Thermoregulatory, behavioural and neurochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related stimulant drugs

Emily Jaehne (B.Sc. Hons)

Discipline of Pharmacology, School of Medical Sciences

The University of Adelaide

(Faculty of Health Science)

February, 2010

A thesis submitted for the degree of Doctor of Philosophy

Table of Contents

List of Figures

List of Tables

Abstract

3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') is an amphetamine derivative widely used in rave party and club scenes. In some users, MDMA causes fatalities, most often due to acute hyperthermia which leads eventually to multi-organ failure. Other structurally related drugs, including methamphetamine and para-methoxyamphetamine (PMA), as well as structurally unrelated cocaine, have also been associated with death due to hyperthermia, and are also often taken with or instead of MDMA. Harm minimisation advice to prevent this acute hyperthermia depends on appropriate thermoregulatory behaviour by drug users, an aspect of thermoregulation which had not been studied with respect to MDMA previously.

The purpose of this thesis was to use a novel behavioural thermoregulation model in rats to investigate the effects of MDMA and other stimulant drugs on behavioural thermoregulation and related physiological parameters, as well as investigating residual neurochemical changes caused by these substances.

The behavioural thermoregulation model used throughout most of this thesis involved rats being administered a drug, immediately prior to being confined to a set ambient temperature (30 ± 1º or 21.5 ± 1.5ºC) for 30 minutes. Rats were then immediately allowed access to a thermally graded runway (11-41ºC) where they were able to choose their preferred temperature for a further 4 hours. The final study consisted of giving rats a drug in their home cages at an elevated ambient temperature.

Firstly, a dose-response study was conducted using MDMA, PMA, methamphetamine and cocaine. All drugs lead to a dose dependent increase in core temperature at high ambient temperature, and this led to animals seeking the cool end of the runway after MDMA, methamphetamine and cocaine administration, but not after PMA. Methamphetamine was the most potent drug at increasing core temperature, followed by MDMA and PMA, then

cocaine as the least potent, however, MDMA and PMA showed steeper slopes on the dose-response curves than methamphetamine and cocaine.

The second study consisted of rats receiving MDMA at 30 or 21.5ºC for three consecutive days a week for one week or 6 weeks before being tested in the thermal gradient. The main findings of this study were that heart rate (HR) response to MDMA progressively decreased with repeated dosing over 6 weeks at both ambient temperatures, and that there was a difference in core temperature between rats treated for 6 weeks compared to 1 week when they were in the thermal gradient.

The third study looked at the effects of MDMA in an animal model of depression, the Flinders Sensitive Line (FSL) rat. We showed that FSL rats were much more sensitive to the effects of MDMA at a high ambient temperature compared to Sprague-Dawley controls, however there were limited differences in behaviour in the thermal gradient between the strains. Pharmacokinetic analysis showed that there was no difference in blood or brain concentrations of MDMA, or its metabolite 3,4-methylenedioxyamphetamine (MDA) which could have explained the different responses. These concentrations also showed that the dosing regimens used throughout this thesis led to similar plasma concentration as those reported in human users.

The final study was a pilot study done to see if proteomics could be a useful method to investigate the effects of MDMA and other stimulants on the brain after administration at a high ambient temperature. Rats were administered MDMA, methamphetamine or a combination, and several changes in protein expression were found. These were mostly evident in rats treated with MDMA which was in contrast to the effects on neurotransmitter concentration and acute hyperthermia, which was only seen in rats treated with MDMA and methamphetamine together.

Three of the four results chapters in this thesis have been published or have been accepted for publication, while the fourth has been prepared as a manuscript ready for publication.

Declaration

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

I give consent to this copy of my thesis when deposited in the University of Adelaide Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Jaehne E. J., Salem A. and Irvine R. J. (2007) Pharmacological and behavioral determinants of cocaine, methamphetamine, 3,4-methylenedioxymethamphetamine and *para*-methoxyamphetamine-induced hyperthermia. Psychopharmacology (Berl) 194: 41-52

Jaehne E. J., Salem A. and Irvine R. J. (2008) The effect of long-term repeated exposure to 3,4-methylenedioxymethamphetamine on cardiovascular and thermoregulatory changes. Psychopharmacology (Berl) 201: 161-70

Jaehne E. J., Majumder I., Salem A. and Irvine R. J. (2009) Increased effects of 3,4-methylenedioxymethamphetamine (ecstasy) in a rat model of depression.

Addiction Biology (accepted 13/10/2009)

.....Emily Joy Jaehne, / /2010

Statement of Authorship and Contribution

Jaehne E. J., Salem A. and Irvine R. J. (2007) Pharmacological and behavioral determinants of cocaine, methamphetamine, 3,4-methylenedioxymethamphetamine and *para*-methoxyamphetamine-induced hyperthermia. Psychopharmacol (Berl) 194: 41-52

Impact factor: 3.625 (2006)

Miss Jaehne had a major input in the experimental design, conducted all experimental procedures, statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.

Signed..... Date.....

Dr Salem was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

Signed..... Date.....

Associate Professor Irvine was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

Signed..... Date.....

Jaehne E. J., Salem A. and Irvine R. J. (2008) The effect of long-term repeated exposure to 3,4-methylenedioxymethamphetamine on cardiovascular and thermoregulatory changes. Psychopharmacol (Berl) 201: 161-70

Impact factor: 3.561 (2007)

Miss Jaehne had a major input in the experimental design, conducted all experimental procedures, (most) statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.

Signed..... Date.....

Dr Salem was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

Signed..... Date.....

Associate Professor Irvine was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

Signed..... Date.....

Jaehne E. J., Majumder I., Salem A. and Irvine R. J. (2009) Increased effects of 3,4-methylenedioxymethamphetamine (ecstasy) in a rat model of depression.

Addiction Biology (accepted 13/10/2009)

Impact factor: 4.953 (2008)

Miss Jaehne had a major input in the experimental design, conducted all telemetric studies and part of the pharmacokinetic studies, as well as conducting all of the statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.

Signed..... Date.....

Dr Majumder was involved in the experimental design, conducted neurochemical analyses and part of the pharmacokinetic studies and contributed to the interpretation of the data collected and preparation of the manuscript.

Signed..... Date.....

Dr Salem was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

Signed..... Date.....

Associate Professor Irvine was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

Signed..... Date.....

Jaehne E. J., Colella A. D., Penno M. Hoffmann P. and Irvine R. J.

A behavioural, neurochemical and proteomic analysis after treatment with 3,4 - methylenedioxymethamphetamine and methamphetamine.

Text in Manuscript.

Miss Jaehne had a major input in the experimental design, conducted all telemetric studies and neurochemical analyses, statistical analysis and graphical presentation of this data, and prepared the manuscript for submission.

Signed..... Date.....

Dr Colella was involved in the experimental design, conducted all proteomic procedures and contributed to the interpretation of the data collected and preparation of the manuscript, including writing much of the proteomics sections of the Methods and Results.

Signed Date 05/02/10

Dr Penno conducted statistical analysis of proteomic results and contributed to the interpretation of the data collected and preparation of the manuscript, including writing much of the proteomics sections of the Methods and Results.

Signed..... Date.....

Dr Hoffmann was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

Signed..... Date.....

Associate Professor Irvine was involved in the experimental design, and contributed to the interpretation of the data collected and preparation of the manuscript.

Signed..... Date.....

Acknowledgements

Supervisors

Associate Professor Rod Irvine

Dr Abdallah Salem

Co-Authors

Dr Irina Majumder

Dr Peter Hoffmann, Dr Alexander Colella, Dr Megan Penno

Fellow PhD Students in Pharmacology

Dr Paul Callaghan

Dr Andrea Gordon, Dr Justin Hay, Glynn Morrish, Daniel Barrett, Peter Grace, Dr Irina Majumder, Eloise Gelston

Kate Morefield, Lynlea Simmonds, Intan Omar

Staff Members in Pharmacology

Professor Jason White, Dr Scott Smid

Karen Nunnes-Vaz, Gordon Crabb

Dr Janet Coller, Dr Mark Hutchinson, Dr Femke Buisman-Pijlman

Family and Friends

Lastly, I must thank my family and partner Chris, just for being there and supporting me financially and emotionally for the last few years.

Financial Support

Australian Postgraduate Award scholarship

ASCEPT student travel grants

Faculty of Health Sciences Postgraduate Travelling Fellowship in 2007

Mutual Community Travel Grant in 2008

Abbreviations, prefixes and symbols

MDMA	3,4-methylenedioxymethamphetamine
PMA	para-methoxyamphetamine
MDA	methylenedioxyamphetamine
MDEA	methylenedioxyethamphetamine
5HT	5-hydroxytryptamine/serotonin
5HTT	serotonin transporter
DA	dopamine
CNS	central nervous system
MAO	monoamine oxidase
HHMA/DHMA/N-Me-α-MeDA	3,4-dihydroxymethamphetamine
HHA/DHA/α-MeDA	3,4-dihydroxyamphetamine
6-OH-MDMA	2-hydroxy-4,5-methylenedioxymethamphetamine
COMT	catechol-O-methyl transferase
HMMA	4-hydroxy-3-methoxymethamphetamine
HMA	4-hydroxy-3-methoxyamphetamine
CYP450	cytochrome P450
LMA	locomotor activity
NA	noradrenaline/norepinephrine
POAH	preoptic anterior hypothalamus
LPS	lipopolysaccharide
T_C	core temperature
AMPT	α-methyl-p-tyrsine
DOI	(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopronane
8-OH-DPAT	8-hydroxy-2-(di-N-proylamino)tetralin
5HIAA	5-hydroxyindole acetic acid

HPLC	high performance liquid chromatography
SD	Sprague-Dawley
T_P	preferred temperature
SERT	serotonin reuptake transporter
DOPAC	dihydroxyphenyl acetic acid
FSL	Flinders Sensitive Line
HR	heart rate
ED_{50}	dose of 50% effective response
MAP	mean arterial pressure
T_A	ambient temperature
2-DE	2-dimensional electrophoresis
MS	mass spectrometry
METH	methamphetamine
ACON	aconitate hydratase
UB2V1	ubiquitin-conjugating enzyme E2 variant 1
MEK1	mitogen-activated protein kinase 1
GSTO1	glutathione transferase omega-1
SSRI	selective serotonin reuptake inhibitor
DAT	dopamine transporter