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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

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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 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.

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 H₁ 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 M₁ 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 M₁ 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 H₁ 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 D₂ 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 cannabinoid 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-HT_{2A} 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-HT_{2A} and 5-HT_{2C} 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 D₂, histamine H₁, serotonin 5HT_{1A} and muscarinic M₁ 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 D₂ 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 D₂ 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-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-HT_{1A} 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 muscarinic M₁ receptor expression in certain brain regions. Olanzapine has a high affinity for the M₁ receptor, while aripiprazole and haloperidol have low affinities. The aim of Chapter 4 was to investigate how these antipsychotics affect M₁ receptor mRNA expression in regions of the brain that are implicated in the pathology of schizophrenia. This study showed that the three antipsychotics increased M₁ receptor mRNA expression in the hippocampus. In addition, increases in M₁ 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 M₁ receptor mRNA expression in schizophrenia are unlikely to be a consequence of drug treatment, and implicate the muscarinic M₁ 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 H₁ receptor expression. To the best of this author's knowledge, this study is the first to compare H₁ receptor expression in the rat brain following short and long-term administration of olanzapine, aripiprazole and haloperidol. Results showed that olanzapine significantly down-regulated H₁ 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-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	Cingulate 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

Chapter 1. Literature review and overview of the study

1.1 Introduction

Schizophrenia is a complicated and disabling brain disorder, which affects approximately 1% of the population, and is one of the most costly diseases to afflict humans (Jablensky et al., 1992, Hyman, 2000). Schizophrenia has features of positive and negative symptoms, cognitive dysfunction and a decline in psychosocial function. Positive symptoms include hallucinations and delusions, while negative symptoms include depression and social withdrawal. Cognitive dysfunction includes deficits in attention as well as in memory and executive function (Kane et al., 2002, Freedman, 2003, Patel et al., 2006). To date, the etiological factors or pathogenic processes in schizophrenia remain unclear. It is generally accepted that a number of factors are involved, including genetic, environmental and brain developmental components (van Erp et al., 2002, Torrey and Yolken, 2003, Rapoport et al., 2005).

Currently, schizophrenia can not be completely cured, although several clinical treatment methods have been employed, including pharmacological intervention, psychotherapy, electrical shock therapy and neurosurgical therapy (Johns and Thompson, 1995, Scott and Dixon, 1995, Matthews and ElJamel, 2003). Pharmacological intervention through the use of antipsychotic drugs remains a key component of therapy, while other methods are used as adjunct treatments.

Antipsychotic drugs are generally divided into two types: typical and atypical antipsychotics (Nasrallah, 2003).

Typical antipsychotics, such as haloperidol and chlorpromazine, are also called conventional or first generation antipsychotics (Sedvall et al., 1995, Terry Jr et al., 2006). They potently antagonise dopamine D₂ receptors and are effective in controlling the positive symptoms, but have little effect on the negative symptoms of schizophrenia (Kapur and Mamo, 2003). They can produce serious extra-pyramidal symptoms (EPS) such as tardive dyskinesia, akathisia and dystonia, as well as raise prolactin levels (Purdon et al., 2001, Montejo, 2008).

Atypical antipsychotics, such as clozapine, olanzapine, quetiapine, risperidone and ziprasidone are referred to as unconventional or second generation antipsychotics (Sedvall et al., 1995, Terry Jr et al., 2006). They have less affinity to D₂ receptors than typical antipsychotics and exert their effects on multiple receptors, such as serotonin 5-HT_{2A}, 5-HT_{2C}, 5-HT_{1A}, histamine H₁ and muscarinic M₁ (Fernández-Novoa and Cacabelos, 2001, Kapur and Mamo, 2003, Matsumoto et al., 2005). These drugs are effective in treating not only the positive symptoms of schizophrenia, but also to some extent the negative symptoms and cognitive deficits (Javitt, 1999, Purdon et al., 2001). An important advantage of atypical antipsychotics is the reduced incidence of EPS compared to typical antipsychotics (Kapur and Mamo, 2003). However, atypical antipsychotics induce other prominent side-effects, such as body weight gain/obesity. Clinical observations have identified olanzapine and clozapine as the atypical

antipsychotic drugs with the highest risk of producing weight gain (Allison et al., 1999, Eder et al., 2001, Nasrallah, 2008). For example, a study suggested that 24-37% of olanzapine-treated patients experienced a $\geq 7\%$ gain in body weight (Haddad, 2005).

Aripiprazole is a relatively new atypical antipsychotic that was introduced in 2002. It was developed as a partial agonist of the dopamine D₂ receptor, as well as the 5-HT_{1A} receptor, and an antagonist of 5-HT_{2A} receptors (Bowles and Levin, 2003, Shapiro et al., 2003, DeLeon et al., 2004). Previous studies have shown that aripiprazole does not induce EPS like typical antipsychotics, or body weight gain like some atypical antipsychotics (Kane et al., 2002, Marder et al., 2003). In addition, aripiprazole has been recommended as a first-line of therapy for the treatment of schizophrenia (DeLeon et al., 2004, Cassano et al., 2007).

As discussed above, compared to typical antipsychotics, significant progress has been made by atypical antipsychotic in efficacy and EPS, but appear to have some unresolved questions remain. However, the mechanisms for their efficacy in treating the negative symptoms and improving cognitive deficits in schizophrenia are unclear. In addition, some atypical antipsychotics induce body weight gain, and the reasons for this are unknown. Furthermore, the mechanisms underlying the lower risk of EPS during aripiprazole treatment compared to typical antipsychotics, as well as the lower body weight gains compared to other atypical antipsychotics remain unknown. Moreover, post-mortem studies have revealed abnormal neurotransmission in schizophrenia, such as dopamine, serotonin,

histamine and muscarinic acetylcholine systems (Nakai et al., 1991, Joyce et al., 1993, Crook et al., 2000, Suhara et al., 2002). However, as schizophrenia patients normally have a long history of antipsychotic treatment, the question raised is whether these changes represent the primary neuropathology of schizophrenia or a secondary effect of antipsychotic treatment. Therefore, studying the mechanisms of drug actions in the brain at a molecular level will provide important information for the design of new antipsychotics drugs, with greater efficacy and fewer side-effects. Understanding the mechanisms of drug action is also important to assist in clarifying whether the results of post-mortem studies are pathological changes or secondary pharmacological effects of antipsychotic treatment.

Rapid progress in molecular biological techniques has enabled investigation of the molecular background of various diseases. Currently, *in situ* hybridisation and radioligand autoradiography techniques have been widely applied in the study of schizophrenia at a molecular level. These studies have pinpointed many functionally related gene groups in the aetiology and treatment of the disease. Specifically, the altered expression of several receptor mRNAs and binding densities have been observed in post-mortem studies of schizophrenia, such as dopamine D₂, histamine H₁, muscarinic M₁ and serotonin 5-HT_{1A} receptors (Fernández-Novoa and Cacabelos, 2001, Kapur and Mamo, 2003, Matsumoto et al., 2005, Newell et al., 2007c). In this study, using *in situ* hybridisation and receptor autoradiography techniques, D₂, H₁, M₁ and 5-HT_{1A} receptor mRNA expression and binding densities were investigated in rat brains following short-

(1 week) and long-term (12 weeks) treatment with aripiprazole, olanzapine and haloperidol.

1.2 Review of the literature

Since the incidental discovery in the late 1950s that chlorpromazine had therapeutic effects in schizophrenia, a number of antipsychotics have been approved and clinically applied to the treatment of schizophrenia (Kapur and Mamo, 2003). The general dosage range, efficacy for treating positive and negative symptoms of schizophrenia and side-effects of some commonly prescribed antipsychotics are listed in Table 1.1. From this table, it is clear that antipsychotic drugs share some common characteristics, despite their typicality. Typical antipsychotics can control positive symptoms but produce EPS, while atypical antipsychotics are effective at controlling the positive and negative symptoms, however, some atypical antipsychotics may lead to body weight gain/obesity.

1.2.1 The pharmacological effects of antipsychotics

It is widely accepted that antipsychotics achieve their pharmacological effects, as well as undesirable side-effects, by targeting different receptor systems of brain. Antipsychotics possess antagonistic and agonistic properties at various receptors such as dopamine D_2 , serotonin $5HT_{2A}$, $5HT_{2C}$ and $5HT_{1A}$, α_{1-2} , muscarinic M_1 and histamine H_1 . Various antipsychotics have different affinities to these receptors in the brain (Table 1.2). The affinity of a drug is its ability to bind to its specific biological targets, such as receptors, enzymes and transporter systems.

Table 1.1 Effects and side-effects of commonly used antipsychotics administered at therapeutic doses

Property	Typical antipsychotic	Atypical antipsychotics					
	Haloperidol	Clozapine	Olanzapine	Quetiapine	Risperidone	Ziprasidone	Aripiprazole*
Usual dose range mg/day	1–7.5mg	200–600mg	5–20mg	300–750mg	2–6mg	80–160mg	10–30mg
Effects							
Positive symptoms	+++	++++	+++	++	+++	+++	++
Negative symptoms	0/+	++	+	+	+	+	+
Side-effects							
Extrapyramidal symptoms	++++	0/+	0/+	0/+	++	+	0/+
Weight gain	0/+	+++	+++	++	++	+	0/+
Type 2 diabetes mellitus	+	++	++	+	+	+	+
Prolactin increase	++	0/+	0/+	0/+	++	0/+	0/+
Anti-cholinergic	0/+	+++	+	0/+	0/+	0/+	0
Postural hypotension	+	+++	0/+	++	+++	+	+
Sedation	+	+++	+++	++	++	++	++

++++, very high; +++, high; ++, moderate; +, low; 0, negligible. * Aripiprazole is a relatively new atypical antipsychotic.

Adapted from the articles (Bridler and Umbricht, 2003, Gardner et al., 2005)

Receptor affinities of antipsychotics may play important roles in their pharmacological effects. Previous studies suggest that antipsychotic receptor affinity may be used to predict the drug's efficacy (Kroeze et al., 2003). The combination of specificities and affinities of antipsychotics with receptors may determine their pharmacological effects.

1.2.2 The selection of antipsychotic drugs

Numerous different antipsychotic drugs are utilised in the clinic to control the symptoms of schizophrenia. As previously mentioned and shown in Table 1.1, typical antipsychotics are used to treat positive symptoms of schizophrenia, but produce EPS, whereas atypical antipsychotics have shown efficacy to treat the positive, negative and cognitive domains of the disease, but can induce metabolic disorders such as weight gain/obesity. Haloperidol and olanzapine were selected for the present study as representatives of typical and atypical antipsychotics respectively. The new generation atypical antipsychotic aripiprazole was also selected for this study as it has similar pharmacological effects as other atypical antipsychotics, but with an improvement in the weight gain/obesity side-effect (DeLeon et al., 2004).

As discussed in Section 1.2.1, antipsychotics achieve their specific pharmacological effects, and unexpected side-effects, by targeting specific neurotransmitter receptors in the brain. Therefore, the affinities of antipsychotics with specific receptors are vital to their pharmacological mechanisms. The antipsychotics chosen for the present study were also based on their affinities to

Table 1.2 Receptor affinities of commonly used antipsychotics.

Receptor	Typical antipsychotic	Atypical antipsychotics					
	Haloperidol	Clozapine	Olanzapine	Quetiapine	Risperidone	Ziprasidone	Aripiprazole*
D ₁	210	85	31	455	460	525	265
D ₂	0.7	126	11	160	4	5	0.45
D ₃	2	473	49	340	10	7	0.8
D ₄	3	35	27	1600	9	32	44
5HT _{1A}	2600	875	>7100	>830	210	3	4.4
5HT _{2A}	45	16	4	295	0.5	0.4	3.4
5-HT _{2C}	1500	16	23	1500	25	1	15
α ₁	6	7	19	7	0.7	10	57
α ₂	360	8	230	87	3	–	–
H ₁	440	6	7	11	20	47	61
M ₁	>1500	1.9	1.9	120	>10,000	>1000	>10,000

All values are reported as K_i (nM). D₁, dopamine₁; D₂, dopamine₂; D₃, dopamine₃; D₄, dopamine₄; 5HT_{1A}, serotonin_{1A}; 5HT_{2A}, serotonin_{2A}; 5HT_{2C}, serotonin_{2C}; H₁, histamine₁; M₁, muscarinic₁; * Aripiprazole is a D₂ partial agonist.

Adapted from Delcon et al (2004)

specific receptors that are implicated in the pathology and treatment of the schizophrenia, such as D₂, 5-HT_{1A}, H₁ and M₁ receptors.

D₂ receptor antagonism in the mesolimbic dopaminergic pathway is related to the improvement of the positive symptoms of schizophrenia, but D₂ receptor blockade in the nigrostriatal dopaminergic pathway produce EPS (Kapur and Mamo, 2003). Interestingly, haloperidol and aripiprazole have similar high affinities for D₂ receptors (Table 1.2), haloperidol causes EPS but aripiprazole does not (Kapur and Mamo, 2003). Therefore, it is important to understand whether these drugs have different effects in the two aforementioned dopaminergic pathways and whether this difference contributes to their different pharmacological effects. Moreover, olanzapine and aripiprazole both increase dopamine release in the prefrontal cortex and hippocampus via 5-HT_{1A} receptors (Ichikawa et al., 2001, Li et al., 2004). However, unlike aripiprazole, olanzapine has no significant affinity with 5-HT_{1A} receptors (Table 1.2). It is not known whether these antipsychotics achieve their pharmacological effects by changing 5-HT_{1A} receptor mRNA expression and binding densities in the specific brain regions. In addition, olanzapine has a high affinity for muscarinic M₁ receptors, but haloperidol and aripiprazole do not (Table 1.2). It was previously proposed that the muscarinic and dopamine systems have an important inverse relationship (Tandon et al., 1989, Bernard et al., 1992, Gomeza et al., 1999). Both haloperidol and aripiprazole have a similar affinity for the dopamine D₂ receptor, however, aripiprazole is a D₂ partial agonist, while haloperidol is a D₂ antagonist. In

addition, evidence shows that aripiprazole has functional selective activity at D₂ receptors (Shapiro et al., 2003). Therefore, it is important to understand if the three antipsychotics have different effects on M₁ receptors. Furthermore, the affinity of antipsychotics for the H₁ receptor may predict body weight gain (Kroeze et al., 2003). One of the three antipsychotics selected in this study, olanzapine has the highest affinity for H₁ receptors and causes body weight gain in patients (Nasrallah, 2003, DeLeon et al., 2004). Therefore, it is important to understand whether olanzapine targets H₁ receptors in specific brain regions related to appetite and food intake. More detailed information on the roles of these receptors in the aetiology and treatment of schizophrenia will be introduced in the following sections.

Pharmacological studies of aripiprazole, olanzapine and haloperidol have been partly addressed in previous works in the neurotransmission systems mentioned above, especially the dopaminergic and serotonergic systems. However, the pharmacological mechanisms of these antipsychotics, particularly their chronic effects on the dopaminergic, serotonergic, histaminergic and muscarinic systems, are still not completely understood.

1.2.3 Antipsychotics and the dopaminergic system

The aetiology of schizophrenia pertaining to the dopaminergic system, as well as the effects of antipsychotic treatment on this system, has been studied for more than half a century. However, there are some questions that still remain unanswered.

1.2.3.1 The dopaminergic system

Dopaminergic neurons are located in the midbrain region and send major projections to various other brain regions via four main pathways in the brain: the nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular pathways (Fig. 1.1) (Vallone et al., 2000, Stahl, 2003). The mesolimbic and mesocortical pathways originate from the ventral tegmental area. The mesolimbic pathway, which is involved in motivated behaviour, emotion and reward, projects to the ventral striatum, nucleus accumbens, amygdaloid complex, piriform cortex, and olfactory tubercle (Stahl, 2003). The mesocortical pathway is related to learning, memory and reward, and projects to the limbic cortical regions of the brain, such as the prefrontal cortex (PFC) and the cingulate cortex (Le Moal and Simon, 1991, Stahl, 2003). The nigrostriatal pathway originates from the substantia nigra pars compacta and projects to the dorsal part of the striatum and caudate putamen (CPu), and plays a fundamental role in the control of posture and motor activity (Stahl, 2003). The tuberoinfundibular pathway originates from the arcuate nucleus of the medio-basal hypothalamus and projects to the anterior pituitary. Dopamine inhibits prolactin secretion in this pathway (Vallone et al., 2000).

Dopamine receptors are G-protein coupled receptors and can be divided into five distinct receptor subtypes (Vallone et al., 2000). The dopamine D₂, D₃, and D₄ receptors belong to the D₂-like receptor family (Sibley and Monsma, 1992, Sokoloff and Schwartz, 1995), and have high affinities for dopamine D₂ receptors, and are located in most dopaminoreceptive regions of the brain, including the motor

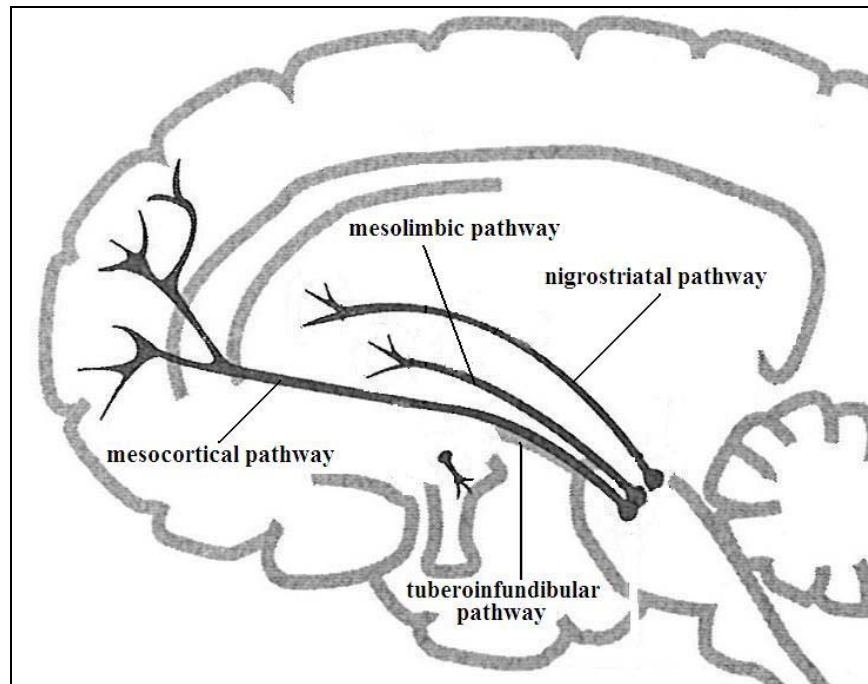


Fig. 1.1 Diagram of the human brain showing four major dopamine pathways: the mesolimbic, nigrostriatal, mesocortical and tuberoinfundibular pathways. Adapted from Stahl (2003).

and limbic structures (Vallone et al., 2000). These receptors are expressed in both pre- and postsynaptic neurons, serving as the main dopamine autoreceptor in presynaptic neurons. Dopamine D₃ and D₄ receptors are predominantly expressed in the subcortical limbic system and are rare in the dorsal striatum and neocortex (Vallone et al., 2000). Previous studies have shown that D₂ receptors play an important role in the pharmacological efficacy of antipsychotics (Kapur and Mamo, 2003). The dopamine D₁ and D₅ receptors belong to D₁-like receptor family. Previous studies have shown that D₁-like receptor subtypes are less likely to be involved in mediating the effects of antipsychotic drugs (Florijn et al., 1997).

1.2.3.2 The dopaminergic hypothesis of schizophrenia

The dopaminergic hypothesis predicts that abnormal activity in dopamine neurons is the key cause of schizophrenia. The hyperactivity of dopamine neurons in the mesolimbic pathway has been linked to the positive symptoms of schizophrenia, while decreased activity of these neurons in the mesocortical pathway may underlie the negative symptoms of schizophrenia (Stahl, 2000). This hypothesis was developed from the observation that drugs such as amphetamine and cocaine, which increase levels of dopamine in the brain, induce or worsen the symptoms of schizophrenia (Breier et al., 1997, Serper et al., 2000). In addition, the efficacy of early antipsychotics, chlorpromazine and haloperidol, to treat schizophrenia symptoms is related to their ability to block dopamine D₂ receptors in the brain (Seeman et al., 1975, Seeman and Lee, 1975, Creese et al., 1976). In addition, post-mortem studies have shown abnormalities in the density of D₂ receptor in the

striatal, temporal and anterior cingulate cortices in schizophrenia patients (Wong et al., 1986, Goldsmith et al., 1997, Suhara et al., 2002). Although the studies in schizophrenia and antipsychotic treatment to date have achieved important progress, the roles of the dopaminergic system in the pathophysiology of schizophrenia and its treatment are still not completely understood.

1.2.3.3 The effects of antipsychotic drugs on dopamine D₂ receptors

Accumulated evidence has supported the idea that typical antipsychotics, such as haloperidol, act mainly as D₂ receptor antagonists. Typical antipsychotics control the positive symptoms of schizophrenia, and produce EPS, by blocking dopamine transmission in the mesolimbic and nigrostriatal pathways respectively (Burt et al., 1977, Chipkin et al., 1987). However, their effects on the D₂ receptor mRNA transcript appear to be controversial, as previous studies have suggested that antipsychotics have either no effect or a small increase in D₂ receptor mRNA expression in the striatal regions of the brain (Coirini et al., 1990, Angulo et al., 1991, Matsunaga et al., 1991, Fox et al., 1994).

Atypical antipsychotics, such as olanzapine, are thought to have a similar efficacy as typical antipsychotics in the control of positive symptoms (Kapur and Mamo, 2003). The benefits of atypical antipsychotics lay in their improvement of negative symptoms and cognitive function, as well as decreased risk of EPS (Geddes et al., 2000, Kapur and Mamo, 2003, Nasrallah, 2003). However, the mechanisms of the therapeutic effects of atypical antipsychotics and their associated EPS decreased are unclear.

A possible explanation for the efficacy of atypical antipsychotics to improve the negative symptoms and cognitive function deficit of schizophrenia is the dopamine theory. The theory is based on the hypothesis that dopaminergic hyperactivity in the mesolimbic pathway may be related to the onset of positive symptoms, while the decreased activity of dopamine neurons in the mesocortical pathway may produce negative symptoms and cognitive deficits (Stahl, 2000). It suggests that atypical antipsychotics down-regulate D₂ receptors in the mesolimbic pathway to control positive symptoms and up-regulate these receptors in the mesocortical pathway to improve the negative symptoms and cognitive function deficits (Stahl, 2000). However, whether atypical antipsychotics can achieve dual effects in the two dopaminergic tracts is unclear and even doubtful. A second explanation for atypical antipsychotic efficacy to treat negative symptoms and cognitive function deficits of schizophrenia is their possible action on non-D₂ receptors, such as serotonin 5-HT_{2A}, 5-HT_{1A} and muscarinic M₁ receptors. For example, evidence suggests that olanzapine may increase dopamine release via 5-HT_{1A} receptors in the prefrontal cortex and hippocampus, as this effect was significantly inhibited by the 5-HT_{1A} receptor antagonist, WAY-100635 (Ichikawa et al., 2001). Studies in our laboratory have shown that olanzapine decreased 5-HT_{2A} receptor mRNA expression in the striatum and limbic systems, while clozapine significantly increased [³H]pirenzepine binding (a muscarinic M₁/M₄ receptor antagonist) in the dentate gyrus of rat brain (Huang et al., 2006, Zavitsanou et al., 2007).

As a relatively new atypical antipsychotic drug, aripiprazole, is considered to be a dopamine partial agonist, exerting its effects on both presynaptic autoreceptors and postsynaptic D₂ receptors (DeLeon et al., 2004). A partial agonist property ensures that the ligand can not elicit the maximum tissue response, even if it can occupy all relevant receptors. The definitive advantages of aripiprazole associated with dopamine D₂ partial agonism in the schizophrenia treatment are yet to be determined. However, a study has suggested that dopamine synthesis, release and dopaminergic neurotransmission is reduced when D₂ partial agonism is targeted to the dopamine autoreceptors (Wolf and Roth, 1990). In addition, aripiprazole has both partial agonist and antagonist activity at the dopamine D₂ receptor (Shapiro et al., 2003, Hirose et al., 2004). Furthermore, aripiprazole is also a partial agonist to serotonin 5-HT_{1A} receptor and an antagonist of 5-HT_{2A} receptors (Table 1.2), and is therefore referred to as ‘a dopamine and 5-HT stabilizer’. However, evidence indicates that aripiprazole acts predominantly on dopamine D₂ receptors at therapeutic doses (Urban et al., 2007, Wood and Reavill, 2007).

The mechanisms underlying atypical antipsychotics, lower risk or elimination of EPS than the typical antipsychotics, are unclear. Currently, there are several explanations: first, atypical antipsychotics, such as olanzapine and clozapine, show a preference to block limbic cortical dopamine D₂ receptors over striatal dopamine D₂ receptors. This is in contrast to haloperidol, which shows high D₂ receptor occupancy in both brain regions (Bigliani et al., 2000, Xiberas et al., 2001). However, there are inconsistent reports that atypical antipsychotics (such as clozapine) have a relatively low D₂ receptor occupancy in both the striatum and

the extrastriatal regions of the brain (Talvik et al., 2001). Therefore, it is important to understand the effects of olanzapine on the dopaminergic system. The second explanation is due to the effects of atypical antipsychotic drugs on multiple receptors, as a common characteristic of these drugs is their broad receptor binding profiles. Olanzapine may decrease EPS by its effect on neurotransmitter systems other than dopamine, such as the serotonergic and muscarinic systems. For example, a study from our group found that olanzapine down-regulated 5-HT_{2A} receptor mRNA expression in the striatum, which may induce a decrease in EPS (Huang et al., 2006). Furthermore, previous studies suggest that antipsychotics drugs with intrinsic muscarinic antagonist properties are associated with decreased EPS (Snyder et al., 1974a, Snyder et al., 1974b). Olanzapine has a high affinity to muscarinic M₁ receptors (Table 1.2), therefore, it is possible olanzapine may decrease EPS through interaction with the muscarinic system. The third explanation involves the theory of rapid dissociation, which proposes that dopamine D₂ receptor blockade by atypical agents only lasts long enough to induce antipsychotic actions, but not long enough to cause motor side-effects (Kapur and Seeman, 2001). A faster dissociation rate from dopamine D₂ receptors results in a lower overall affinity to the receptor subtype and contributes to the atypical antipsychotic activity of the commonly prescribed atypical antipsychotic drugs. Clozapine and quetiapine have a lower D₂ receptor occupancy at therapeutic doses (<60%), which is evidenced in previous studies using PET scan technology (Farde et al., 1992, Kapur et al., 1999, Kapur et al., 2000). Other PET studies have reported a D₂ receptor occupancy rate of 70% or higher in most other typical and atypical antipsychotics (Farde et al., 1988). Therefore, the rapid

dissociation hypothesis relating to the effect of antipsychotics on dopamine D₂ can only explain the pharmacological mechanisms of clozapine and quetiapine, but it does not completely explain the atypical properties of other atypical antipsychotics such as olanzapine.

Interestingly, aripiprazole is similar to haloperidol in its high affinity to the dopamine D₂ receptor (Table 1.2), despite the fact that aripiprazole has a lower risk of EPS than haloperidol. For example, PET images from normal volunteers showed that aripiprazole produces very high levels of dopamine D₂ receptor occupancy (40–95%), with virtual saturation of dopamine D₂ receptors at clinically relevant doses (Yokoi et al., 2002). The D₂ receptor partial agonism exhibited by aripiprazole may underlie its pharmacological mechanisms, and improved EPS (Wood and Reavill, 2007). In addition, aripiprazole may exhibit functional selectivity at dopamine D₂ receptors, meaning it activate different signalling pathways through its effects on a single receptor (Mailman, 2007). For example, a previous study reported that aripiprazole acted as a potent partial agonist in D₂ receptor-mediated signalling responses, such as the potentiation of arachidonic acid release, and as a weak partial agonist using MARK (mitogen-activated protein kinase), but lacked agonist activity on receptor internalisation (Urban et al., 2007). Whether aripiprazole's functional selective activity contributes to its decreased risk of EPS is unknown. Furthermore, aripiprazole exerts its effects on a broad range of receptors such as the serotonergic and muscarinic systems, which may also explain its reduced EPS incidence.

In summary, typical antipsychotics (such as haloperidol) control the positive symptoms of schizophrenia, and produce EPS, by blocking dopamine D₂ receptors in the mesolimbic and nigrostriatal dopamine pathways in schizophrenia patients. Atypical antipsychotics such as olanzapine may have similar mechanisms to haloperidol in controlling positive symptoms. However, the mechanisms underlying olanzapine's improvement in the reduction of negative symptoms, cognitive function and decreased incidence of EPS remain unclear. Unlike haloperidol, olanzapine may exert differential effects on dopamine D₂ receptors located in the mesolimbic and nigrostriatal dopamine pathways. In addition, the action of olanzapine on other neurotransmission systems, such as the serotonergic and muscarinic signalling pathways, may explain its broader efficacy in treating the multiple symptoms of schizophrenia and superior tolerability compared to typical antipsychotics. Compared to haloperidol and olanzapine, the new antipsychotic, aripiprazole may use different mechanisms to improve the symptoms of schizophrenia and cognitive function deficits, and decrease EPS. Aripiprazole may utilise its dopamine D₂ partial agonist properties, or functionally selective activity at D₂ receptors to produce more specific effects in the mesolimbic and nigrostriatal dopamine pathways.

1.2.4 Antipsychotics and the serotonergic system

The pathological theory of schizophrenia has focused on the dopamine system for more than half a century. However, due to the fact that the dopamine theory cannot completely explain the aetiology and treatment of schizophrenia, increasing attention has been directed towards the possible role of other

neurotransmission systems in the neuropathology of schizophrenia and antipsychotic treatment, such as the serotonergic, glutamatergic, histaminergic and muscarinic systems (Kapur and Mamo, 2003, Iwabuchi et al., 2005, Matsumoto et al., 2005, du Bois and Huang, 2007).

In particular, the serotonin system has received increasing attention, due to the fact that most atypical antipsychotics display relatively low antagonistic effects at the dopamine D₂ receptor, but a high affinity for serotonergic receptors, particularly the serotonin 5-HT_{2A} receptors (Meltzer et al., 2003). Therefore, the 5-HT_{2A} receptor has been the main focus of serotonin research in the pathogenesis and treatment of schizophrenia. The role of 5-HT_{2A} receptors in the aetiology and treatment of schizophrenia have been addressed by a number of workers (Burnet and Harrison, 1995, Huang et al., 2006, Arranz et al., 2007, Sáiz et al., 2007). However, 5-HT_{2A} antagonism alone (such as that induced by ritanserin) showed no effects in the improvement of schizophrenia symptoms (Bantick et al., 2001). Therefore, attention has been shifted to other serotonin receptors in the pharmacological treatment of schizophrenia. This section of the review will focus on the role of serotonin 5-HT_{1A} receptors in antipsychotic treatment.

1.2.4.1 The serotonergic system

Serotonin (5-HT) neurons originate in the raphe nucleus of the midbrain, where they project to several brain regions, such as the cingulate cortex, hippocampus and amygdala (Wilson and Molliver, 1991). There are more than 14 structurally and pharmacologically distinctive mammalian 5-HT receptor subtypes in the

central nervous system (CNS), that have been classified into seven receptor families (5HT₁₋₇) (Barnes and Sharp, 1999). The 5-HT receptor families are mostly G-protein coupled metabotropic receptors, excluding the 5-HT₃ receptor, which is a ligand-gated ion channel (Barnes and Sharp, 1999). The major 5-HT receptors that are involved in the aetiology of schizophrenia and the action of antipsychotics are 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, 5-HT₆ and 5-HT₇ receptors (Meltzer and Nash, 1991, Sleight et al., 1996, Frederick and Meador-Woodruff, 1999, Wood et al., 2001, Arranz et al., 2007). It also includes the 5-HT transporters (Meltzer et al., 2003).

The 5-HT_{1A} receptor is highly expressed in the limbic system, including the hippocampus, cingulate cortex and raphe nuclei. Lower levels of 5-HT_{1A} receptors are found in the amygdala, thalamus, striatum, and adult cerebellum (Yocca et al., 1992, Burnet et al., 1997, Hall et al., 1997, Parsey et al., 2006). Serotonin 5-HT_{1A} receptor agonists inhibit serotonin neuronal activity in the hippocampus, frontal cortex and other brain regions, as this effect can be blocked by a 5-HT_{1A} antagonist, WAY-100635 (Sprouse and Aghajanian, 1988, Barnes and Sharp, 1999). It is already known that the cingulate cortex, hippocampus and amygdala are related to cognitive function, emotion, learning and memory (Vogt et al., 1992, Vizi and Kiss, 1998). It is important to understand whether atypical antipsychotics affect 5-HT_{1A} receptor expression in these brain regions.

Serotonin 5-HT_{1A} receptors have a series of specific physiological roles. For example, 5-HT_{1A} agonists have similar properties as 5-HT_{2A} receptor antagonists.

In fact, serotonin 5-HT_{1A} receptor subtypes are considered the functional antagonist of 5-HT_{2A} receptors (Meltzer et al., 2003). In addition, 5-HT_{1A} receptors play an important role in controlling the activity of monoaminergic neurons such as dopamine neurons (Barnes and Sharp, 1999). Furthermore, 5-HT_{1A} receptors are involved in the regulation of dopamine release in the prefrontal cortex and hippocampus, which have significant associations with the negative symptoms and cognitive function deficits of schizophrenia (Meltzer et al., 2003). Finally, the serotonin 5-HT_{1A} agonist, 8-OH-DPAT, is also associated with antagonism of haloperidol-induced catalepsy in the rat (Lucas et al., 1997).

1.2.4.2 The involvement of the 5-HT_{1A} receptors in schizophrenia

The negative symptoms and cognitive deficits observed in schizophrenia have been attributed to the hypoactivity of dopamine in the prefrontal cortex (Kapur and Remington, 1996, Knable and Weinberger, 1997, Castner et al., 2000). However, studies have proposed that 5-HT_{1A} agonists may improve symptoms of schizophrenia by reducing serotonergic function to disinhibit dopamine release through mesocortical projections (i.e. from the ventral tegmental area to the prefrontal cortex). These effects may be achieved by reducing serotonergic transmission through 5-HT_{1A} agonist action on autoreceptors located in the raphe nuclei (Kapur and Remington, 1996). Furthermore, a study has shown a potent 5-HT_{1A} agonist, MKC-242, increased dopamine release in the prefrontal cortex and hippocampus (Sakaue et al., 2000). In addition, post-mortem studies showed alterations in serotonin 5-HT_{1A} receptors in numerous brain regions, such as the prefrontal, temporal and cingulate cortices and amygdala in schizophrenia patients

(Hashimoto et al., 1991, Joyce et al., 1993, Yasuno et al., 2004). These results suggest that 5-HT_{1A} receptor activation is significantly involved in negative symptoms and cognitive deficits observed in schizophrenia and its treatments.

1.2.4.3 The effects of antipsychotic drugs on serotonin 5-HT_{1A} receptors

Previous studies have shown that atypical antipsychotics such as aripiprazole, olanzapine and clozapine, regardless of their 5-HT_{1A} affinity, promote cortical dopamine release via 5-HT_{1A} receptors, which may play important roles in the improvement of the negative symptoms and cognitive function deficits of schizophrenia (Rollema et al., 1997, Ichikawa et al., 2001, Li et al., 2004). To date, it is not clear if atypical antipsychotics change 5-HT_{1A} receptor expression in the human brain. In animal studies, researchers have investigated the expressions of 5-HT_{1A} receptors following chronic administration of clozapine and haloperidol (at 14 days and 21 days) in rat brain (Burnet et al., 1996a, Ase et al., 1999). Both studies reported that haloperidol did not affect the expression of 5-HT_{1A} receptors in the rat. In addition, the density of serotonin 5-HT_{1A} receptor binding sites was not significantly affected by clozapine following 14 days treatment (Burnet et al., 1996a). However, after 21 days, there was a significant reduction in 5-HT_{1A} receptor binding sites in the frontal, parietal and temporal cortex in the drug treated group compared to the controls (Ase et al., 1999). It was suggested that clozapine may improve the negative symptoms and cognitive function deficits through its high affinity to the 5-HT_{1A} receptor, as 5-HT_{1A} receptor expression is increased in these brain regions in schizophrenia (Hashimoto et al., 1991). Haloperidol does not possess this binding property, which is in agreement with

the clinical characteristics of clozapine and haloperidol, where clozapine improves negative symptoms and cognitive deficit but haloperidol does not.

To date, no study has investigated whether aripiprazole and olanzapine affect serotonin 5-HT_{1A} receptor expression in the brain. Aripiprazole has a high affinity for 5-HT_{1A} and 5-HT_{2A} receptors, acting as a partial agonist and antagonist at these receptors respectively (DeLeon et al., 2004, Zhu et al., 2004). Therefore, it is important to understand whether aripiprazole targets the 5-HT_{1A} receptor to achieve its efficacy in improvement of negative symptoms and cognitive function deficits. In addition, olanzapine lacks an appreciable affinity for 5-HT_{1A} receptors (Table 1.2). However, its ability to increase dopamine release in the prefrontal cortex and hippocampus may be via 5-HT_{1A} receptors (Ichikawa et al., 2001). It is unknown whether olanzapine can change 5-HT_{1A} receptor expression in these specific brain regions.

In summary, compared to typical antipsychotics, the significant progress in treatment using atypical antipsychotics such as aripiprazole and olanzapine is in ameliorating the negative symptoms and cognitive function deficits of schizophrenia. Both atypical antipsychotics increase dopamine release in the prefrontal cortex and hippocampus either by directly or indirectly acting upon 5-HT_{1A} receptors, which may contribute to the improvement of the negative symptoms and cognitive function deficits of schizophrenia. However, it is not clear whether chronic treatment with aripiprazole and olanzapine can induce

changes in serotonin 5-HT_{1A} receptor expression in specific brain regions such as the hippocampus.

1.2.5 Antipsychotics and the muscarinic acetylcholine system

It has been discussed that the hypotheses of dopamine and serotonin cannot completely explain the aetiology and treatment of schizophrenia (section 1.2.3 and 1.2.4). There is an inverse relationship between dopamine and muscarinic systems (Bernard et al., 1992, Gomeza et al., 1999). Furthermore, down-regulated M₁ receptor expression is found in the brain of schizophrenia patients (Crook et al., 2001, Deng and Huang, 2005, Newell et al., 2007a). However, it is unclear whether these changes are the real pathology of schizophrenia or secondary effects of medication. Therefore, this section will focus on the potential role of muscarinic receptors, especially M₁ receptors, in the aetiology of schizophrenia and antipsychotic treatments.

1.2.5.1 The muscarinic acetylcholine system

Acetylcholine (ACh) is the primary neurotransmitter involved in learning, memory, attention and motor control in the central nervous system (Caulfield, 1993, Wess et al., 2003, Volpicelli and Levey, 2004, Wess, 2004). There are two types of acetylcholine receptors, nicotinic and muscarinic receptors, both of which are involved in the pathophysiology of schizophrenia (Martin et al., 2007, Raedler et al., 2007). The basal forebrain is the main source of cholinergic afferents, and cholinergic neurons in this region project to all layers of the cerebral cortex (Mesulam, 1995). In the nucleus basalis, acetylcholine, dopamine, serotonin,

GABA and noradrenaline synapses on cholinergic cell bodies, suggesting that multiple neurotransmitter systems can modulate cholinergic function (Smiley and Mesulam, 1999, Smiley et al., 1999).

Central muscarinic acetylcholine receptors are involved in regulating a large number of cognitive, behavioural, sensory, motor and autonomic functions (Eglen et al., 1999, Felder et al., 2000, Felder et al., 2001, Wess et al., 2003). Five muscarinic acetylcholine receptor subtypes have been identified M_{1-5} that mediate either excitatory or inhibitory neurotransmission (Caulfield, 1993). Muscarinic M_1 , M_3 and M_5 receptors are coupled to G_q proteins and are referred to as M_1 -like receptors. M_2 and M_4 are coupled to G_i/o proteins and are referred to as M_2 -like receptors. M_1 receptors are mainly located in the cerebral cortex, hippocampus and striatum accumbens complex. Expression of muscarinic M_2 , M_3 and M_4 receptors in these brain regions are moderate, while expression of the M_5 is low in these areas (Vilaró et al., 1990, Levey, 1993, Caulfield and Birdsall, 1998, Berkeley et al., 2001, Hamilton and Nathanson, 2001, Krejci and Tucek, 2002, Porter et al., 2002).

1.2.5.2 The involvement of the M_1 receptors in schizophrenia

Increasing evidence suggests that central muscarinic activity is involved in the pathophysiology and pharmacotherapy of schizophrenia. For example, muscarinic acetylcholine receptors are involved in the regulation of cognition, memory activities and sleep, which are altered in schizophrenia patients (Hyde and Crook, 2001). In addition, an increase in muscarinic cholinergic activity is associated

with the negative symptoms of schizophrenia, while a decrease in muscarinic cholinergic activity is associated with the positive symptoms of the disease (Tandon et al., 1991). An *in vivo* study found that muscarinic receptors were significantly down-regulated in most brain regions in schizophrenia, including the frontal cortex and caudate-putamen (Raedler et al., 2003). Coinciding with these findings, a series of post-mortem studies indicated a decrease in [³H]pirenzepine binding sites, a ligand with a high affinity for M₁ and M₄ receptors (M₁/M₄ receptors, mainly binding to M₁ receptors, although it has affinity for both receptors), in regions of the brain including the anterior cingulate cortex, prefrontal cortex, hippocampal formation and caudate-putamen in schizophrenia (Dean et al., 1996, Crook et al., 2000, Crook et al., 2001, Zavitsanou et al., 2004). Reductions in muscarinic M₁ receptors were also identified in the superior temporal gyrus and posterior cingulate cortex in schizophrenia following research done in our laboratory (Deng and Huang, 2005, Newell et al., 2007c). Pharmacological studies suggest that muscarinic M₁ receptors are involved in mediating higher cognitive processes, such as learning and memory (Fisher et al., 1996, Iversen, 1997). Furthermore, a study has demonstrated that M₁ receptor knockout mice exhibit cognitive function impairments (Anagnostaras et al., 2003). In addition, a reduction in muscarinic M₁ receptor activity leads to an increase in locomotor activity (Miyakawa et al., 2001, Wess et al., 2003). The above evidence supports the idea that the muscarinic cholinergic system is implicated in the pathophysiology of schizophrenia. However, as schizophrenia patients generally have a long history of antipsychotic treatment, it is not clear whether alterations in muscarinic M₁ receptors are a result of the neuropathology

of schizophrenia, or whether these changes represent a secondary effect of antipsychotic treatment.

1.2.5.3 The effects of antipsychotic drugs on muscarinic M₁ receptors

Atypical antipsychotics, such as clozapine and olanzapine, have anti-muscarinic properties (Bymaster et al., 2003). Clozapine and olanzapine increase the levels of the M₁ receptors in the rat cortex as revealed by [³H]pirenzepine binding studies (Crook et al., 2001, Terry Jr et al., 2006). In addition, haloperidol also increases M₁ receptor expression in the cortex (Crook et al., 2001, Terry Jr et al., 2006). Generally, the therapeutic actions and side-effects of antipsychotic drugs can be attributed to their relative affinities for specific neurotransmitter receptors (van Rossum, 1966). Olanzapine has a high affinity for muscarinic M₁ receptors, while haloperidol has no significant affinity to M₁ receptors (Table 1.2). However, both antipsychotics up-regulate muscarinic M₁ receptor binding densities in the cortex (Crook et al., 2001). These results suggest that direct blockade of M₁ receptors may not be important in the pharmacological mechanisms of antipsychotic drug action in muscarinic M₁ receptor neurotransmission. The notion that overactivity of the dopaminergic system is the main pathophysiological mechanism of schizophrenia, and that D₂ receptor blockade plays an important role in the mechanisms of antipsychotic treatment has been discussed (see section 1.2.3). It is important to note that the muscarinic system has significant interactions with the dopaminergic system in the brain (Bernard et al., 1992, Gomeza et al., 1999). The anatomical and physiological evidence of interactions between acetylcholine and dopamine neurons has been identified (Lehmann and Langer, 1983, Le Moine et

al., 1990, Bernard et al., 1992, Levey, 1993). Therefore, the effects of olanzapine and haloperidol on muscarinic M₁ receptor binding densities may be a downstream effect of changes in the action of other receptors, such as the dopamine D₂ receptor. In order to confirm the effects of olanzapine and haloperidol on muscarinic M₁ receptor neurotransmission, it is important to investigate the effects of these drugs on M₁ receptor mRNA expression in the brain.

As in the case of haloperidol, aripiprazole lacks appreciable affinity to muscarinic M₁ receptors. However, unlike haloperidol, aripiprazole is a D₂ partial agonist, and recently it has been found to exhibit functional selectively effects on D₂ receptors (Lawler et al., 1999, Shapiro et al., 2003, DeLeon et al., 2004, Mailman, 2007, Urban et al., 2007). To this author's knowledge, there are no published studies that examine the effects of aripiprazole treatment on muscarinic M₁ receptor expression in the brain. It is currently unknown if the specific action of aripiprazole at dopamine D₂ receptors may contribute to the drug's specific effects on M₁ receptor expression. It is particularly important to understand the effects of antipsychotics on the muscarinic M₁ receptors in those brain regions that have been indicated in pathological changes of M₁ receptors in schizophrenia, such as the hippocampus (Crook et al., 2000).

In summary, the muscarinic acetylcholine system, especially muscarinic M₁ receptors, has been implicated in the pathophysiology and pharmacotherapy of schizophrenia. Currently, autoradiography studies suggest that olanzapine and

haloperidol may exert their antagonistic effects on M₁ receptors through downstream mediators, such as the dopaminergic system. In order to confirm the effects of olanzapine and haloperidol on M₁ receptors, it is important to investigate how the two antipsychotics affect M₁ receptor mRNA expression in the rat brain. Aripiprazole has high affinities to dopamine D₂ and serotonin 5-HT_{1A} receptors, but has no significant affinity to muscarinic M₁ receptors. Since changes in M₁ receptors could be the result of downstream effects, it is important to examine the effects of aripiprazole on M₁ receptor neurotransmission.

1.2.6 Antipsychotics and the histaminergic system

Despite their apparent superiority in treating the negative and cognitive domains of schizophrenia compared to typical antipsychotics, a major downfall of some atypical antipsychotics is their propensity to induce weight gain/obesity (Nasrallah, 2003). This side-effect may lead to poor compliance in adhering to medication regimes and other adverse health effects, such as diabetes and cardiovascular disease.

Body weight gain is normally due to increased energy intake or decreased energy expenditure. Several nuclei of the hypothalamus are important in the regulation of food intake and energy expenditure, including the ventromedial hypothalamus (VMH) and arcuate nucleus (Arc) (Hillebrand et al., 2002, King, 2006). Neurotransmitters such as serotonin and histamine are considered prime candidates as regulators of the control of food intake and body weight gain within the hypothalamus (Huang et al., 2004, Yoshimatsu, 2008). For example, serotonin

5-HT_{1A} receptors have been implicated in food intake and body weight gain (Dryden et al., 1996, Park et al., 1999, López-Alonso et al., 2007), while studies from our laboratory have shown that olanzapine decreases the level of serotonin 5-HT_{2A} receptor mRNA expression in hypothalamic nuclei relevant to the regulation of energy balance, such as VMH and Arc (Huang et al., 2006). The histamine H₁ receptor also plays an important role in regulating energy balance (Masaki and Yoshimatsu, 2006). This study will focus on the effects of the histamine H₁ receptor in the regulation of body weight gain following antipsychotic treatment.

1.2.6.1 The histaminergic system

Histaminergic cell bodies are located exclusively in the tuberomammillary nucleus of the posterior hypothalamus, with axons projecting extensively throughout the brain, including the limbic system and neocortex (Watanabe et al., 1984, Haas and Panula, 2003, Mochizuki et al., 2004). There are four histamine receptor subtypes H₁₋₄, all of which are G protein-coupled receptors (Hill et al., 1997). H₁ and H₂ receptors are found in the hypothalamus, cerebral cortex, and limbic system (Nakai et al., 1991, Lintunen et al., 1998, Brown et al., 2001, Dai et al., 2007). H₃ receptors are present on the axon terminals of histamine-containing neurons, where they modulate histamine synthesis and release (Arrang et al., 1983). H₄ receptors are widely distributed in the brain, and high levels of H₄ receptors have been identified in the spinal cord, while low levels of H₄ receptors are present in the hypothalamus (Strakhova et al., 2009).

Histaminergic receptors have been implicated in many physiological, pathophysiological and behavioural processes (Sakata et al., 1991, Hill et al., 1997, Morisset et al., 2000, Hough, 2001). For example, histamine H₁ receptors are involved in modulating various neurophysiologic functions, including feeding, energy expenditure, sleeping, wakefulness, locomotor activity, emotion, memory and learning and aggressiveness (Monnier et al., 1970, Kalivas, 1982, Haq et al., 1996, Haas and Panula, 2003, Masaki et al., 2004, Masaki and Yoshimatsu, 2006). Previous studies have suggested that H₁ receptor activity decreases food intake (Sakata et al., 1988a, Sakata et al., 1988b). Neuronal histamine has an anti-obesity action. For example, anti-histaminergic drugs increase feeding behaviour, while histidine, the biological precursor of histamine, suppresses feeding in rats (Orthen-Gambill, 1988). In addition, histamine H₁ receptor knockout mice gradually develop mature-onset obesity accompanied by hyperphagia and altered diurnal feeding patterns (Masaki et al., 2003, Masaki et al., 2004).

1.2.6.2 The involvement of the H₁ receptors in schizophrenia

Previous research has indicated that H₁ receptor antagonism affects cognition, motor activity and induces sedation (Haas and Panula, 2003). Sleep disturbance, motor activity, eating and drinking disorders are often present in schizophrenia patients (Nakai et al., 1991). Therefore, it is possible that H₁ receptors are involved in the pathophysiology of schizophrenia. A study reported a dysfunction in the central and peripheral histaminergic system of schizophrenia patients (Fernández-Novoa and Cacabelos, 2001). For example, blood histamine levels were decreased in schizophrenia patients compared to healthy subjects

(Fernández-Novoa and Cacabelos, 2001). Previous studies have also indicated that the binding potentials of histamine H₁ receptors in some brain areas, such as the frontal and prefrontal cortices, are significantly lower in schizophrenia patients compared to control subjects (Nakai et al., 1991, Iwabuchi et al., 2005). Furthermore, behavioural and electrophysiological studies have shown that H₁ receptor gene knockout mice have selective cognitive dysfunction (Dai et al., 2007).

1.2.6.3 The role of the histamine H₁ receptors in antipsychotic-induced weight gain

A meta-analysis indicated that H₁ receptor blockade is the primary cause of antipsychotic-induced weight gain (Matsui-Sakata et al., 2005). Clinical observations indicate that atypical antipsychotics such as olanzapine and clozapine increase appetite and produce body weight gain/obesity (Wirshing et al., 1999). In addition, these factors positively correlate with the drug's H₁ receptor affinity (Wetterling, 2001). In fact, meta-analyses show that an antipsychotic drug's H₁ receptor affinity may predict its short-term weight gain for typical and atypical antipsychotic drugs (Kroeze et al., 2003). As shown in Table 1.2 of the three antipsychotics selected for the present study, olanzapine has a higher affinity than aripiprazole for the H₁ receptor, while haloperidol has the lowest. This is consistent with clinical observations that olanzapine causes significant hyperphagia and body weight gain/obesity, while haloperidol and aripiprazole do not (Allison et al., 1999, Kasper et al., 2003). The side-effect of olanzapine to

increase hyperphagia and body weight gain has been modelled in the female rat (Arjona et al., 2004, Cooper et al., 2005, Huang et al., 2006).

The various regions of the hypothalamus that are implicated in the control of energy regulation, such as the ventromedial hypothalamic nucleus (VMH), arcuate hypothalamic nucleus (Arc) and paraventricular hypothalamic nucleus (PVN), are regions rich in histaminergic H₁ receptors. It has been suggested that histamine affects food intake via H₁ receptors in the VMH and the PVN of the hypothalamus (Fukagawa et al., 1989, Sakata et al., 1991, Brown et al., 2001, Magrani et al., 2004, Masaki and Yoshimatsu, 2006). However, whether H₁ receptors play a role in olanzapine-induced weight gain is unclear. In particular, the effects of olanzapine's H₁ antagonistic properties in specific regions of the hypothalamus, including the VMH, PVN and Arc are unknown.

In summary, body weight gain/obesity is a detrimental side-effect of some atypical antipsychotics such as olanzapine. Body weight gain is normally caused by increased food intake or decreased energy expenditure. The hypothalamus, which is rich in histamine H₁ receptors, is important in the regulation of energy balance. Olanzapine is a H₁ receptor antagonist and has high affinity for H₁ receptors. Previous studies have shown that H₁ receptor blockade in the VMH and PVN is implicated in body weight gain/obesity. Furthermore, studies suggest that the affinity of antipsychotics with H₁ receptors may predict body weight gain. However, it is not clear whether atypical antipsychotics, such as olanzapine, produce body weight gain by altering H₁ receptor expression in specific nuclei of

the hypothalamus that are involved in the regulation of food intake, such as VMH, PVN and Arc.

Overall to summarise, the symptoms of schizophrenia are primarily controlled by pharmacological intervention. Antipsychotics achieve their pharmacological effects by regulating neurotransmitter systems in the brain. Due to the effects of antipsychotics on neurotransmission, the question was raised as to whether pathological changes in the post-mortem brain of schizophrenia patients are due to the real pathology of the disease, or are secondary to medication. In addition, the mechanisms underlying the propensity of antipsychotics to produce a series of side-effects (EPS, body weight gain/obesity, etc) are not known. Investigation of receptor expression patterns in animals treated with antipsychotics will provide important information for etiological studies of schizophrenia and the development of new antipsychotic drugs.

1.3 Aims of the study

General aim and hypothesis:

The aim of this study was to investigate the molecular mechanisms of the pharmacological effects and side-effects of various antipsychotics (aripiprazole, olanzapine and haloperidol) in a rat model. It was hypothesised that these antipsychotics would have differential effects on receptor expression in the various brain regions implicated in the symptoms of schizophrenia, as well as those regions relating to EPS and body weight gain/obesity side-effects.

Furthermore, it was also hypothesised that receptor expression would be affected by the duration of antipsychotic treatment.

Specific Aims:

1. To investigate whether, unlike olanzapine and haloperidol, aripiprazole selectively affects neurotransmission in the mesolimbic and nigrostriatal dopaminergic pathways – a mechanism that may contribute to its long-term drug efficacy and low risk of extrapyramidal side-effects (Chapter 2).
2. To investigate whether the various atypical antipsychotics, olanzapine and aripiprazole, differentially modulate 5-HT_{1A} receptor expression in various brain regions such as the hippocampus, cingulate cortex and amygdala, which may contribute to the improvement of negative symptoms and cognitive function deficits observed in schizophrenia (Chapter 3).
3. To investigate whether olanzapine, aripiprazole and haloperidol affect M₁ receptor expression in those brain regions that exhibit M₁ receptor expression alterations in schizophrenia patients, such as the hippocampus. These findings will help to clarify whether changes in M₁ receptor expression are a result of the real pathology of schizophrenia or due to secondary effects of medication (Chapter 4).
4. To investigate whether H₁ receptor blockade in the hypothalamus is one of the mechanisms by which olanzapine induces the side-effect of body weight gain/obesity (Chapter 5).

1.4 General methods

1.4.1 Animals

A total of 101 female Sprague Dawley rats weighing 220–250g were obtained from the Animal Resources Centre (Perth, WA, Australia). This project studied female rats only as previous studies indicated that female rats are more sensitive to antipsychotics in inducing body weight gain than male rats (Pouzet et al., 2003, Arjona et al., 2004). Upon arrival, rats were divided into four drug treatment groups (Fig. 1.2). Rats were housed individually in environmentally controlled conditions (temperature 22°C; light cycle from 07:00 h to 19:00 h and dark cycle from 19:00 h to 07:00 h), with *ad libitum* access to water and standard laboratory chow. After a 1 week familiarisation period, they were treated with aripiprazole (2.25 mg/kg/day, Otsuka, Japan), olanzapine (1.5 mg/kg/day, Eli Lilly, USA), haloperidol (0.3 mg/kg/day, Sigma, Australia) or vehicle (control), either short- (1 week) or long-term (12 weeks). Dosage selection will be explained in the following paragraph. The daily dosage was divided into three equal amounts and rats were treated three times a day (06:00 h, 14:00 h, 22:00 h) orally by specially prepared drug pellets, as described previously (Huang-Brown and Guhad, 2002, Han et al., 2008b, Han et al., 2009). Figure 1.3 illustrates treatment delivery. The minimum number of rats per group was 12. Body weight, food and water intake were recorded weekly. In the chronic study group, open field testing was performed 1 week and 11 weeks after drug treatment (Fig. 1.4 A). All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

Outline of experimental design

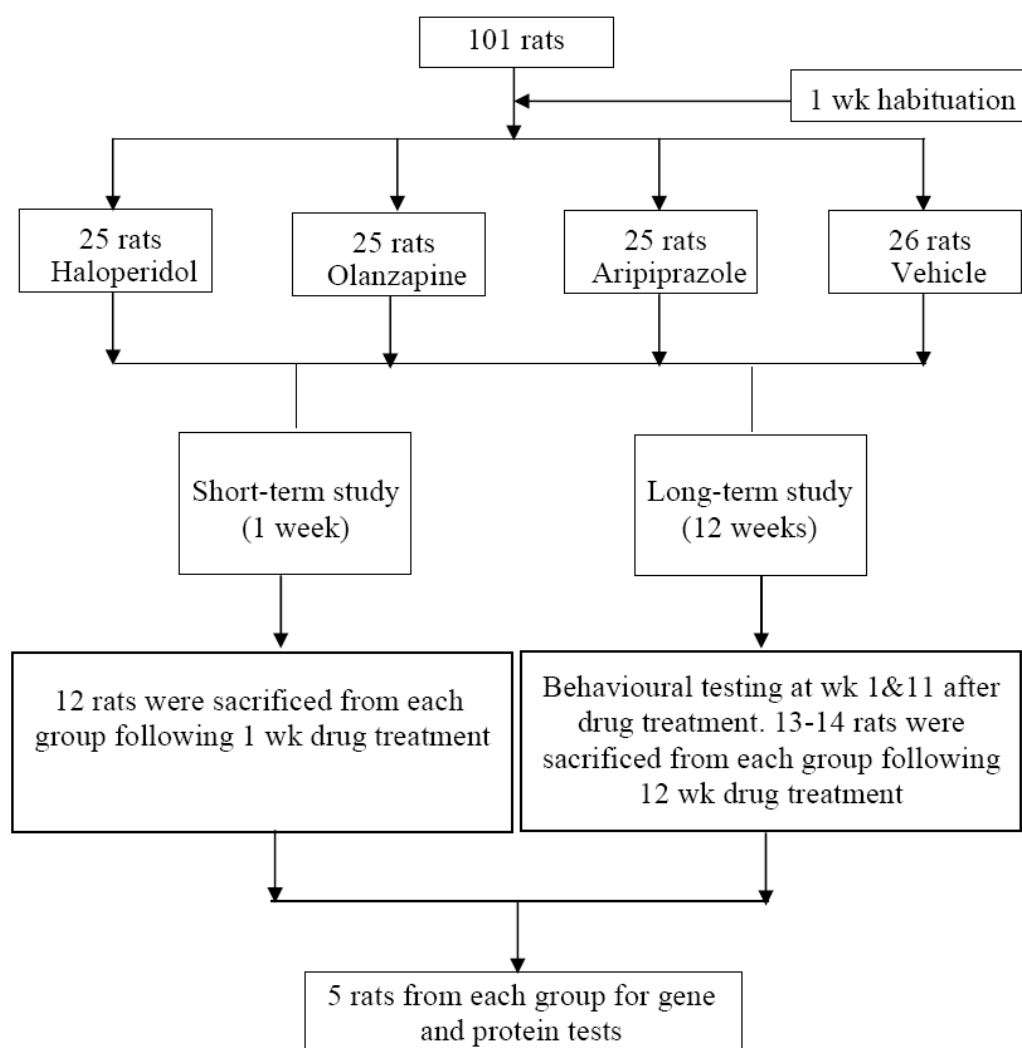


Fig. 1.2 Flowchart showing the experiment design and assignment of rats. After one week of habituation, 101 female Sprague Dawley rats were divided into four groups. They were treated with aripiprazole (2.25 mg/kg/day), olanzapine (1.5 mg/kg/day), haloperidol (0.3 mg/kg/day) and vehicle (Control), either short- (1 week) or long-term (12 weeks). The daily dosage was divided into three equal amounts and rats were treated three times a day orally using the sweet ‘cookie-dough’ pellet method (see Fig. 1.3), modified from Huang-brown and Guhad (2002). The minimum number of rats per group was 12. After 1 week of drug treatment, rats for short-term studies were sacrificed. In the chronic study group, open field tests were performed 1 week and 11 weeks after drug treatment (see Fig. 1.4 A and B). After 12 weeks of drug treatment, rats for the chronic study were sacrificed. Five brains were used for detecting gene and protein expressions for each group.



Fig. 1.3 Administration of antipsychotic drug treatment to a female Sprague Dawley rat using the sweet ‘cookie-dough’ pellet method, modified from Huang-Brown and Guhod (2002).

1.4.2 The dosage selection of antipsychotic drugs

The drug dosages used in the literature for aripiprazole, olanzapine and haloperidol treatment vary significantly in animal studies. For example, aripiprazole has recently been used at doses ranging from 2 to 3 mg/kg (Bruins Slot et al., 2005, Kalinichev et al., 2005, Li et al., 2005, Schwabe and Koch, 2007), while haloperidol has frequently been utilised at a dose of 0.3mg/kg (Pouzet et al., 2003, Wiley, 2008). In addition, doses of olanzapine ranging from 1.2mg/kg (Arjona et al., 2004, Huang et al., 2006) to 2.0 mg/kg (Cooper et al., 2005) have consistently been used in the literature and have been shown to produce the obesity phenotype in rats (Arjona et al., 2004, Cooper et al., 2005, Huang et al., 2006). Clinical studies have shown that the efficacy of antipsychotics requires dopamine D₂ receptor occupancy over 60%. Therefore, the selected dosage in this study (ie. aripiprazole, 2.25mg/kg; olanzapine, 1.5mg/kg; haloperidol, 0.3mg/kg) all share a D₂ occupancy of approximately 70–80% in rats (Kapur and Mamo, 2003, Natesan et al., 2006a, Natesan et al., 2006b). In addition, the doses used in this study have been used in the above mentioned literature, and are shown to be pharmacologically and behaviourally effective (Pouzet et al., 2003, Arjona et al., 2004, Natesan et al., 2006a).

1.4.3 Open field test

Open field testing was used study to determine whether antipsychotic drugs influence locomotor activity, in relation to energy expenditure. A single rat was placed in the centre of a grey rectangular arena (100 × 100 cm² with 40 cm high walls) and behaviour was recorded for 5 minutes (Fig. 1.4). Video recordings of

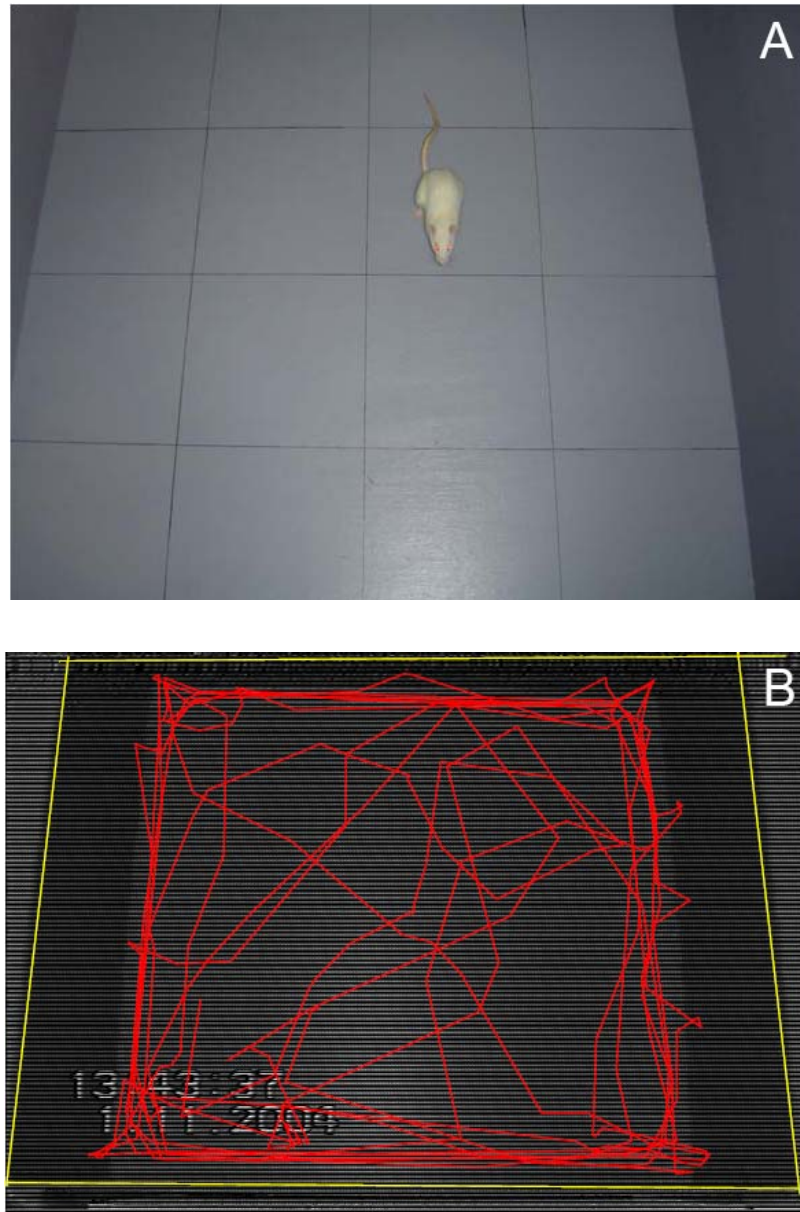


Fig. 1.4 A. Rat performing in the open field test shown crossing between zones in the open field. B. An Ethovision trace showing rat activity in the open field test.

rat behaviour were analysed using Noldus software, EthoVision Color-Pro (Noldus, Netherland). The following behavioural activities were examined: total distance travelled, distance travelled in the perimeter, latency to leave the centre of the arena, frequency of crossing between central/perimeter aspects of the arena, frequency of entering and total time spent in the central and the peripheral zones. Figure 1.4 *B* is an example of the track travelled by a rat during open field testing using Noldus software, EthoVision Color-Pro (Noldus, Netherland).

1.4.4 Histology

At the completion of drug treatment, all rats were sacrificed using carbon dioxide 48h after the last drug treatment. Periovary, perirenal and inguinal fat masses were dissected and weighed for comparison and statistical analysis. Brain tissue was also removed and frozen using liquid nitrogen, then stored at -80°C until biochemical analysis. Five brains from each group were used for detecting receptor mRNA expression and binding density studies. Coronal brain sections ($14\text{ }\mu\text{m}$) were cut at -17°C with a cryostat, and thaw-mounted onto poly-lysine-coated slides. Figure 1.5 shows the location of sections of rat brain collected, and main nuclei that were detected using *in situ* hybridization and autoradiography techniques. Sections for *in situ* hybridization were fixed in ice-cold phosphate-buffer containing 4% paraformaldehyde. Acetylation was carried out in 0.25% acetic anhydride in 0.1M triethanolamine buffer (pH 8.0) for 10 min. Sections were then dehydrated in ethanol and stored at -20°C until use. Neuroanatomical structures were identified according to a standard rat brain atlas (Paxinos and Watson, 1997).

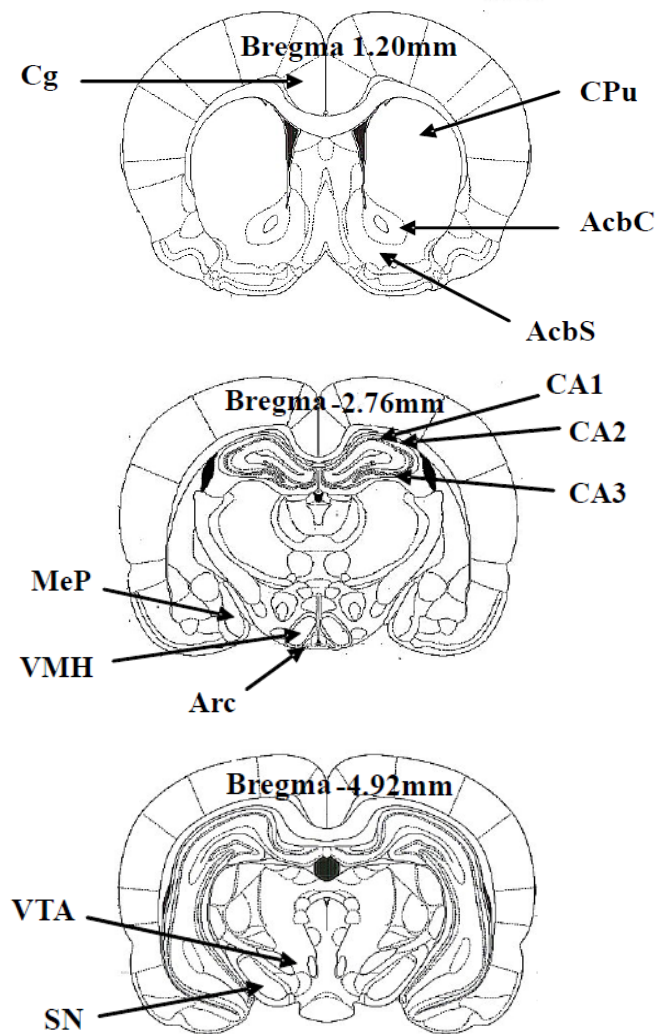


Fig. 1.5 Diagrams of the rat brain showing the location of nuclei detected in this study. Adapted from the reference Paxinos and Watson (1997). Abbreviations: Arc, arcuate hypothalamic nucleus; CA1-3, fields CA1-3 of hippocampus; Cg, cingulate cortex; CPu, caudate-putamen; MeP, medial posterodorsal nuclei of posterior amygdala; AcbC, nucleus accumbens core; AcbS, nucleus accumbens shell; VMH, ventromedial hypothalamic nucleus; VTA, ventral tegmental area; SN, substantia nigra.

1.4.5 *In situ* hybridisation

Specific antisense hybridisation probes were selected to examine the expression of the dopamine D₂ receptor and tyrosine hydroxylase (TH) (described in Chapter 2), serotonin 5-HT_{1A} receptors (described in Chapter 3), muscarinic M₁ receptors (described in Chapter 4) and histamine H₁ receptors (described in Chapter 5). No sequences bearing significant homology to the designed probes were found in the Gene Bank (NCBA). All oligonucleotide probes were terminally labelled with 10-fold molar excess of [³⁵S] dATP (specific activity: 1000 Ci/mmol, Amersham, Buckinghamshire, UK) and terminal transferase (Promega, Madison, WI, USA), and purified over a MicroSpin G-50 column (Amersham, UK). The probe concentration was 10⁷ pcm of [³⁵S]-labelled probes in 750 µl hybridisation solution. Hybridisation was carried out by incubating sections in the hybridisation buffer (50% deionized formamide, 4 x SSC, 10% dextran sulfate, 1 x Denhardt's solution, 0.2% sheared salmon sperm DNA, 0.1% long-chain polyadenylic acid, 0.012% heparin, 20 mM sodium phosphate, pH7.0, 10⁶/75 µl of labeled probe and 5% DTT) at 37°C for 16 hours. Non-specific hybridization was determined by including 100-fold molar excess of non-labeled probes in the respective hybridization solution. After hybridization, sections were washed in 1 x SSC buffer at 55°C three times for 20 minutes each followed by two washes in 1 x SSC at room temperature for 1 h each. Finally, sections were dipped sequentially in Milli-Q water, 70% ethanol and 95% ethanol before air-drying and exposure to Hyper-β-max film (Amersham, UK). Methodology for film exposure is detailed in Chapters 2, 3, 4 and 5, and films were developed using standard procedures.

Sections containing positive signals were dipped in emulsion solution (Amersham, UK) and then exposed (detailed in Chapters 2, 3, 4 and 5). This allowed a further examination of positive signals at the cellular level and confirmation of the results from the film. As in our previous work (Huang et al., 2004), all films were analysed using a computer-assisted image analysis system, Multi-Analyst, connected to a GS-690 Imaging Densitometer (Bio-Rad, USA). Quantification of mRNA expression levels in various brain regions was performed by measuring the average density of each region. Values were then compared against a ^{14}C -labelled autoradiographic standard (Amersham, UK).

1.4.6 Receptor autoradiography

The dopamine D_2 receptor, dopamine transporter (DAT), serotonin $5\text{-HT}_{1\text{A}}$ receptor and histamine H_1 receptor binding tests were performed as detailed in Chapters 2, 3, and 5. Autoradiographic images of the D_2 receptor and DAT were produced using a beta image camera (BioSpace, Paris, France) as previously described (Deng and Huang, 2005). Slides were exposed for 3.5 h at a high-resolution setting. A series of sections with a known amount of ligand was used as a standard in all scans. Quantitative analysis of these images was performed with β -Image Plus (Version 4; BioSpace). The density of the binding signal was first expressed in counts per minute per square millimetre (cpm/mm^2) of area selected, and was then converted into femtomole (fmole) of radioligand bound per milligram (mg) tissue equivalent (fmole/mg TE) by comparing with the standards. The slides used for serotonin $5\text{-HT}_{1\text{A}}$ and histamine H_1 receptor binding were exposed to Kodak BioMax MR film (see details in Chapter 3 and Chapter 5).

Films were then developed using standard procedures and all films were analysed using a computer-assisted image analysis system, Multi-Analyst, connected to a GS-690 Imaging Densitometer (Bio-Rad, USA). The optical measurements of 5-HT_{1A} and H₁ binding densities were converted to fmoles [³H]ligand per mg tissue equivalent, according to the calibration curve obtained from the tritium standards. The specific binding values were obtained by subtracting non-specific values from the total binding values.

1.4.7 Statistical analysis

According to previous studies (Huang et al., 2005), a power calculation was conducted using the JMP 5.1 computer program package. At a power of 80%, at least 12 rats were required in each group for behavioural testing, and five rats were required in each group for the changes in neurochemical analysis to be significantly different at an alpha level of 0.05.

Data were analysed using SPSS (Chicago, IL, USA). All data were analysed for normal distribution using a Kolmogorov-Smirnov Test. Data were analysed by analysis of variance (ANOVA), followed by Tukey-Kramer-HSD post-hoc analyses (see Chapters 2, 3, 4 and 5 for details). Correlations were carried out using Pearson's correlation tests (see Chapters 2, 3, 4 and 5 for details). Data were expressed as means \pm SEM. A *P*-value of less than 0.05 was regarded as statistically significant.

1.5 Summary of this thesis

The pharmacological effects of an antipsychotic are obtained by its receptor-binding properties. Blockade of the dopamine D₂ receptor is essential to most antipsychotic activities, especially for controlling the positive symptoms of schizophrenia and producing EPS. It is generally accepted that dopamine D₂ antagonism explains the effects of typical antipsychotics such as haloperidol. However, emerging data suggests that this may not be the only mechanism of action of all antipsychotic drugs. The improvements of negative symptoms and cognitive function deficits, decreased risk of EPS, increased appetite and body weight gain/obesity observed following treatment with olanzapine and other atypical antipsychotics may reflect the action of these drugs at receptors other than D₂, such as serotonin 5-HT_{1A}, muscarinic M₁, histamine H₁ and other receptors. Individual atypical antipsychotics exhibit different affinities to these neurotransmitter receptors, and can be equal to, or exceed their affinity for D₂ receptors (Table 1.2).

The binding profiles of antipsychotics to specific receptors may partially explain their pharmacological mechanisms of efficacy and side-effects. However, the binding profiles of an antipsychotic drug cannot completely explain its effects. For example, aripiprazole has a similar high affinity for D₂ receptors as haloperidol, with a high level of D₂ receptor occupancy (>90%) at the therapeutic dose (Grunder et al., 2003). It produces long-term efficacy in controlling the symptoms of schizophrenia, however, differing from haloperidol, aripiprazole has a low risk for inducing EPS. In addition, aripiprazole has a high affinity for the

serotonin 5-HT_{1A} receptor, while olanzapine has no significant affinity to this receptor (Table 1.2), however both antipsychotics increase dopamine release via 5-HT_{1A} receptors in the prefrontal cortex (Ichikawa et al., 2001, Li et al., 2004). It is not clear whether these antipsychotics may directly affect 5-HT_{1A} receptor expression. Olanzapine has a high affinity for muscarinic M₁ receptors and a weaker affinity for the D₂ receptors, while both aripiprazole and haloperidol have a high affinity for D₂ receptors but a weaker affinity for M₁ receptors (Table 1.2). Previous studies have shown that olanzapine and haloperidol affect M₁ receptor binding densities (Crook et al., 2001, Terry Jr et al., 2006). It is important to understand how these antipsychotics affect M₁ receptor mRNA expression.

A recent study suggested the affinity of antipsychotics for the H₁ receptor may predict body weight gain/obesity (Kroeze et al., 2003). Olanzapine has high affinity for the H₁ receptor, but aripiprazole and haloperidol have not (Table 1.1). Consistent with clinical study, olanzapine causes significant body weight gain/obesity, but aripiprazole and haloperidol do not (Nasrallah, 2008). It is important to compare whether olanzapine has different effects than aripiprazole and haloperidol on the H₁ receptor expression in the hypothalamus.

Four studies were conducted in this project in order to answer the above questions. The results were presented in four chapters.

1.5.1 Aripiprazole differentially affects mesolimbic and nigrostriatal dopaminergic transmission: implications for long-term drug efficacy and low extrapyramidal side-effects

Aripiprazole has been used to effectively treat schizophrenia in the clinic, however, its mechanisms of action are not clear. This study examined how short- and long-term aripiprazole treatment affects dopaminergic transmission in the mesolimbic and nigrostriatal pathways. For comparison, the effects of haloperidol and olanzapine treatment were also examined. Aripiprazole significantly increased D₂ receptor and decreased tyrosine hydroxylase (TH) mRNA expression in the ventral tegmental area (VTA) after 1 week and 12 weeks treatment, but had no effect in the substantia nigra (SN) and nucleus accumbens (Acb). Aripiprazole also decreased dopamine transporter (DAT) binding density in the Acb (after both 1 week and 12 weeks treatment) and VTA (after 1 week treatment). In contrast, haloperidol significantly increased D₂ receptor binding density and decreased DAT binding density in the Acb and caudate putamen (CPu) after both 1 and 12 weeks of treatment, and decreased DAT binding in the VTA after 12 weeks treatment. Olanzapine had less widespread effects, namely an increase in D₂ receptor mRNA in the VTA after 12 weeks treatment and decreased DAT binding in the Acb after 1 week of treatment. These results suggest that aripiprazole has selective effects on the mesolimbic dopaminergic pathway. Selectively reducing dopamine synthesis in the VTA is a possible therapeutic mechanism for the long-term efficacy of aripiprazole in controlling schizophrenia symptoms with reduced extrapyramidal side-effects.

1.5.2 The effects of antipsychotic drugs administration on 5-HT_{1A} receptor expression in the limbic system of the rat brain

Increasing evidence suggests that 5-HT_{1A} receptors are involved in the pathophysiology and treatment of schizophrenia. This study investigated 5-HT_{1A} receptor mRNA expression and binding density in female rats treated with aripiprazole, olanzapine, haloperidol or vehicle for 1 or 12 weeks. This study showed that aripiprazole significantly increased the binding density of 5-HT_{1A} receptors in the CA1 region of the hippocampus, and in the medial posterodorsal nuclei of posterior amygdala (MeP), compared to the control group after 1 week of treatment. Olanzapine significantly decreased the binding density of 5-HT_{1A} receptors in Layers I-IV of the cingulate cortex after 1 week treatment. Neither of these antipsychotic drugs affected 5-HT_{1A} receptor binding density after 12 weeks drug treatment. As expected, haloperidol did not have any significant effect on 5-HT_{1A} binding density after 1 or 12 weeks treatment. Serotonin 5-HT_{1A} receptor mRNA expression was not altered by antipsychotic treatment in any brain region. The results indicate that aripiprazole and olanzapine have differential effects on 5-HT_{1A} protein expression, which may contribute to their distinct profiles in improving negative symptoms and cognitive deficits in schizophrenia. However, aripiprazole and olanzapine may produce adaptation and desensitisation of 5-HT_{1A} receptor expression after long-term treatment.

1.5.3 Effects of antipsychotic medication on muscarinic M₁ receptor mRNA expression in the rat brain

Alterations in muscarinic M₁ receptor binding and mRNA expression have been revealed in the post-mortem brains of schizophrenia patients. As most patients in these studies had been treated with antipsychotics, medication effects cannot be excluded as a possible explanation for the results. Using *in situ* hybridisation technique, this study investigated M₁ receptor mRNA expression in rats treated with the typical antipsychotic haloperidol and the atypical antipsychotics olanzapine and aripiprazole for 1 or 12 weeks. Compared to the control group, haloperidol significantly increased M₁ receptor mRNA expression in the CA1, CA2 and CA3 regions of the hippocampus after both 1 and 12 weeks of treatment, and increased M₁ mRNA expression in the substantia nigra compacta after 1 week of treatment. Olanzapine significantly increased M₁ receptor mRNA expression in the hippocampus (CA1, CA2 and CA3) and substantia nigra compacta after 12 weeks of treatment, but not after 1 week. Aripiprazole significantly increased M₁ receptor mRNA expression in the hippocampus (CA1) after 1 and 12 week treatments, and increased M₁ receptor mRNA expression in the nucleus accumbens after 1 week treatment. Despite their different affinities for muscarinic M₁ receptors, all three antipsychotic medications induced a similar trend of change in M₁ mRNA expression in selected brain regions. These data suggest that the decreased M₁ receptor binding and mRNA expression observed in schizophrenia patients is unlikely to be a consequence of drug treatment and implicates muscarinic M₁ receptors in the pharmacotherapy of the disease.

1.5.4 Short- and long-term effects of antipsychotic drug treatment on weight gain and H₁ receptor expression

The pharmacological mechanisms by which some atypical antipsychotics, such as olanzapine, produce body weight gain/obesity side-effect are not clear. Body weight gain/obesity has an important relationship with energy intake and expenditure. This study investigated body weight gain, food intake, open-field activity, and brain histamine H₁ receptor mRNA and protein expression in rats treated with three antipsychotics for 1 or 12 weeks. Administration of olanzapine for 1 week led to an increase in body weight gain and in fat deposition compared to controls. In the 12 week olanzapine treatment group, accumulative food intake was significantly higher in the first 7 weeks of treatment compared to controls, while body weight gain was significantly greater in the first 8 weeks compared to control. Using *in situ* hybridisation, we found that olanzapine treatment, but not aripiprazole or haloperidol treatment, significantly reduced H₁ receptor mRNA expression in the arcuate hypothalamic nucleus (Arc) and ventromedial hypothalamic nucleus (VMH) compared to controls. Quantitative autoradiography data showed a reduction in VMH H₁ receptor binding density after 1 and 12 weeks olanzapine treatment. There were significant negative correlations between the levels of H₁ receptor mRNA expression, and body weight gain and energy efficiency in the Arc and VMH after 1 and 12 week antipsychotic treatment in all groups. In addition, H₁ receptor mRNA expression in the Arc showed a significant negative correlation with food intake and fat mass in all groups. Furthermore, there were negative correlations between H₁ receptor binding density in the VMH, and total fat mass and body weight gain after 1 week antipsychotic treatment. The

present study suggests that down-regulated VMH and Arc H₁ receptor expression may be a key factor contributing to olanzapine-induced body weight gain/obesity. It implies that new antipsychotic development may need to avoid or eliminate H₁ receptor targeting in the hypothalamus in order to remove/reduce this side-effect risk.

In brief, these results show that antipsychotics achieve their pharmacological effects, and produce side-effects, by altering specific neurotransmitter receptor expression in the brain. The binding profiles of antipsychotics with specific receptors cannot completely predict the properties of antipsychotics. In addition, alterations in some receptor expression (such as 5-HT_{1A} receptor) by antipsychotic treatment may produce adaptation and desensitisation after long-term usage.

Chapter 2. Aripiprazole differentially affects mesolimbic and nigrostriatal dopaminergic transmission: implications for long-term drug efficacy and low extrapyramidal side-effects

Published in *International Journal of Neuropsychopharmacology* (Mei Han, Xu-Feng Huang and Chao Deng “Aripiprazole differentially affects mesolimbic and nigrostriatal dopaminergic transmission: implications for long-term drug efficacy and low extrapyramidal side-effects”, *Journal of Neuropsychopharmacology*, Vol. 12, 2009, 941–952. Cambridge University Press Copyright © 2009 CINP; “Reprinted with permission of Cambridge University Press”).

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Chapter 3. The effects of antipsychotic drugs administration on 5-HT_{1A} receptor expression in the limbic system of the rat brain

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Chapter 4. Effects of antipsychotic medication on muscarinic M₁ receptor mRNA expression in the rat brain

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Chapter 5. Short-and long-term effects of antipsychotic drug treatment on weight gain and H₁ receptor expression

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Chapter 6. Conclusions and recommendations

6.1 Overall conclusion

This study compared the changes in neurotransmitter receptor expression (D_2 , H_1 , $5-HT_{1A}$ and M_1 receptors) in the rat brain following short- and long-term administration of typical (haloperidol) and atypical (olanzapine and aripiprazole) antipsychotic drugs. The results suggest that antipsychotics achieve their pharmacological effects and produce side-effects through alteration in the expression of these specific neurotransmitter receptors in brain regions associated with the neuropathology and treatment of schizophrenia.

An exciting finding was that aripiprazole, unlike other antipsychotics, has selective effects on the mesolimbic, but not nigrostriatal, dopaminergic pathway. Selectively reducing dopamine synthesis in the VTA of the mesolimbic pathway is a possible therapeutic mechanism for the long-term efficacy of aripiprazole in controlling schizophrenia symptoms with reduced EPS. A possible explanation for the mechanisms behind the actions of aripiprazole is that as a D_2 partial agonist, aripiprazole may have more robust actions in dopamine neurons of the mesolimbic pathway than in the nigrostriatal pathway due to the different sensitivity to dopamine (see Chapter 2 for details). Another possible explanation may be due to aripiprazole's functionally selective properties (Urban et al., 2007), meaning that aripiprazole can act as a potent agonist at D_2 receptor-mediated signalling responses in the VTA, but lacks agonist activity on D_2 receptor in the

SN. In addition, this study confirmed previous findings that haloperidol achieves its pharmacological effects, and produces EPS, by blocking D₂ receptors in the NAc and CPu, respectively. Olanzapine did not show significant effects on D₂ receptor expression in the mesolimbic and nigrostriatal dopaminergic pathways. This indicates that olanzapine might achieve its pharmacological effects by acting on other neurotransmitter receptors, such as serotonin 5-HT_{2A} and 5-HT_{2C}, and muscarinic M₁ receptors.

This study indicated that aripiprazole and olanzapine have different effects on 5-HT_{1A} protein expression after short-term (1 week) drug treatment. These effects may be achieved by either directly or indirectly targeting 5-HT_{1A} receptors, which may partially explain why the two atypical antipsychotics can improve the negative symptoms and cognitive function deficits of schizophrenia. However, these effects may induce adaptation and desensitisation after long-term (12 weeks) treatment, which suggests that long-term administration of these antipsychotic drugs is not associated with alterations in receptor binding or mRNA expression, indicating secondary compensation (either at the level of second messenger systems or changes in neurotransmitter levels). Adaptation and desensitisation may also be achieved through compensation by other neurotransmitter pathways such as the dopaminergic, muscarinic or serotonergic systems. In addition, haloperidol did not induce alteration in 5-HT_{1A} receptor expressions in the rat brain. This finding is consistent with the drug's pharmacological profile, as haloperidol shows no significant improvement of the negative symptoms and cognitive function deficits in schizophrenia treatment.

This study showed that olanzapine, aripiprazole and haloperidol increased muscarinic M₁ receptor mRNA expression, but the changes differ in magnitude and brain regions affected. Therefore, it is unlikely that a decrease in M₁ receptor binding density previously identified in schizophrenia patients is a direct consequence of drug treatment (Zavitsanou et al., 2004, Deng et al., 2007, Newell et al., 2007a). Rather, increasing M₁ receptor production (a property shared by the three drugs utilised in the present study) may contribute to their therapeutic efficacy in treating schizophrenia. Furthermore, previous studies suggest that dopamine and muscarinic systems have significant and extensive interactions (Bernard et al., 1992, Gomeza et al., 1999). Of all the antipsychotics used in the present study, only olanzapine has high affinity for the M₁ receptor. Indeed, haloperidol and aripiprazole have a low affinity for the M₁ receptor, however, both drug exhibit a high affinity for the dopamine D₂ receptor. Therefore, it is suggested that antipsychotic-induced up-regulation of muscarinic M₁ mRNA expression is unlikely to be a direct effect of the M₁ antagonistic properties of the drugs, but rather a result of downstream effect, possibly from the dopamine system. In addition, this study showed that aripiprazole, olanzapine and haloperidol up-regulate M₁ receptor mRNA expression in the hippocampus, a pivotal structure of the limbic system. This result indicates that M₁ receptor mRNA up-regulation may be at least partially responsible for improvements in the limbic-related symptoms, such as emotional and cognitive functions, observed in antipsychotic-treated schizophrenia patients.

This study was the first to report an olanzapine-induced down-regulation of the H₁ receptor exclusively in the VMH and Arc of hypothalamus, which may be a key factor contributing to olanzapine-induced body weight gain/obesity. The study revealed that olanzapine-induced weight gain is largely due to an increase in energy intake coupled with a lack of increase in spontaneous locomotor activity. Other factors contributing to the energy balance equation may require further investigation, such as resting metabolic rate and active/willingness activities. The exact mechanism by which olanzapine, as a H₁ receptor antagonist, down-regulates H₁ receptor in the VMH and Arc is not clear. Normally, administration of an antagonist will induce up-regulation of its down-stream receptors (Abbas et al., 2007, Han et al., 2008b). However, recently an exception was seen in a number of G-protein-coupled receptors, such as the serotonin 5-HT_{2A} and 5-HT_{2C} receptors (Huang et al., 2006). Aripiprazole and haloperidol did not affect the expression of the H₁ receptor, which is consistent with their lack of significant affinity for the H₁ receptor (Table 1.2). The present study also confirmed the previous finding that H₁ receptor affinity may predict the body weight gain liability of antipsychotic drugs (Kroeze et al., 2003).

6.2 Recommendations for further research

Based on the findings of this thesis, recommendations for further study are listed below.

- 1) This study showed that aripiprazole, olanzapine and haloperidol have different effects on the mRNA expression of neurotransmitter receptors and binding

densities, including dopamine D₂, serotonin 5-HT_{1A}, histamine H₁ and muscarinic M₁ receptors. Targeted alteration to various neurotransmitter receptors may contribute to the specific pharmacological properties of the drugs. Furthermore, the involvement of other neurotransmission systems, such as glutamate and Gamma-aminobutyric acid (GABA), in the pathology of schizophrenia and its treatments (Newell et al., 2005, Newell et al., 2007c), makes it important to examine the effects of antipsychotics on these neurotransmitter receptors.

2) The present works examined the effects of antipsychotics on neurotransmitter receptors in the healthy female rat, which may not necessarily reflect the clinical scenario in human schizophrenia patients who have the pathophysiological status. Therefore, an important step forward would be to investigate the effects of antipsychotic drugs in animal models of schizophrenia. For example, a perinatal insult to the rat brain by phencyclidine (PCP) treatment can result in behavioural deficits, that persist into adulthood, which are relevant to the symptoms of psychiatric disorders like schizophrenia (du Bois et al., 2008). The PCP rat model is well established in our laboratory. In addition, studies have found some significant changes in neurotransmitter receptors, such as NMDA and muscarinic M₁/M₄ receptors, in PCP-treated rats and mice (Newell et al., 2007b, du Bois et al., 2009a, du Bois et al., 2009b). Similar to the PCP rat/mouse model, neuregulin-1 (NRG-1) mutant mice mimic some aspects of schizophrenia. For example, these mice display late-onset altered locomotor activity, sensory gating deficits and cognitive dysfunction (O'Tuathaigh et al., 2007, Duffy et al., 2008, Tomiyama et al., 2009). The NRG-1 gene has a close regulatory relationship with N-methyl-D-

aspartate (NMDA) receptors, which are important to the neuropathology of schizophrenia (Ozaki et al., 1997). Recently, functional magnetic resonance imaging (fMRI) has shown that schizophrenia patients with NRG-1 mutations have altered brain function in a region-specific manner, particularly in the prefrontal cortex (Kircher et al., 2009). Therefore, it would be of interest to examine the effects of antipsychotics on neurotransmitter receptor expression in the PCP-treated, or NRG-1 mutant mice models. The results from these studies would provide further insight into the mechanisms of antipsychotics in treat schizophrenia.

3) As discussed above, aripiprazole may selectively decrease dopamine synthesis in the VTA but not the SN. However, it is not clear whether the underlying mechanism can be attributed to the D₂ partial agonist property of aripiprazole, or its functional selectivity. Further *in-vivo* studies are justified in order to reveal the selective mechanisms exhibited by aripiprazole, and provide an important guide for developing new, highly targeted antipsychotic drugs.

4) Aripiprazole and olanzapine changed 5-HT_{1A} receptor binding density in a region-specific manner following short-term drug treatment (1 week). Treatment with these antipsychotic drugs may induce adaptation or desensitisation, as the above effects disappeared with long-term drug treatment (12 weeks). However, the mechanism for this adaptation is not clear. Serotonin originates in the raphe nucleus of the midbrain (Barnes and Sharp, 1999), and previous studies have shown that chronic administration of 5-HT_{1A} receptor agonists can induce

desensitisation of these receptors in the median raphe nucleus (Kreiss and Lucki, 1997, Blier and Ward, 2003). It would be valuable to examine whether aripiprazole and olanzapine induce adaptation or desensitisation in 5-HT_{1A} receptors in the raphe nucleus in an *in-vivo* study.

5) The present study indicated that aripiprazole, olanzapine and haloperidol increase muscarinic M₁ receptor mRNA expression in different brain regions. Aripiprazole and haloperidol have no significant affinity to the M₁ receptor, therefore it is possible that their effects are mediated indirectly via other receptor systems. Interestingly, these antipsychotics increase M₁ receptor mRNA expression in the mesolimbic and the nigrostriatal pathways. Previous studies have identified an inverse relationship between the dopaminergic and muscarinic systems (Bernard et al., 1992, Gomeza et al., 1999). Therefore, the exact mechanisms behind the effects of antipsychotics on M₁ receptor mRNA expression need to be further investigated.

6) This study did not observe any effect of antipsychotics on M₁ receptor mRNA expression in the cerebral cortex examined. Previous studies reported inconsistent effects of antipsychotics on M₁ receptor binding densities in this region (Crook et al., 2001, Zavitsanou et al., 2007). Further investigation of the effects of antipsychotics on cortical M1 receptors using functional assay methodology, for example, [³⁵S]GTP autoradiography, may produce a more conclusive result in future studies.

7) This study suggested that the body weight gain/obesity side-effect of olanzapine may be related to a reduction in H₁ receptor expression in the VMH and Arc. These hypothalamic regions include a number of key neurotransmitter receptors that regulate body weight gain and food intake, such as neuropeptide Y (NPY), pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) receptors. The relationship between H₁ receptors and other neurotransmitter receptors in the hypothalamus should be investigated in order to obtain a deeper understanding the involvement of histamine H₁ receptors in weight regulation. In addition, in order to confirm the involvement of H₁ receptors, located in the VMH and Arc, in olanzapine-induced body weight gain/obesity, the following experiments are necessary: (1) the use of an H₁ receptor agonist to treat body weight gain induced by olanzapine in order to test whether with a H₁ agonist it is possible to correct the levels of H₁ receptor expression in the VMH and Arc, (2) to test drug withdrawal effects in order to investigate whether olanzapine-induced body weight gain and H₁ receptor expression levels return to basal levels on cessation of drug treatment.

In conclusion, this study has shown that aripiprazole, olanzapine and haloperidol treatment can alter the expression of several neurotransmitter receptors, including dopamine D₂, serotonin 5-HT_{1A}, muscarinic M₁ and histamine H₁ receptors, in those brain regions that are related to the symptoms of schizophrenia and pharmacological effects and side-effects of antipsychotics. The binding profiles of antipsychotics for specific receptors cannot completely predict the level of their therapeutic efficacies, as there are significant interactions among various

neurotransmission systems in the brain. In addition, the changes in some receptor expression caused by antipsychotic treatment may produce adaptation and desensitisation after long-term use. Finally, this study also has the benefit of assisting in the development of new highly targeted antipsychotic drugs, as well as providing insights for etiological studies of schizophrenia.

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