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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

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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

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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 collegues 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.

Han, M., Huang, X.F., and Deng, C. Aripiprazole differentially affects mesolimbic and nigrostriatal dopaminergic transmission: implications for long-term drug efficacy and low extrapyramidal side-effects. *International Journal of Neuropsychopharmacology*, 12: 941–952, 2009.

Han, M., Deng, C., Burne, T., Newell, K.A., and Huang, X.F. Short-and long-term effects of antipsychotic drug treatment on weight gain and H_1 receptor expression, *Psychoneuroendocrinology*, 33: 569–580, 2008.

Han, M., Newell, K.A., Zavitsanou, K., Deng, C., and Huang, X.F. Effects of antipsychotic medication on muscarinic M_1 receptor mRNA expression in the rat brain. *Journal of Neuroscience Research*, 86: 457–464, 2008.

Publications in Conference Proceedings

Han, M., Deng, C., Zavitsanou, K., Tan, Y.Y., and Huang, X.F. Effects of antipsychotics on muscarinic M_1 receptor mRNA expression in the rat brain. *Proceeding of the 7th IBRO World Congress of Neuroscience*, 274, 2007.

Han, M., Deng, C., Newell, K.A., and Huang, X.F. Histamine H_1 mRNA expression is decreased in the rat hypothalamus following olanzapine treatment. *Schizophrenia Bulletin*, 33(2): 317, 2007.

Han, M., Deng, C., Tan, Y.Y., and Huang, X.F. Differential effects of antipsychotics on dopamine transmission in the mesolimbic and nigrostriatal pathways in rats. *Sixth International Symposium on Frontiers in Life Sciences Molecular Basis of Disease, Prevention and Treatment*, September 20-23, Qingdao, China 2006. *Cell Biology International*, 30 (8): S6, 2006.

Han, M., Deng, C., Tan, Y.Y., and Huang, X.F. Aripiprazole treatment increases D_2 receptor mRNA expression in the ventral tegmental area of rat brain. *Proceedings of the Australian Neuroscience Society*, 26:105, 2006.

Additional Publications

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

du Bois, T., Deng, C., **Han, M.,** Newell, K.A., and Huang, X.F. Excitatory and inhibitory neurotransmission is chronically altered following perinatal NMDA receptor blockade. *European Neuropsychopharmacology*, 19: 256–265, 2009.

du Bois, T., Newell, K.A., **Han, M.,** Deng, C., and Huang, X.F. Perinatal PCP treatment alters the developmental expression of prefrontal and hippocampal muscarinic receptors. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 33: 37–40, 2009.

Weston-Green K., Huang, X.F., **Han, M.,** and Deng, C. The effects of antipsychotics on the densities of cannbinoid receptors in the dorsal vagal complex of rats: Implications for olanzapine-induced weight gain. *International Journal of Neuropsychopharmacology*, 11: 827–835, 2008.

Deng, C., Weston-Green K., **Han, M.,** and Huang, X.F. Olanzapine treatment decreases the density of muscarinic M2 receptors in the dorsal vagal complex of rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 31: 915–920, 2007.

Deng, C., **Han, M.,** and Huang, X.F. No changes in densities of cannabinoid receptors in the superior temporal gyrus in schizophrenia. *Neuroscience Bulletin*, 23: 341–347, 2007.

Zavitsanou, K., Nguyen, V.H., **Han, M.,** and Huang, X.F. Effects of typical antipsychotic drugs on rat brain muscarinic receptor. *Neurochem Research*. 32(3): 525–32, 2007.

Huang, X.F., **Han, M.,** Huang, X., Zavitsanou, K., and Deng, C. Olanzapine differentially affects 5-HT2A and 2C receptor mRNA expression in the rat brain. *Behavioural Brain Research*, 171: 355–362, 2006.

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.

Huang, X., Deng, C., **Han, M.,** Zavitsanou, K., and Huang, X.F. Central 5-HT2 receptor mRNA expression pattern following chronic olanzapine treatment in rats. *Proceeding of the Australian Neuroscience Society*, 26:139, 2006.

Tan, Y.Y., Huang, X., **Han, M.,** and Huang, X.F. Effects of chronic clozapine treatment on 5-HT2A and 5-HT2C receptor mRNA expression in the rat limbic system, *Proceeding of the Australian Neuroscience Society*, 26: 102, 2006.

Zavitsanou, K., Nguyen, V.H., **Han, M.,** Katsifis, A., and Huang, X.F. Effects of typical and atypical antipsychotic drugs on rat brain muscarinic receptors, *Proceeding of the Australian Neuroscience Society*, 26: 104, 2006.

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 D_2 receptor binding density, but decreased DAT binding density in the NAc, CPu and VTA. Olanzapine had less widespread effects on D_2 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-HT_{1A} 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-HT_{1A} receptors, but olanzapine has not. Therefore, the aim of Chapter 3 was to examine whether these antipsychotics affect 5-HT_{1A} receptor expression. The results showed that aripiprazole increased 5-HT_{1A} 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-HT_{1A} 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-HT_{1A} 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_{1A} receptor expression after long-term treatment.

Schizophrenia patients exhibit a decrease, or no change, in muscarinc M_1 receptor expression in certain brain regions. Olanzapine has a high affinity for the M_1 receptor, while aripiprazole and haloperidol have low affinities. The aim of Chapter 4 was to investigate how these antipsychotics affect M_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_1 receptor mRNA expression in the hippocampus. In addition, increases in M_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_1 receptor mRNA expression in schizophrenia are unlikely to be a consequence of drug treatment, and implicate the muscarinic M_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_1 receptor expression. To the best of this author's knowledge, this study is the first to compare H_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_1 receptor mRNA expression and

binding density in the ventromedial hypothalamic nucleus (VMH), and H_1 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 H_1 receptor expression in the VMH or Arc. Histamine H_1 receptor mRNA expression in the VMH and Arc were negatively correlated to body weight gain and energy efficiency, while H_1 receptor mRNA expression in the Arc showed negative correlations to food intake and total fat mass. In addition, there was a negative relationship between H_1 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 H_1 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-HT_{1A}) 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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LIST OF ABBREVIATIONS

5-HT	Serotonin
AcbC	Nucleus accumbens core
AcbS	Nucleus accumbens shell
ANOVA	Analysis of variance
ARP	Aripiprazole
Arc	Arcuate hypothalamic nucleus
CA1	CA1 region of hippocampus
CA2	CA2 region of hippocampus
CA3	CA3 region of hippocampus
CART	Cocaine- and amphetamine-regulated transcript
Cg	Cigulate cortex
CNS	Central nervous system
CONT	Control
CPu	Caudate-putamen
DAT	Dopamine transporter
DM	Dorsomedial hypothalamic nucleus
DG	Dentate gyrus
HB	Habenular nucleus
EPS	Extrapyramidal symptoms
FBW	Final body weight
GABA	Gamma-aminobutyric acid
HPD	Haloperidol
IBW	Initial body weight
LHA	Lateral hypothalamic area
MAPK	Mitogen-activated protein kinase
MeP:	Medial posterodorsal nuclei of posterior amygdala
MePV	Medial amygdaloid nucleus, posteroventral part
MRI	Magnetic resonance imaging
NAc	Nucleus accumbens
NAcC	Nucleus accumbens core
NAcS	Nucleus accumbens shell
NMDA	N-methyl-D-aspartate
NRG-1	Neuregulin-1
NPY	Neuropeptide Y
PCP	Phencyclidine
PET	Positron emission tomography
POMC	Pro-opiomelanocortin
PVN	Paraventricular hypothalamic nucleus
OLZ	Olanzapine
RT	Reticular thalamic nucleus
SEM	Standard error of the mean
SRI	Schizophrenia Research Institute
SN	Substantia nigra
SNC	Substantia nigra compacta
TE	Tissue equivalent

TH	Tyrosine hydroxylase
VMH	Ventromedial hypothalamic nucleus
VTA	Ventral tegmental area
ZI	Zona incerta