2010

Effects of antipsychotic drugs on the expression of neurotransmitter receptors in the rat brain

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EFFECTS OF ANTIPSYCHOTIC DRUGS ON THE
EXPRESSION OF NEUROTRANSMITTER
RECEPTORS IN THE RAT BRAIN

A thesis submitted in fulfilment of the
requirements for the award of the degree

DOCTOR OF PHILOSOPHY

From

SCHOOL OF HEALTH SCIENCES
UNIVERSITY OF WOLLONGONG

By

MEI HAN

2010
CERTIFICATION

I, Mei Han, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Health Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged below. This document has not been submitted for qualifications at any other academic institution.

Mei Han

2009
ACKNOWLEDGEMENTS

I would like to express my appreciation to several people who have given me assistance and support throughout my PhD studies. This thesis would not appear in its present form without your kind assistance and support.

I am grateful to have such encouraging and supportive supervisors Dr Chao Deng and Professor Xu Feng Huang. I am greatly indebted to you for helping me to overcome obstacles during my studies. In particular, thanks for your support in the preparation of my published papers and thesis. Without your continuous commitment and motivational guidance it would be impossible for me to finish this project.

I give my sincere thanks to Dr Kelly Anne Newell and Dr Teresa Marie du Bois for their tremendous assistance and continuous support during the course of my study, especially for their help in the preparation of my published papers.

Thank you to Mrs Katrina L. Weston-Green, Dr Kelly Anne Newell, Dr Teresa Marie du Bois and Dr Mandy Reid for enthusiastic editorial reading of my thesis.

I would like acknowledge Dr Tracy Maddocks for allowing me to successfully carry out animal experiments.
Thanks to Dr Thomas Burne for his help in analysing and interpretation of all the behavioural data.

I would also like to thank Dr YingHua Yu and other people in research group. Their enthusiastic help was important for the completion of this thesis.

Acknowledgement is also given to The University Research Committee, University of Wollongong and the Schizophrenia Research Institute (SRI) for providing the scholarship enabling me to conduct my research.

And finally, I would like to take the opportunity to express my deep gratitude to my parents for their support and encouragement. A special thank also goes to my husband Zhengyi Jiang and my daughter Fan Jiang for their tremendous support, encouragement, patience and help during my PhD study.

Thank you all!
STATEMENTS

According the guidelines of the University of Wollongong thesis committee, I have chosen to present my PhD thesis in ‘Publication Format’. This includes four series of experiments, from which three were published in peer reviewed journals and one has been accepted for publication in *Neuroscience*. I am the first author in all four publications. I would like to state that I am the primary designer of these experiments. I have carried out all experiments and performed data analysis and written up these papers. Furthermore, I have published additional seven research papers and nine conference abstracts together with my colleagues during the course of my PhD study.
PUBLICATIONS

The following publications and presentations have arisen directly from the work conducted for this thesis.

Publications in Refereed Journals

Han, M., Huang, X.F., du Bois, T., and Deng, C. The effects of antipsychotic drugs administration on 5-HT$_{1A}$ receptor expression in the limbic system of the rat brain. *Neuroscience*, in press, accepted for publication on 17 September, 2009.


Publications in Conference Proceedings


**Additional Publications**

The following publications have arisen from other projects that I have involved in throughout my doctoral study.


Weston-Green K., Deng, C., **Han, M.**, and Huang, X.F. Effects of Antipsychotic Drugs on Weight Gain and CB1 Receptors in the Dorsal Vagal Complex of Rats. *Proceedings of the 7th IBRO World Congress of Neuroscience*, 144, 2007.

du Bois, T., Deng C, Hsh, C.W., **Han M**, Li Y, Tan, Y.Y., and Huang, X.F. Effects of perinatal NMDA receptor antagonist treatment on dopaminergic system development and behaviour. *Proceeding of the 7th IBRO World Congress of Neuroscience*, 234, 2007.


ABSTRACT

Currently, the control of schizophrenia symptoms is primarily through pharmacological intervention. However, antipsychotics can cause several side-effects, such as extrapyramidal symptoms (EPS) and body weight gain/obesity, which severely affect patient compliance to continue with medication. In addition, due to the effects of antipsychotics on neurotransmission, it is unclear whether central pathological changes observed in post-mortem tissue in schizophrenia are the real pathology of the disease or are a result of the effects of antipsychotic drugs. The aim of this study was to investigate the molecular mechanisms of the pharmacological efficacy and side-effects of antipsychotic drugs. To achieve this aim, this study examined the expression of dopamine D$_2$, histamine H$_1$, serotonin 5HT$_{1A}$ and muscarinic M$_1$ receptors in the rat brain following short-term (1 week) and long-term (12 weeks) treatment with aripiprazole, olanzapine and haloperidol.

Aripiprazole and haloperidol both have a high affinity for dopamine D$_2$ receptors, however aripiprazole has a lower risk of EPS than haloperidol. The aim of Chapter 2 was to understand the mechanism underlying why aripiprazole, unlike haloperidol, has a therapeutic effect but does not induce significant EPS. Results showed that aripiprazole selectively increased D$_2$ receptor mRNA expression and decreased tyrosine hydroxylase mRNA expression (TH; a rate-limiting enzyme for the synthesis of dopamine) in the ventral tegmental area (VTA), but not the substantia nigra (SN). Aripiprazole also decreased dopamine transporter (DAT) binding density in the nucleus accumbens (NAc) and VTA. Consistent with
previous findings, haloperidol significantly increased D2 receptor binding density, but decreased DAT binding density in the NAc, CPu and VTA. Olanzapine had less widespread effects on D2 receptor expression and DAT binding density. These results suggest that aripiprazole may control schizophrenia symptoms through a novel mechanism: that is, by selectively reducing dopamine synthesis in the VTA but not SN. This may contribute to the long-term efficacy of aripiprazole in controlling schizophrenia symptoms with reduced EPS.

It has been previously reported that aripiprazole and olanzapine increased dopamine release in the prefrontal cortex via the serotonin 5-HT1A receptor, which may partially explain why these drugs can improve the negative symptoms and cognitive functional deficits associated with schizophrenia. It is interesting that aripiprazole has a high affinity for 5-HT1A receptors, but olanzapine has not. Therefore, the aim of Chapter 3 was to examine whether these antipsychotics affect 5-HT1A receptor expression. The results showed that aripiprazole increased 5-HT1A binding density in the CA1 region of the hippocampus and medial posterodorsal nuclei of the posterior amygdala (MeP), while olanzapine down-regulated the binding density of 5-HT1A receptors in the cingulate cortex. However, these changes were not apparent after 12 weeks of drug treatment. This study suggests that aripiprazole and olanzapine have different effects on the binding density of 5-HT1A receptors. The results indicate that aripiprazole and olanzapine have differential effects on 5-HT1A protein expression, which may contribute to their distinct profiles in improving negative symptoms and cognitive
deficits in schizophrenia. However, they may induce adaptation and desensitisation in serotonin 5-HT\textsubscript{1A} receptor expression after long-term treatment.

Schizophrenia patients exhibit a decrease, or no change, in muscarinic M\textsubscript{1} receptor expression in certain brain regions. Olanzapine has a high affinity for the M\textsubscript{1} receptor, while aripiprazole and haloperidol have low affinities. The aim of Chapter 4 was to investigate how these antipsychotics affect M\textsubscript{1} receptor mRNA expression in regions of the brain that are implicated in the pathology of schizophrenia. This study showed that the three antipsychotics increased M\textsubscript{1} receptor mRNA expression in the hippocampus. In addition, increases in M\textsubscript{1} receptor mRNA expression were also observed in the SN following treatment with haloperidol and olanzapine, and in the NAc following treatment with aripiprazole. These results suggest that alterations of M\textsubscript{1} receptor mRNA expression in schizophrenia are unlikely to be a consequence of drug treatment, and implicate the muscarinic M\textsubscript{1} receptor as a contributor to the therapeutic effects of schizophrenia treatments.

The aim of Chapter 5 was to investigate whether the body weight gain/obesity side-effect of olanzapine was produced by regulating histamine H\textsubscript{1} receptor expression. To the best of this author’s knowledge, this study is the first to compare H\textsubscript{1} receptor expression in the rat brain following short and long-term administration of olanzapine, aripiprazole and haloperidol. Results showed that olanzapine significantly down-regulated H\textsubscript{1} receptor mRNA expression and
binding density in the ventromedial hypothalamic nucleus (VMH), and H₁ receptor mRNA expression in the arcuate hypothalamic nucleus (Arc). Consistent with their low risk of weight gain/obesity side-effect, aripiprazole and haloperidol had no effect on H₁ receptor expression in the VMH or Arc. Histamine H₁ receptor mRNA expression in the VMH and Arc were negatively correlated to body weight gain and energy efficiency, while H₁ receptor mRNA expression in the Arc showed negative correlations to food intake and total fat mass. In addition, there was a negative relationship between H₁ receptor binding densities in the VMH and total fat mass and body weight gain. This study suggests that an olanzapine-induced down-regulation of histamine H₁ receptor expression in regions of hypothalamus involved in the regulation of food intake (the Arc and VMH) may be a key factor contributing to olanzapine-induced body weight gain/obesity.

In conclusion, this study revealed that the effects of antipsychotics on specific neurotransmitter receptors contribute to the mechanisms of their pharmacological efficacy and side-effects. The binding profiles of antipsychotics for specific receptors cannot completely predict the level of their therapeutic efficacies and side-effects. Furthermore, the changes in expression of some receptors (such as 5-HT₁₅A) by antipsychotic treatment may produce the adaptation and desensitisation after long-term use. These results have also provided significant information which may assist with the development of new antipsychotic drugs.
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT</td>
<td>Serotonin</td>
</tr>
<tr>
<td>AcbC</td>
<td>Nucleus accumbens core</td>
</tr>
<tr>
<td>AcbS</td>
<td>Nucleus accumbens shell</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ARP</td>
<td>Aripiprazole</td>
</tr>
<tr>
<td>Arc</td>
<td>Arcuate hypothalamic nucleus</td>
</tr>
<tr>
<td>CA1</td>
<td>CA1 region of hippocampus</td>
</tr>
<tr>
<td>CA2</td>
<td>CA2 region of hippocampus</td>
</tr>
<tr>
<td>CA3</td>
<td>CA3 region of hippocampus</td>
</tr>
<tr>
<td>CART</td>
<td>Cocaine- and amphetamine-regulated transcript</td>
</tr>
<tr>
<td>Cg</td>
<td>Cingulate cortex</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CONT</td>
<td>Control</td>
</tr>
<tr>
<td>CPu</td>
<td>Caudate-putamen</td>
</tr>
<tr>
<td>DAT</td>
<td>Dopamine transporter</td>
</tr>
<tr>
<td>DM</td>
<td>Dorsomedial hypothalamic nucleus</td>
</tr>
<tr>
<td>DG</td>
<td>Dentate gyrus</td>
</tr>
<tr>
<td>HB</td>
<td>Habenular nucleus</td>
</tr>
<tr>
<td>EPS</td>
<td>Extrapyramidal symptoms</td>
</tr>
<tr>
<td>FBW</td>
<td>Final body weight</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>HPD</td>
<td>Haloperidol</td>
</tr>
<tr>
<td>IBW</td>
<td>Initial body weight</td>
</tr>
<tr>
<td>LHA</td>
<td>Lateral hypothalamic area</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MeP:</td>
<td>Medial posterodorsal nuclei of posterior amygdala</td>
</tr>
<tr>
<td>MePV</td>
<td>Medial amygdaloid nucleus, posteroventral part</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NAc</td>
<td>Nucleus accumbens</td>
</tr>
<tr>
<td>NAcC</td>
<td>Nucleus accumbens core</td>
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<td>NAcS</td>
<td>Nucleus accumbens shell</td>
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<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<tr>
<td>NRG-1</td>
<td>Neuregulin-1</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>PCP</td>
<td>Phencyclidine</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular hypothalamic nucleus</td>
</tr>
<tr>
<td>OLZ</td>
<td>Olanzapine</td>
</tr>
<tr>
<td>RT</td>
<td>Reticular thalamic nucleus</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SRI</td>
<td>Schizophrenia Research Institute</td>
</tr>
<tr>
<td>SN</td>
<td>Substantia nigra</td>
</tr>
<tr>
<td>SNC</td>
<td>Substantia nigra compacta</td>
</tr>
<tr>
<td>TE</td>
<td>Tissue equivalent</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Name</td>
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<tr>
<td>--------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>TH</td>
<td>Tyrosine hydroxylase</td>
</tr>
<tr>
<td>VMH</td>
<td>Ventromedial hypothalamic nucleus</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
</tr>
<tr>
<td>ZI</td>
<td>Zona incerta</td>
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