Calcium Channel Distribution in the Arterial Vascular Tree and its Relation to Function

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A thesis submitted for the degree of

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DECLARATION

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Christine J Ball

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PUBLICATIONS

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Wilson D, Ball C, Turner S, Saint D, Beltrame J. Response to Is Combined L- and T-Channel Blockade Better Than L-Channel Blockade in Therapy. *Hypertension* 2009; 54:e4. (Appendix 2)

PUBLISHED ABSTRACTS

Ball C, Saint D, Wilson D, Beltrame J. Heterogeneity in vasomotor responses to L- and T-type calcium channel blockers. *Journal of Molecular and Cellular Cardiology* 2008;44(4):817-818

Ball C, Saint D, Beltrame J, Wilson D. The role of L- and T- channels in the large and microvasculature. *Heart, Lung and Circulation* 2008;17(3):S241

Ball C, Saint D, Wilson D, Beltrame J. Heterogeneity in vasomotor responses to L- and T-type calcium channel blockers. *Heart, Lung and Circulation* 2007;16(2): S212-S213

Ball C, Saint D, Beltrame J. The effect of efonidipine hydrochloride on human subcutaneous microvascular constrictor responses. *Journal of Molecular and Cellular Cardiology* 2006;41(4):733

PRESENTATIONS AT NATIONAL

AND INTERNATIONAL CONFERENCES

2009

- National Heart Foundation of Australia, Brisbane

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- International Society for Heart Research Congress, Greece
- International Society for Heart Research, Adelaide

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- International Society for Heart Research, New Zealand
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- National Health and Medical Research Congress, Melbourne
- International Society for Heart Research, Canberra
- The Queen Elizabeth Hospital Research Day, Adelaide
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- Cardiac Society of Australia and New Zealand, Perth
- The Queen Elizabeth Hospital Research Day, Adelaide

AWARDS AND SCHOLASRHIPS

2008

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- Frontiers in Vascular Medicine Young Investigator Finalist

2006

- The Queen Elizabeth Hospital Research Day Oral Presentation Award Recipient

2005

- The University of Adelaide, Faculty of Health Sciences Postgraduate Research Scholarship

ABBREVIATIONS

5HT	5-Hydroxytrypamine (commonly known as Serotonin)
ACh	Acetylcholine
Ang-II	Angiotensin II
ANP	Atrial Natriuretic Peptide
ATP	Adenosine Triphosphate
BK	Bradykinin
Ca^{++}	Ionic Calcium
CaM	Calmodulin
cAMP	Cyclic Adenosine Monophosphate
CCB	Calcium Channel Blocker
cDNA	Complimentary Deoxyribonucleic Acid
cGMP	Cyclic Guanosine Monophosphate
cGRP	Calcitonin Gene-Related Peptide
Cl^{-}	Ionic Chloride
CSFP	Coronary Slow Flow Phenomenon
DAG	Diacyl Glycerol
DNA	Deoxyribonucleic Acid
E_{max}	Maximal Contractile Response
EC_{50}	Concentration Required for 50% Maximal Response
EDHF	Endothelium Derived Hyperpolarising Factor
EDRF	Endothelium Derived Relaxing Factor
eNOS	Endothelial Nitric Oxide Synthase

Et-1	Endothelin-1
K^+	Ionic Potassium
KCl	Potassium Chloride
KPSS	Potassium Physiological Salt Solution
HVA	High Voltage-Activated
iNOS	Inducible Nitric Oxide Synthase
IP_3	1,4,5-triphosphate
LVA	Low Voltage-Activated
MLC	Myosin Light Chain
MLCK	Myosin Light Chain Kinase
MLCP	Myosin Light Chain Phosphatase
mRNA	Messenger Ribosomal Nucleic Acid
nNOS	neuronal NOS
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NPY	Neuropeptide Y
OD	Optical Density
PCR	Polymerase Chain Reaction
PE	Phenylephrine
PKC	Protein Kinase C
PLC	Phospholipase C
RNA	Ribosomal Nucleic Acid
ROCC	Receptor-Operated Ca^{++} Channel
ROK	Rho-kinase

RyR	Ryanodine Receptor
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SOCC	Store-Operated Ca^{++} Channel
SR	Sarcoplasmic Reticulum
TBS-T	Tris Buffered Saline with Tween 20
VOCC	Voltage-Operated Ca^{++} Channel
VSM	Vascular Smooth Muscle
VSMC	Vascular Smooth Muscle Cell

ABSTRACT

Clinical evidence in microvascular disease suggests that T-type Ca^{++} channel blockers (CCBs) have benefits over conventional L-type CCBs, however the basis for this remains largely unknown. The objective of this study was to examine vascular reactivity utilising both pharmacological and molecular techniques. This thesis is composed of three sections including (A) an Introduction, (B) Functional Vascular Studies and (C) Molecular Vascular Studies.

Section A summarised fundamental principles of the vasculature including an outline of the vascular system, vascular physiology, vascular cell biology, regulation of cytosolic Ca^{++} and vascular pathophysiology.

Section B utilised isolated vessels and wire myography to determine the effect of pre-treatment with L-type CCBs (verapamil and nifedipine) and combined L- and T-type CCBs (mibefradil and efonidipine) on endothelin-1 (Et-1) and K^+-mediated contractile responses in large (rat aorta) and small (rat mesenteric and human subcutaneous) vessels. All four CCBs inhibited both Et-1 and K^+-mediated contractile responses to a similar extent in large rat vessels, however in rat microvessels the combined L- and T-channel blockers produced significantly greater inhibition of contraction than L-channel blockers alone. The significance of this differential T-channel effect in microvessels was further supported by: (1) demonstration of divergent CCB responses in human microvessels, (2) incremental inhibition of constrictor responses with a combined L- and T- CCB despite maximal

L-channel blockade, (3) utilisation of structurally diverse CCBs with varied affinity for L- and T-channels, (6) use of pharmacodynamically and therapeutically appropriate CCB concentrations, (7) confirmation of contractile agonist independent responses, (8) consistent results even in the presence of an altered microvascular physiology in the form of chronic Et-1 activation and (9) exclusion of an endothelium-dependent mechanism.

Section C utilised the molecular techniques of quantitative polymerase chain reaction (PCR) and ratiometric western blotting to examine the distribution of the pore-forming subunits $Ca_v1.2$, $Ca_v3.1$ and 3.2 in both large (rat aorta) and small (rat mesenteric) vessels. The PCR data was equivocal with no difference noted in the distribution of the L- and T-channels between large and small vessels. In contrast to this, quantitative western blot analysis revealed that while there is a similar distribution of the three subunits in the large vessel, there is a significantly increased expression of both T-channel pore-forming subunits in microvessels ($Ca_v3.1$: 112 ± 38%*, $Ca_v3.2$: 168 ± 48%* relative to L-channel expression, *$p<0.05$).

Considered together these 'functional' and 'structural' studies indicate the important role of the Ca^{++} T-channel in regulating contractile responses in the microvasculature and their therapeutic potential.