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Acute and chronic toxicity of methamphetamine exposure in cultured neuronal cells

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Declaration

I declare that this thesis is my own account of my research and has not been previously submitted for a degree at any tertiary educational institution.

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Abstract

Methamphetamine is a highly addictive psychostimulant drug with serious health consequences that include long-term neurotoxic effects. While the neurotoxic mechanisms are still not fully understood, monoamine release, production of reactive oxygen species and excitotoxicity are believed to be involved. There is currently no effective treatment to prevent these effects. Using metabolomic analysis to explore the effect of methamphetamine on neuronal cells with dose and time may help to elucidate the biochemical pathways affected, and provide an insight into methamphetamine neurotoxicity.

A B50 neuroblastoma cell culture model was used in these experiments. Cell viability was assessed by lactate dehydrogenase assay and Trypan blue exclusion testing after 48 hours exposure to 1 mM methamphetamine. A dose curve was conducted exposing cells to a range of methamphetamine doses (100 nM, 1 µM, 10 µM, 100 µM and 1 mM) over 48 hours. A time course examined the 6-, 24- and 48-hour time points after B50 exposure to 1 mM methamphetamine. A gas chromatography-mass spectrometry metabolomic method was used to analyse the treated cells and cell media of the dose curve and time course. The metabolites found to contribute most to the variance between the samples were chosen for further study.

Methamphetamine caused observable damage to B50 cells and cell viability which was found to be dose-dependent by Trypan blue testing, however, LDH results were inconclusive. The metabolites found to change over dose and time during methamphetamine exposure included amino acids, carbohydrates and fatty acids. The dose curve showed a build-up of carbohydrates, a decrease in octadecenoate and alterations to many amino acids with increasing dose. The results from the time course found an increase in L-glutamate and related metabolites, an increase in antioxidant amino acids and a decrease in carbohydrates over time. The changes suggest glutamate release, reactive oxygen species and disturbances to energy utilisation may be involved in the effect of methamphetamine upon neuronal cells.

The study has confirmed that methamphetamine causes dose-dependent damage and death of neurons. Methamphetamine exposure resulted in quantifiable biochemical changes over dose and

time with the metabolite changes reflecting the known mechanisms of methamphetamine neurotoxicity. The result of this study furthers our understanding of neurochemical processes in response to methamphetamine and could potentially lead to the identification of therapeutic targets.

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List of abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
ATP	Adenosine triphosphate
B50	B50 rat neuroblastoma cell line
Ca^{2+}	Calcium
CNS	Central nervous system
DAT	Dopamine transporter
DMEM	Dulbecco's modified Eagle's medium
EAA	Excitatory amino acid
GABA	γ-aminobutyric acid
GC	Gas chromatography
IL-1	Interleukin 1
LC	Liquid chromatography
LDH	Lactate dehydrogenase
m/z	Mass to charge ratio
MAO	Monoamine oxidase
MS	Mass spectrometry
MSTFA	N-methyl-N-(trimethylsilyl)trifluroacetamide
NMDA	N-methyl-$_D$-aspartate
NMR	Nuclear magnetic resonance
nNOS	Nitric oxide synthase
P2P	Phenyl-2-propanone
PBS	Phosphate buffered solution
PCA	Principal component analysis
PKC	Protein kinase C
ROS	Reactive oxygen species
SERT	Serotonin transporter
VMAT-2	Vesicular monoamine transporter

Index to units

^{o}C	Degrees centigrade
µg	Microgram
µL	Microlitre
µm	Micrometre
µM	Micromolar
cm	Centimetre
g	Gram
g	G-force
m	Metre
mg	Microgram
mL	Millilitre
mm	Millimetre
mM	Millimolar
rpm	Revolutions per minute

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