The use of combined telemetry and microdialysis techniques to assess 3,4-methylenedioxymethamphetamine (MDMA, ‘Ecstasy’) effects in rats

Intan Omar (BHlthSc(Hons))

Discipline of Pharmacology, School of Medical Sciences,
Faculty of Health Sciences
The University of Adelaide

Thesis submitted in fulfillment of the requirements of
Master of Clinical Science

June 2015
Table of Contents

List of Tables ........................................................................................................................................ iv

List of Figures ........................................................................................................................................ v

Thesis Abstract ....................................................................................................................................... vii

Declaration ............................................................................................................................................... ix

Acknowledgments .................................................................................................................................. x

Publications arising from the thesis ...................................................................................................... xi

Published abstract .................................................................................................................................... xi

Abbreviations .......................................................................................................................................... xii

Chapter 1 Research Background ........................................................................................................ 1

1.1 3,4-methylenedioxymethamphetamine (MDMA, ‘Ecstasy’) ......................................................... 1

1.1.1 History of origins .......................................................................................................................... 1

1.1.2 Epidemiological studies ............................................................................................................... 4

1.2 Mechanisms of action of MDMA ................................................................................................. 5

1.2.1 Neuropharmacology .................................................................................................................... 5

1.2.1.1 Animal studies ......................................................................................................................... 5

1.2.2 Brain regions ............................................................................................................................... 10

1.3 Effects of MDMA in humans ....................................................................................................... 12

1.3.1 Psychological effects ................................................................................................................... 12

1.3.2 Physiological effects ................................................................................................................... 13

1.3.3 Long-term effects ....................................................................................................................... 15

1.4 Effects of MDMA in animals ....................................................................................................... 17

1.4.1 Disruption of thermoregulation ................................................................................................. 17

1.4.2 Behavioural effects ..................................................................................................................... 19

1.4.3 Cardiovascular effects ............................................................................................................... 20

1.4.4 Long-term effects ....................................................................................................................... 21
1.5 Pharmacokinetics of MDMA
1.5.1 Humans
1.5.2 Animals
1.5.3 MDMA metabolites

1.6 Appraisal of methodological approaches used to assess MDMA effects in animal models
1.6.1 Telemetry
1.6.1.1 History
1.6.1.2 Design methodology of telemetric system
1.6.2 Microdialysis
1.6.2.1 History
1.6.2.2 Design methodology of microdialysis system
1.6.2.3 Ethical implications of experimental design
1.6.3 Combined telemetry and microdialysis

1.7 Aims and hypotheses

Chapter 2 MDMA-induced hyperthermia: The influence of methodological approaches used to measure core body temperature

2.1 INTRODUCTION

2.2 MATERIALS AND METHODS
2.2.1 Animals
2.2.2 Rectal temperature measurement
2.2.3 Radiotelemetry
2.2.4 Behavioural score
2.2.5 Drug treatments
2.2.6 Chemicals and reagents
2.2.7 Data analysis

2.3 RESULTS
2.3.1 Core body temperature
2.3.2 Behavioural response
2.3.3 Survival rate
Chapter 3 The effects of systemic administration of MDMA, and central perfusion of MDMA and MDA into the striatum, on core body temperature, heart rate, locomotor activity and striatal serotonin concentration

3.1 INTRODUCTION

3.2 MATERIALS AND METHODS

3.2.1 Animals

3.2.2 Radiotelemetry

3.2.3 Brain surgery for probe implantation

3.2.4 Experimental protocol

3.2.5 High Performance Liquid Chromatography (HPLC) with electrochemical detection (ED)

3.2.6 Reverse dialysis recovery

3.2.7 Drugs preparation and administration

3.2.8 Chemicals and reagents

3.2.9 Data analysis

3.3 RESULTS

3.3.1 HPLC

3.3.3 Core body temperature

3.3.4 Heart rate

3.3.5 Locomotor activity

3.3.6 Standards validation

3.3.7 Extracellular 5-HT and 5-HIAA concentrations

3.4 DISCUSSION

Chapter 4 General Discussion

REFERENCES
List of Tables

Table 1. 1: History of MDMA (adapted from Freudenmann et al. 2006).............................4

Table 1. 2: Affinity of MDMA for major recognition sites in the rat brain. Derived from Battaglia et al. (1988a)..................................................................................................................7

Table 1. 3: MDMA effects – Roles of different brain regions........................................11

Table 1. 4: Relative potencies of amphetamine derivatives at selected receptors in the brain, with respect to MDMA. Adapted from Battaglia et al. (1988). ......................................27

Table 1. 5: Neurotoxicity of MDMA metabolites. Adapted from Capela et al (2009). ......28

Table 1. 6: Summary of a number of MDMA studies.....................................................29

Table 1. 7: Studies using telemetry to assess the effects of MDMA in animal models.......33

Table 1. 8: Examples of tissue analysed by microdialysis. Adapted from Chefer et al. (2009).................................................................................................................................37

Table 1. 9: Examples of compounds analysed by microdialysis. Adapted from Chefer et al. (2009).................................................................................................................................38

Table 1. 10: Previous microdialysis studies looking at the effects of MDMA.................40

Table 1. 11: Studies of MDMA using combined telemetry and microdialysis techniques..41

Table 2. 1: Survival rate (%) at each time points..............................................................62

Table 3. 1: Accuracy and precision data for assay validation, n=4. Validity required accuracy and precision to be within ±15%...............................................................81
List of Figures

Figure 1. 1: Chemical structures of MDMA and related amphetamine derivatives.............2

Figure 1. 2: MDMA pharmacological mechanism of action at the neuronal serotonergic terminal and synapse. ......................................................................................................................... 6

Figure 1. 3: Pathways of MDMA metabolism. Adapted from Capela et al (2009). ............26

Figure 1. 4: Diagram of a telemetric technique setup..............................................................32

Figure 1. 5: Diagram of a microdialysis technique setup (www.accessscience.com)...........37

Figure 1. 6: The diagram of combined telemetry and microdialysis techniques setup. Adapted from Rodsiri et al. (2011)...........................................................................................................43

Figure 1. 7: The advantages and disadvantages of telemetry and microdialysis techniques. ........................................................................................................................................44

Figure 2. 1: Mean core temperature change measured using rectal probe following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.........................54

Figure 2. 2: Mean core temperature change measured using telemetry following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.........................55

Figure 2. 3: Mean core temperature change measured using rectal probe and telemetry following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta........57

Figure 2. 4: Behavioural response in rats measured using rectal probe following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta.........................59

Figure 2. 5: Behavioural response in rats measured using telemetry following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta..............................60

Figure 2. 6: Behavioural response in rats measured using rectal probe and telemetry following administration of saline and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta........61

Figure 3. 1: Reverse dialysis recovery for (a)100µM MDMA and (b)5µM MDA...............76
Figure 3. 2: Core temperature response following administration of 100µM MDMA, 5µM MDA, control (aCSF), and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta. 77

Figure 3. 3: Heart rate response following administration of 100µM MDMA, 5µM MDA, control (aCSF), and 10mg/kg MDMA i.p at high (29 ± 1°C) Ta. 79

Figure 3. 4: Locomotor activity following administration of 100µM MDMA, 5µM MDA, control (aCSF), and 10mg/kg MDMA i.p. at high (29 ± 1°C) Ta. 80

Figure 3. 5: Effect of 100µM MDMA, 5µM MDA, control (aCSF), and 10mg/kg MDMA i.p. on striatal 5-HT at high (29 ± 1°C) Ta. 83

Figure 3. 6: Effect of 100µM MDMA, 5µM MDA, control (aCSF), and 10mg/kg MDMA i.p. on striatal 5-HIAA at high (29 ± 1°C) Ta. 84
Thesis Abstract

3,4-methylenedioxymethamphetamine (MDMA, ‘Ecstasy’) is known to produce hyperthermia and adverse cardiovascular effects in humans following consumption, which can be life threatening. In animals, MDMA also produces similar effects as seen in humans such as increase in core body temperature (Tc) which has been linked to chronic neurotoxicity. Currently, clinical treatment of these adverse effects is inadequate mainly due to limited understanding of the mechanism involved in the acute MDMA-induced adverse effects. Due to ethical reasons, MDMA studies in humans are limited and studies have relied on the use of animal models to investigate MDMA effects. Therefore, it is important to assess MDMA-induced effects using appropriate techniques to relate the findings from animals to humans.

The general aims of this thesis were to investigate effects of different methods used to measure core body temperature and behaviour following MDMA administration and the validity of combined telemetry and microdialysis techniques to assess MDMA and its active metabolite, 3,4-methylenedioxymphetamine (MDA) effects on body temperature (Tc), behaviour, heart rate (HR), locomotor activity (LMA), and 5-HT extracellular levels in the rat striatum.

The first part of this thesis looked at the influence of methodological approaches used to assess changes in core body temperature and behaviour following MDMA administration. A number of studies used rectal probe measurement which requires handling and restraining of rats which results in confounding effects on the parameters measured including Tc and behaviour. Telemetry has been developed to measure these behavioural parameters without the necessity of handling the rats. The use of rectal probe caused potentiation of 10mg/kg (i.p.) MDMA-induced increase in core body temperature.
compared to the use of telemetry to measure Tc during the first 60 minutes following MDMA administration and has also resulted in a lower survival rate. These results demonstrate the importance of using appropriate techniques when measuring these parameters to avoid confounding effects and that telemetry provides a more accurate assessment of MDMA-induced change in core body temperature.

The second part of the thesis looked at the validity of combined telemetry and microdialysis techniques to investigate effects of systemic administration of MDMA and central administration of MDMA and MDA on Tc, HR, LMA and 5-HT extracellular levels in the striatum. Systemic administration of 10mg/kg (i.p.) MDMA produced significant increase in Tc, HR, LMA and 5-HT extracellular levels in the striatum whereas central administration of 100µM MDMA only produced significant increase in 5-HT extracellular levels. Central administration of 5µM MDA produced no significant changes in the parameters measured, which suggests that MDA, at concentration used in this study, does not play a major role in MDMA-induced increase in 5-HT extracellular levels in the striatum and the occurrence of hyperthermia.

In summary, this thesis has demonstrated that a combined telemetry and microdialysis technique provides a better approach to assess MDMA effects in rats, allowing central administration of drugs, and simultaneous measurement of physiological and neurochemical parameters. The combined techniques provided a better tool to investigate the effects of MDMA particularly looking at the relationship between the physiological and neurochemical effects in animal models.
Declaration

I, Intan Sofia Omar certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution 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 Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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, and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Intan Omar

30th June 2015
Acknowledgments

Supervisors
Dr Abdallah Salem
Associate Professor Rod Irvine

Fellow postgraduate students in Pharmacology
Dr Irina Majumder, Dr Emily Jaehne, Dr Liang Liu, Eloise Gelston, Dr Yue Wu
Chang Chen, Jake Gordon, Heilie Kwok, Lauren Nicotra, Nicole Sumracki, Benjamin Harvey, Jacob Thomas, Zaipul Md, Yibai Li

Staff members in Pharmacology
Dr Scott Smid, Dr Femke Buisman-Pjilman, Dr Janet Coller, Dr Mark Hutchinson
Karen Nunnes-Vaz, Gordon Crabb

Family
I would like to express my gratitude to husband Saifuddin Khalid, my children Khalid Umar and Sofeeyya Aleena, my parents Omar Awang and Norzaili Ahmad, and my family for their support and encouragement during my postgraduate journey.

Friends
Siti Sulaiman, Raudhah Muhamad, Izzati Nadijah Jailani, Zahratul Hamra, Ahlul Zilal

Financial Support
Adelaide Graduate Fee Scholarship
Publications arising from the thesis

Published abstract

• ‘Ecstasy’: Where in the brain does it work? Postgraduate Research Conference, Faculty of Health Sciences, University of Adelaide, August 2011.

Abbreviations

°C – degree Celcius

4-MTA – 4-methylthioamphetamine

5-HIAA – 5-hydroxyindoleacetic acid

5-HT – serotonin (5-hydroxytryptamine)

aCSF – artificial cerebrospinal fluid

ANOVA – analysis of variance

AUC – area under the curve

CH$_3$OH – methanol

cm – centimetre

C$_{\text{max}}$ – peak concentration

COMT – catechol O-methyl transferase

CYP – cytochrome P450

DA – dopamine

DOB – 2,5-dimethoxy-4-bromoamphetamine

DOPAC – 3,4-dihydroxyphenylacetic acid

EDTA – ethylenediaminetetraacetic acid

g – gram

h – hour

HHA – 3,4-dihydroxyamphetamine

HHMA – 3,4-dihydroxymethamphetamine

HMA – 4-hydroxy-3-methoxyamphetamine

HMMA – 4-hydroxy-3-methoxymethamphetamine

HPLC – high performance liquid chromatography

HPLC-ED – high performance liquid chromatography with electrochemical detection

i.m. – intramuscular
i.p. – intraperitoneal
kg – kilogram
M – mol/litre
MAO – monoamine oxidase
MAOI – monoamine oxidase inhibitor
MDA – 3,4-methylenedioxymphetamine
MDE – 3,4-methylenedioxyethylamphetamine
MDMA – 3,4-methylenedioxymethamphetamine (Ecstasy)
METH – methamphetamine
mg – miligram
min – minute
ml – mililitre
mm – mililitre
NaCl – sodium chloride
NaH$_2$PO$_4$ – sodium dihydrogen phosphate
OSA – octanesulphonic acid
PMA – para-methoxyamphetamine
s – second
SD – Sprague-Dawley
SEM – standard error of mean
V – volt
µl – microlitre