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

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February, 2010

A thesis submitted for the degree of Doctor of Philosophy
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Abstract

3,4-Methylenedioxyamphetamine (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 21.5°C 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.

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

Emily Jaehne, PhD Thesis 2009
Statement of Authorship and Contribution

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........................................
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.....................................
Addiction Biology (accepted 13/10/2009)

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

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Signed..................................... Date..................................
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Signed.................................. Date...............................
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A be havioural, ne urochemical a nd pr oteomic a nalys is a fter t reatment w ith 3,4 -m ethylenedioxym ethamphetam ine and methamphetam ine.

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..............................

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.

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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 $\alpha$-methyl-p-tyrsine
DOI (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropionane
8-OH-DPAT 8-hydroxy-2-(di-N-propylamino)tetratin
5HIAA 5-hydroxyindole acetic acid
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>SD</td>
<td>Sprague-Dawley</td>
</tr>
<tr>
<td>$T_p$</td>
<td>preferred temperature</td>
</tr>
<tr>
<td>SERT</td>
<td>serotonin reuptake transporter</td>
</tr>
<tr>
<td>DOPAC</td>
<td>dihydroxyphenyl acetic acid</td>
</tr>
<tr>
<td>FSL</td>
<td>Flinders Sensitive Line</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
</tr>
<tr>
<td>$ED_{50}$</td>
<td>dose of 50% effective response</td>
</tr>
<tr>
<td>MAP</td>
<td>mean arterial pressure</td>
</tr>
<tr>
<td>$T_A$</td>
<td>ambient temperature</td>
</tr>
<tr>
<td>2-DE</td>
<td>2-dimensional electrophoresis</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>METH</td>
<td>methamphetamine</td>
</tr>
<tr>
<td>ACON</td>
<td>aconitate hydratase</td>
</tr>
<tr>
<td>UB2V1</td>
<td>ubiquitin-conjugating enzyme E2 variant 1</td>
</tr>
<tr>
<td>MEK1</td>
<td>mitogen-activated protein kinase 1</td>
</tr>
<tr>
<td>GSTO1</td>
<td>glutathione transferase omega-1</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
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