep described in Chapter 8. Thus, the sheep were prepared with full instrumentation for haemodynamic measurements as described in Chapters 6 and 8, with intravascular catheters in the ascending aorta, coronary sinus and inferior vena cava as described in Chapters 2 and 6. The pre - experimental preparation of the sheep and the control recordings of haemodynamic parameters were as described in Chapters 6 and 8.

9.2.2. Study design

9.2.2.1 Drugs and drug administration

The doses of drugs and the preparation of the injection solutions were those described in Chapter 8. In brief, the doses of lignocaine were 50, 75 and 100 mg, and of pethidine were 100, 200 and 300 mg. In each case, a quantity of the drug was diluted with 20 ml of 0.9% saline such that the desired dose was contained in 10 ml, and a total of 10 ml of the solution was injected via the inferior vena cava catheter over 1 sec using the Angiomat injector (see 8.2.).

9.2.2.2 Blood sample collection

Following the injection of the drugs (time = 0), blood samples were taken from the ascending aorta and coronary sinus catheters at the following time intervals: every 5 sec for the first minute, every 15 sec for the second minute, every 30 sec

to 5 min and every 60 sec until 15 min. The blood sampling times were therefore nominally 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 360, 420, 480, 540, 600, 660, 720, 780, 840, 900 sec after drug injection.

To achieve such rapid blood sampling, the "flush and withdrawal" method of blood sampling described in Chapter 5 was used for both arterial and coronary sinus blood collection during the first 120 sec after drug injection. From 120 sec onwards, 0.5 ml blood samples were collected using the "two stopcock" sampling method described previously (see 2.4). It was not always possible to collect a blood sample at the nominal sample time due to the catheter "valving" on the vessel wall. This could often be prevented or minimised by slightly moving the head of the sheep which moved the catheter bundle entering the neck of the sheep, and slightly moved the tips of the catheters in relation to the vessel walls within the sheep. The following method was used to accurately record the actual times when samples were taken. Vocal cues for the scheduled sample collection times described above, including a count - down for the drug injection, were pre - recorded onto an audio tape. This tape was played back and used to time and coordinate the experiments. Whenever a blood sample was taken, the sample number was spoken, together with any other comments concerning the progress of the experiment, and recorded by a second audio tape player recording the sounds in the laboratory. The resulting tape of nominal and actual samples times and comments was decoded after each experiment.

The 3 ml blood samples taken using the "flush and withdrawal" blood sampling method, and the 0.5 ml blood samples taken using the "two stopcock" method were transferred into 10 ml soda glass tubes, and 1.5 ml Eppendorf microtubes, respectively. To determine the exact volume of each blood sample, these tubes were weighed both before and after the addition of the blood sample. The

blood samples were stored at -20°C until they were assayed using the methods described previously (see 2.5).

9.2.3 Left coronary artery blood flow

The mean and phasic left coronary artery blood flow velocities were recorded continuously for the 10 min after drug injection, and for 30 sec at 15 min after drug injection, as described previously (see 8.2.3). Because the blood flow velocities in the left coronary artery did not change significantly from 2 min onwards after the injection of the drugs (8.3), the blood flow velocities were assumed not to change between 10 and 15 min after the drug injections.

Left coronary artery blood flow velocities corresponding to the times of blood sample collection were obtained by averaging the blood flow velocities in the 5 sec intervals immediately before each blood sampling time. These flow velocities were entered into the spreadsheet program (see 2.6) and converted to left coronary artery blood flow rates (ml/min) after the coronary artery Doppler flow probes were calibrated *in vitro* (see 6.5.2)

9.2.4 Drug assay

Lignocaine and pethidine were assayed using the methods described previously (2.5). The blood drug concentration in each 3 ml sample collected by the "flush and withdrawal" method was corrected for the dilution which occurred during sampling, and the actual volume sampled, using Equation 5.1.

The blood drug concentrations in samples collected by the "two stopcock" sampling method were corrected for the actual volume sampled using the following equation:

$$C_{corrected} = C_{assay} \times \frac{0.5}{V_{sample}} \qquad \ldots(9.1)$$

where $C_{corrected}$ is the corrected blood drug concentration, C_{assay} is the assayed blood drug concentration, V_{sample} is the actual volume sampled and 0.5 is the nominal sample volume.

9.2.5 Calculation of myocardial drug uptake and elution using mass balance principles

The basis for the use of mass balance principles to describe regional drug distribution has been discussed previously (1.5). The equations to describe the rate and extent of myocardial drug uptake and elution were adapted from Upton et al., (1988a) and are as follows:

Net drug flux (mg/min) for the myocardium ($J_{net.m}$)

$$J_{net.m} = Q_{myo} \times (C_a - C_{cs}) \qquad ...(9.2)$$

Where Q_{myo} is the myocardial blood flow rate (ml/min). In this study, this is represented by the left coronary artery blood flow rate. C_a is the arterial blood drug concentration (converted to mg/ml), representing blood drug concentration entering the left coronary artery, and C_{cs} is the simultaneously measured coronary sinus blood drug concentration (converted to mg/ml). $J_{net.m}$ is the rate (mg/min) of drug uptake into (positive values) or elution from (negative values) the myocardium.

Net mass (mg) of drug in the myocardium ($M_{net.m}$)

$$M_{net.m} = \int_0^t J_{net.m} \cdot dt \qquad ...(9.3)$$

In this study, the $M_{net.m}$ was calculated as the integral of $J_{net.m}$ over the 15 min period after the drug injections. It is known that drugs such as lignocaine (Upton et al., 1985) and pethidine (Upton R.N., unpublished observations) are not

metabolized or eliminated by the myocardium, and it was shown in Chapters 3 and 4 that the loss of drug by direct diffusion from the surface of the heart and by lymph drainage from tissues is negligible. Therefore the $M_{net.m}$ represents the amount (mass) of drug remaining in the myocardium perfused by the left coronary artery and drained by the coronary sinus (see 6.2) at a given time.

9.2.5.1 Mean myocardial drug concentration

The mean concentration of the drug in the region of the myocardium studied (see 6.2) was calculated from the mass of drug ($M_{net.m}$) in the myocardium and the mass of this myocardial tissue. The latter was calculated from the total mass of the heart of each sheep multiplied by the proportion of the myocardial tissue perfused by the left coronary artery as determined in 10 other sheep hearts (see 6.2) Thus, the myocardial tissue drug concentration was calculated using the following equation:

$$C_{myo} = \frac{M_{net.m}}{M_{myo}} \qquad \ldots(9.4)$$

Where C_{myo} is the calculated myocardial tissue drug concentration (μg/g), the unit of $M_{net.m}$ is converted to μg and M_{myo} is the myocardial tissue mass (g) which is perfused by the left coronary artery. It is apparent that Equation 9.2 requires simultaneous arterial and coronary sinus blood drug concentrations. During the first 2 min after drug injection, it was not possible in some studies to take the blood samples at the nominated blood sampling time (as described in 9.2.2). In these cases, the blood drug concentration at the nominated sampling time was calculated from a straight line drawn between the concentrations of the samples immediately before and after the nominated time. A linear relationship between these concentrations was assumed in the absence of any suitable theoretical alternative, and because any error was thought to be negligible as a result of the rapid frequency of blood sampling.

9.2.6 Statistical analysis

The Paired t - test was used to compare peak drug concentrations in arterial and venous blood and the calculated myocardial drug concentrations, the times of the peak drug concentrations, and the blood flow rates between two different doses. The Two sample t - test was used to compare the AUC_{cs}/AUC_{art} between two dose studies. Linear regression was used to analyse the relationship between peak arterial blood drug concentrations and the doses used. One Factor Repeated Measures Analysis of Variance was used to examine the trend of the peak arterial blood drug concentration, peak myocardial uptake flux and peak myocardial mass to dose ratios of the two drugs. The dose factor was partitioned into the linear and quadratic trend components by means of orthogonal polynomial contrasts (Winer, 1971). $P < 0.05$ was considered statistically significant. Statistical methods used for analysis of different data are also described in the legends to each Table.

9.3 Results

The results obtained from the sheep with pericardial adhesion and abnormally low myocardial contractility and cardiac output discussed in Chapters 6 and 8 was excluded. In another sheep, the blood sampling catheter had come out of the coronary sinus and was in the right atrium during the 75 mg lignocaine, 100 and 200 mg pethidine studies. This was deduced from the time - courses of the blood drug concentrations which were uncharacteristic for the coronary sinus, but were similar to those of the pulmonary artery blood drug concentrations which were measured in each sheep as part of a study not described in this thesis. The results from these experiments were also excluded. Therefore, the numbers of studies (n) for statistical analysis for lignocaine 50, 75 and 100 mg doses were 5, 4 and 5, and for pethidine 100, 200 and 300 mg doses were 4, 4, and 5, respectively.

9.3.1. Arterial and coronary sinus blood drug concentrations

Figs. 9.1 and 9.2 show the time - courses of the mean lignocaine and pethidine concentrations, respectively, in arterial and coronary sinus blood after intravenous

bolus injections. It is found that in all the studies, the arterial blood drug concentrations were higher than the coronary sinus blood drug concentrations for approximately 1 min after drug injections; this relationship reversed after this time. The peak arterial blood concentrations of the two drugs and the times at which these peaks were reached are listed in Table 9.1. For the two drugs, significantly higher peak arterial blood drug concentrations were observed with the use of higher doses, except for the 100 mg dose of lignocaine when the peak arterial blood drug concentration was not statistically higher than that for the 75 mg dose. The linear relationships between the dose and the resultant peak arterial blood drug concentrations of lignocaine and pethidine are shown in Fig. 9.3. The coefficients of correlation (r) for the two drugs were 0.716 and 0.927, respectively, suggesting that peak arterial blood drug concentrations increased as higher doses of the drugs were used. The ratios of peak arterial blood drug concentrations to the drug doses used for lignocaine and pethidine are also shown in Table 9.1. Significant linear trends for peak arterial drug concentration to dose ratios were only found for pethidine (Fig. 9.4A and 9.4E).

It is also apparent that in all the studies, the times of peak arterial blood drug concentrations were approximately 15 - 20 sec after drug injections. The times of the peak coronary sinus blood drug concentrations were more variable and were between 80 and 130 sec after drug injections (Table 9.1).

The areas under the arterial (AUC_{art}) and coronary sinus (AUC_{cs}) blood drug concentration curves to 15 min and the AUC_{cs}/AUC_{art} following the use of the different doses of the two drugs are listed in Table 9.2. In 1 of the 4 studies of the 200 mg dose of pethidine, and 4 of the 5 studies of the 300 mg dose of pethidine, the calculated AUC_{cs} were greater than AUC_{art}. This is shown by the mean ratios of AUC_{cs}/AUC_{art} which were greater than 1 for these doses (Table 9.2).

The major metabolites of lignocaine and pethidine, i.e. MEGX and norpethidine, respectively (see 2.5), were not detected in the blood samples collected during the 15 min study periods.

9.3.2 Cardiac output and left coronary artery blood flow rates

Because cardiac output is one of the determinants of initial mixing of drug in the blood and therefore the time - course of the arterial blood drug concentrations (Upton et al., 1990b), the mean cardiac outputs during the first 20 sec period after the drug injections are listed in Table 9.3. It is apparent that only the cardiac outputs for the 100 mg doses of lignocaine were significantly lower than those for the 75 mg doses, and the coefficients of variation of the mean cardiac outputs for this time period were large. The time - courses of left coronary artery blood flows during the control periods and the 15 min after the bolus injections of different doses of lignocaine and pethidine have been described in Chapter 8 and are shown in Figs. 8.1 and 8.5. There were no significant changes in coronary artery blood flow after any of the doses of lignocaine, while all doses of pethidine caused transient increases of flow in the first 30 to 60 sec after drug injection. Left coronary artery blood flow rates in the first minute after drug administration, during which time rapid myocardial drug uptake was observed, are also listed in Table 9.3.

9.3.3 Myocardial net drug flux

The time - courses of the myocardial net drug fluxes ($J_{net.m}$) for different doses of lignocaine and pethidine are shown in Figs. 9.5 and 9.6, respectively. The time - course of $J_{net.m}$ reflects the direction and rate of drug uptake. As expected from the arterial and coronary sinus blood drug concentration changes, the myocardial net drug uptake fluxes lasted for relatively short periods and were followed by prolonged periods of slower drug elution from the myocardium (negative net drug fluxes).

The peak myocardial drug uptake flux, the times of the peak net drug uptake flux and the times of the start of net drug elution (i.e. when the flux first became negative) are listed in Table 9.4. It is apparent that the times of peak myocardial uptake flux were approximately 10 to 20 sec after drug administration (Table 9.1), while the times of the start of drug elution from the myocardium do not coincide

with the either peak arterial or coronary sinus blood drug concentrations (Table 9.1 and 9.4). The peak myocardial uptake flux to dose and to peak arterial blood drug concentration ratios are listed in Table 9.5, and One Factor Repeated Measures Analysis of Variance showed significant linear trends for the myocardial uptake flux to dose and to peak arterial concentration ratios of pethidine (Fig. 9.4F and 9.4G).

9.3.4 Net drug mass in the myocardium

The time - courses of the net drug mass in the myocardium ($M_{net.m}$) are shown in Fig. 9.7 A and B for lignocaine and pethidine, respectively. The $M_{net.m}$ is the total amount of drug in the area of the myocardium studied, i.e. the myocardial tissue perfused by the left coronary artery. The peak drug mass represents the maximum amount of drug in the myocardium being studied. To examine the relationships between dose and myocardial drug uptake, the ratios of the peak myocardial net drug masses for lignocaine and pethidine and the corresponding doses are listed in Table 9.6. A significant linear trend for peak myocardial drug mass to dose ratio was found only for the different doses of pethidine, using One Factor Repeated Measures Analysis of Variance (Fig. 9.4H). In these studies, the $M_{net.m}$ was also used for the calculation of the myocardial drug concentrations (Eq. 9.4).

9.3.5 The calculated myocardial drug concentrations

The time - courses of the myocardial lignocaine and pethidine concentrations ($\mu g/g$) after intravenous bolus injection of the different doses of the two drugs are shown in Fig. 9.8 A and B, respectively. The peak myocardial drug concentrations and the times when these concentrations were reached are listed in Table 9.7. It is apparent that the peak myocardial drug concentrations occurred later than the peak arterial blood drug concentrations and earlier than the peak coronary sinus blood drug concentrations (comparing Table 9.7 and Table 9.1). The time - courses of the myocardial drug concentration were plotted on a logarithmic axis (Fig. 9.9), but this did not produce linear plots for any of the doses or drugs, suggesting the time - courses were not well described by simple exponential

functions. For comparison, the time - courses of the myocardial drug concentrations at 1, 2, 5, 10 and 15 min after drug injections were expressed as percentages of the peak myocardial drug concentrations. These data are shown in Table 9.8. It is apparent that at 10 and 15 min after drug injections the myocardial drug concentrations of pethidine had decreased more rapidly than those of lignocaine.

To determine if there was a linear relationship between the arterial or coronary sinus blood drug concentrations and the myocardial drug concentrations, the time - courses of the ratios of the myocardial drug concentrations to the simultaneous arterial and coronary sinus blood concentrations of lignocaine and pethidine were calculated and are shown in Figs. 9.10 and 9.11, respectively. It is evident that no linear relationship was found between either arterial or venous blood drug concentrations and myocardial drug concentrations. It would also be expected that in the presence of equilibrium or pseudo - equilibrium between the blood and the myocardium drug concentrations, these ratios would be constant. However, this was not observed.

9.4 Discussion

9.4.1 Blood drug concentrations

As outlined in Chapter 1, there is some ambiguity in the literature concerning the definition of a drug bolus. Although administration of drug as a bolus can refer to administration ranging from an almost instantaneous injection to an injection over several minutes, it has been recognised that drug injection rate can be a major determinant of the peak blood drug concentration and the resultant disposition of drug in tissues (Crawford, 1966; Rutten et al., 1989; Upton et al., 1990b), and therefore the initial effects of drugs (Gillies and Lees, 1989). In the study presented here, the use of the Angiomat injector (see 8.2.2) enabled accurate control of drug injection volume and rate which minimised variations in pharmacokinetic and pharmacodynamic measurements due to the influences of these factors.

In contrast to many studies of blood drug concentrations after a bolus which used the "back extrapolation" method to determine the theoretical initial, instantaneous drug concentration in blood (Chiou, 1979), the development and use of the rapid, accurate blood sampling method described in Chapter 5 enabled the characterisation of the rapid increase and decrease of the drug concentrations in arterial blood after rapid intravenous drug administration (Figs 9.1 and 9.2). It is apparent that even though the drugs were injected into a large central venous blood vessel (IVC) over 1 sec, "instant mixing" of the drugs in the "central blood pool" did not occur. Instead, the peak arterial blood drug concentrations occurred approximately 15 to 20 sec after the injections (Figs. 9.1 and 9.2, Table 9.1) which is significantly later than the time 0 assumed in conventional pharmacokinetic studies (Loo and Riegelman, 1969; Wagner, 1976; Chiou, 1979). In fact, with slower injection rates, or injection of drug into a peripheral vein, the peak blood drug concentrations will occur even later than those in these studies (Upton et al., 1990b), suggesting that the "back extrapolation" method is not valid.

Peak arterial blood drug concentrations of lignocaine and pethidine increased with the use of higher doses (Fig. 9.3). However, the linear trend of the peak arterial concentration to dose ratios of pethidine suggests that as higher doses of the drug were used, disproportionate (to the doses used) increases of peak arterial drug concentrations occurred (Fig. 9.4 A and E). It has been reported that drugs such as lignocaine and pethidine administered as intravenous bolus injections underwent rapid and saturable uptake into and slow elution from the lungs. (Tucker and Boas, 1971; Junod, 1976; Post, 1979; Persson et al., 1988). This may explain the disproportionate increases in peak arterial pethidine concentrations as higher doses were used, because during the first 20 sec after pethidine injections the cardiac outputs did not differ between doses (Table 9.3). However, this phenomenon was not observed for lignocaine. Although the reason is not clear, the dose difference might not have been be large enough to show the damping effect of saturable lung drug uptake on peak arterial blood lignocaine concentrations at the lower doses.

9.4.2 Myocardial pharmacokinetics

9.4.2.1 Myocardial drug uptake

Rate of myocardial drug uptake

In this study, myocardial blood flow was measured and used to determine the actual rate and extent of myocardial drug uptake and elution (Upton et al., 1988a). Positive net drug fluxes indicating net myocardial drug uptake were found during the initial 60 sec after the drug injections (Figs. 9.5 and 9.6). This period of rapid myocardial drug uptake is consistent with findings in other studies of the myocardial uptake of drugs such as propranolol (Kates and Jaillion, 1980), verapamil (Keefe and Kates, 1982) and amiodarone (Kannan et al., 1984), in which myocardial drug concentrations were determined by biopsy and the maximum concentrations were found to occur within minutes of drug administration. These durations of the net drug uptake flux were substantially shorter than the previously reported rapid "distribution" half - lives of drug concentrations in blood after intravenous drug administrations (Benowitz et al., 1974a), which are believed to represent the rate of drug uptake and disposition into "well - perfused" tissues such as the myocardium.

The times of the peak uptake fluxes of lignocaine and pethidine into the myocardium corresponded well with the times of peak arterial blood drug concentrations (Table 9.1 and 9.4), which would be expected to be one of the determinants of myocardial drug uptake. It is apparent for the pooled data shown in Table 9.4 that the peak net drug uptake fluxes for the 75 mg doses of lignocaine were significantly higher than those for the 50 mg dose and that the peak net uptake fluxes of pethidine increased significantly as dose increased. However, the absence of any linear trend for the ratios of these peak myocardial uptake fluxes to dose and to peak arterial blood drug concentrations for lignocaine (Fig. 9.4B and 9.4C) suggest that the rate of myocardial uptake is proportional to both dose used (when injection rate is constant) and to peak arterial drug concentration (Table 9.5). For pethidine,

the linear trend of ratios for these peak myocardial uptake fluxes to doses and to the peak arterial blood drug concentrations suggest that the rate of myocardial uptake of the drug was also fluenced by the coronary artery blood flow. This is shown by the significantly higher coronary artery blood flow rates as higher doses of the drug were used. Linear relationships between myocardial blood flow and myocardial lignocaine and procainamide uptake have been confirmed in studies in which myocardial drug concentrations were determined in biopsied myocardial tissues (Wenger et al., 1978; Zito et al., 1981). Based on the high plasma clearance and extensive extravascular distribution of pethidine, its disposition in the body has also been suggested to be largely perfusion - controlled (Mather et al., 1975). This is supported by the significant relationships between coronary artery blood flow change induced by pethidine and the rate of myocardial drug uptake of this drug shown in this study. However, it is possible that other factors such as the extent and avidity of drug binding in blood may also play important roles in the rate and extent of myocardial drug uptake.

Extent of myocardial drug uptake

The net masses of lignocaine and pethidine in the myocardium increased as higher doses were used (Fig. 9.7). The peak drug masses of lignocaine in the myocardium were proportional to the doses used, as shown by the peak mass to dose ratios listed in Table 9.6 and the lack of any linear trend for myocardial peak mass to dose ratios of the drug (Fig. 9.4D). For pethidine, the linear trend for myocardial peak mass to dose ratios suggest that myocardial drug uptake was disproportionately increased as higher doses of the drug were used (Fig. 9.4H). However, both proportionate and disproportionate increases in the myocardial uptake of the drugs at different doses suggest that, over the dose ranges used, uptake of lignocaine and pethidine into the myocardium was not saturated.

The myocardial concentrations of lignocaine and pethidine reached peak values at approximately 1 min after the drug injections (Table 9.7), which is earlier than in studies of the maximal myocardial concentrations of lignocaine (2.4 ± 0.2 min) and mexiletine (5.5 ± 0.6 min) in humans (Horowitz et al., 1986). This may reflect the influence of injection rate (injection over 10 sec was used in the human studies) on the time and magnitude of the peak arterial blood drug concentrations which subsequently influenced the myocardial drug uptake. The calculated peak myocardial lignocaine concentrations for the doses used were lower than the reported myocardial lignocaine concentrations from studies in dogs (16 ± 4.3 μg/g) and pigs (28.6 ± 4.3 μg), in which myocardial drug concentrations were measured in myocardial tissues obtained by *in vivo* biopsy (Vogt et al., 1988). This may be due to the relatively higher doses (3 mg/kg) and the maintenance infusion (100 mg/min) used in the reported study, to species differences in myocardial drug uptake (Vogt et al., 1988), or to the higher blood drug concentrations due the use of anaesthesia (Mather et al., 1986a).

9.4.2.2 Myocardial drug elution

The elution of both drugs from the myocardium started approximately 1 min after injection (Table 9.4). For each dose of lignocaine, the AUC_{cs} was always significantly smaller than AUC_{art} during the 15 min period, while there were no statistically significant differences between the AUC_{cs} and AUC_{art} after the injection of each dose of pethidine (Table 9.2). There were no significant differences among the AUC_{cs}/AUC_{art} for the different doses of lignocaine or pethidine, but these ratios for the 100 mg dose of pethidine were higher than those for the 100 mg dose of lignocaine, and the ratios for the 200 and 300 mg doses of pethidine were almost all significantly higher than the ratios for each dose of lignocaine (Table 9.2). Because these calculations are not dependent on coronary artery blood flow, the higher AUC_{cs}/ AUC_{art} may indicate that the elution of pethidine from the myocardium is more rapid than that of lignocaine. The rapid elution of pethidine from the myocardium is also indicated by the

rapid decrease in the calculated myocardial drug concentrations shown in Table 9.8. The ratios of the myocardial drug concentrations to the peak myocardial drug concentrations at 10 and 15 min after drug administration were not statistically different between different doses of lignocaine or pethidine (see columns 4 and 5, Table 9.8). By pooling the data for the different doses of each drug, these ratios were 34 ± 13 and 26 ± 14% at 10 and 15 min for pethidine, and 50 ± 21 and 46 ± 22% for lignocaine, respectively; these pooled myocardial drug concentrations at 10 and 15 min after drug administration were statistically different between lignocaine and pethidine ($P < 0.05$ and 0.01 for 10 and 15 min, respectively).

The reasons for the differences between the rates of elution of lignocaine and pethidine are open to conjecture. It is known that the arterial blood concentrations of pethidine decrease more rapidly than lignocaine in the sheep (Mather et al., 1986b), and it may be possible the rate of decrease of the arterial drug blood concentrations limits the rate of myocardial elution. Alternatively, it may be that the differences were due to the reported specific binding of lignocaine to myocardial tissue (Lullmann et al., 1979; Lloyd and Taylor, 1975), or to the higher lipophilicity of pethidine (Upton et al., 1987).

9.4.3 Relationships between myocardial and blood drug concentrations

In contrast to predictions based on traditional pharmacokinetic concepts, the myocardial concentrations of lignocaine did not reach equilibrium or "pseudo - equilibrium" with either arterial or coronary sinus blood drug concentrations during the 15 min period after the drug injections, as shown by the continuously increasing ratios of myocardial to arterial and to coronary sinus blood drug concentration ratios (Figs. 9.10 and 9.11). After different doses of pethidine, the ratios of the myocardial to coronary sinus blood drug concentrations all increased, but in a less obvious manner than those for lignocaine. The ratio of the myocardial and arterial blood drug concentrations seemed more variable: it increased for the 100 mg doses, was rather constant for the 200 mg doses and

increased and then decreased for the 300 mg doses. The reasons for this are not clear, but it would be expected that factors influencing initial myocardial drug uptake and subsequent elution such as drug binding in blood and tissue, and tissue blood flow rate, would play important roles.

Further discrepancies are apparent upon examining the times of the peak coronary sinus blood drug concentrations. These did not coincide with the times of the peak myocardial drug concentrations nor the times of the start of drug elution from the myocardium (Table 9.1, 9.4 and 9.7). The peak coronary sinus blood drug concentrations would be expected to be determined by drug lipophilicity, tissue binding, ionization and coronary blood flow rates. These observations, with those of Figures 9.10 and 9.11, confirm that regional venous blood did not reach equilibrium with myocardial drug concentrations over the time - scale studied.

9.4.4 Use of mass balance principles to study myocardial pharmacokinetics

To date, only a limited number of methods are available for the study of myocardial pharmacokinetics, and each of these methods, such as *in vitro* myocardial preparations, or *in vivo* and post - mortem myocardial tissue biopsy, have shortcomings (Horowitz and Powell, 1986; Upton, 1990a; see 1.2). It is now becoming recognised that methods which influence the physiological status of the body, e.g. anaesthesia and myocardial biopsy, may also influence drug distribution and drug effect, and should be avoided.

Simultaneous sampling of arterial and coronary sinus blood has been used to describe drug uptake into and elution from the myocardium in experimental animals, but factors such as failure to measure myocardial blood flow, failure to match the blood sampling regimen to the periods of rapid changes in blood drug concentration, and failure to define the region of the myocardium studied have limited the interpretation of the results of these studies (Marks et al., 1964; Selden and Neill, 1975; Hayward et al., 1983). In a recent report, mass balance principles were used to study short term myocardial drug uptake in patients with ischaemic

heart disease. Simultaneous arterial and coronary sinus blood samples were collected and coronary sinus blood flow rates were measured intermittently. However, myocardial drug content was expressed per unit of coronary sinus blood flow, because the mass of the myocardial tissue under study was not known (Horowitz et al., 1986).

On the basis of studies presented in the previous chapters, the rate and extent of myocardial drug uptake was calculated using mass balance principles. The careful identification of the myocardial tissue under study (see 6.2) enabled the calculation of myocardial drug concentrations. Myocardial blood flow in each experiment was continuously recorded by a Doppler flowmeter in unpremedicated, conscious, unrestrained sheep in a manner which would not influence the normal physiological status of the animals.

The need to continuously measure blood flow to the myocardium, rather than assuming that myocardial blood flow is a fixed proportion of the cardiac output, cannot be over emphasised (Runciman et al., 1984a). This is shown by the fact that the AUC_{cs} were larger than the AUC_{art} in 4 of the 5 studies of 300 mg pethidine (Table 9.2). If a constant coronary artery blood flow was assumed, this would suggest that the myocardium was synthesizing pethidine. In fact, the measured coronary artery blood flow (significantly increased during the initial 0.5 to 1 min, Chapter 8) used to calculate the time - course of the net drug masses in the myocardium, provided the positive mass balance between myocardial drug uptake and elution (Fig. 9.8).

This preparation for the study of myocardial drug uptake and elution is still not perfect in that coronary sinus blood is assumed to be homogeneous (Horowitz et al., 1986). However, a 7F catheter with a large inner diameter was used for blood sampling collection to minimize the likelihood of sampling only one tributary of the coronary sinus. Secondly, the sheep preparation uses relatively invasive techniques that cannot be used in human studies. Thirdly, only the total drug concentration in the region of the myocardium under study can be calculated by

this method. Also, little is revealed about drug uptake and elution at cellular level. Nevertheless, these data represent studies which are one step closer to measuring the concentrations of drugs at their sites of action after short term intravenous use. Further studies of the factors which determine the concentrations of drugs at their "sites of action" and therefore control drug effect are indicated. In the next chapter, myocardial pharmacokinetics are related to myocardial pharmacodynamics to examine the usefulness of these total myocardial drug concentrations.

Table 9.1 Peak drug concentrations in arterial and coronary sinus blood, the time after injection at which these peaks occurred, and the ratios of the peak arterial blood concentration to dose after intravenous bolus injections of lignocaine and pethidine (mean ± S.D., the number of studies for each dose group are the same as those listed in the other Tables of Chapter 9).

Lignocaine	**50 mg**	**75 mg**	**100 mg**
Arterial blood			
Peak concentration (μg/ml)	18.2 ± 4.7	32.6 ± 6.8[a]	43.9 ± 18.5[a]
Peak time (sec)	16 ± 2	15 ± 0	16 ± 4
Peak concentration/dose	0.036 ± 0.009	0.043 ± 0.009	0.044 ± 0.018
Coronary sinus blood			
Peak concentration (μg/ml)	2.3 ± 1.2	3.2 ± 2.1	3.2 ± 2
Peak time (sec)	83 ± 7	129 ± 43	85 ± 38
Pethidine	**100 mg**	**200 mg**	**300 mg**
Arterial blood			
Peak concentration (μg/ml)	27.8 ± 4.6	66.8 ± 13.3[b]	114.5 ± 23.1[b]
Peak time (sec)	20 ± 6	16.3 ± 2.5	15 ± 0
Peak concentration/dose	0.028 ± 0.005	0.033 ± 0.007	0.038 ± 0.008
Coronary sinus blood			
Peak concentration (μg/ml)	2.7 ± 1	7 ± 1.5[b]	13.7 ± 2.5[b]
Peak time (sec)	125 ± 37	91 ± 23	80 ± 29

[a]Significantly higher than the 50 mg dose.

[b]Significantly higher than those of the lower dose(s).

Paired t - test was used for statistical analysis of the peak blood drug concentrations, and analysis of the peak times.

Table 9.2 The areas under the arterial and coronary sinus blood drug concentration curves to 15 min (AUC_{art} and AUC_{cs}, respectively) and the ratio of AUC_{cs}/AUC_{art}, after the different doses of lignocaine and pethidine (mean S.D.).

Lignocaine	**AUC_{art}** ((μg/ml) x sec)	**AUC_{cs}** ((μg/ml) x sec)	**AUC_{cs}/AUC_{art}**
50 mg (n = 5)	907 ± 342[a]	734 ± 345	0.79 ± 0.14
75 mg (n = 4)	1068 ± 431[a]	886 ± 353	0.83 ± 0.05
100 mg (n = 5)	1241 ± 480[a]	900 ± 428	0.70 ± 0.11
Pethidine	**AUC_{art}** ((μg/ml) x sec)	**AUC_{cs}** ((μg/ml) x sec)	**AUC_{cs}/AUC_{art}**
100 mg (n = 4)	1131 ± 275	993 ± 246	0.88 ± 0.1[b]
200 mg (n = 4)	2555 ± 294	2611 ± 225	1.02 ± 0.09[c]
300 mg (n = 5)	4298 ± 934	4691 ± 1188	1.10 ± 0.08[d]

[a]AUC_{art} significantly greater than AUC_{cs}.

[b]Significantly greater than that for 100 mg lignocaine.

[c]Significantly greater than that for 75 and 100 mg of lignocaine.

[d]Significantly greater than those for all the doses of lignocaine.

Two Sample t - test was used for analysis of the diferences between AUC_{cs} and AUC_{art} of each dose study and Paired t - test was used for analysis of AUC_{cs}/AUC_{art} between different doses.

Table 9.3 Cardiac output (CO, mean S.D.), coefficients of variation (CV) of cardiac output during the first 20 sec, and coronary artery blood flow rates (CBF, mean S.D.) during the first minute after the different doses of lignocaine and pethidine.

Lignocaine	**CO** (L/min)	**CV** (%)	**CBF** (ml/min)
50 mg (n = 20*)	6.38 ± 2.98	46.7	139 ± 30[a]
75 mg (n = 16*)	6.81 ± 1.61	23.6	121 ± 23
100 mg (n = 20*)	5.83 ± 1.25	21.4	122 ± 21
Pethidine	**CO** (L/min)	**CV** (%)	**CBF** (ml/min)
100 mg (n = 16*)	8.10 ± 2.79	34.5	147 ± 43
200 mg (n = 16*)	7.25 ± 1.86	25.1	167 ± 48[b]
300 mg (n = 20*)	7.53 ± 3.31	44.0	196 ± 68[b]

*Cardiac output and left coronary artery blood flow were measured every 5 sec during the 20 sec periods, thereforefor generating 4 measurements for each study, and 16 measurements for doses studied in 4 sheep, and 20 measurements for doses studied in 5 sheep.

[a]Statistically higher than the 75 mg dose.

[b]Statistically higher than the lower doses.

Paired t - test was used for statistical analysis.

Table 9.4 The peak net drug uptake flux, the times at which the peak uptake flux occured, and the times of the start of the drug elution after the different doses of lignocaine and pethidine.

Lignocaine	**Peak uptake flux** (mg/min)	**Time of peak** (sec)	**Start of elution** (sec)
50 mg (n = 5)	2.3 ± 0.4	17 ± 5	67 ± 23
75 mg (n = 4)	4.3 ± 1.7[a]	18 ± 5	60 ± 13
100 mg (n = 5)	5.8 ± 3.4	16 ± 4	63 ± 14
Pethidine	**Peak uptake flux** (mg/min)	**Time of peak** (sec)	**Start of elution** (sec)
100 mg (n = 4)	5.1 ± 2.6	21 ± 4	74 ± 16
200 mg (n = 4)	15.1 ± 4.6[a]	16 ± 3	54 ± 8
300 mg (n = 5)	27.5 ± 8.7[a]	16 ± 3	53 ± 12[b]

[a]Significantly higher than the lower doses (Paired t - test).

[b]Significantly earlier than the 100 mg dose (Paired t - test).

Table 9.5 The relationships between the peak net drug uptake fluxes and the drug dose, and between the peak net drug uptake fluxes and their corresponding peak arterial blood drug concentrations for lignocaine and pethidine (mean ± S.D.)

Lignocaine	Peak uptake flux/dose	Peak uptake flux/Peak arterial
50 mg (n = 5)	0.045 ± 0.01	0.127 ± 0.02
75 mg (n = 4)	0.057 ± 0.02	0.129 ± 0.03
100 mg (n = 5)	0.058 ± 0.03	0.124 ± 0.03
Pethidine	**Peak uptake flux/dose**	**Peak uptake flux/Peak arterial**
100 mg (n = 4)	0.045 ± 0.01	0.164 ± 0.03
200 mg (n = 4)	0.075 ± 0.02	0.224 ± 0.05
300 mg (n = 5)	0.092 ± 0.03[a]	0.241 ± 0.05[a]

[a]Significantly higher than the 100 mg dose of pethidine (Paired t - test).

Table 9.6 Peak myocardial net drug mass expressed as percentages of the different doses of lignocaine or pethidine.

Lignocaine	**Peak mass/dose (%)**
50 mg (n = 5)	1.38 ± 0.70
75 mg (n = 4)	1.47 ± 0.72
100 mg (n = 5)	1.34 ± 0.64
Pethidine	
100 mg (n = 4)	1.65 ± 0.27
200 mg (n = 4)	1.87 ± 0.77
300 mg (n = 5)	2.04 ± 0.41[a]

[a]Statistically higher than the lower doses of pethidine (Paried t - test).

Table 9.7 The calculated peak myocardial concentrations of lignocaine and pethidine and the times after injection at which these peaks occurred.

Lignocaine	**Peak concentration** ($\mu g/g$)	**Time of peak** (sec)
50 mg (n = 5)	3.83 ± 1.9	66 ± 23
75 mg (n = 4)	6.02 ± 2.93[a]	55 ± 9
100 mg (n = 5)	7.44 ± 3.5[a]	59 ± 14
Pethidine	**Peak concentration** ($\mu g/g$)	**Time of peak** (sec)
100 mg (n = 4)	9.04 ± 1.76	75 ± 12.5
200 mg (n = 4)	20.46 ± 8.55[b]	58 ± 5
300 mg (n = 5)	34.09 ± 7.42[b]	46 ± 11[b]

[a]Significantly higher than that of 50 mg dose (Paired t - test)

[b]Significantly higher than that of 100 mg dose (Paired t - test)

Table 9.8 The myocardial drug concentrations as percentages of the peak myocardial drug concentrations at different times after drug injection for the different doses of lignocaine and pethidine.

	Time after drug injection (min)				
	1	**2**	**5**	**10**	**15**
Lignocaine					
50 mg (n = 5)	96 ± 7	91 ± 5	64 ± 15	55 ± 28	52 ± 30
75 mg (n = 4)	99 ± 1	88 ± 10	51 ± 6	35 ± 9	31 ± 10
100 mg (n = 5)	100 ± 0.1	92 ± 6	70 ± 14	57 ± 19	54 ± 20
Pooled	98 ± 4	90 ± 7	62 ± 13	50 ± 21	46 ± 22
Pethidine					
100 mg (n = 4)	98 ± 2	95 ± 4	65 ± 13	41 ± 14	33 ± 14
200 mg (n = 4)	100 ± 0	90 ± 4	57 ± 9	34 ± 11	26 ± 12
300 mg (n = 5)	98 ± 3	82 ± 9	50 ± 9	31 ± 10	25 ± 11
Pooled	99 ± 2	89 ± 8	57 ± 11	34 ± 13[a]	26 ± 14[a]

[a]Significantly lower than the corresponding values for lignocaine (Two Sample t - test).

Figure 9.1

The time - courses of lignocaine concentrations in arterial (open circles) and coronary sinus blood (open diamonds) after the intravenous bolus injection of 50, 75 or 100 mg doses of lignocaine, shown in (A), (B) and (C), respectively. The smaller graphs in the upper right hand corner of each panel show the data regraphed on a scale which better shows the peak arterial blood drug concentrations.

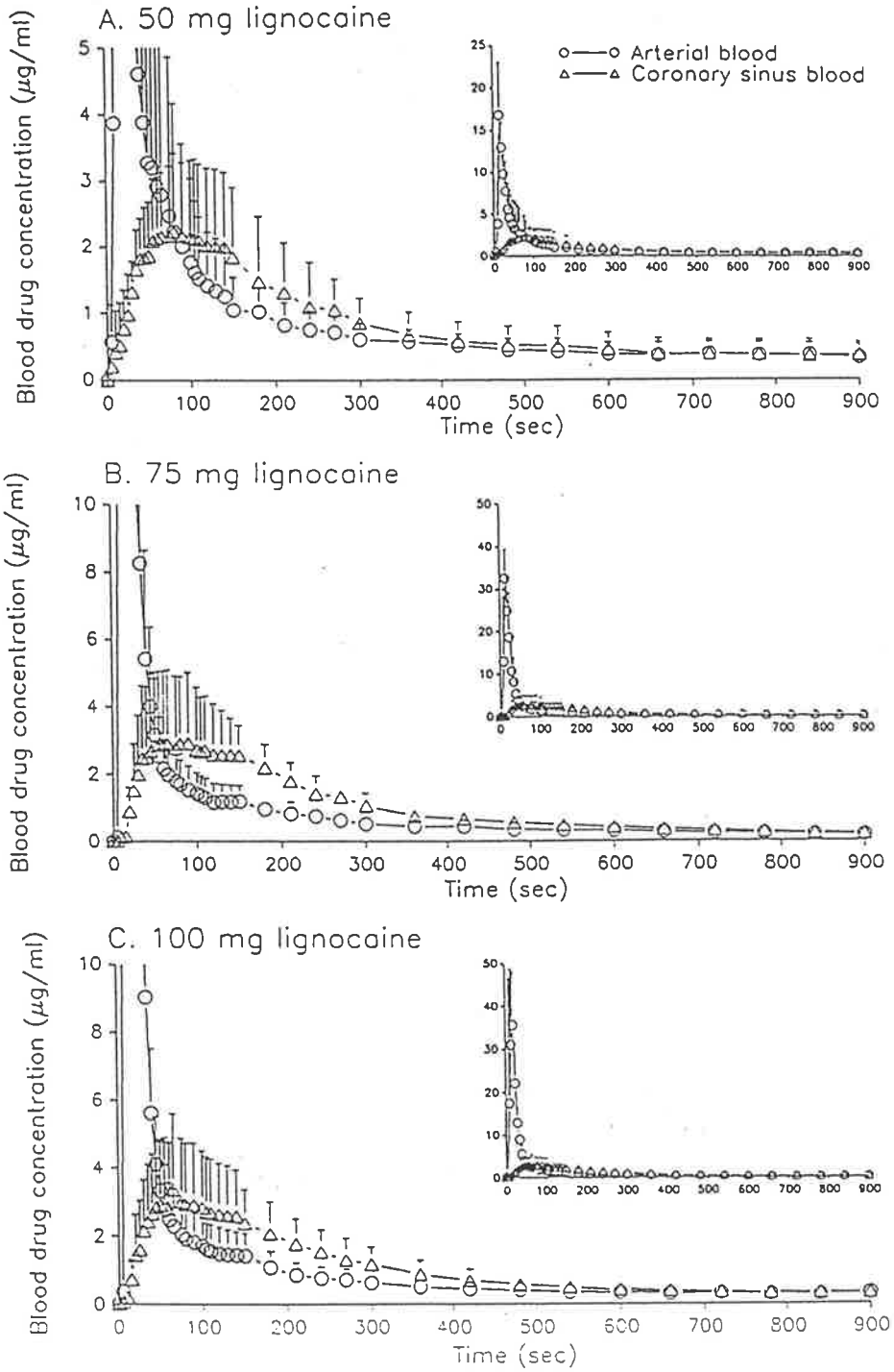

A. 50 mg lignocaine
Arterial blood
Coronary sinus blood
Blood drug concentration (μg/ml)
Time (sec)
B. 75 mg lignocaine
Blood drug concentration (μg/ml)
Time (sec)
C. 100 mg lignocaine
Blood drug concentration (μg/ml)
Time (sec)

Figure 9.2

The time - courses of pethidine concentrations in arterial (open circles) and coronary sinus (open diamonds) blood after the intravenous bolus injections of 100, 200 or 300 mg doses of pethidine, shown in (A), (B) and (C), respectively. The smaller graphs in the upper right corner of each panel show the data regraphed on a scale which better shows the peak arterial blood drug concentrations.

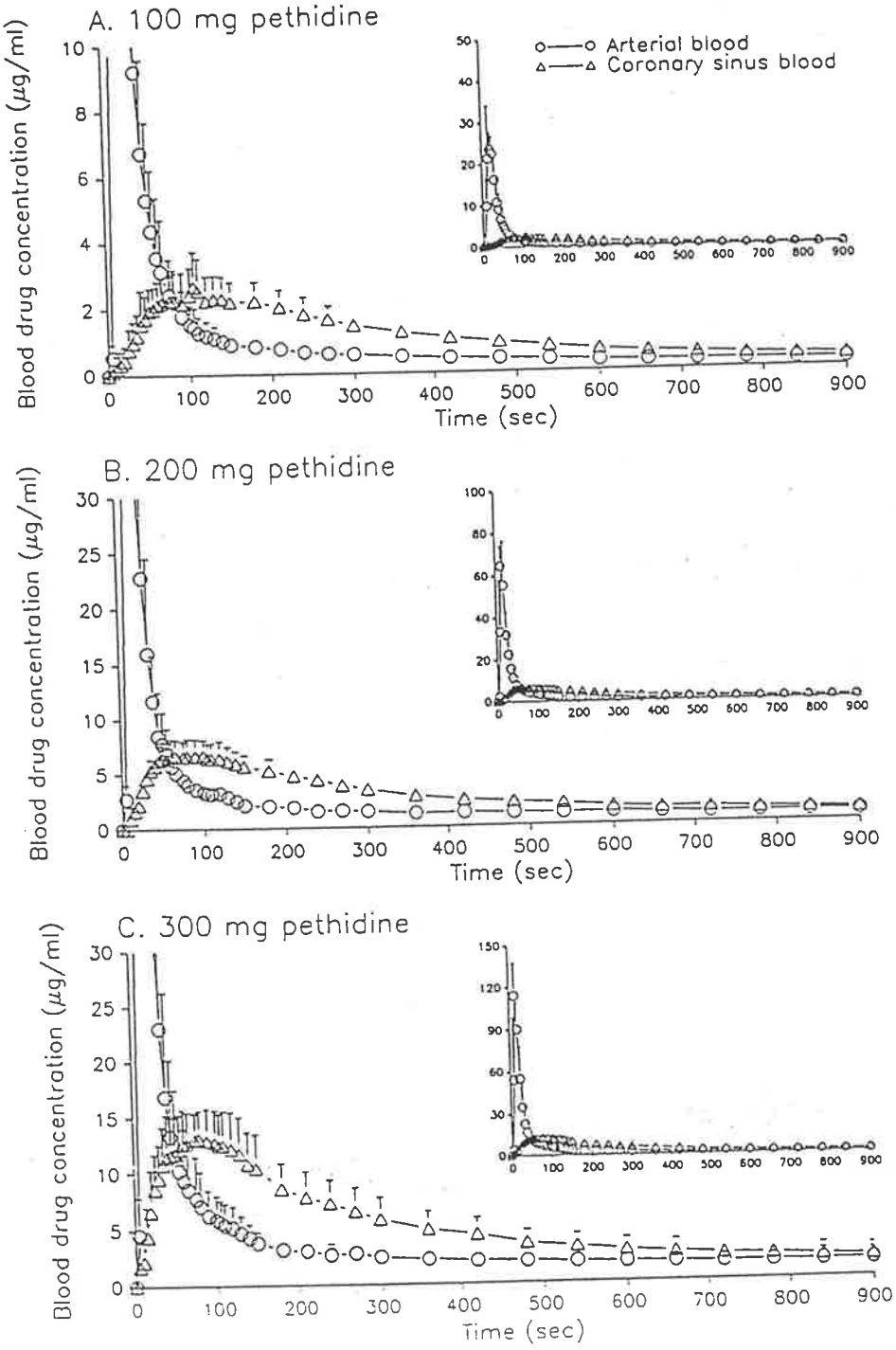
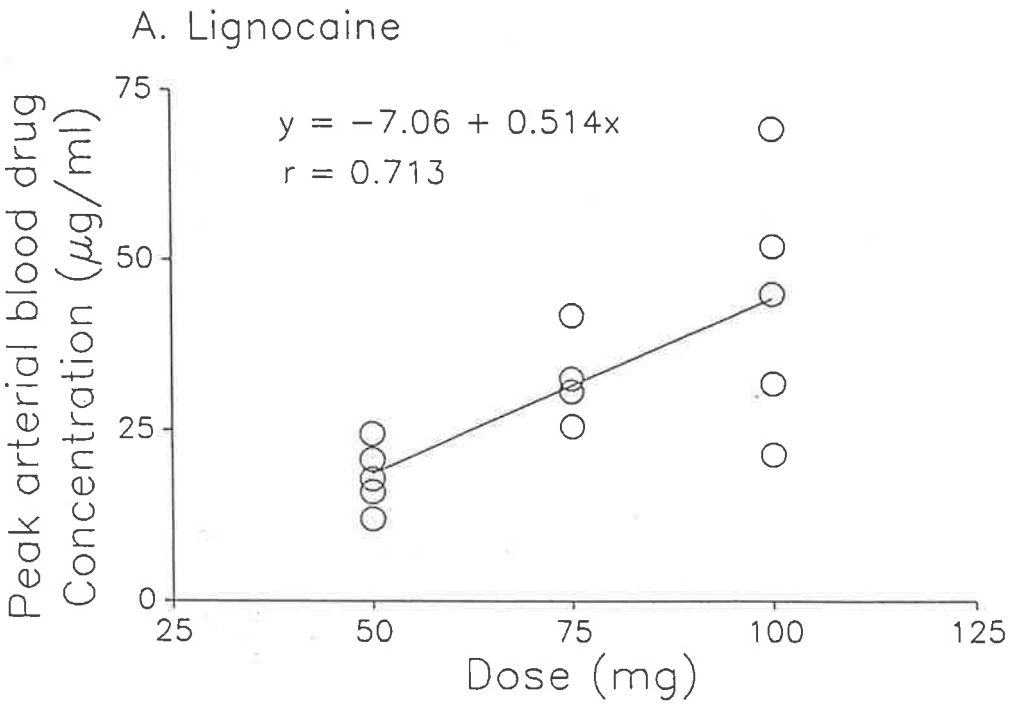

A. 100 mg pethidine
Arterial blood
Coronary sinus blood
Blood drug concentration (μg/ml)
Time (sec)
B. 200 mg pethidine
Blood drug concentration (μg/ml)
Time (sec)
C. 300 mg pethidine
Blood drug concentration (μg/ml)
Time (sec)

Figure 9.3

Linear relationship between the peak arterial blood drug concentrations and the drug doses used. (A) Lignocaine (n = 14): Y is peak arterial blood drug concentration and X the dose used. (B) Equivalent of (A) but for pethidine (n = 13). The coefficients of correlation (r) are both statistically significant (T_r are 3.52 and 8.18 for lignocaine and pethidine, respectively, $P < 0.05$ for both drugs).


A. Lignocaine
y = −7.06 + 0.514x
r = 0.713
Peak arterial blood drug Concentration (μg/ml)
75
50
25
0
25
50
75
100
125
Dose (mg)

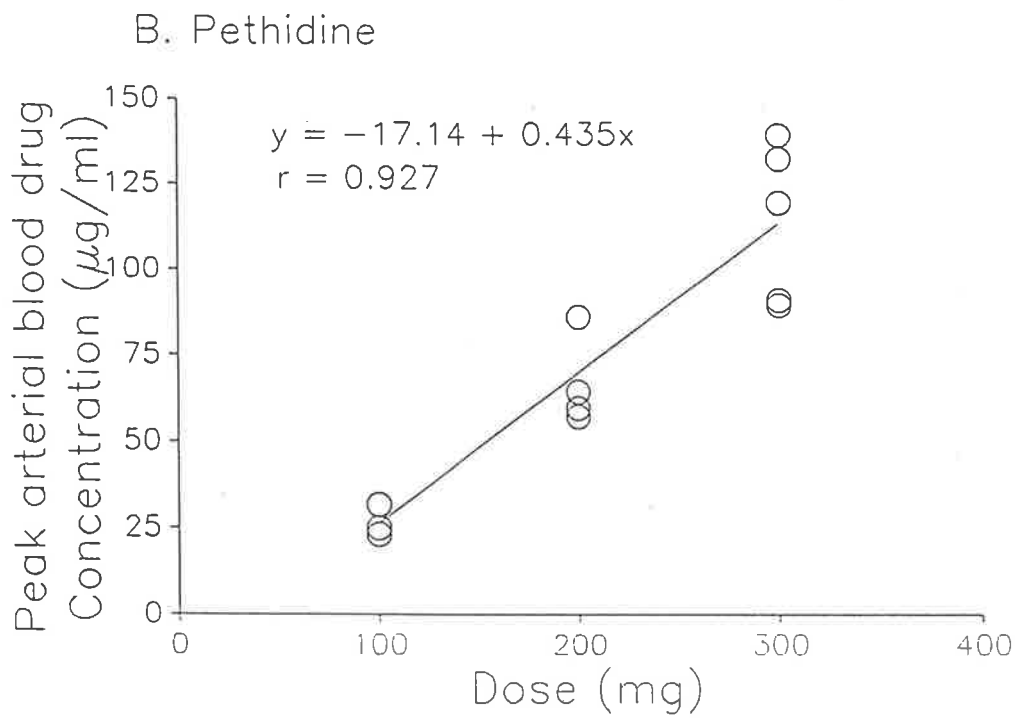

B. Pethidine
y = −17.14 + 0.435x
r = 0.927
Peak arterial blood drug Concentration (μg/ml)
150
125
100
75
50
25
0
0
100
200
300
400
Dose (mg)

Figure 9.4

The trends of peak arterial blood drug concentration/dose (A, E), peak myocardial uptake flux/dose (B, F), peak myocardial uptake flux/peak arterial blood drug concentration (C, G) and peak myocardial drug mass/dose (D, H) in relation to doses of lignocaine and pethidine used. Statistically significant linear trends for these ratios and the doses were only found for pethidine (One Factor Repeated Measures Analysis of Variance).

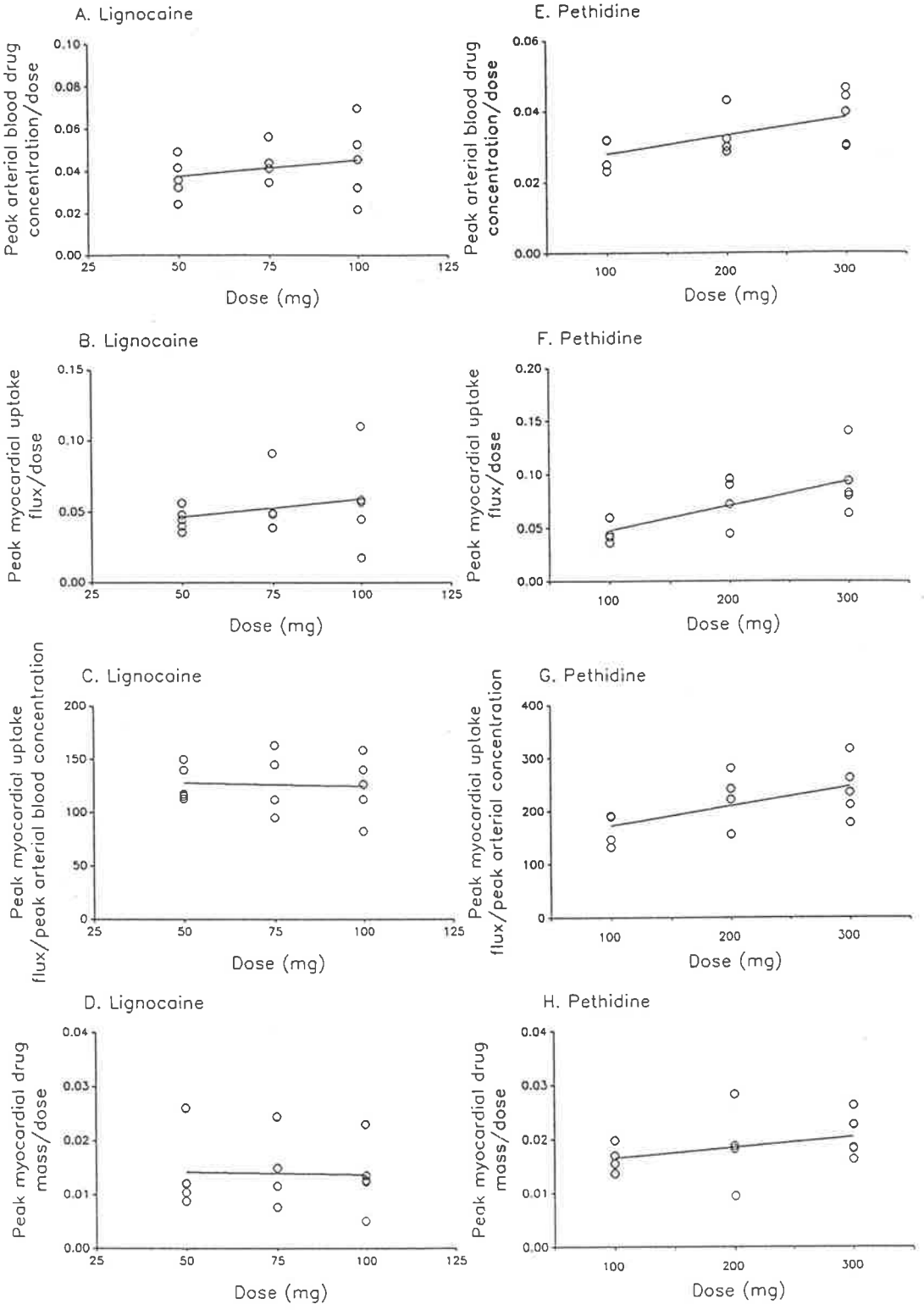

A. Lignocaine
Peak arterial blood drug concentration/dose
0.10
0.08
0.06
0.04
0.02
0.00
25
50
75
100
125
Dose (mg)
E. Pethidine
Peak arterial blood drug concentration/dose
0.06
0.04
0.02
0.00
100
200
300
Dose (mg)
B. Lignocaine
Peak myocardial uptake flux/dose
0.15
0.10
0.05
0.00
25
50
75
100
125
Dose (mg)
F. Pethidine
Peak myocardial uptake flux/dose
0.20
0.15
0.10
0.05
0.00
100
200
300
Dose (mg)
C. Lignocaine
Peak myocardial uptake flux/peak arterial blood concentration
200
150
100
50
0
25
50
75
100
125
Dose (mg)
G. Pethidine
Peak myocardial uptake flux/peak arterial concentration
400
300
200
100
0
100
200
300
Dose (mg)
D. Lignocaine
Peak myocardial drug mass/dose
0.04
0.03
0.02
0.01
0.00
25
50
75
100
125
Dose (mg)
H. Pethidine
Peak myocardial drug mass/dose
0.04
0.03
0.02
0.01
0.00
100
200
300
Dose (mg)

Figure 9.5

The time - courses of the calculated net drug fluxes for the myocardium after intravenous bolus injections of 50, 75 or 100 mg of lignocaine, shown by (A), (B) and (C), respectively. The smaller graphs in the upper right corner of each panel shows the data regraphed on a scale which better shows the negative net drug fluxes.

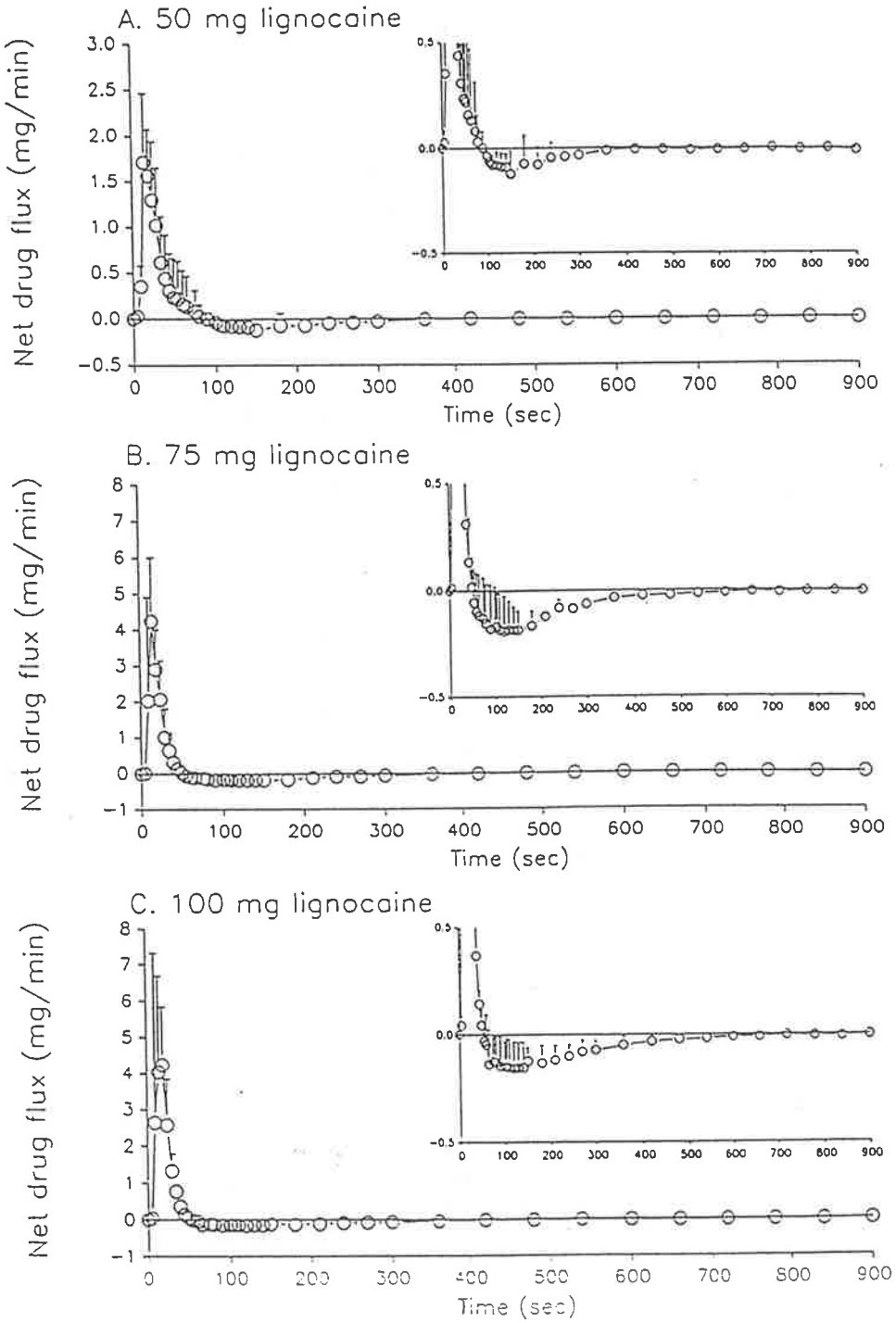

A. 50 mg lignocaine
Net drug flux (mg/min)
Time (sec)
B. 75 mg lignocaine
Net drug flux (mg/min)
Time (sec)
C. 100 mg lignocaine
Net drug flux (mg/min)
Time (sec)

Figure 9.6

The time - courses of the calculated net drug fluxes for the myocardium after the intravenous bolus injections of 100, 200 or 300 mg doses of pethidine, shown by (A), (B) and (C), respectively. The smaller graphs in the upper right corner of each panel shows the data regraphed on a scale which better shows the negative net drug fluxes.

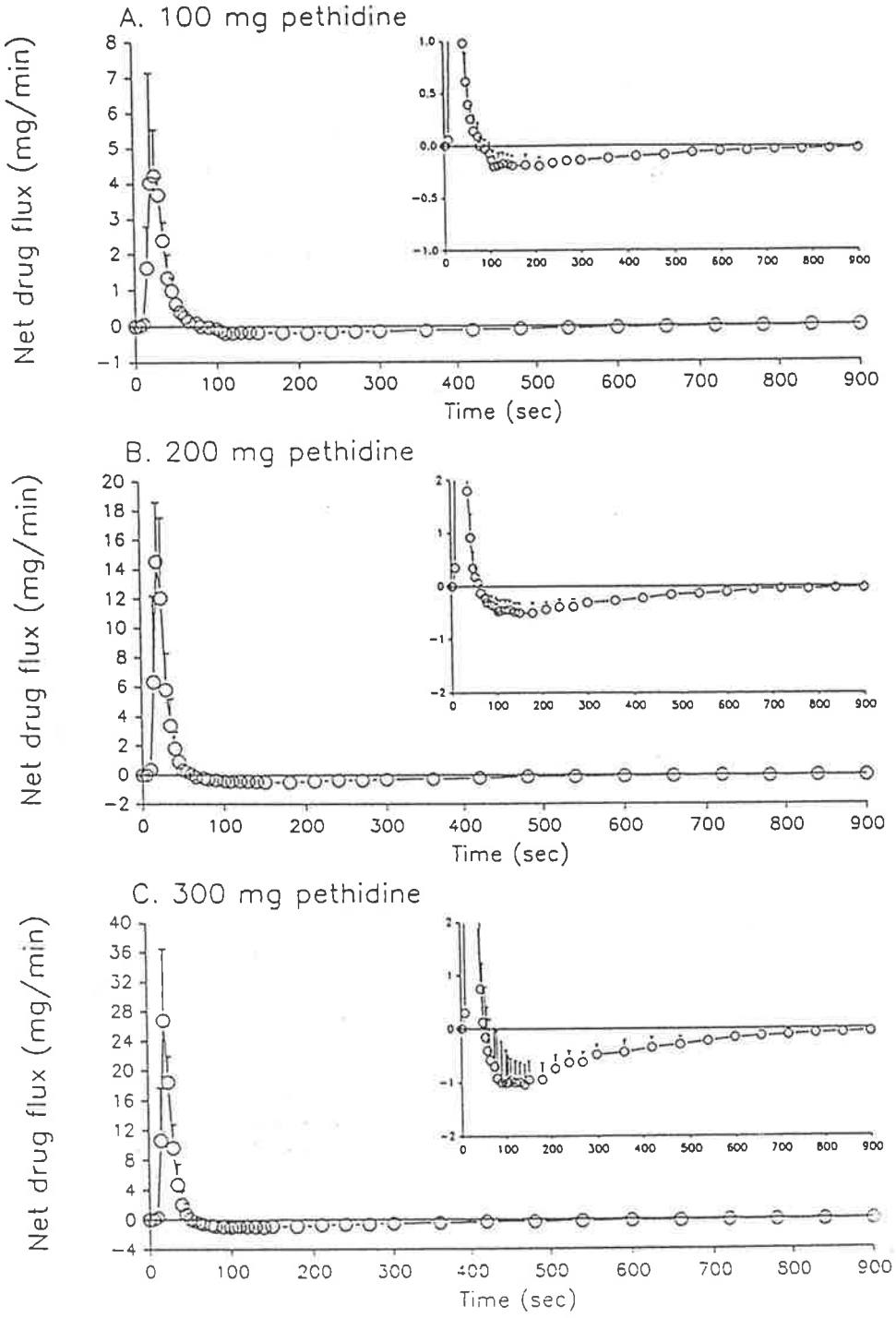
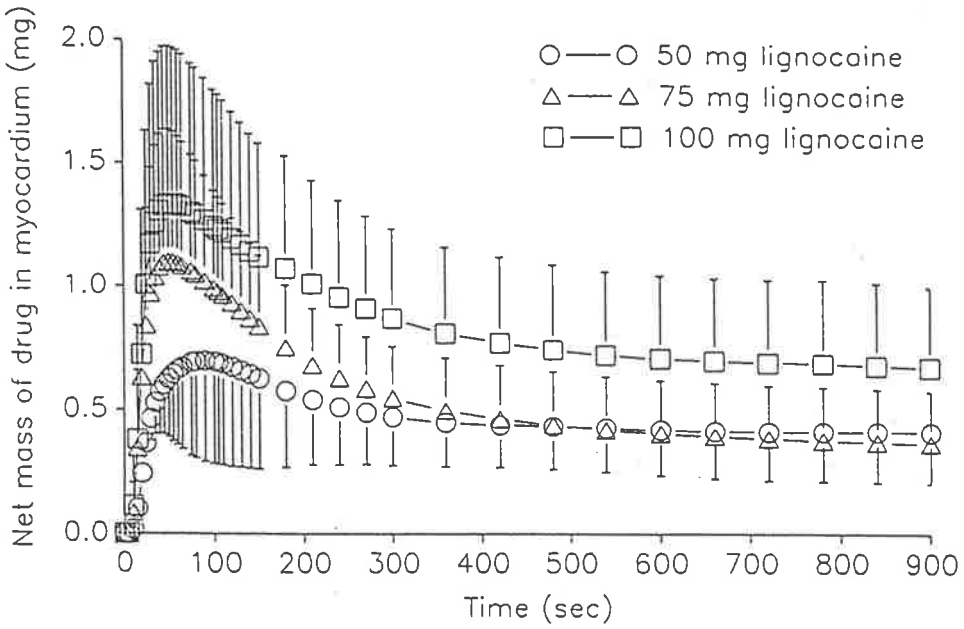

A. 100 mg pethidine
Net drug flux (mg/min)
Time (sec)
B. 200 mg pethidine
Net drug flux (mg/min)
Time (sec)
C. 300 mg pethidine
Net drug flux (mg/min)
Time (sec)

Figure 9.7

Time - courses of the calculated net drug masses of lignocaine (A) and pethidine (B) in the myocardium. In (A) the open circles, open triangles and open squares represent the net drug mass in the myocardium after 50, 75 and 100 mg doses of lignocaine. In (B), the open circles, open triangles and open squares represent the net drug mass in the myocardium after 100, 200 and 300 mg doses of pethidine.

A. Lignocaine



B. Pethidine

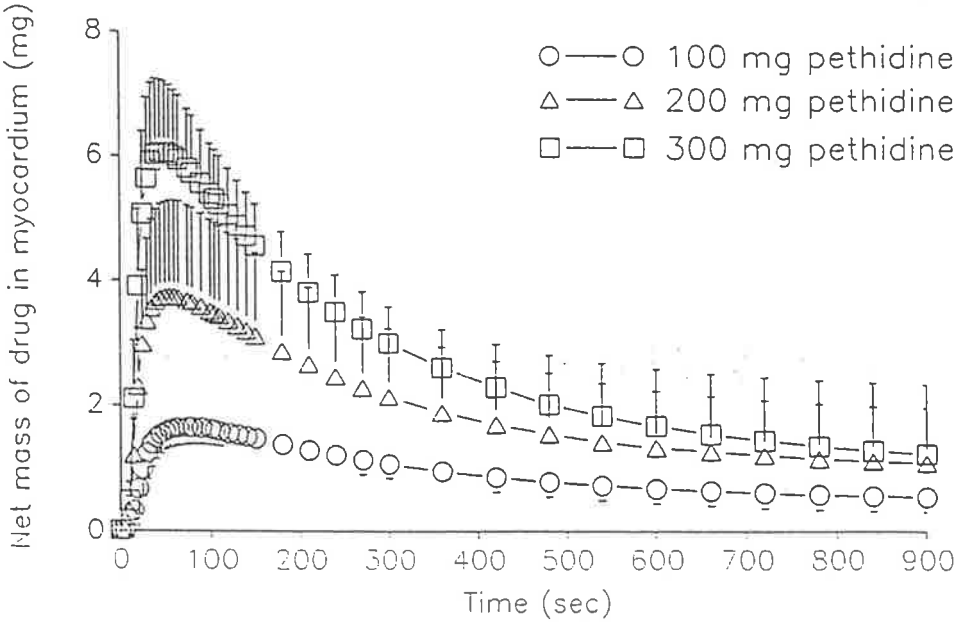


Figure 9.8

The time - courses of the calculated myocardial concentrations of lignocaine (A) and pethidine (B) after intravenous bolus drug injections. In (A), the open circles, open triangles and open squares represent the myocardial lignocaine concentrations after 50, 75 and 100 mg doses of lignocaine. In (B), the open circles, open triangles and open squares represent the myocardial pethidine concentrations after 100, 200 and 300 mg doses of pethidine. The error bars of myocardial drug concentrations after the 100 mg doses of pethidine were so small at later times that they can not be shown on the graph.

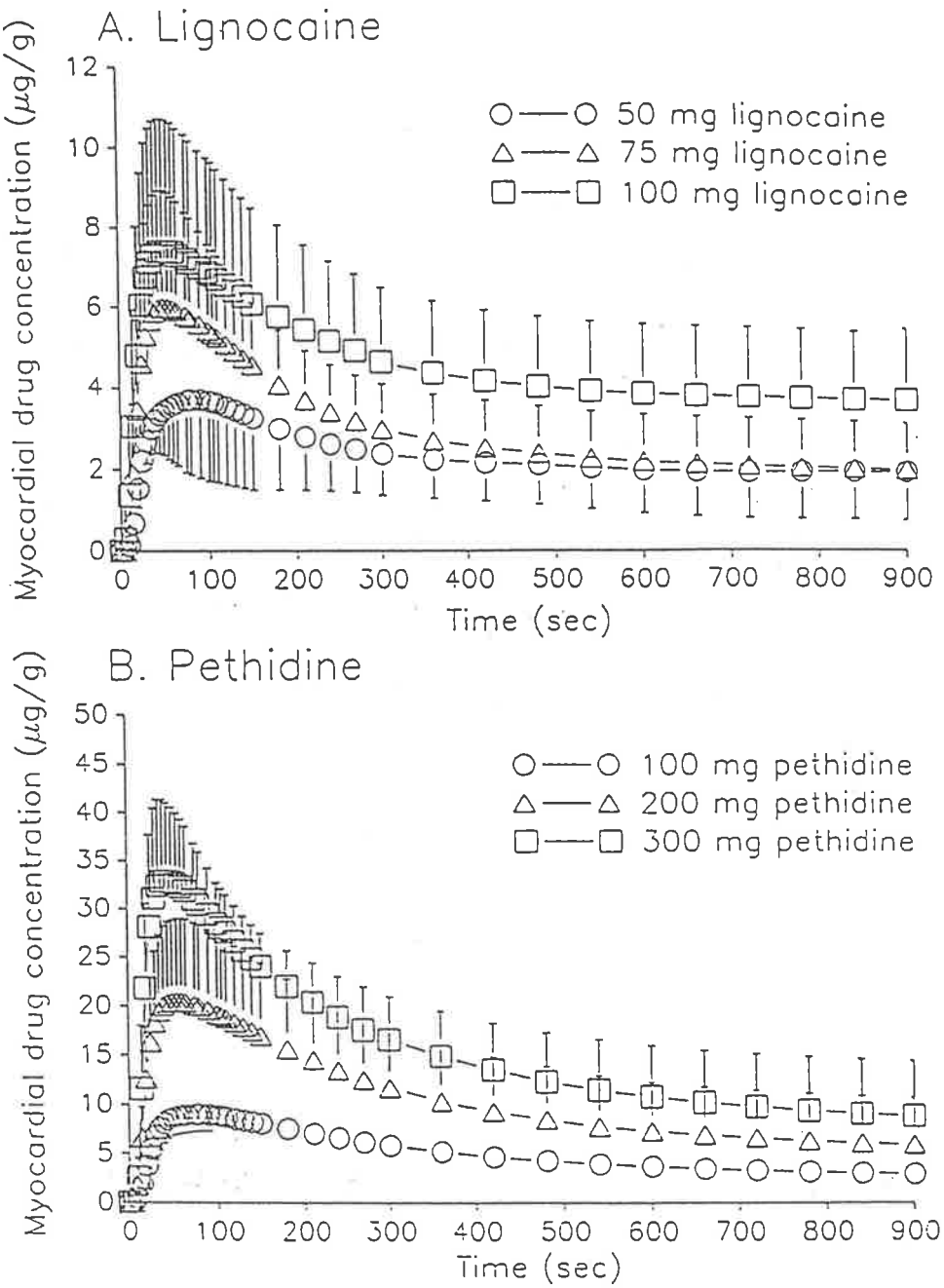
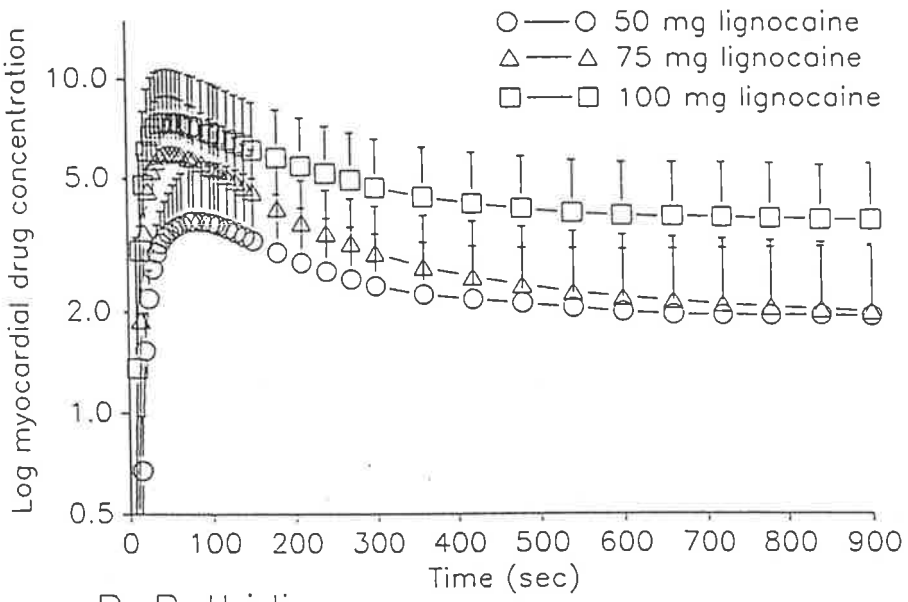

A. Lignocaine
Myocardial drug concentration (μg/g)
12
10
8
6
4
2
0
0
100
200
300
400
500
600
700
800
900
Time (sec)
50 mg lignocaine
75 mg lignocaine
100 mg lignocaine
B. Pethidine
Myocardial drug concentration (μg/g)
50
45
40
35
30
25
20
15
10
5
0
0
100
200
300
400
500
600
700
800
900
Time (sec)
100 mg pethidine
200 mg pethidine
300 mg pethidine

Figure 9.9

The equivalent of Fig. 9.8 except drug concentrations are plotted with a logarithmic concentration axis. The time - courses of the calculated myocardial concentrations of lignocaine (A) and pethidine (B) after intravenous bolus drug injections. In (A), the open circles, open triangles and open squares represent the myocardial lignocaine concentrations after 50, 75 and 100 mg doses of lignocaine. In (B), the open circles, open triangles and open squares represent the myocardial pethidine concentrations after 100, 200 and 300 mg doses of pethidine.

A. Lignocaine



B. Pethidine

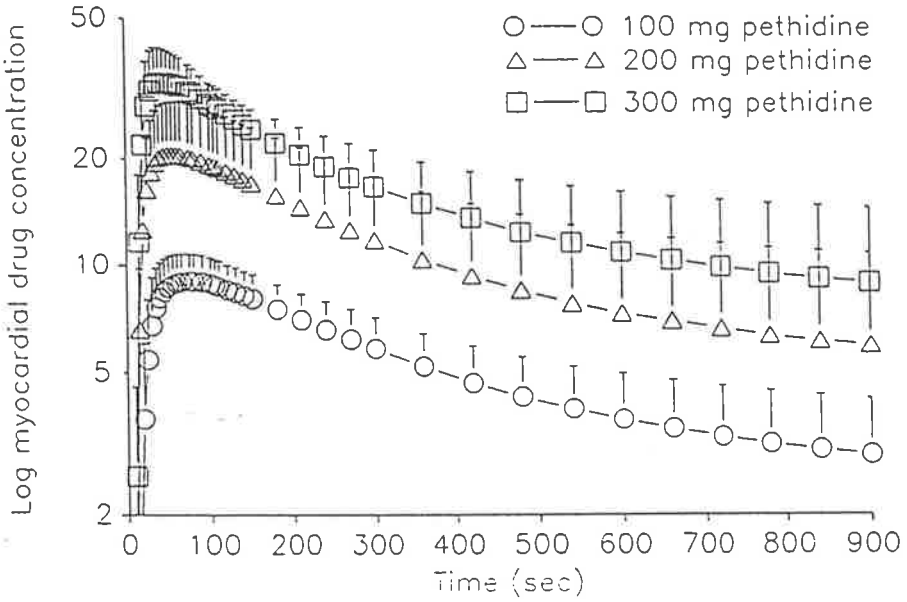


Figure 9.10

The time - courses of the ratios of the calculated myocardial lignocaine concentrations and the arterial (open circles) or coronary sinus (open triangles) blood concentrations after intravenous bolus injections of 50, 75 and 100 mg of lignocaine shown in (A), (B) and (C), respectively.

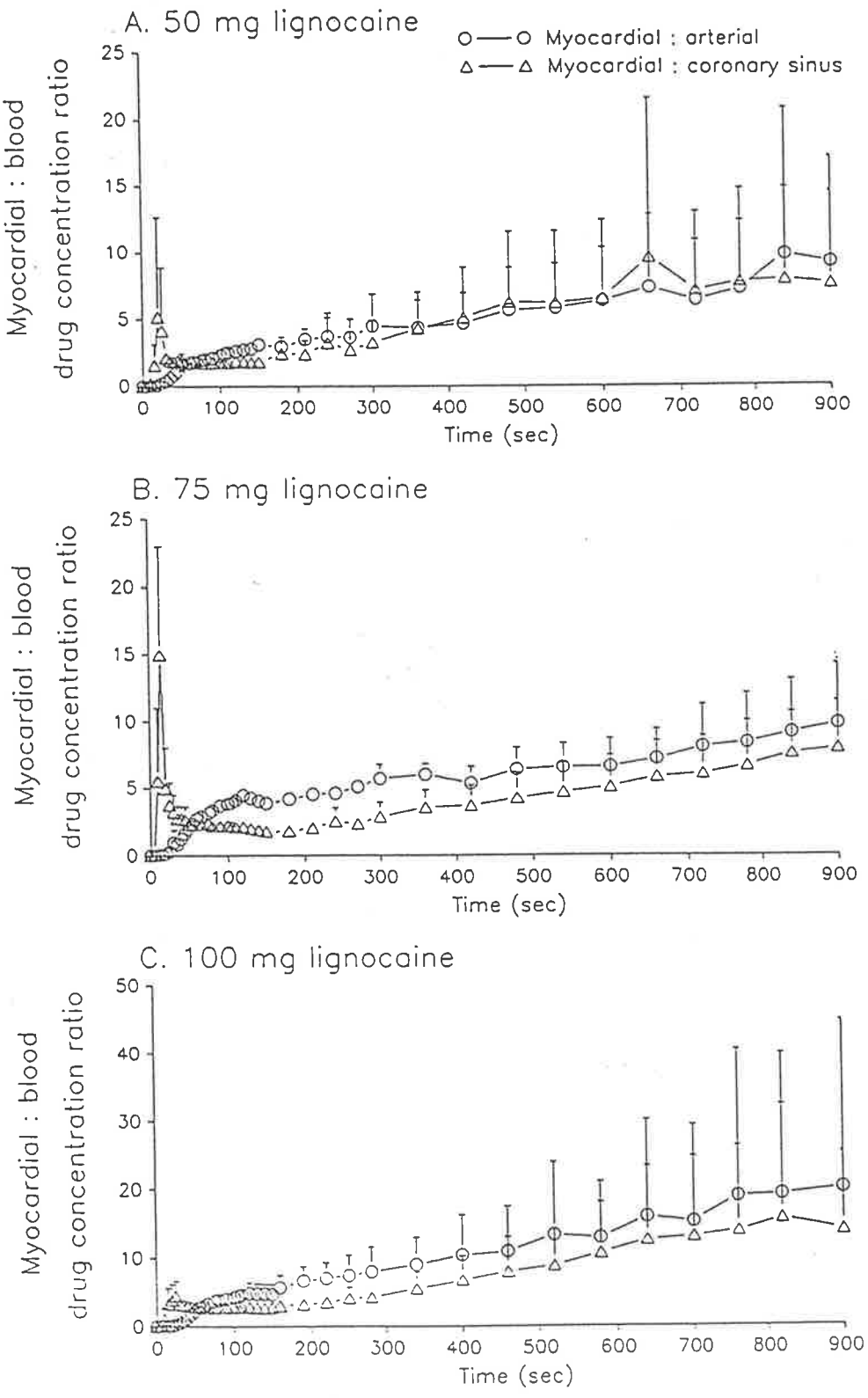
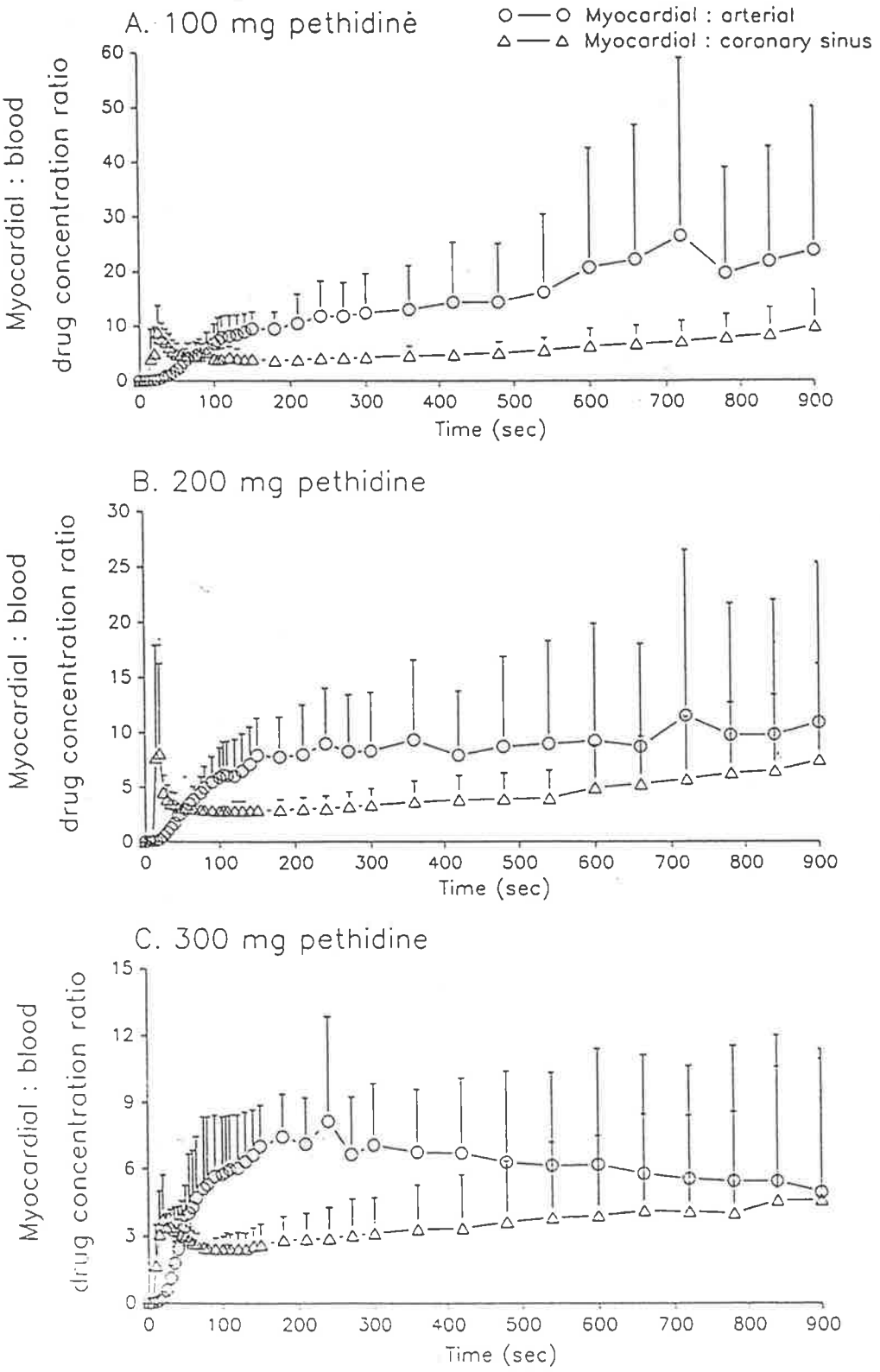

A. 50 mg lignocaine
o—o Myocardial : arterial
Δ—Δ Myocardial : coronary sinus
Myocardial : blood
drug concentration ratio
25
20
15
10
5
0
0
100
200
300
400
500
600
700
800
900
Time (sec)
B. 75 mg lignocaine
Myocardial : blood
drug concentration ratio
25
20
15
10
5
0
0
100
200
300
400
500
600
700
800
900
Time (sec)
C. 100 mg lignocaine
Myocardial : blood
drug concentration ratio
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
900
Time (sec)

Figure 9.11

The time - courses of the ratios of the calculated myocardial pethidine concentrations and the arterial (open circles) and coronary sinus (open triangles) blood concentrations after intravenous bolus injections of 100, 200 and 300 mg of pethidine shown in (A), (B) and (C), respectively.


A. 100 mg pethidine
O—O Myocardial : arterial
Δ—Δ Myocardial : coronary sinus
Myocardial : blood
drug concentration ratio
0
10
20
30
40
50
60
0
100
200
300
400
500
600
700
800
900
Time (sec)
B. 200 mg pethidine
Myocardial : blood
drug concentration ratio
0
5
10
15
20
25
30
0
100
200
300
400
500
600
700
800
900
Time (sec)
C. 300 mg pethidine
Myocardial : blood
drug concentration ratio
0
3
6
9
12
15
0
100
200
300
400
500
600
700
800
900
Time (sec)

CHAPTER 10: RELATIONSHIPS BETWEEN THE MYOCARDIAL PHARMACOKINETICS AND PHARMACODYNAMICS OF LIGNOCAINE AND PETHIDINE.

10.1 Introduction

In studies of the relationships between the kinetics and dynamics of drugs, it is not uncommon for the time - course of drug effects to be compared with the time - course of the systemic (e.g. arterial or peripheral venous) blood drug concentrations (Gerstenblith 1972; Holford and Sheiner, 1981; Liem et al., 1988). The results of these studies have often shown an "anti - clockwise hysteresis" between drug concentration and drug effect, indicating that the drug effects lagged behind systemic drug concentrations (see Chapter 1.3). This is often attributed to a lack of equilibrium between the measured drug concentrations in blood and the drug concentrations at the sites of drug effect.

Compartmental pharmacokinetic models have been developed and used successfully to compensate for this lack of equilibrium between drug concentrations in blood, tissues and sites of drug effect. For example, a compartmental model was constructed with the heart located in a peripheral compartment (represented by drug concentrations of saliva) in which procainamide effects on the heart were better related to the predicted concentrations in this peripheral compartment than the central compartment containing the blood pool (Galeazzi et al., 1976). Alternatively, in the study of other drugs using pharmacokinetic - pharmacodynamic modelling, a hypothetical "effect" compartment has been constructed as part of the compartmental model, and its rate constants were adjusted so that the "apparent" drug concentrations in the compartment could be related to the effects without hysteresis (Sheiner et al., 1979; Stanski et al., 1984).

While both of these methods have some use in predicting the time - course of drug effects in response to changes in the dose regimen of a drug (provided there are no other physiological or pharmacological changes in a patient), these methods assume that it is not possible to measure or rationally predict drug concentration at a site

closer to the site of drug effect. More importantly, neither of these compartmental methods can be physiological representations of, for example, the myocardial pharmacokinetics of drugs, as the compartments have no anatomical reality, and the rate constants are not related to the physical processes which may influence drug disposition (Hull, 1990).

It has been proposed that the magnitudes of drug effects on the myocardium are a function of the myocardial drug concentrations (Horowitz et al., 1986). It has also been proposed that the study of the relationships between myocardial pharmacokinetics and pharmacodynamics would provide insight into the mechanisms of drug action, the ability to predict the magnitude of drug effects and establish a more rational basis for the more efficient, safer use of drugs (Upton, 1990; Holford and Sheiner, 1981; Colburn, 1987).

In Chapter 9, it was shown that even in a "well - perfused" tissue such as the myocardium there was a lack of equilibrium between the calculated myocardial drug concentrations and the rapidly changing arterial and coronary sinus blood drug concentrations for up to 15 min after intravenous drug administration. It was also found in Chapter 8 that the principle haemodynamic effects caused by intravenous injections of lignocaine and pethidine were dose dependent reductions of myocardial contractility, as indicated by the decreases of LV dp/dt_{max}. Although the heart rates changed significantly after the administration of pethidine, neither previous studies nor present results have confirmed that this is a direct effect of pethidine on the myocardium. Therefore, in this chapter the relationships between the effects of lignocaine and pethidine on myocardial contractility and their concentrations in arterial and coronary sinus blood and the myocardium were examined in order to determine which of these concentrations, if any, were in equilibrium with the drug concentrations at the sites of drug effect within the myocardium.

10.2 Materials and methods

The data concerning the myocardial pharmacodynamics of lignocaine and pethidine described in Chapter 8 were correlated with the simultaneously determined blood

and myocardial drug concentration data presented in Chapter 9. Therefore, the sheep preparation, study design, data acquisition and methods for pharmacokinetic and pharmacodynamic analysis were as described in those chapters.

It is known that LV dp/dt_{max} is a reliable index of myocardial contractility in man when factors such as left ventricular end - diastolic pressure, arterial diastolic pressure and heart rate are constant (Mason, 1969). The pilot study described in Chapter 6 showed that in conscious, unrestrained sheep changes in heart rate from 100 to 190 beats/min caused less than an 8% change in LV dp/dt_{max}. Furthermore, of the other factors, only the aortic diastolic blood pressure changed during the studies, in that it increased for 1 to 2 min after different doses of pethidine. Therefore, it is concluded that LV dp/dt_{max} is a reasonable index of myocardial contractility in these studies.

The following three comparisons between various measurements of drug concentrations and myocardial contractility were made.

10.2.1 Comparison of the times of peak drug concentrations and maximum depression of myocardial contractility

The times at which the peak arterial, coronary sinus or calculated myocardial drug concentrations occurred were compared to the times of the maximum depression of myocardial contractility for the different doses of lignocaine and pethidine. Note that the maximum depression of myocardial contractility described here refers to the observed maximum decrease of LV dp/dt_{max} under non steady - state drug concentration conditions, and that this differs from the maximum drug effect (E_{max}) determined as part of dose - response studies under steady state drug concentration conditions (Holford and Sheiner, 1981; see 1.3).

10.2.2 Comparisons of the drug concentrations at the beginnings and ends of the periods of depression of myocardial contractility

To perform this analysis, a quantitative definition of the duration of the drug induced depression of myocardial contractility was needed. For this purpose, the

coefficient of variation of LV dp/dt_{max} during each control period (30 sec) was calculated and for all the experiments this was found to range between 0 and 2.7%. As described previously, changes in heart rate from 100 to 190 beats/min caused less than an 8% change in LV dp/dt_{max}. In view of this, the period of depression of myocardial contractility was arbitrarily defined as that period during which there was a greater than 10% reduction in myocardial contractility. Drug concentrations at the beginning and end of this period were compared for each dose of lignocaine and pethidine.

10.2.3 Concentration - effect relationships

The arterial blood, coronary sinus blood and myocardial drug concentrations were each plotted against the depression of myocardial contractility induced by the two drugs in time order to examine for hysteresis or nonlinearity in the concentration - effect relationships (see 1.3). To quantitate the magnitude of hysteresis in these plots, each plot was divided into two sections at the point of the peak drug concentrations (Fig. 10.1). The area under each section of the curve (AUC) was calculated using the trapezoidal rule. Hysteresis was considered to be present when the differences between the AUC of these two sections of the hysteresis loop were statistically significant.

10.2.4 Data analysis

The data were entered into a spreadsheet program (see 2.6) for analysis. Two Sample t - test was used for the analysis of the AUC of the two sections of the hysteresis loops, the differences between the times of peak drug concentrations and maximum drug effect, and the differences between blood or myocardial drug concentrations at the beginnings and the ends of the periods of depression of myocardial contractility. $P < 0.05$ was considered statistically significant. Linear regression was used for the correlation between myocardial drug concentration and the percent decreases of myocardial contractility (LV dp/dt_{max}).

10.3 Results

The data of the sheep with pericardial adhesions, low cardiac output and low LV dp/dt_{max} were not included in statistical analysis. Those pharmacokinetic data from 75 mg lignocaine, 100 and 200 mg pethidine studies on the sheep in which the coronary sinus blood sampling catheter was in the right atrium also were not included. Therefore, the number for drug effect studies of each dose was 5 and for pharmacokinetic studies of 50, 75, and 100 mg lignocaine and 100, 200, and 300 mg pethidine was 5, 4, and 5 and 4, 4, and 5, respectively.

The time - courses of the mean arterial blood, coronary sinus blood and myocardial drug concentrations and the time - courses of the mean percent decreases of LV dp/dt_{max} after the intravenous bolus injection of the different doses of lignocaine and pethidine are shown in Figs. 10.2 and 10.3, respectively. The time - course of the decrease in LV dp/dt_{max} after 300 mg pethidine (Fig. 10.3 C) was inconsistent with the lower doses and was relatively variable - this was presumably due to the excitatory central nervous system effects observed for this dose of pethidine (Sugioka et al., 1957; Chapter 8). With this exception however, it is apparent from Figs. 10.2 and 10.3 that the time - courses of the reductions in LV dp/dt_{max} did not closely follow the time - courses of the arterial blood drug concentrations, but were similar to the time - courses of the coronary sinus blood and calculated myocardial drug concentrations.

10.3.1 The times of peak drug concentrations and maximum depression of myocardial contractility

These times for the peak arterial blood, coronary sinus blood and myocardial drug concentrations are listed in Table 10.1. It is apparent that for all the doses of both drugs, the times when the peak arterial blood drug concentration were reached were approximately 20 to 30 sec earlier than the times of maximum decreases in LV dp/dt_{max} (Figs. 10.2 and 10.3). These time differences were all statistically significant. The times of the peak coronary sinus blood drug concentrations for all the doses of lignocaine and pethidine were approximately 30 to 70 sec later than

the times of maximum depression of LV dp/dt_{max}, and these time differences were also statistically significant. With the exception of the 100 mg doses of both lignocaine and pethidine, the times at which the calculated peak myocardial drug concentrations occurred were not statistically different to the times of the maximum depression of myocardial contractility (Table 10.1). For the 100 mg doses, the mean times of peak lignocaine and pethidine myocardial concentrations were significantly later than the mean times of maximum myocardial depression (17 and 19 sec, respectively).

10.3.2 Drug concentrations at the beginnings and ends of periods of depression of myocardial contractility

The arterial and coronary sinus blood and the calculated myocardial drug concentrations at the beginnings and ends of the periods of depression of myocardial contractility are shown in Figs. 10.4 and 10.5 for lignocaine and pethidine, respectively. The arterial blood drug concentrations at the beginnings of these periods of depression of myocardial contractility were all statistically higher than those at the ends of those periods, but no such differences were observed for the coronary sinus blood or myocardial drug concentrations.

10.3.3 Concentration - effect relationships

The percentage decreases of LV dp/dt_{max} plotted against the corresponding arterial blood drug concentrations are shown in Fig. 10.6, against the coronary sinus blood drug concentrations in Fig. 10.7 and against the calculated myocardial drug concentrations in Fig. 10.8. The AUC of the two sections of each of the concentration - effect curves are shown in Fig. 10.9. Significant anti - clockwise hysteresis was always present when the arterial concentrations were used (Figs. 10.6 and 10.9). For the coronary sinus blood drug concentrations, significant clockwise hysteresis was found for the 100 mg dose of pethidine, but no significant hysteresis was found for any other studies (Figs. 10.7 and 10.9). There were no significant hystereses between the calculated myocardial drug concentrations and LV dp/dt_{max} depression (Figs. 10.8 and 10.9).

A linear pharmacodynamic model (Holford and Sheiner, 1981; Schwinghammer and Kroboth, 1988; also see 1.3 and Equation 1.1) was used to describe the myocardial drug concentration - effect relationships of the two drugs. Because the 300 mg dose of pethidine caused obvious CNS effects to the conscious, unrestrained sheep, and this presumably modified time - courses of drug effect on LV dp/dt_{max} (see 8.3), the concentration - effect data of 300 mg pethidine was not included in this analysis. The data for the three doses of lignocaine and the two doses of pethidine (100 and 200 mg) were pooled together, respectively. Linear regressions between the myocardial drug concentrations and the percent depressions of LV dp/dt_{max} were performed and are shown in Fig. 10.10. The correlation coefficients (r) of the regressions for lignocaine and pethidine were 0.918 and 0.858, respectively. The slopes (S) of the linear regressions for the pooled data of lignocaine and pethidine were 4.4 and 1.7, respectively. The mean and S.D. of the slopes (S) of each individual study are listed in Table 10.2. The intercepts of the regressions are -4.5 and -0.5 for lignocaine and pethidine, respectively.

10.4 Discussion

It is apparent from Figs. 10.3, 10.6, 10.7 and 10.8 that the concentration - contractility relationship for the 300 mg dose of pethidine was qualitatively different to those for the other studies. As mentioned in Chapter 8, this dose of pethidine caused noticeable excitatory effects on the central nervous system manifested by the sheep bleating and kicking. It is likely that these CNS effects also indirectly affected myocardial contractility. This dose of pethidine is therefore not included in the following discussion. However, these data serve to illustrate the complexity of action of intravenously administered drugs that have both myocardial and CNS effects. As also shown by the work of Rutten et al., (1989), drugs such as local anaesthetics and analgesics can cause initial direct myocardial depression which is subsequently "over - ridden" by increases in contractility caused by CNS stimulation or fitting. It may be possible that the treatment of acute local anaesthetic toxicity with anti - convulsants, such as diazepam, may have a detrimental effect on myocardial performance if it

"unmasks" the intrinsic depression caused by the local anaesthetic. Certainly, more work in this area is indicated.

The concept of therapeutic or toxic blood drug concentrations is based on the assumption that drug concentrations in blood are in equilibrium or pseudo - equilibrium with drug concentrations in the tissues of drug effect. However, there are two major areas of concern with this assumption. Firstly, in situations in which drugs are administered as intravenous bolus injections or short intravenous infusions, such as for the control of arrythmias using antiarrhymic drugs (Carson et al., 1979; Lucchesi and Patterson, 1984), drug concentrations in both blood and tissues change so rapidly that this equilibrium is not achieved, even for well - perfused organs such as the heart, as shown in Chapter 9. Secondly, in many studies little consideration is given to the influence of blood sampling site on pharmacokinetic - pharmacodynamic relationships. Many workers use peripheral venous blood drug concentrations, but the composition of this blood is dependent on where in the heterogeneous venous circulation the blood sample was taken from - for example the concentrations of pethidine in the hepatic vein and inferior vena cava of sheep during constant rate infusions were approximately 1% and 50% of the pulmonary artery concentrations (Mather et al., 1986b). When these two factors act simultaneously, it is not surprising that the "therapeutic drug concentrations" may be of no use for predicting the time - course of the effects of drugs such as antiarrythmics (Koch - Weser, 1972; Gerstenblith, 1972; Allen et al., 1977; Hashimoto et al., 1982; 1984; Liem et al., 1988).

Although peripheral or central venous blood drug concentrations were not measured in the studies presented here, drug concentrations were measured at three sites relevant to the myocardial pharmacokinetics and pharmacodynamics of the drugs - the arterial blood, the myocardium and the coronary sinus blood. The usefulness of the drug concentrations from these sites will be discussed in turn.

It has recently been proposed that arterial blood drug concentrations should be used for pharmacokinetic - pharmacodynamic studies because arterial blood carries drugs

to their sites of action in the body (Chiou, 1989a; 1989b). In the studies presented here there was a poor relationship between the arterial blood drug concentration and the depression of myocardial contractility. Significantly higher arterial blood drug concentrations were observed at the beginnings than at the ends of the periods of depression of myocardial contractility caused by the drugs (Figs. 10.4 and 10.5). Also, the maximum decreases in myocardial contractility were recorded significantly later than the peak arterial blood drug concentrations (Figs. 10.2 and 10.3, Table 10.1). Furthermore, the concentration - contractility relationships showed statistically significant *anticlockwise* hysteresis, indicating that the depression of myocardial contractility lagged behind the arterial blood drug concentrations.

These findings agree with those of others addressing similar problems. Hysteresis between the arterial plasma concentrations of propofol, procainamide, lignocaine and their depressant effects on myocardial contractility after intravenous bolus administration have also been reported (Binnion 1969; Liem et al., 1988; Coetzee et al., 1989). Furthermore, the myocardial effects of drugs such as antiarrythmics (Galeazzi et al., 1976; Reiter et al., 1982) and some anaesthetics (Christensen et al., 1982) were found to be poorly correlated with either arterial or systemic venous blood drug concentrations. The lack of equilibrium between arterial blood and myocardial drug concentrations observed in Chapter 9 is the most likely explanation for these observations.

By the argument of Horowitz and Powell (1986), it would be anticipated that the myocardial concentrations of lignocaine and pethidine would be better indices of the myocardial depressant effects of these drugs than their arterial blood concentrations. However, it should be remembered that methods based on mass balance principles determine the total myocardial drug concentration which may in itself be inadequate if these drugs act on very specific sites within the myocardium that are not in equilibrium with the total myocardial drug concentration. Nevertheless, for all the doses of both lignocaine and pethidine, good correlations were found between the myocardial drug concentrations and the depression in myocardial contractility, with

no significant hysteresis in the concentration - effect plots (Figs. 10.8 and 10.9). No statistical differences were found between the myocardial concentrations of lignocaine or pethidine at the beginnings and at the ends of the periods of depression of myocardial contractility. Furthermore, there were few differences in these values between different doses, with the exception of the 200 mg dose of pethidine for which the myocardial concentration at the beginning of the period of depression of myocardial contractility was significantly higher than that of the 100 mg dose. This consistency provides reasonable justification for the pooling of these data to identify "threshold" myocardial concentrations which induce a greater than 10% depression of myocardial contractility. These "threshold" values are 4.5 ± 2.4 μg/g and 8.2 ± 3.8 μg/g for lignocaine and pethidine, respectively.

The threshold myocardial lignocaine concentration is within the range of its reported therapeutic blood concentrations (Gerstenblith et al., 1972; Schnittger et al., 1978). It is also consistent with the conclusions from a study using a tissue biopsy method which showed that the concentrations of lignocaine in the ischaemic myocardium required to prevent ventricular arrhythmias (8 μg/g) after acute myocardial infarction were in the toxic concentration range (Vogt et al., 1988). The threshold myocardial concentration of pethidine presented here is approximately 10 times higher than the clinical effective analgesic blood concentrations of pethidine in patients (0.5 to 0.7 μg/ml; Mather and Meffin, 1978; Austin et al., 1980; Edwards et al., 1982), and is much higher than the effective analgesic blood concentration (0.93 μg/ml) in experimental sheep (Nolan et al., 1988). However, it is apparent from Fig 9.10 and Fig. 9.11 that at the ends of the studies, the ratios of myocardial to blood pethidine concentration was approximately were 5 - 20. Thus, the myocardial depression caused by pethidine may be of clinical importance under circumstances of low blood drug concentrations after quick intravenous bolus administration, although this remains to be determined.

Another noticeable attribute of the myocardial drug concentration - effect curves is that during the first 20 to 30 sec after drug injection (represented by the first 4 to 6

points on the curves in Fig. 10.10), the plot appears to curve upwards. After this period, the concentration - effect relationship is essentially linear. This initial upward curve may represent the time required for the drug to reach and act at the relevant sites (Roux et al., 1989; Schlepper, 1989; Almotrefi and Dzimiri, 1990) or simply the time required for the drug to reach different parts of the heart before the global deterioration of myocardial contractility appeared.

Because, in this study neither the drug concentrations nor the effects reached steady state or their maximum value, a linear pharmacodynamic model was used to describe the myocardial drug concentration - effect relationship (see 1.3 and Equation 1.1). The fact that myocardial drug concentrations at the beginnings and the ends of the periods of the depression of myocardial contractility were not statistically different for different doses of lignocaine, and only in the 200 mg pethidine study, the myocardial drug concentrations at the beginning of the period of contractility depression was higher than that of the 100 mg dose, justifies the pooling of the data of different doses of each drug for analysis. It is apparent that the myocardial concentration - contractility relationship is essentially linear (Fig. 10.9; Holford and Sheiner, 1981). Using this model, the predicted drug effect at zero myocardial drug concentration (E_0, i.e. the intercept of the regressions) for both lignocaine and pethidine were within 5% of the control values of LV dp/dt_{max} which is less than the predetermined term of myocardial contractility depression, i.e. more than 10% decrease in LV dp/dt_{max}.

In summary, these data show that the total myocardial drug concentration determined using mass balance principles is in equilibrium with the sites of drug effect in the myocardium causing depression of contractility, and that the magnitude and duration of the depression is a direct concentration - dependent effect on the myocardial tissue.

Although the calculated myocardial drug concentrations were in equilibrium with the depression of contractility for the drugs used in this chapter, they are still relatively difficult to measure in patients (Horowitz et al., 1986). As a simpler alternative, it

has been suggested that the effluent regional venous blood from the tissue or organ in which a drug exerts its effects could be used for the study of regional pharmacodynamics (Chiou, 1989a; 1989b). This is an extension of the concept of venous equilibrium used in many physiological pharmacokinetic models in which the tissue concentrations of a drug are related to the effluent venous concentrations by a constant ratio - the partition coefficient. In the studies presented here, although the coronary sinus blood drug concentrations were related relatively closely to the myocardial depressant effects of lignocaine and pethidine, i.e. significant hysteresis was only found in drug concentration - effect relationship of 100 mg dose of pethidine (Fig. 10.7), and no statistical differences in the coronary sinus drug concentrations at the beginnings and ends of the periods of myocardial depression (Fig. 10.4), the fact that the coronary sinus blood drug concentrations were not in equilibrium with the calculated myocardial concentrations (see 9.4, Fig. 9.8 and Fig.9.9), and the times of peak coronary sinus blood drug concentrations were significantly later than the times of the maximum depression of myocardial contractility suggest that coronary sinus blood drug concentrations are not reliable indices myocardial drug effect and the use of calculated myocardial drug concentrations is preferable after short term intravenous use of drugs. However, in situations in which the blood drug concentration changes are slower than those which occur after short term intravenous administration, the use of regional venous blood concentrations as an index of drug effect may have some merit. Nevertheless, a thorough knowledge of the relationships between regional venous and tissue drug concentrations would be an important prerequisite - for example the regional venous drug concentrations of the hindquarters of sheep bear little relationship to the tissue drug concentrations in the hindquarters after 180 min constant intravenous infusion (Upton et al., 1991b).

In conclusion, the depressant effects of lignocaine and pethidine on global myocardial contractility were linearly related to the calculated myocardial concentrations of these drugs. This further supports the argument that the reductions in myocardial contractility are because the drugs exert a direct negative

inotropic effect on the myocardium. This is of particular significance for pethidine because its *in vivo* effects on myocardial contractility are still controversial. Further study of the relationships between the myocardial and blood concentrations of such drugs, and of their effects, rather than the use of over - simplified, poorly based assumptions will have a number of benefits. The understanding of the efficacy of drugs administered using different dose regimens and under different pathological conditions, such as those often encountered in clinical use of antiarrhythmic drugs and anaesthetic agents, will be increased. Furthermore, such studies will provide data for the establishment of improved pharmacokinetic and pharmacodynamic models. Such models, if sufficiently developed, may help to predict the magnitude of drug effects from readily available data - such as cardiac output and drug injection rate - or may be used to design dose regimens to rapidly achieve therapeutic effects while at the same time minimising the potential for life threatening side - effects.

Table 10.1 The times of the peak arterial and coronary sinus blood and myocardial drug concentrations and times of the maximum depression of myocardial contractility after the i.v. bolus injection of lignocaine or pethidine (mean ± S.D., for maxmium drug effects of each dose of the drugs, n = 5; for peak drug concentrations of 50, 75 and 100 mg lignocane and 100, 200 and 300 mg pethidine doses , n = 5, 4, 5 and 4, 4, 5, respectively).

	Time (sec) of peak drug concentrations			Time (sec) of maximum depression of LV dp/dt_{max}
	Arterial	**Coronary sinus**	**Myocardium**	
Lignocaine				
50 mg	16 ± 2[a]	83 ± 7[b]	66 ± 23	54 ± 11
75 mg	15 ± 0[a]	129 ± 43[b]	55 ± 9	51 ± 5
100 mg	16 ± 4[a]	85 ± 38[b]	59 ± 14[b]	42 ± 4
Pethidine				
100 mg	20 ± 6[a]	125 ± 37[b]	75 ± 13[b]	56 ± 11
200 mg	16 ± 3[a]	91 ± 23[b]	58 ± 5	59 ± 12
300 mg	15 ± 0[a]	80 ± 29[b]	46 ± 11	46 ± 31

[a]Significantly earlier than the time of maximum depression (Two Sample t - test).

[b]Significantly later than the time of maximum depression (Two Sample t - test).

Table 10.2 The slopes (S) of the linear regressions between myocardial drug concentrations and percent decrease of LV dp/dt_{max} for the different doses of lignocaine and pethidine (mean ± S.D.).

Lignocaine	**Slope (S)**
50 mg	4.5 ± 2.7
75 mg	6.0 ± 2.4
100 mg	5.7 ± 2.7
Pethidine	**Slope (S)**
100 mg	3.3 ± 0.9
200 mg	2.4 ± 1.2

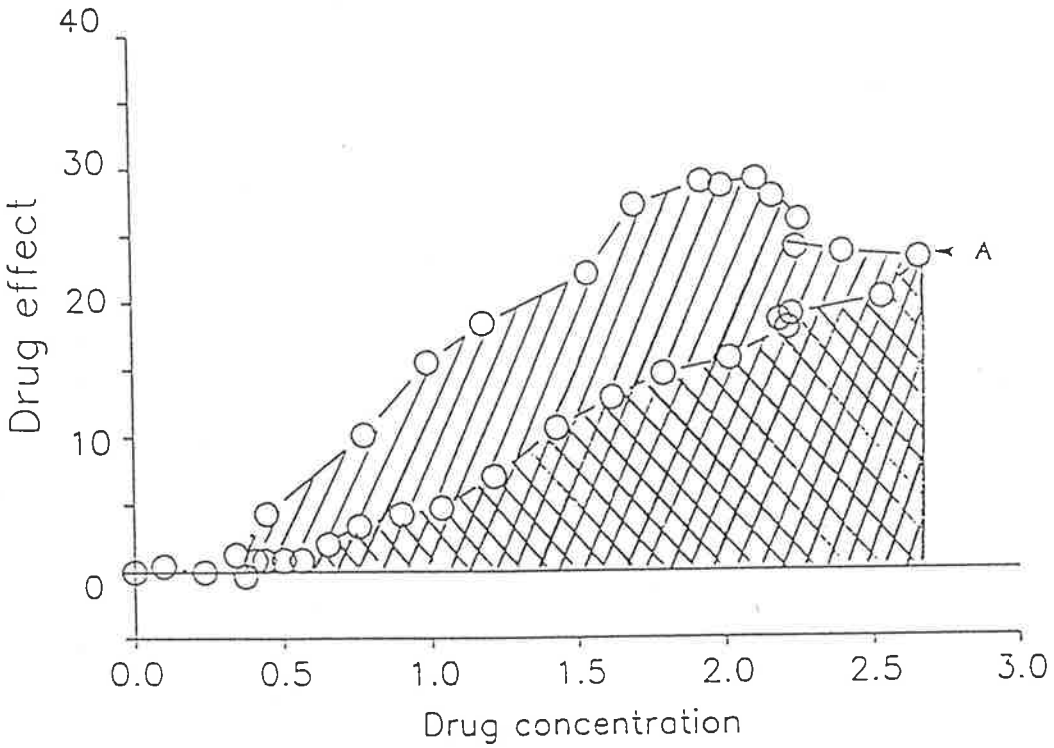


Figure 10.1

An example of hysteresis in a plot of drug concentration verses drug effect. The plot was divided into two sections at the highest drug concentration (A) and the two areas under the sections (the hatched area plus the corssly hatched area and the crossly hatched area, respectively) were calculated using the trapezoidal rule. If the difference between the areas (the hatched area) was statistically significant, hysteresis was considered to be present.

Figure 10.2

The time - courses of lignocaine concentrations in arterial blood (open circles), coronary sinus blood (open triangles) and the myocardium (open squares) shown with the time - courses of the percent decreases of myocardial contractility (LV dp/dt_{max} , open diamonds) from the control values after the 50 mg (A), 75 mg (B) and 100 mg (C) doses of lignocaine, respectively. The vertical axis on the left side represents the blood or myocardial drug concentrations and on the right side represents percent decreases of LV dp/dt_{max} .

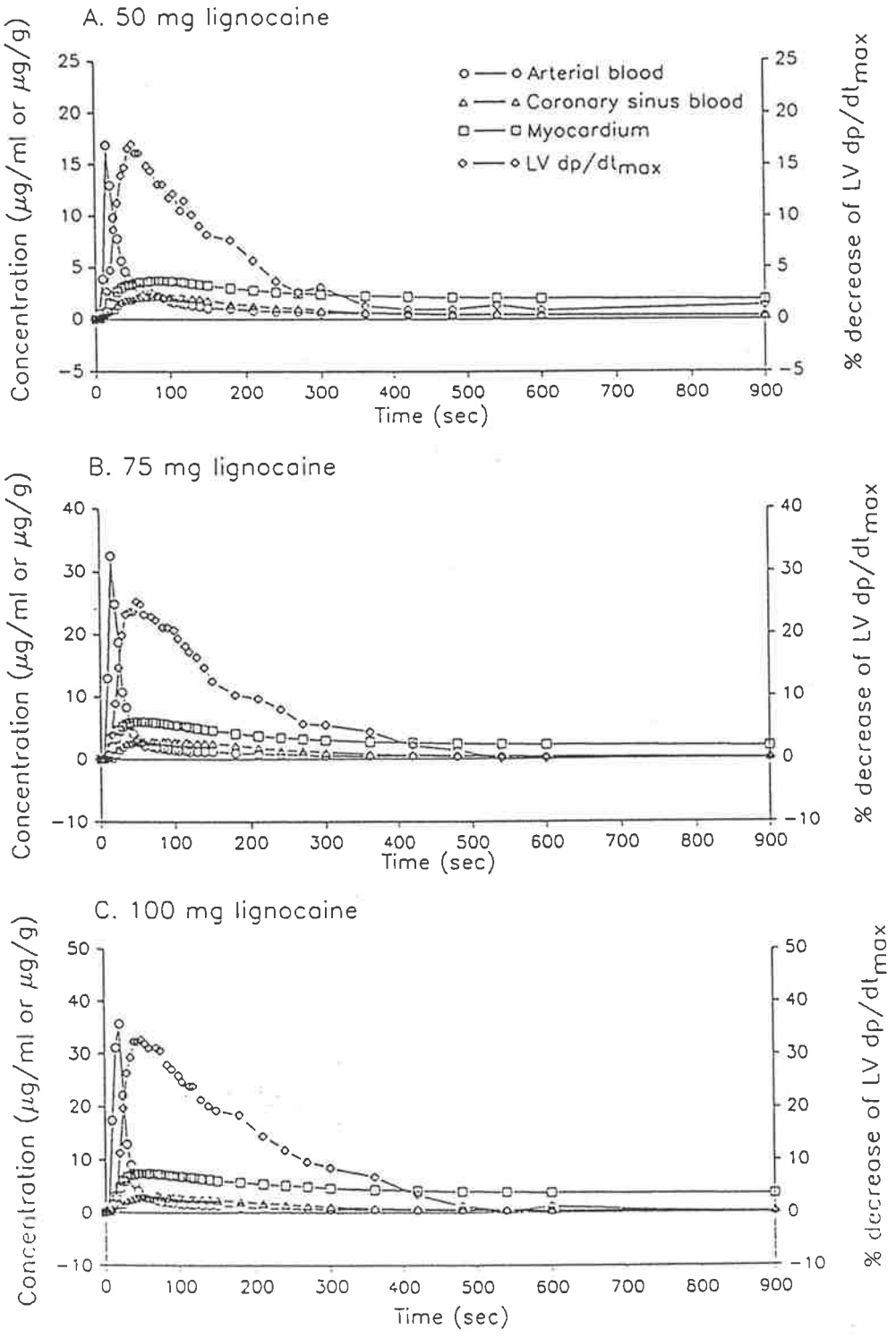

A. 50 mg lignocaine
Arterial blood
Coronary sinus blood
Myocardium
LV dp/dtmax
Concentration (μg/ml or μg/g)
% decrease of LV dp/dtmax
Time (sec)
B. 75 mg lignocaine
Concentration (μg/ml or μg/g)
% decrease of LV dp/dtmax
Time (sec)
C. 100 mg lignocaine
Concentration (μg/ml or μg/g)
% decrease of LV dp/dtmax
Time (sec)

Figure 10.3

The time - courses of pethidine concentrations in arterial blood (open circles), coronary sinus blood (open triangles) and the myocardium (open squares) and the time - courses of the percent decreases in myocardial contractility (LV dp/dt_{max}, open diamonds) from the control values after the 100 mg (A), 200 mg (B) and 300 mg (C) doses of pethidine, respectively. The vertical axis on the left side represents the blood or myocardial drug concentrations, and on the right side represents the percent decreases of LV dp/dt_{max}.

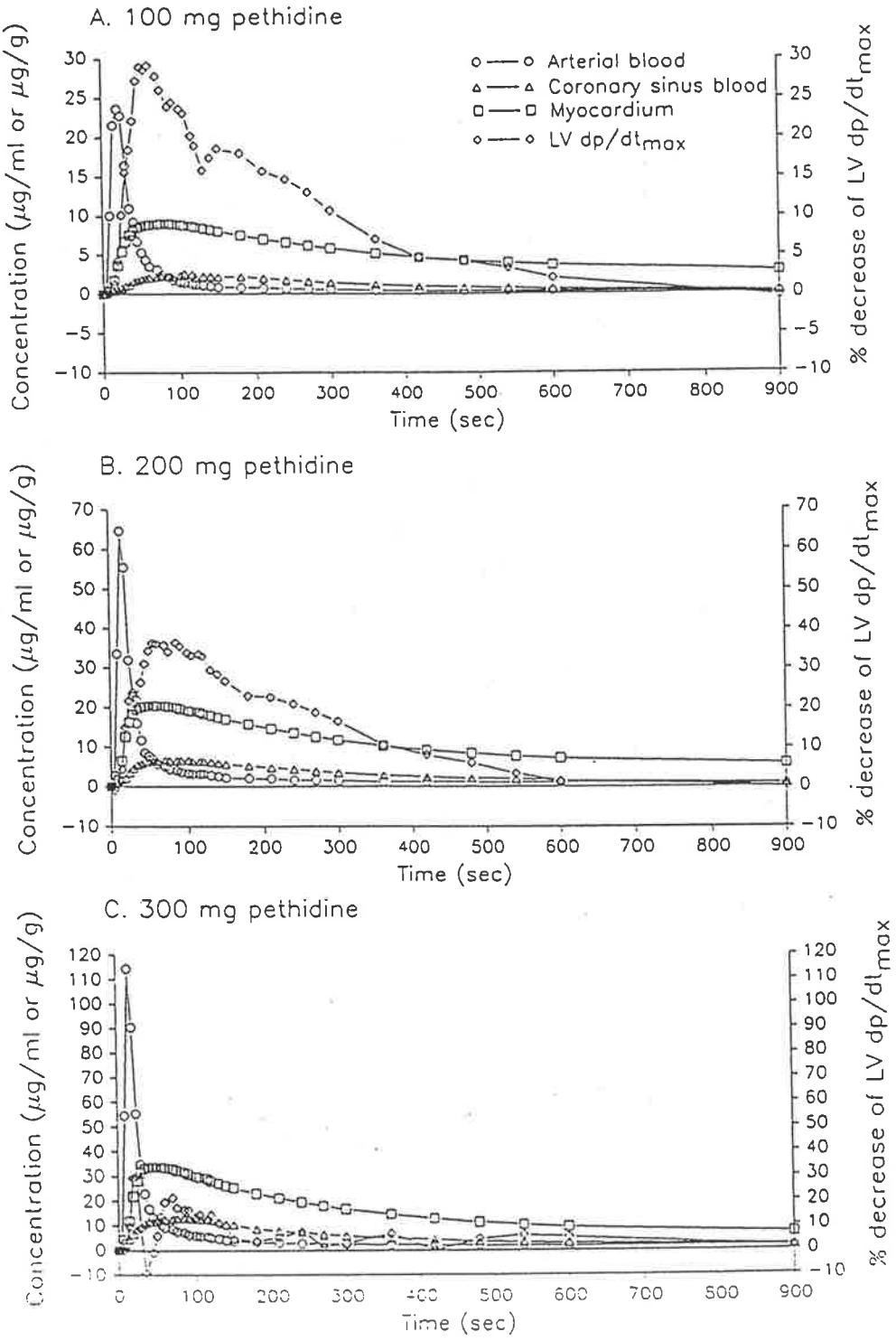

A. 100 mg pethidine
Arterial blood
Coronary sinus blood
Myocardium
LV dp/dtmax
Concentration (μg/ml or μg/g)
% decrease of LV dp/dtmax
Time (sec)
B. 200 mg pethidine
Concentration (μg/ml or μg/g)
% decrease of LV dp/dtmax
Time (sec)
C. 300 mg pethidine
Concentration (μg/ml or μg/g)
% decrease of LV dp/dtmax
Time (sec)

Figure 10.4

The mean (± S.D.) of lignocaine concentrations in arterial blood (A), coronary sinus blood (B) and the myocardium (C) at the beginnings (open bars) and ends (hatched bars) of the periods of myocardial contractility depression after the different doses of lignocaine. Asterisks represent statistically significant differences between drug concentrations at the beginnings and ends of the periods.

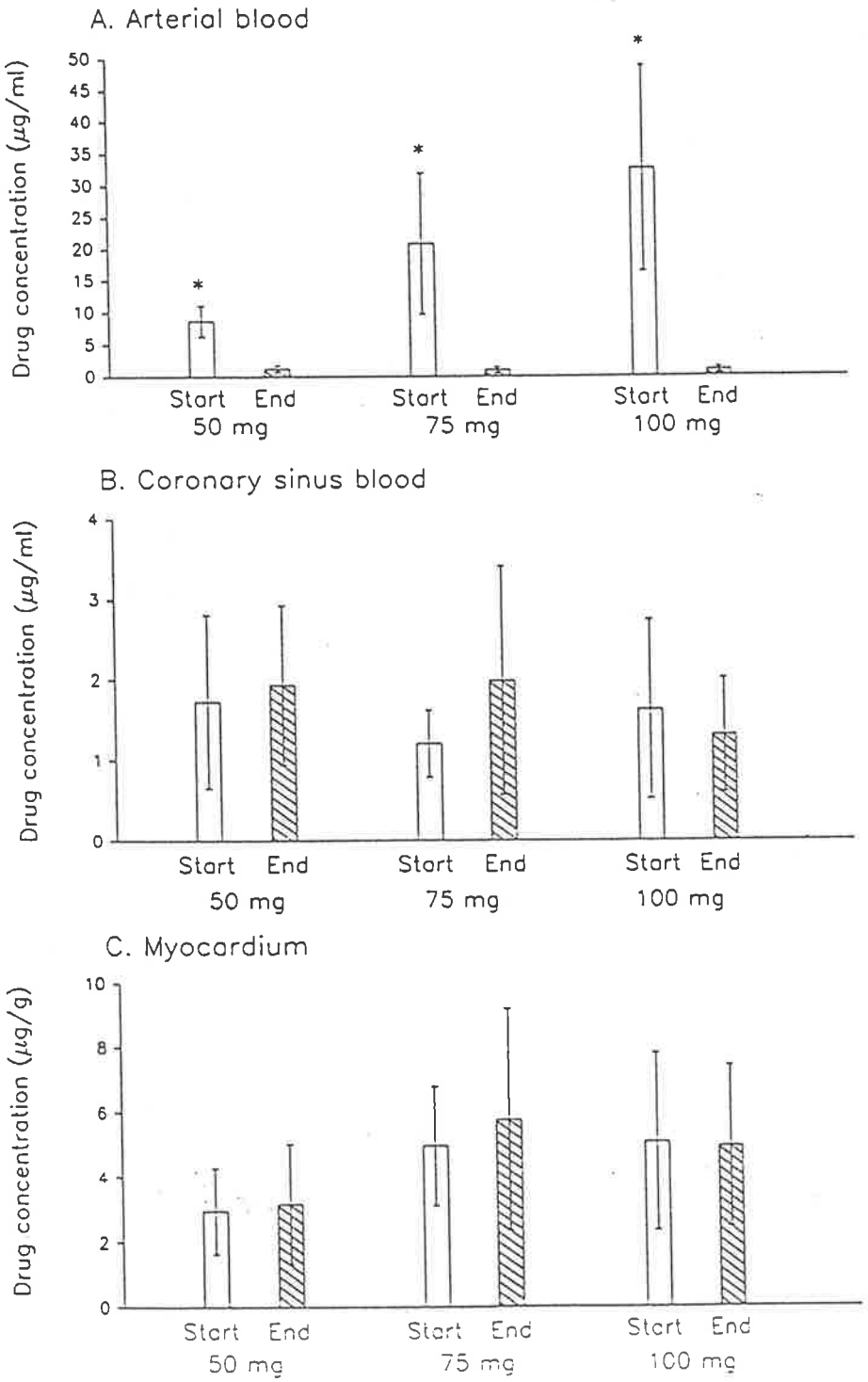

A. Arterial blood
Drug concentration (μg/ml)
50
45
40
35
30
25
20
15
10
5
0
*
*
*
Start End
50 mg
Start End
75 mg
Start End
100 mg
B. Coronary sinus blood
Drug concentration (μg/ml)
4
3
2
1
0
Start End
50 mg
Start End
75 mg
Start End
100 mg
C. Myocardium
Drug concentration (μg/g)
10
8
6
4
2
0
Start End
50 mg
Start End
75 mg
Start End
100 mg

Figure 10.5

The mean (± S.D.) of pethidine concentrations in arterial blood (A), coronary sinus blood (B) and the myocardium (C) at the beginnings (open bars) and ends (hatched bars) of the periods of myocardial contractility depression after different doses of pethidine. Asterisks represent statistically significant differences between drug concentrations at the beginnings and ends of the periods.

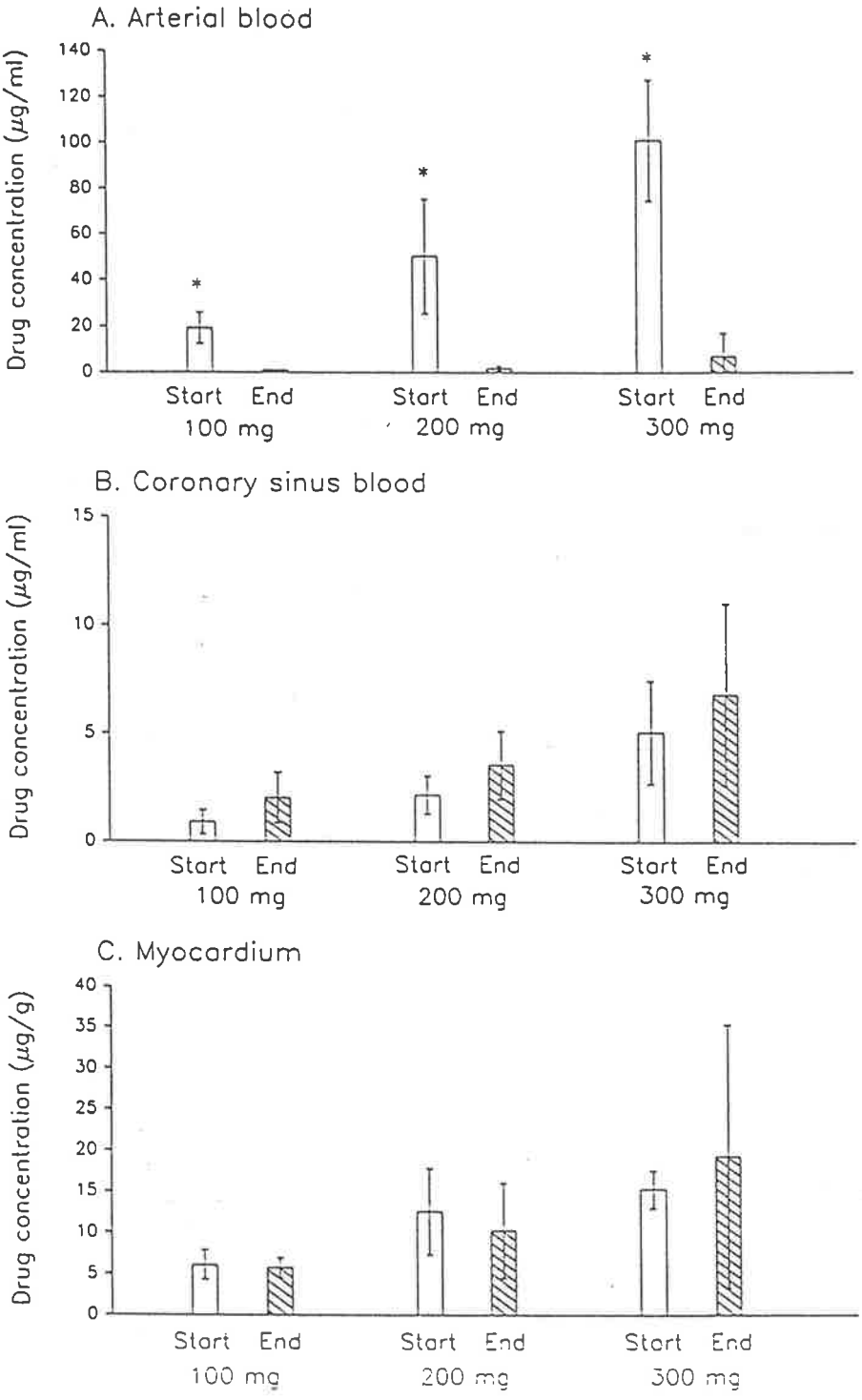

A. Arterial blood
Drug concentration (μg/ml)
140
120
100
80
60
40
20
0
*
*
*
Start End
100 mg
Start End
200 mg
Start End
300 mg
B. Coronary sinus blood
Drug concentration (μg/ml)
15
10
5
0
Start End
100 mg
Start End
200 mg
Start End
300 mg
C. Myocardium
Drug concentration (μg/g)
40
35
30
25
20
15
10
5
0
Start End
100 mg
Start End
200 mg
Start End
300 mg

Figure 10.6

The relationships between the arterial blood drug concentrations and the percentage decreases of myocardial contractility (LV dp/dt_{max}) after 50 mg, 75 mg and 100 mg doses of lignocaine (A, B and C, respectively) and 100 mg, 200 mg and 300 mg of doses pethidine (D, E and F, respectively). The arrows show the time sequences of the concentration - effect relationships. Anti - clockwise hystereses were found for all studies.

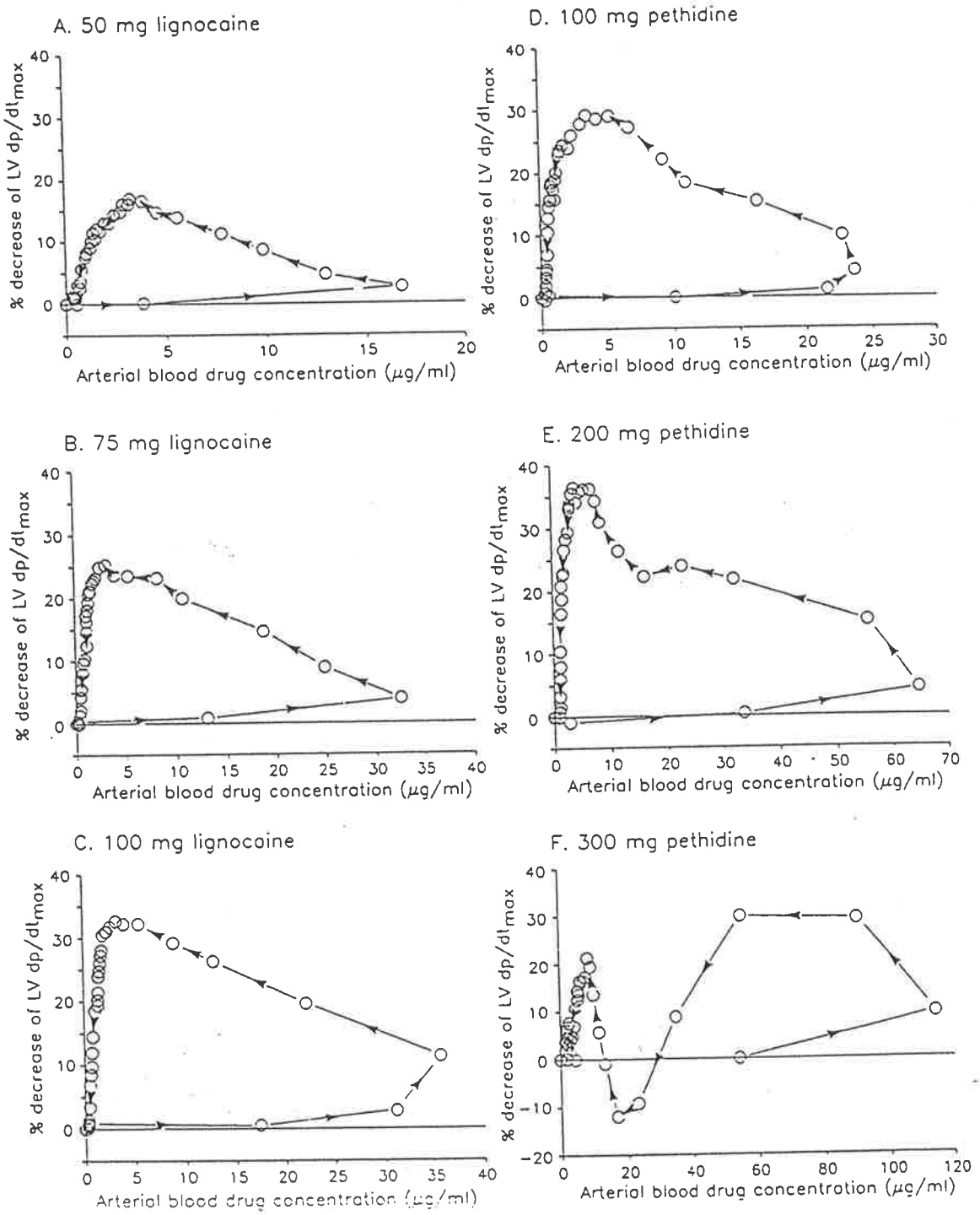

A. 50 mg lignocaine
% decrease of LV dp/dt max
Arterial blood drug concentration (μg/ml)
B. 75 mg lignocaine
% decrease of LV dp/dt max
Arterial blood drug concentration (μg/ml)
C. 100 mg lignocaine
% decrease of LV dp/dt max
Arterial blood drug concentration (μg/ml)
D. 100 mg pethidine
% decrease of LV dp/dt max
Arterial blood drug concentration (μg/ml)
E. 200 mg pethidine
% decrease of LV dp/dt max
Arterial blood drug concentration (μg/ml)
F. 300 mg pethidine
% decrease of LV dp/dt max
Arterial blood drug concentration (μg/ml)

Figure 10.7

The relationships between the coronary sinus blood drug concentrations and the percentage decreases of myocardial contractility (LV dp/dtmax) after 50 mg, 75 mg and 100 mg doses of lignocaine (A, B and C, respectively) and 100 mg, 200 mg and 300 mg doses of pethidine (D, E and F, respectively). Arrows show the time sequences of the concentration - effect relationships. Significant clockwise hysteresis was found for the 100 mg pethidine studies.

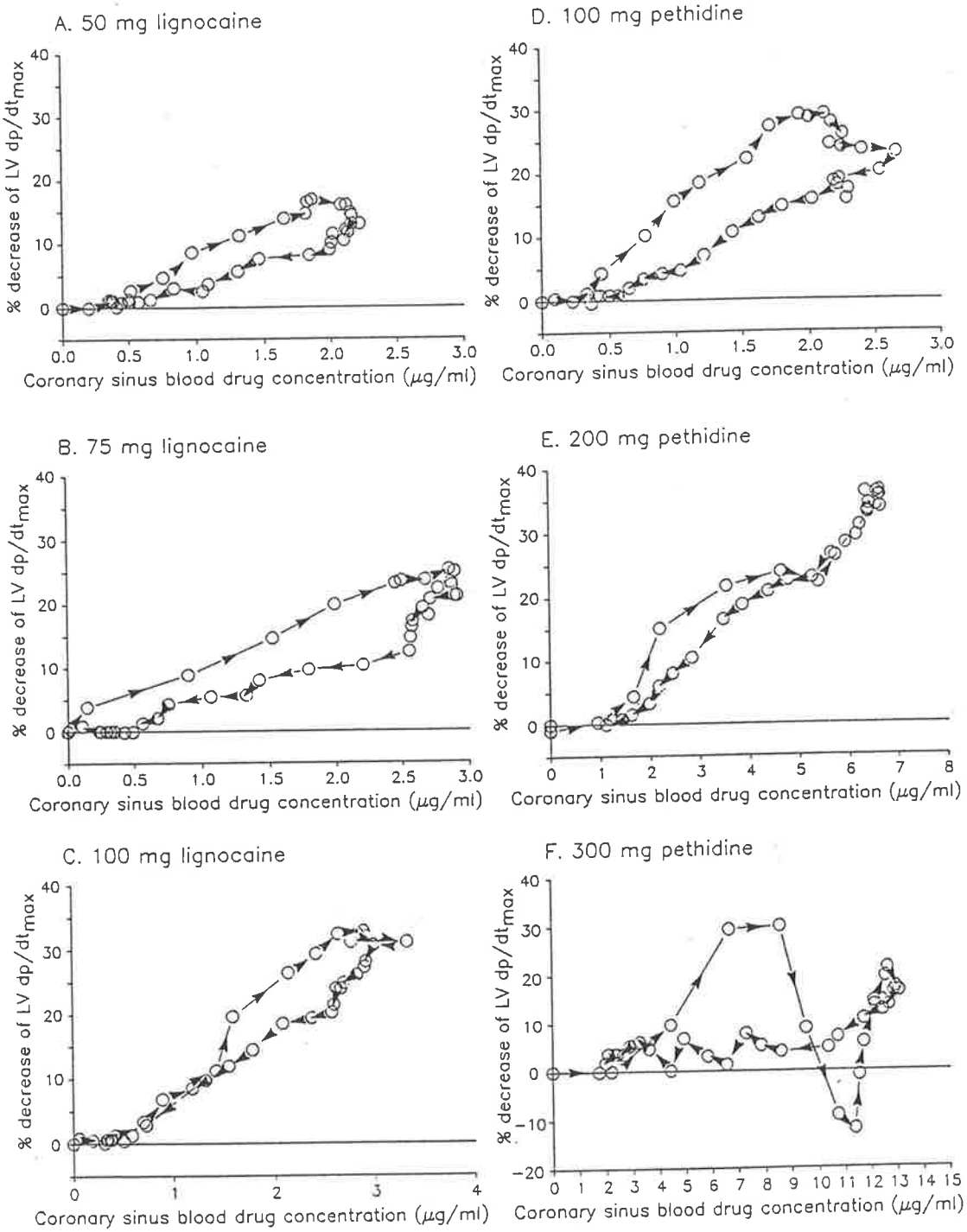

A. 50 mg lignocaine
% decrease of LV dp/dtmax
Coronary sinus blood drug concentration (μg/ml)
B. 75 mg lignocaine
% decrease of LV dp/dtmax
Coronary sinus blood drug concentration (μg/ml)
C. 100 mg lignocaine
% decrease of LV dp/dtmax
Coronary sinus blood drug concentration (μg/ml)
D. 100 mg pethidine
% decrease of LV dp/dtmax
Coronary sinus blood drug concentration (μg/ml)
E. 200 mg pethidine
% decrease of LV dp/dtmax
Coronary sinus blood drug concentration (μg/ml)
F. 300 mg pethidine
% decrease of LV dp/dtmax
Coronary sinus blood drug concentration (μg/ml)

Figure 10.8

The relationships between the myocardial drug concentrations and the percentage decreases of myocardial contractility (LV dp/dt_{max}) after intravenous injections of 50 mg, 75 mg and 100 mg doses of lignocaine (A, B and C, respectively) and 100 mg, 200 mg and 300 mg doses of pethidine (D, E and F, respectively). Arrows show the time sequences of the concentration - effect relationships. No significant hysteresis was found for any of the studies.

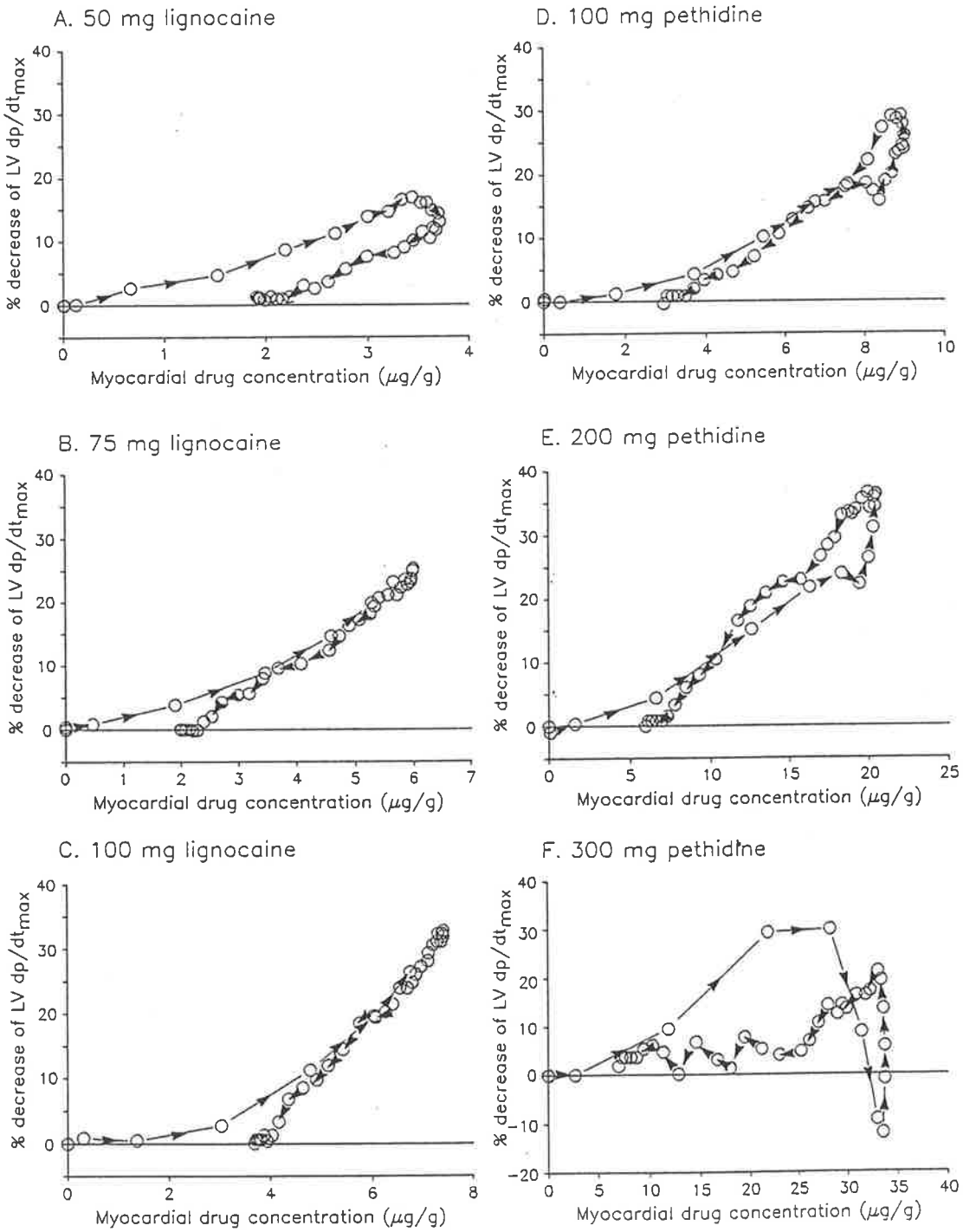

A. 50 mg lignocaine
B. 75 mg lignocaine
C. 100 mg lignocaine
D. 100 mg pethidine
E. 200 mg pethidine
F. 300 mg pethidine
% decrease of LV dp/dtmax
Myocardial drug concentration (μg/g)

Figure 10.9

The areas under the arterial blood drug concentration - effect curves (A), coronary sinus blood drug concentration - effect curves (B) and myocardial drug concentration - effect curves (C) of lignocaine (left side of the panels and left vertical axis) and pethidine (right side panels and right vertical axis), respectively. The open boxes represent the mean (± S.D,) areas under the sections between zero and the peak drug concentrations, and the hatched boxes represent the areas under the sections from the peak drug concentrations to the concentrations at the end of the study. Asterisks represent statistical differences between the areas under the two sections of each concentration - effect curve.

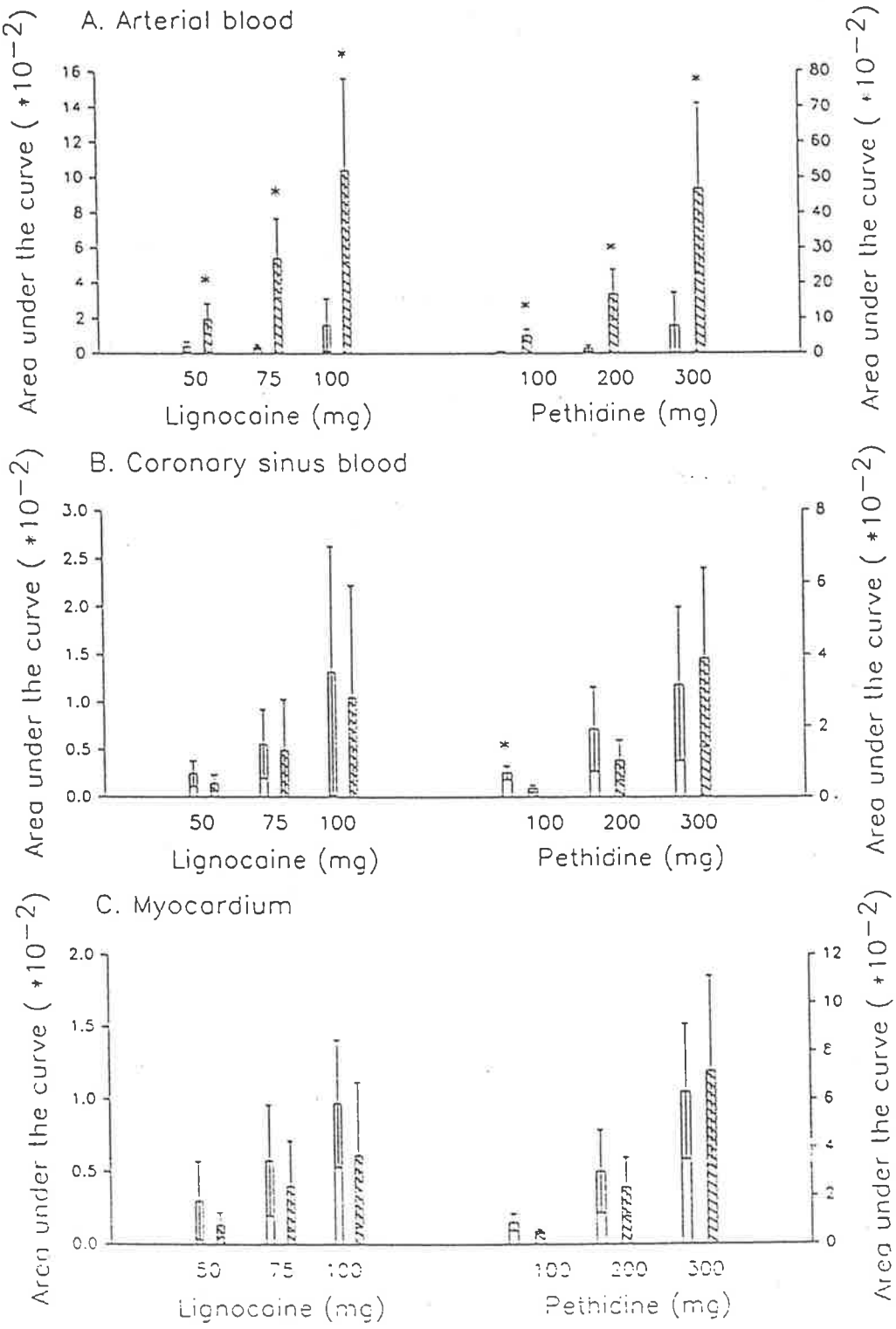
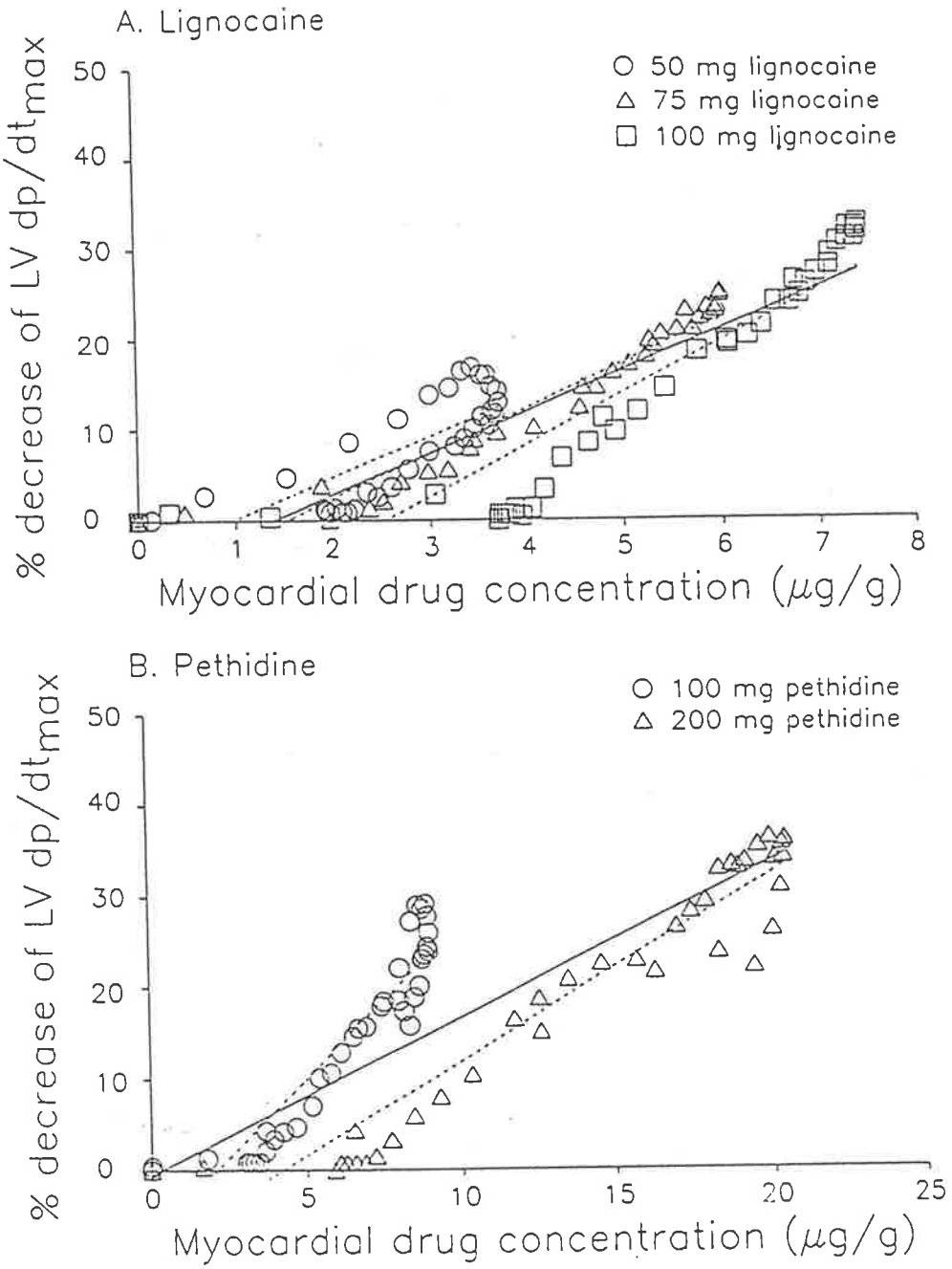

A. Arterial blood
Area under the curve (*10−2)
Lignocaine (mg)
Pethidine (mg)
B. Coronary sinus blood
Area under the curve (*10−2)
Lignocaine (mg)
Pethidine (mg)
C. Myocardium
Area under the curve (*10−2)
Lignocaine (mg)
Pethidine (mg)

Figure 10.10

(A) Linear regressions between the mean myocardial lignocaine concentration and the mean percent decrease of myocardial contractility (LV dp/dt_{max}). The open circles, triangles or squares represent data from 50 mg, 75 mg or 100 mg dose studies, respectively. The long - dashed lines are the linear regressions for each dose study. The solid line is the linear regression for the pooled data of all the doses with an intercept of -4.45, slope of 4.44 and correlation coefficient of 0.918.

(B) The equivalent to (A) but for the pethidine 100 mg (open circles) and 200 mg (open triangles) studies. The intercept, slope and correlation coefficient of the linear regression for the pooled data were -0.5, 1.71 and 0.858, respectively.


A. Lignocaine
○ 50 mg lignocaine
△ 75 mg lignocaine
□ 100 mg lignocaine
% decrease of LV dp/dtmax
0
10
20
30
40
50
0
1
2
3
4
5
6
7
8
Myocardial drug concentration (μg/g)
B. Pethidine
○ 100 mg pethidine
△ 200 mg pethidine
% decrease of LV dp/dtmax
0
10
20
30
40
50
0
5
10
15
20
25
Myocardial drug concentration (μg/g)

SECTION IV - CHAPTER 11: GENERAL CONCLUSIONS AND DISCUSSION.

11.1 Overview

Studies of pharmacokinetics and pharmacodynamics seek to understand the relationships between the dose, concentrations and effects of drugs so that the therapeutic effects of the drugs can be achieved in a predictable manner while concomitantly minimising toxic effects. While systemic pharmacokinetic studies provide information about the time - courses of drug concentrations in fluids such as arterial and venous blood, regional pharmacokinetics concerns the study of the movement of drug between blood and the tissues of the body. Regional pharmacokinetics provides a vital link between systemic pharmacokinetics and pharmacodynamics (Upton, 1990).

This thesis addressed regional pharmacokinetics and pharmacodynamics in the myocardium, which is an important site of action for many drugs. Some studies have suggested that the myocardial drug concentration is the major determinant of the magnitude of drug effects on the myocardium (Horowitz and Powell, 1986). Much of the existing information about myocardial drug disposition has been acquired from *in vitro* studies. The acknowledged differences between the conclusions of *in vitro* and *in vivo* studies suggested that *in vivo* studies of myocardial pharmacokinetics were necessary. However, there are few methods available for the study of myocardial pharmacokinetics *in vivo* (Horowitz and Powell, 1986), and many have limitations, such as the need for general anaesthesia, which could alter either myocardial pharmacokinetics or pharmacodynamics (Runciman and Mather, 1986). Furthermore, modelling methods for studying myocardial drug disposition have in general made many simplifying assumptions about myocardial drug disposition that ignore the basic physiology of the heart.

In view of these criticisms, the overall aim of this thesis was to investigate the application of mass balance principles to the study of myocardial pharmacokinetics and pharmacodynamics. Methods based on mass balance principles have the advantage of being suitable for studies of regional pharmacokinetics in conscious,

unrestrained animals such as the chronically catheterised sheep preparation (Runciman et al., 1984a), are suitable for serial studies in animals and are compatible with simultaneous measurements of pharmacodynamics. A review of the literature showed that these methods have been used in limited numbers of experiments in both animals and patients (Selden and Neill, 1975; Horowitz et al., 1986). However, because the method does not allow the direct measurement of myocardial drug concentrations, it was felt that is was important to thoroughly examine some of the prerequisites and assumptions needed for the use of the method.

11.2 Section II

11.2.1 Validation of the use of mass balance principles

The studies reported in this section of the thesis were designed primarily to experimentally validate some general assumptions made for the application of mass balance principles, particularly to the myocardium.

11.2.1.1 The direct diffusion of drugs from the surface of organs

Experience with *in vitro* pharmacokinetic preparations such as tissue slices have shown that the rate of diffusion of drugs in the absence of blood perfusion is relatively slow. Nevertheless, there is no reason why the anatomical boundaries of organs should also be the boundaries for drug diffusion if suitable concentration gradients exist. Significant rates of such diffusion of drugs out of organs would invalidate mass balance calculations which assume all drug transport is via the vasculature. To quantitate this effect, in the studies of Chapter 3 the *in vivo* rate of the diffusion of lignocaine, procainamide and pethidine from the surfaces of the heart and a kidney of anaesthetised sheep was examined. A method was developed whereby the organs could be artificially "enveloped" and surrounded by saline so that the rate and the amount of drug which transferred into this saline could be measured. It was found that the rates of diffusion into the saline were in accordance with the

lipophilicities of the drugs, but that the rates of diffusion were so low as to be negligible in mass balance calculations.

11.2.1.2 Drug transport in lymph

The removal of drugs from organs by lymph was postulated as another process which could influence mass balance calculations. To quantitate this, methods of simultaneously studying drug concentrations in the lymph and blood from the hindquarters of both anaesthetised and conscious, chronically catheterised sheep were developed. It was found in the studies of Chapter 4 that the lymph flow rates from the hindquarters of anaesthetised and conscious, unrestrained sheep were 11.4 ± 5.6 and 31 ± 12.2 ml/min, respectively, and that the concentrations of lignocaine, procainamide and pethidine in lymph were approximately equal to those in blood. It was concluded that the rate of drug transport from organs by lymph is negligible compared to that by blood and would therefore have a negligible effect on mass balance calculations.

11.2.1.3 Rapid blood sampling methods

Measurement of rapid drug uptake and elution using mass balance principles requires accurate and faithful characterisation of the time - courses of drug concentrations in the afferent and efferent blood of a region (Chiou, 1979; 1980). Many blood sampling methods based on the fraction collection principle can achieve sampling rates as rapid as one sample every 1 - 2 sec. However, by reviewing the early studies of dye kinetics it became apparent that these methods may be inaccurate due to dispersion of blood in the sampling catheter. The *in vitro* and *in vivo* studies described in Chapter 5 showed that laminar and/or turbulent flow of blood in blood sampling catheters meant that fraction collection methods could not be used to accurately characterise rapidly changing blood drug concentrations. A new rapid blood sampling method (the "flush and withdrawal" method) was developed, and its accuracy established *in vitro*.

11.2.2 Other implications

Although the principle aims of the studies of Section II were to establish the validity of mass balance principles for the study of myocardial pharmacokinetics, the studies of Chapters 3 and 4 have wider implications; these are discussed below.

11.2.2.1 Drug diffusion between highly and poorly perfused tissues

It is known that tissues with a low blood flow, such as infarcted myocardium, can achieve relatively high drug concentrations. This drug accumulation in ischaemic tissue has been attributed to the slow elution of drug from these tissues (Wenger et al., 1978; Zito et al., 1980; 1981), but the mechanism of drug accumulation in the infarcted myocardium was not sufficiently explained. The studies in Chapter 3 showed that drugs can diffuse along concentration gradients from tissues of high drug concentration such as the myocardium into the pericardial fluid. Although the rate of drug diffusion was relatively slow, comparatively high drug concentrations could eventually be achieved in tissues or parts of the body with no perfusion. This diffusion of drugs across apparently large distances may be an alternative mechanism by which drugs can enter areas such as ischaemic myocardium. Further investigations in this area are warranted.

11.2.2.2 Drug concentrations in lymph as an insight into drug disposition in the interstitial space

Drug disposition in the interstitial fluid is important for two reasons. Firstly, the interstitial space constitutes a large volume for drug distribution within tissues (Lullmann et al., 1979). Secondly, many drugs exert their effects on putative receptors on the surface of parenchymal cells which are surrounded by interstitial fluid. Many studies have shown that lymph is essentially interstitial fluid and the study of lymph drug concentrations would be an ideal model for investigation of drug disposition in the interstitial space. Therefore, the study of drug concentrations in lymph is of significance to both pharmacokinetic and

pharmacodynamic studies in that it can provide further insight into drug disposition within tissues and the relationships between drug effect and observed drug concentrations. It was concluded that an understanding of the factors influencing the concentration of drugs in lymph, such as the status of the regional microcirculation, tissue pH, capillary permeability and drug binding to proteins in lymph would be important for the complete characterisation of the regional pharmacokinetics of a drug.

11.3 Section III

The studies in Section III concerned the development and utilisation of a chronically instrumented sheep preparation for the integrated study of myocardial pharmacokinetics and pharmacodynamics.

11.3.1 Development of a chronically instrumented sheep preparation

In accordance with the requirements for the use of mass balance principles, a great deal of effort was expended defining the region perfused by the left coronary artery and drained by the coronary sinus. A previous study had shown that the left ventricular wall and the ventricular septum of the heart of sheep were drained by the coronary sinus. The studies described in Chapter 6 showed that these regions of myocardium were also perfused by the left coronary artery. The tissue weight of this region of the myocardium was quantified so that the calculated mass of drug in the myocardium could be expressed as the myocardial drug concentration.

The use of mass balance principles to determine myocardial drug uptake also requires the accurate and online measurement of myocardial blood flow. To do this, the chronically catheterised sheep preparation first developed by Runciman et al., (1984a) was modified with Doppler flow probes on the left coronary artery and main pulmonary artery to measure *in vivo* blood flow velocities in these vessels. Methods for the *in vivo* and *in vitro* calibration of these Doppler flow probes were developed and validated. The method was found to be reliable and suitable for measuring cardiac output and left coronary artery blood flow in conscious, unrestrained sheep.

11.3.2 Haemodynamic effects of the intravenous injection of small volumes of hypotonic solutions

During control studies to select a suitable vehicle for diluting drugs for intravenous injection, it was found that the intravenous injection of pure Water for Injection B.P. and other hypotonic solutions had severe deleterious effects on the myocardial function and haemodynamic status of the chronically instrumented sheep. This included significant decreases in myocardial contractility, cardiac output, stroke volume, coronary blood flow and mean arterial blood pressure and increases in heart rate. This finding is of major concern because the haemodynamic effects of such solutions have not been reported previously, and because there are no specifications in the major pharmacopoeias for the tonicity of small volume (< 100 ml) solutions for intravenous injection.

Further investigation revealed that the intensity of the depression of myocardial function was related to the injection rate, and, more importantly, inversely related to the tonicity of the solutions. After further study it was concluded that the depression was related to intravascular haemolysis induced by the hypotonic solutions. The need for further investigation of the haemodynamic effects of hypotonic solutions, particularly with respect to the formulation of drugs for intravenous use, was emphasised. However, the clinical implications of these findings are yet to be determined.

11.3.3 Myocardial pharmacokinetics and pharmacodynamics

The myocardial pharmacokinetics and pharmacodynamics of lignocaine and pethidine were investigated in 6 chronically instrumented conscious, unrestrained sheep. To facilitate discussion, the pharmacodynamics and pharmacokinetics of the drugs will be considered separately, followed by a discussion of the relationship between kinetics and dynamics.

11.3.3.1 Myocardial pharmacodynamics of lignocaine and pethidine

Lignocaine and pethidine are drugs widely used in clinical medicine. However, it was apparent from previous studies that several points concerning their effects on the myocardium were still not clearly understood.

Based on findings that cardiac output and blood pressure were not depressed during episodes of central nervous system (CNS) toxicity due to the intravenous administration of local anaesthetics, it was commonly believed that the cardiovascular system was more tolerant to the effects of these agents than the CNS. To test this, the effects of relatively low doses of lignocaine (50, 75 and 100 mg) on cardiovascular function were studied. It was found that these doses caused no discernible CNS effects, but caused dose - dependent reductions in myocardial contractility as indicated by effects on the maximum rate of left ventricular pressure rise (LV dp/dt_{max}). This effect was linearly related to the doses used. There were no significant changes in the more commonly monitored haemodynamic parameters such as cardiac output and blood pressure. This finding questioned the previously reported conclusions concerning the relative toxicities of lignocaine to the CNS and cardiovascular system.

Although the majority of previous reports supported the notion that pethidine has negative inotropic effects on the myocardium, the results of recent *in vivo* and *in vitro* studies have created controversy. In the studies described in Chapter 8, it was found that although CNS stimulating effects were not apparent after 100 and 200 mg doses of pethidine, these doses caused myocardial depression as indicated by reductions in LV dp/dt_{max}. For the 300 mg dose, excitatory effects on the CNS were apparent, and it was found that the changes in LV dp/dt_{max} were more variable than for the lower doses. It was concluded that although the excitatory CNS effects of drugs such as lignocaine and pethidine can mask the intrinsic cardiovascular depression caused by these drugs, pethidine does cause dose - related myocardial depression at low doses.

11.3.3.2 Myocardial pharmacokinetics of lignocaine and pethidine

The myocardial pharmacokinetics of lignocaine and pethidine were studied in conscious, unrestrained sheep using mass balance principles. In contrast to the assumptions made in many pharmacokinetic studies, the calculated drug concentrations in the "well - perfused" myocardium did not reach pseudo - equilibrium with either the arterial or the coronary sinus blood drug concentrations during the 15 min period after drug injection. The rapid uptake of these drugs into the myocardium lasted for approximately 1 min and was followed by a prolonged period of slower elution from the myocardium. The elution of pethidine was more rapid than that of lignocaine, which may be related to the higher lipophilicity of pethidine. The duration of net drug uptake into the myocardium was considerably shorter than the previously reported fast distribution half - lives of the drugs.

The importance of measuring coronary artery blood flow in studies of myocardial drug uptake was shown in the pethidine studies in which coronary blood flow increased significantly in the first few minutes after injection. It was found that the AUC_{cs} exceeded the AUC_{art} for the 200 and 300 mg doses. Consideration of these data in isolation would imply production of pethidine by the myocardium. However when the AUC's were converted to drug mass terms using coronary blood flow, the transient changes in blood flow altered the calculation such that it was possible to mass balance pethidine across the myocardium.

11.3.3.3 Relationships between pharmacokinetics and pharmacodynamics

In view of the consistency of the reductions in myocardial contractility (LV dp/dt_{max}) induced by the intravenous injections of lignocaine and pethidine, the relationships between contractility and the concentrations of lignocaine or pethidine in arterial and coronary sinus blood and the myocardium were examined. In contrast to the theory of Chiou (1989a; 1989b), during the non steady - state conditions which occurred after intravenous bolus administration,

the arterial drug concentrations were not in equilibrium with changes in myocardial contractility. Marked anti - clockwise hysteresis in the concentration - effect relationships showed that the changes in contractility lagged behind the arterial drug concentrations. When the myocardial drug concentrations were compared to changes in contractility, no such hysteresis was found. Furthermore, the myocardial drug concentrations at the beginnings and ends of the periods of myocardial depression were not statistically different. The threshold myocardial drug concentrations of lignocaine and pethidine which caused 10% reductions in contractility were found to be 4.5 ± 2.4 μg/g and 8.2 ± 3.8 μg/g, respectively. The pooled results of the three doses of lignocaine and the two lower doses of pethidine showed that the depression in myocardial contractility for each drug was linearly related to its myocardial concentrations. The correlation coefficients of the regressions were 0.918 and 0.858, respectively.

Although coronary sinus blood drug concentrations and the drug effect showed no significant hysteresis except for the 100 mg dose of pethidine, the coronary sinus blood concentrations peaked significantly later than the maximum depression in contractility. Together with the knowledge that coronary sinus drug concentrations were not in equilibrium with myocardial drug concentrations, it is suggested that under conditions after a short intravenous bolus drug administration, the venous equilibrium theory does not apply.

11.3.4 Implications of the studies of myocardial pharmacokinetics and pharmacodynamics

It was shown that myocardial drug concentrations determine the timing, intensity and duration of the myocardial depressant effects of lignocaine and pethidine. In the majority of the pharmacokinetic and pharmacodynamic studies reported in the literature, and in clinical drug monitoring, arterial or venous drug concentrations are used because these are the more readily available blood samples. However, these pharmacokinetic - pharmacodynamic studies show that myocardial drug

concentrations determined the magnitude of the drug effect on the myocardium. Therefore, any factor which may influence myocardial drug uptake and/or elution such as changes in coronary artery blood flow, myocardial mechanical activity or myocardial biochemistry, can alter the blood concentration - effect relationship. Such changes are frequently encountered in clinical situations in which intravenous bolus injections of cardio - active drugs are administered.

Myocardial pharmacokinetic - pharmacodynamic studies represent the first step in gaining an understanding of the global myocardial concentration - effect relationships of drugs which exert their effects on the myocardium. Further studies of the relationship between the blood and myocardial drug concentrations and the major factors influencing myocardial drug disposition may help to predict myocardial drug effects from blood drug concentrations.

11.4 Future directions for research

11.4.1 Further validation of mass balance principles in studies of myocardial pharmacokinetics

Methods based on mass balance principles provide only an indirect estimation of the myocardial concentrations of drugs, and must therefore be rigourously validated before their wide - spread use. We plan to further validate these methods with studies in anaesthetised sheep in which the global myocardial drug concentrations calculated using mass balance principles will be compared with the drug concentrations determined simultaneously by direct biopsies of myocardial tissue (Anderson et al., 1980).

11.4.2 Further investigations of the relationships between myocardial pharmacokinetics and pharmacodynamics

11.4.2.1 Myocardial pharmacokinetics of drugs with different physiochemical properties

It is known that the physiochemical properties of drugs such as lipophilicity, protein and tissue binding and pKa influence their disposition. In the studies presented in this thesis, the short term myocardial pharmacokinetics of two

basic amine drugs were examined. Studies of more clinically important basic amine antiarrhythmic and beta - receptor blocking drugs with different lipophilicities and pKa's such as flecainide, metoprolol and atenolol would provide more information concerning the influence of the physicochemical properties of drugs on their myocardial pharmacokinetics. Such studies are planned.

11.4.2.2 The effects of pathophysiology on myocardial pharmacokinetics

Drugs such as antiarrhythmics and anaesthetics are often used in emergency and intensive care medicine where pathophysiological states such as anaemia, tachycardia, hypotension, decreased coronary artery blood flow and acidosis are often encountered. It is certain that the myocardial pharmacokinetics of drugs will be altered under these circumstances (Lloyd and Taylor, 1975; 1976; Horowitz et al., 1986; Nancarrow et al., 1987; Jones et al., 1989). These conditions could be induced in the chronically instrumented sheep preparation by interventions such as cardiac pacing, controlled blood loss, coronary artery constriction, the induction of metabolic acidosis using electro - anaesthesia, and by making the sheep breathe hypoxic gas mixtures. Myocardial drug uptake and elution under these conditions can be studied using the methods described in this thesis. This knowledge would be important for understanding drug disposition and effect under pathophysiological conditions, and may eventually facilitate the more efficient and safe use of drugs in critically ill patients.

11.4.3 The measurement of interstitial and intracellular myocardial drug concentrations

Pilot investigations have shown that it may be possible to cannulate the lymph vessels draining the myocardium of sheep. This would allow the measurement of interstitial drug concentrations in the heart of sheep. As discussed previously (see 11.2.2.2), these concentrations would be expected to be in equilibrium with the drug concentrations in fluid surrounding receptors on the surface of myocardial cells.

Other methods may be suitable for calculating intracellular myocardial drug concentrations. If the volumes of the intravascular and interstitial spaces, and of the total tissue, can be measured using the dye dilution principle with various indicators (Bradbury, 1973), a knowledge of the total drug mass in the myocardium, and in the intravascular and interstitial spaces, may allow indirect calculations of intracellular myocardial drug concentrations. Although technically difficult, such studies may be vital for greater understanding of the effects of drugs on the heart.

11.4.4 Modelling of myocardial pharmacokinetics and pharmacodynamics

In the past, models of myocardial pharmacokinetics and pharmacodynamics have been based on many simplifying assumptions, and have rarely been validated with data concerning the myocardial concentrations of drugs. By contributing to the pool of information concerning the physiological basis of drug uptake and elution in the myocardium, the studies in this thesis and further investigations may pave the way for more sophisticated regional pharmacokinetic and pharmacokinetic - pharmacodynamic models using directly measurable parameters that represent biological processes (Lullman, 1979; Upton, 1991a), and myocardial drug disposition and effect may be able to be predicted from the more readily measurable blood drug concentrations.

11.4.5 Modelling pharmacokinetics and pharmacodynamics in the other regions

Analogous studies in different regions or organs with different drugs will extend our basic understanding of the processes involved in pharmacokinetics and pharmacodynamics, will facilitate the development of a robust overall model, and may also have practical implications for clinical medicine. Studies correlating opioid CNS toxicity with analgesic effect, and anaesthetic agent CNS effect with myocardial depression are planned.

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The data of Chapters 3, 4, 5, 7, 8, and 9 are stored (ASCII) in the disk attached for the purpose that other workers in this field may have access to the individual runs/experiments while reading the thesis. Those data of Chapters 3, 7, 8 and 9 are not published at the time of this thesis writing and will be used in further publication and modeling of myocardial pharmacokinetics and pharmacodynamics.