Reducing olanzapine-induced side effects by betahistine: a study in the rat model

Jaimei Lian

University of Wollongong

2010

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REDUCING OLANZAPINE-INDUCED SIDE EFFECTS
BY BETAHISTINE: A STUDY IN THE RAT MODEL

A thesis submitted in (partial) fulfilment of the
requirements for the award of the degree

MASTER OF SCIENCE - RESEARCH

from

UNIVERSITY OF WOLLONGONG

by

JIAMEI LIAN, MBBS

SCHOOL OF HEALTH SCIENCES

2010
CERTIFICATION

I, Jiamei Lian, declare that this thesis, submitted in partial fulfilment of the requirements for the award of Master of Science by Research, in the School of Health Sciences, University of Wollongong, is entirely my own work unless otherwise referenced or acknowledged. This manuscript document has not been submitted for qualifications at any other academic institution.

Jiamei Lian

August 2010
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I love you all!
PUBLICATIONS

This following publications and presentations have arisen directly or indirectly from the work conducted in this thesis.

Journal Paper


Conference Abstracts


ABSTRACT

Schizophrenia is a devastating mental disorder influencing functions of the central nervous system. Olanzapine, an atypical antipsychotic drug, is extremely efficient in treating both the positive and negative symptoms, as well as the cognitive deficits of schizophrenia. However, it induces some serious side effects, such as body weight gain/obesity, hormonal dysfunction and other metabolic disorders. Since the antagonistic affinities to histamine H₁ receptors of antipsychotic drugs are thought to be one of the main indicators of antipsychotic-induced weight gain/obesity, it is proposed that a histamine H₁ receptor agonist may have potential to reduce antipsychotic-induced weight gain side effects.

This project aimed to investigate whether a combined treatment of betahistine (a histamine H₁ receptor agonist and H₃ receptor antagonist) with olanzapine could improve the body weight/obesity side effects induced by olanzapine in a rat model. Forty-nine female Sprague Dawley rats were divided into four groups and fed orally with olanzapine (3 mg/kg/day) and/or betahistine (8 mg/kg/day), or vehicle for two weeks. Body weight, food intake, water intake and locomotor activity were measured. An intra-peritoneal glucose challenge test was conducted. Blood samples were taken for measuring appetite hormones including plasma insulin and PYY. White and brown fat tissue was collected and weighed. Brain tissue was obtained for histamine H₁ receptor binding experiments in key regions of the brain involved in food intake and body weight regulation.
Rats treated solely with olanzapine exhibited significant body weight gain and increased food intake. However, sole beta histine treatment had no effect on weight gain and food intake. Co-treatment of olanzapine with beta histine significantly reduced (-33%) weight gain and feeding efficiency compared to sole olanzapine treatment. Olanzapine treatment reduced locomotor activity and increased white fat tissue; however beta histine had no influence on these parameters. These results suggested that co-treatment with beta histine partially improves the body weight gain side effect induced by olanzapine.

The hormone measurements in this project revealed that olanzapine tended to decrease fasting plasma insulin levels, and that co-treatment of olanzapine and beta histine can improve insulin levels. No change was detected in plasma PYY levels.

The H₁ receptor binding experiments revealed a tendency for beta histine to increase H₁ receptor binding density in the ventromedial hypothalamic nucleus (VMH), and significantly decreased H₁ receptor binding density in the dorsal vagal complex (DVC), with no significant effect in the arcuate hypothalamic nucleus (Arc). There was no difference in H₁ receptor binding in these nuclei following olanzapine treatment, possibly due to the influence of overnight fasting.

In conclusion, the findings of this project revealed that olanzapine-induced body weight gain could partially be reduced by co-treatment with beta histine, which confirms our hypothesis that use of an H₁ receptor agonist can improve antipsychotic-induced weight gain/obesity side effects. These findings have
important implications for clinical trials involving the use of betahistine to improve olanzapine-induced weight gain/obesity side effects. Furthermore, the finding of this study may aid in the development of a new treatment strategy for schizophrenia patients.
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<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HT</td>
<td>Serotonin</td>
</tr>
<tr>
<td>Ach</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AFI</td>
<td>Accumulated food intake</td>
</tr>
<tr>
<td>AgRP</td>
<td>Agouti-related peptide</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AP</td>
<td>The area postrema</td>
</tr>
<tr>
<td>Arc</td>
<td>Arcuate hypothalamic nucleus</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under curve</td>
</tr>
<tr>
<td>AWI</td>
<td>Accumulated water intake</td>
</tr>
<tr>
<td>BAT</td>
<td>Brown adipose tissue</td>
</tr>
<tr>
<td>BWG</td>
<td>Body weight gain</td>
</tr>
<tr>
<td>CAFE</td>
<td>Comparison of atypicals for first-episode of psychosis</td>
</tr>
<tr>
<td>CART</td>
<td>Cocaine and amphetamine regulated transcript</td>
</tr>
<tr>
<td>CATIE</td>
<td>Clinical antipsychotic trials of intervention effectiveness study</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
</tr>
<tr>
<td>DMV</td>
<td>The dorsal motor nucleus of the vagus</td>
</tr>
<tr>
<td>DSM-IV-TR</td>
<td>Forth version of American psychiatric association’s standardised criteria-diagnostic and statistical manual of mental disorders</td>
</tr>
<tr>
<td>DVC</td>
<td>Dorsal vagal complex</td>
</tr>
<tr>
<td>EPS</td>
<td>Extra pyramidal symptoms</td>
</tr>
<tr>
<td>FBW</td>
<td>Final body weight</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>IBW</td>
<td>Initial body weight</td>
</tr>
<tr>
<td>FMPH</td>
<td>2-(3-trifluoromethylphenyl)histamine</td>
</tr>
<tr>
<td>ICD-10</td>
<td>Tenth edition of the international statistical classification of diseases and related health problems</td>
</tr>
<tr>
<td>MCH</td>
<td>Melanin-concentrating hormone</td>
</tr>
<tr>
<td>MFI</td>
<td>Median fluorescent intensity</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>NA</td>
<td>Noradrenaline</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>NTS</td>
<td>Nucleus of the tractus solitarius</td>
</tr>
<tr>
<td>PIP2</td>
<td>Phospholipase C and the phosphatidylinositol</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomenocortin</td>
</tr>
<tr>
<td>PYY</td>
<td>Peptide YY</td>
</tr>
<tr>
<td>SD</td>
<td>Sprague Dawley</td>
</tr>
<tr>
<td>UCP1</td>
<td>The uncoupling protein 1</td>
</tr>
<tr>
<td>VMH</td>
<td>Ventromedial hypothalamic nucleus</td>
</tr>
<tr>
<td>WAT</td>
<td>White adipose tissue</td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

With our entry into the twenty-first century, schizophrenia remains a dynamic, multidimensional, heterogeneous and genetically complicated disease with no unequivocally established, contributory environmental factors. Although substantial progress has been achieved in both diagnosis and treatment of the disorder and in understanding the disorder’s neurobiological substrates, a full understanding of its origins and pathogenic mechanisms remains elusive. Schizophrenia is characterised by positive and negative symptoms, as well as cognitive deficits. Antipsychotics have been the mainstay treatment of schizophrenia which is broadly classified as typical and atypical antipsychotics. Typical antipsychotics can ameliorate the positive symptoms of schizophrenia, through blockade of the D₂ dopamine receptor. However, D₂ blockade induces extra-pyramidal symptoms such as tardive dyskinesia. The newer generation (atypical antipsychotic) drugs are effective in relieving the positive and negative symptoms, as well as cognitive deficits of schizophrenia, with reduced risk of extra-pyramidal symptoms. However, atypical antipsychotics cause some other side effects, such as body weight gain/obesity and other metabolic symptoms (Nasrallah, 2008). Metabolic dysfunction is a significant issue that needs to be addressed in both scientific and clinical research, as it may lead to further complications such as diabetes, cardiovascular disease, non-compliance with medication, and premature death (by 20-30 years) (Chagnon et al., 2004; Nasrallah,
2008; Patel et al., 2009). This critical need to control antipsychotic-induced obesity and other metabolic side effects has been addressed in this project.

1.2 Schizophrenia

1.2.1 Overview of schizophrenia

Schizophrenia is a severe, chronic and disabling psychotic disorder affecting the function of the central nervous system, and is one of the most costly diseases to patients and their families (Hyman, 2000). The onset of the disorder is normally during adolescence or early adulthood (Laruelle et al., 2003). World-wide, the prevalence of schizophrenia is approximately one in one hundred individuals (McGrath et al., 2003). It is believed that factors such as genetics and environmental vulnerability can affect multiple neurotransmitter systems, such as the dopaminergic, glutamatergic and muscarinic systems, and cause schizophrenia symptoms (Lewis and Lieberman, 2000). However, to date, the exact aetiology of schizophrenia remains unclear.

1.2.2 Symptoms and diagnosis

The symptoms of schizophrenia are generally separated into: (1) Positive symptoms: the presence of abnormal thoughts and behaviour, such as delusions, hallucinations, and disorganised speech, (2) Negative symptoms: apathy, avolition and poverty of speech, and (3) Cognitive dysfunction: deficits in memory, attention and executive function, (Kuperberg and Heckers, 2000). Diagnosis of schizophrenia is currently based on symptoms, history and a few other objective signs. The American Psychiatric Association’s standardised criteria - Diagnostic and Statistical Manual of
Mental Disorders (version DSM-IV-TR), as well as the World Health Organisation’s Tenth Edition of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) are widely applied to diagnose schizophrenia.

1.3 Antipsychotic drugs

In order to relieve schizophrenia symptoms, several modalities of treatment have been used including psychotherapy, electroconvulsive therapy, neurosurgical therapy, as well as pharmacological intervention (Johns and Thompson, 1995; Scott and Dixon, 1995; Matthews and Eljamel, 2003). Pharmacological intervention has remained the most effective in treating schizophrenia. Over the past 60 years, two generations of antipsychotic drugs have been developed to treat schizophrenia and other psychoses. Antipsychotics, although relatively effective at treating psychotic symptoms, can also produce problematic side effects, including Parkinsonian/extrapyramidal symptoms (Goldstein et al., 2000).

1.3.1 Typical antipsychotic drugs

The first generation of antipsychotics, also called ‘typical’ or conventional antipsychotic drugs, are used to treat psychotic disorders, such as schizophrenia and other mental disorders (acute mania, delusional disorders), mostly by blocking dopamine D2 receptors (Fulton and Goa, 1997; Kapur and Remington, 2001). Since the first typical antipsychotic drug, chlorpromazine, was discovered in the 1950s, numerous typical antipsychotic drugs have been developed and clinically applied to treat schizophrenia, including haloperidol, fluphenazine, loxapine, molindone, thioridazine, zuclopenthixol, prochlorperazine, perphenazine and thiothixene.
Typical antipsychotic drugs help to ameliorate the positive symptoms of schizophrenia, and have little effect on the negative symptoms of schizophrenia (Kapur and Mamo, 2003). However, the most serious problems associated with these drugs are extra-pyramidal symptoms (EPS) such as tardive dyskinesia and akathisia, as well as hyperprolactinemia (Purdon et al., 2001; Montejo, 2008). Over the past 40 years, drug development and refinement has continued in order to overcome these side effects and to treat non-responsive schizophrenia patients more effectively. Thus, a new generation of atypical antipsychotic drugs was gradually introduced, called ‘atypical’ antipsychotics.

### 1.3.2 Atypical antipsychotic drugs

Atypical antipsychotic drugs are also known as second generation antipsychotics, and currently form the first line treatment for schizophrenia (Lieberman et al., 2005). The first atypical antipsychotic drug, clozapine, was discovered in the 1950s, and introduced into the clinic in the 1970s. Gradually, other similar atypical antipsychotics were introduced to the market such as olanzapine, aripiprazole, risperidone, quetiapine and amisulpride. Atypical antipsychotic drugs enhance therapeutic actions for treating the positive and negative symptoms, as well as cognitive deficits of schizophrenia by binding to multiple neurotransmitter receptors, including the dopamine, histamine, muscarinic and serotonergic receptors (Fernández-Novoa and Cacabelos, 2001; Kapur and Mamo, 2003; Matsumoto et al., 2005). They have also been increasingly applied to other mental disorders, such as depression, dementia and bipolar disorder (Centorrino et al., 2002; Tuunainen et al., 2002). There is less risk of developing EPS compared to typical antipsychotic drugs,
and this is due to the atypical drugs having a lower affinity for fewer D<sub>2</sub> receptors (Kapur and Mamo, 2003). However, clinical studies have demonstrated that some atypical antipsychotics cause other prominent side effects such as weight gain and obesity, which may lead to medical and social consequences, such as type II diabetes, hypertension, cardiovascular disease, gallbladder disease, osteoarthritis, sleep apnoea and respiratory problems, and even the relapse of psychosis due to non-compliance (Cooper et al., 2005).

1.3.3 How serious is the weight gain side effect caused by atypical antipsychotics?

Clinical studies have indicated that patients gain 4-5 kg of weight during the first 10 weeks of treatment with some atypical antipsychotics, such as olanzapine and clozapine, and may continue to gain weight throughout the treatment period (Allison et al., 1999; Nasrallah, 2008). A recent CAFE (Comparison of Atypicals for First-Episode Psychosis) study reported that after 12 weeks of treatment, significant weight gain (≥ 7% body weight) occurred in a large number of schizophrenia patients treated with olanzapine (59.8%), compared to risperidone (32.5%) and quetiapine (29.2%). Furthermore, the same investigators found that after 52 weeks of treatment, 80% percent of olanzapine-treated patients, compared to 57.6% risperidone and 50% quetiapine-treated patients, gained ≥ 7% body weight (Patel et al., 2009) (Table 1.1).
Table 1.1 The percentage of patients with significant weight gain (≥ 7% body weight) after 12 and 52 weeks treatment with olanzapine, quetiapine and risperidone.

<table>
<thead>
<tr>
<th></th>
<th>Olanzapine</th>
<th>Quetiapine</th>
<th>Risperidone</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 weeks</td>
<td>59.8%</td>
<td>32.5%</td>
<td>29.2%</td>
</tr>
<tr>
<td>52 weeks</td>
<td>88.6%</td>
<td>69.7%</td>
<td>63.3%</td>
</tr>
</tbody>
</table>

(Based on data from Patel et al., 2009)

It has also been demonstrated that antipsychotic-induced weight gain is the main cause of non-compliance and discontinuation of treatment, often resulting in the relapse of psychosis, which can induce further toxicity in the brain (Silverstone et al., 1988; Myers and Rosen, 1999). More importantly, some of these side effects are associated with increased morbidity and mortality, as well as reduced quality of life (Green et al., 2000). Although the underlying mechanisms are unclear, atypical antipsychotic drugs have been found to disrupt glucose metabolism and increase the risk of diabetes mellitus (Starrenburg and Bogers, 2009). It is reported that the prevalence of diabetes mellitus and obesity in schizophrenia patients is 1.5-2.0 times higher than the general population. Atypical antipsychotic treatment has been identified as an additional significant risk factor for metabolic complications (Boehm et al., 2004). Atypical antipsychotics are known to decrease insulin levels following short-term treatment, while long-term treatment may result in insulin resistance and diabetes (Chiu et al., 2006; Haupt, 2006; Chintoh et al., 2008b). It is particularly noted that children and adolescents are more sensitive than adults to atypical antipsychotic-induced weight gain/obesity, and other metabolic side effects (Correll, 2008). Therefore, compared to typical antipsychotics, although atypical antipsychotic
drugs have made greater progress in therapeutic effect and reducing the incidence of EPS, they cause serious body weight gain/obesity side effects, and the mechanisms of these side effects remain largely unknown.

1.4 Progress in the neuropharmacological mechanisms of atypical antipsychotics-induced side effects

Antipsychotics have various affinities of binding with diverse neurotransmitter receptors, which may play a significant role in their therapeutic and side effects (Table 1.2). They are antagonists or agonists of many neurotransmitter receptors such as dopamine D₂, serotonin 5HT₂A, 5HT₂C, adrenergic alpha₁-2, muscarinic M₃ and histamine H₁ receptors (Nelson and Richelson, 1984; Nelson et al., 1987; Asai et al., 1994) (Table 1.2). The potency of blockade on these receptors has been utilised to predict the likelihood of adverse effects and drug interactions in clinical practice (Richelson, 1996). Both typical and atypical antipsychotics bind to dopamine D₂ receptors and block D₂ receptors in the dopamine pathways of the brain. Typical antipsychotic drugs are characterised by their strong antagonistic effect on dopamine receptors, particularly the dopamine D₂ receptor (Nasrallah, 2008). For example, haloperidol acts as a high-affinity antagonist for dopamine D₂, D₃ and D₄ receptors, which has been known to cause extra-pyramidal side effects (Burstein et al., 2005) (Table 1.2). On the other hand, the atypical antipsychotic drugs olanzapine and clozapine, have a lower incidence of extra-pyramidal side effects than typical antipsychotics (e.g. chlorpromazine; haloperidol), as they are less potent antagonists of dopamine D₂ receptors (Schotte et al., 1996). Instead, they encompass a wide range of non-dopaminergic G-protein-coupled targets, including histamine,
muscarinic, serotonin, glutamate and α-adrenergic receptors (Bymaster et al., 1996; Casey, 1997; Hale, 1997; Bymaster et al., 1999; Richelson and Souder, 2000). Several meta-analyses have revealed that antagonistic properties of histamine H₁, 5HT₂C and muscarinic receptors are involved in antipsychotic-induced weight gain/obesity (Table 1.2).

Table 1.2 Weight gain and receptor binding affinities for atypical antipsychotic drugs.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Haloperidol</th>
<th>Clozapine</th>
<th>Olanzapine</th>
<th>Quetiapine</th>
<th>Risperidone</th>
<th>Aripiprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT₂C</td>
<td>10000</td>
<td>17</td>
<td>6.8</td>
<td>2502</td>
<td>35</td>
<td>22.4</td>
</tr>
<tr>
<td>5-HT₂A</td>
<td>53</td>
<td>5.4</td>
<td>2</td>
<td>101</td>
<td>0.17</td>
<td>8.7</td>
</tr>
<tr>
<td>D₂</td>
<td>4</td>
<td>256</td>
<td>34</td>
<td>245</td>
<td>6.5</td>
<td>0.66</td>
</tr>
<tr>
<td>H₁</td>
<td>1800</td>
<td>1.2</td>
<td>2</td>
<td>11</td>
<td>15</td>
<td>29.7</td>
</tr>
<tr>
<td>M₃</td>
<td>10000</td>
<td>25</td>
<td>105</td>
<td>10000</td>
<td>10000</td>
<td>4677</td>
</tr>
<tr>
<td>α₁A</td>
<td>12</td>
<td>1.64</td>
<td>115</td>
<td>22</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>α₂A</td>
<td>1130</td>
<td>142</td>
<td>314.1</td>
<td>3630</td>
<td>150.8</td>
<td>74</td>
</tr>
</tbody>
</table>

| Weight Gain (kg/10wks) | 0.48 | 4.00 | 3.51 | 2.61 | 1.67 | 0.71 |

The receptor affinity values were reported as Ki (nM). 5-HT₂C, serotonin₂C; 5-HT₂A, serotonin₂A; D₂, dopamine₂; H₁, histamine₁; M₃, muscarinic₃. (Data adapted from Allison et al., 1999, Kroeze et al., 2003)

The H₁ receptor antagonist properties have been identified as the main predictor for the development of antipsychotic-induced body weight gain/obesity side effects (approximately Clozapine > Olanzapine > Risperidone > Haloperidol > Ziprasidone >
Aripiprazole) (Kroeze et al., 2003; Matsui-Sakata et al., 2005; Correll, 2008; Lian et al., 2010a). It is very interesting that the olanzapine-induced body weight gain/obesity side effect can be successfully controlled by co-administration with aripiprazole in adult schizophrenia patients, without reducing the original olanzapine doses (Henderson et al., 2009). Aripiprazole was developed as a D2 partial-agonist, 5-HT1A partial-agonist, and also 5-HT2A antagonist (DeLeon et al., 2004). However, aripiprazole has a low histaminergic antagonism and does not affect H1 receptor expression, which may account for the effect of aripiprazole in reducing olanzapine-induced obesity (DeLeon et al., 2004; Han et al., 2008).

1.4.1 Neuro-regulation of food intake and body weight gain

Normally, increased energy intake or decreased energy expenditure is the main reason for body weight gain. Body weight is adjusted via the energy homeostasis system. The nuclei of the hypothalamus, such as the ventromedial hypothalamus (VMH) and arcuate nucleus (Arc) as well as the dorsal vagal complex (DVC), assist in the regulation of food intake and energy expenditure (Hillebrand et al., 2002; Matsui-Sakata et al., 2005). There are two neuronal populations in the Arc of the hypothalamus that release neuropeptides in response to the peripheral appetite and adiposity signals: neurons that express appetite inhibiting cocaine- and amphetamine-related transcript (CART) and pro-opiomelanocortin (POMC), and neurons that express appetite stimulating agouti-related peptide (AgRP) and neuropeptide Y (NPY). These anorexigenic (POMC, CART and CRH) and orexigenic (NPY, AgRP and MCH) neuropeptides are involved in this complicated regulation system. However, to date, less attention has been paid to the brainstem regulation of energy
balance (Schwartz et al., 2000), although some recent studies have revealed the importance of the brainstem in detecting and responding to hunger and satiety (Grill and Kaplan, 2002).

The neurotransmitters serotonin (5HT), noradrenaline (NA), acetylcholine (ACh) and histamine receptors have been implicated in the regulation of energy homeostasis (Dryden et al., 1996; Park et al., 1999; Huang et al., 2006; Deng et al., 2010). In particular, 5-HT2 and H1 receptor antagonists are well documented to increase appetite and obesity development (Tecott et al., 1995; Deng et al., 2010). As described in the Table 1.2, the body weight gain/obesity side effect of atypical antipsychotic drugs is related to its histamine H1 receptor binding affinity (Masaki and Yoshimatsu, 2006).

1.4.2 Histamine neurotransmission and atypical antipsychotic drug-induced body weight gain/obesity

1.4.2.1 Histamine H1 receptor and body weight regulation

Histamine exerts its actions through the four histamine receptors, termed H1, H2, H3 and H4 (Masaki and Yoshimatsu, 2006). All of them are G-protein-coupled receptors and widely expressed in the whole body, including the central nervous system, especially in the hypothalamus, cerebral cortex and limbic system (H1 receptors), hippocampus, amygdale and basal ganglia (H2 receptors), basal ganglia (H3 receptors), and hypothalamus and spinal cord (H4 receptors) (Arrang et al., 1983; Lintunen et al., 1998; Brown et al., 2001; Strakhova et al., 2009; Deng et al., 2010).
This project focused on the histamine H₁ receptor, due to its involvement in regulating food intake. The histamine H₁ receptor is expressed in the central nervous system, smooth muscle, the heart and on vascular endothelial cells. It activates phospholipase C and the phosphatidyl-inositol (PIP2) signalling pathways. The histamine H₁ receptor is expressed on hypothalamic neurons as well as neurons of the dorsal vagal complex (DVC) of the brainstem (Han et al., 2008; Poole et al., 2008), which are involved in the regulation of food intake and energy expenditure (Yoshimatsu et al., 2002; Masaki and Yoshimatsu, 2006; Poole et al., 2008). It has been reported that intracerebroventricular injection of the H₁ receptor agonist, 2-(3-trifluoromethylphenyl)histamine (FMPH) inhibits food intake (Sakata et al., 1997), while H₁ receptor knockout mice develop an obese phenotype (Masaki et al., 2004).

1.4.2.2 Effects of atypical antipsychotics on histamine H₁ receptors and body weight gain

Antipsychotic antagonistic affinity for the H₁ receptor has been significantly correlated with increased body weight, adiposity and insulin-resistance (Erhart et al., 1998; Wirshing et al., 1999; Kroeze et al., 2003; Meltzer et al., 2003; Matsui-Sakata et al., 2005). High histamine H₁ receptor antagonistic affinity is a predictor for the development of antipsychotic-induced weight gain (Stahl et al., 2009), especially short-term weight gain (Table 1.2) (Kroeze et al., 2003). A recent study in our laboratory showed that short (1 week) and long (12 weeks) terms of olanzapine treatment significantly reduced H₁ receptor mRNA expression in the hypothalamic arcuate nucleus (Arc) and ventromedial hypothalamic nucleus (VMH). It is interesting that H₁ receptor mRNA expression in the Arc showed a significant
negative correlation with food intake and fat pad mass (Han et al., 2008). It is reported that olanzapine can directly modulate histaminergic neurotransmission, which is correlated with the regulation of feeding behaviour of rats (Davoodi et al., 2008). Clinical evidence also indicates that the H₁ receptor antagonistic property of antipsychotic drugs may play a role in the weight-gain/obesity side effect (Kroeze et al., 2003). Kim and colleagues (2007) found that olanzapine and clozapine activate hypothalamic AMP-kinase via H₁ receptors to increase food intake and body weight gain (Kim et al., 2007). As previously mentioned, although evidence suggests that the high affinity of olanzapine to H₁ receptors may contribute to its weight gain/obesity, the exact mechanism(s) by which this atypical antipsychotic drug induces these side effects remains largely unanswered.

Figure 1.1 The possible mechanism of Histamine H₁ receptor regulate of food intake (DVC: Dorsal vagal complex). In normal conditions, histamine can activate H₁ receptors on the hypothalamic/DVC neurons, which leads to a decrease in food intake. However, olanzapine blocks histamine H₁ receptors on hypothalamic/DVC neurons causing an increase in food intake.
1.5 Atypical antipsychotic drug—Olanzapine

The atypical antipsychotic drug olanzapine was selected in this project due to its proven efficacy in ameliorating both the positive and negative symptoms of schizophrenia, as well as the cognitive deficits of the disorder, with less EPS, but with a high incidence of body weight gain/obesity side effect (DeLeon et al., 2004; Tohen et al., 2005; Nasrallah, 2008; Patel et al., 2009). The Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study found that olanzapine was the drug with the highest compliance, compared to other typical and atypical antipsychotics (Lieberman et al., 2005).

Like other atypical antipsychotic drugs, olanzapine is less likely to cause extrapyramidal side effects, however its most notorious side effects are weight gain and sedation (Allison et al., 1999). Olanzapine has a higher risk of causing weight gain/obesity than typical antipsychotics such as chlorpromazine and haloperidol, and other atypical antipsychotics like risperidone and aripiprazole, due to its high H1 receptor affinity (Allison et al., 1999; Kroeze et al., 2003; Patel et al., 2009) (Table 1.2). It was reported that olanzapine ranks only after clozapine as having one of the highest incidence of weight gain side effect (Allison et al., 1999; Wirshing et al., 1999; Taylor and McAskill, 2000). Moreover, high dosage of olanzapine-treated schizophrenia patients has been shown to induce higher weight gain than clozapine after one year of treatment (Zipursky et al., 2005). Therefore, it is critical to determine the mechanism of olanzapine-induced body weight gain and whether the H1 receptor agonist could relieve this side effect.
In addition, among the various antipsychotic drugs, olanzapine is one of the atypical antipsychotics with the greatest risk of inducing disturbances in glucose regulation and elevated plasma lipid levels, which together indicate the presence of insulin resistance and/or metabolic disturbance in humans (Allison et al., 1999; Zipursky et al., 2005; Haupt, 2006; Iqbal and Thomas, 2007). Iqbal and colleagues reported that olanzapine-treated patients have the highest incidence of diabetes (0.91%) compared to patients treated with other atypical antipsychotic drugs (Iqbal and Thomas, 2007). These results are crucial for understanding the higher risk of olanzapine in producing hyperglycaemia and diabetes in humans.

1.6 Betahistine

The key issue is how to control the antipsychotic-induced body weight gain side effect. Betahistine (C₈H₁₂N₂) is readily available and has been used to treat more than 100 million patients suffering from vertigo and dizziness in Canada and Europe since the 1970s (Tighilet et al., 2007). Recently, it has also been used as an anti-obesity drug in Europe. Betahistine acts as a modulator of the histaminergic system and has both H₁-agonistic and H₃-antagonistic activity in the brain (Yoshida et al., 2000; Fossati et al., 2001), with a higher affinity for the H₃ receptor than the H₁ (Lecklin et al., 1998). The selective H₁ receptor agonist, 2-(3-trifluoromethylphenyl)histamine (FMPH), is unable to cross the blood brain barrier (Malmberg-Aiello et al., 1998), and there is no other highly selective and orally deliverable H₁ agonist on the market. However, the combined histaminergic H₁/H₃ action of betahistine can reduce satiety and the desire to eat fatty foods (Szelag et al., 2001). In addition, a small clinical trial found that betahistine was able to reduce
olanzapine-induced weight gain (Poyurovsky et al., 2005). Furthermore, betahistine was well tolerated and did not change the therapeutic effect of olanzapine (Poyurovsky et al., 2005). Therefore, in this study, we investigated the effects of betahistine on its ability to attenuate the body weight gain side effect induced by olanzapine.

1.7 Hormonal regulation of food intake and body weight gain and the effect of atypical antipsychotics

Energy homeostasis and body weight are regulated through an interaction between the central nervous system and peripheral appetite signals such as insulin, peptide YY (PYY), leptin and ghrelin (Stanley et al., 2005; Esen-Danaci et al., 2008). This study was focused on insulin and PYY. These hormones stimulate POMC/CART neurons (catabolic pathway) and inhibit NPY/AGRP neurons (anabolic pathway) thus reducing food intake and increase energy expenditure. This mechanism works quite well to address the regulation of energy homeostasis and body weight control.

It is reported that atypical antipsychotic drugs, such as olanzapine, could influence the levels of insulin and PYY. To our knowledge, no research has been conducted to determine the effect of betahistine on correcting the abnormal, olanzapine-induced hormone levels.

The pancreatic hormone insulin is positively correlated with long-term energy homeostasis (Woods, 1974). Some studies reported that acute high doses of olanzapine administration had no significant influence on insulin levels, while others showed decreased plasma insulin levels (Chintoh et al., 2008a; Savoy et al., 2010). In
contrast, enhanced insulin levels were found in a chronic olanzapine administration experiment (Perez-Iglesias et al., 2008). Furthermore, another study demonstrated insulin resistance, indicating a direct effect on abnormal glucose level with chronic olanzapine treatment (Chintoh et al., 2008b). Therefore, although it is known that atypical antipsychotics influence appetite hormonal levels, the role of these hormones in antipsychotic-induced body weight gain is still not clear.

Peripheral administration of PYY\textsubscript{3-36} could decrease food intake and body weight gain in rodents and humans (Batterham et al., 2002; Batterham et al., 2003). Plasma PYY levels in obese humans are less than those in non-obese humans (le Roux et al., 2006). A report from our group has shown that olanzapine administration significantly reduced PYY binding density after two hours of drug treatment (Wang and Huang, 2008). However, the exact effect of olanzapine administration on PYY is unknown.

1.8 Animal models of olanzapine-induced weight gain/obesity

Rat models of olanzapine-induced weight gain are well established. One study showed that olanzapine-induced weight-gain resulted at least in part from increased food intake, reduced gross motor activity and enhanced energy efficiency in female rats (Arjona et al., 2004). Other studies have demonstrated that chronic antipsychotic treatment can increase body weight in female rats, but not in male rats (Baptista et al., 1993; Baptista et al., 1998; Baptista et al., 1999; Pouzet et al., 2003; Arjona et al., 2004). It has also been reported that olanzapine, but not clozapine, can increase body weight in female rats only (Albaugh et al., 2006).
The animal (rat) model of olanzapine-induced weight-gain/obesity has been successfully established in our laboratory (Huang et al., 2006). The development of olanzapine-induced body weight gain/obesity in female rats does show similarities with humans in the clinic population (Figure 1.2) (Zipursky et al., 2005; Huang et al., 2006). Figure 1.2A shows the time course for weight gain after olanzapine treatment in human patients for two years with the greatest increase being observed during the first 20-week period (Zipursky et al., 2005). The development of the olanzapine-induced body weight gain can be divided into three stages: firstly, early acceleration stage; secondly, middle new equilibrium stage; and lastly, heavy weight maintenance stage. The rat model established in our laboratory mimics the development of olanzapine-induced body weight gain in humans. It also shows the three stages of development, and especially, similarly to humans, there was a rapid increase in body weight gain in the first two weeks (early acceleration stage) (Figure 1.2B) (Deng et al., 2007; Han et al., 2008; Weston-Green et al., 2008). Therefore, this project focused on the first two weeks of drug treatment.
Figure 1.2 A: Mean weight change (kg) in olanzapine-treated schizophrenia patients. B: Body weight gain (g) in a rodent model of olanzapine treatment over 36 days compared to controls. (A: Adapted from The British Journal of Psychiatry, 187, Zipursky, RB. et al., Course and predictors of weight gain in people with first-episode psychosis treated with olanzapine or haloperidol, pp. 537-543. © 2005, with permission from The Royal College of Psychiatrists. B: Adapted from Behavioural Brain Research, 171, Huang X-F et al., Olanzapine differentially affects 5-HT$_{2A}$and$_{2C}$ receptor mRNA expression in the rat brain, pp. 355-362. © 2006, with permission from Elsevier.)

1.9 Summary

Although the atypical antipsychotic drug olanzapine is highly effective in treating the multiple domains of schizophrenia and is well-tolerated by patients, it has serious side effects such as body weight-gain/obesity and metabolic side effects. Although the exact mechanisms are still not clear, previous studies have revealed that the H$_1$ receptor antagonism may play a key role in causing these side effects. The question of whether betahistine, a histamine H$_1$ receptor agonist is able to improve olanzapine-induced side effects will be addressed in this study. As noted above, olanzapine-induced weight gain and abnormal appetite hormonal levels have been successfully modelled in female rats. In this project, this animal model was used to investigate the body weight gain/obesity side effect induced by olanzapine.
1.10 Aims

General aim:
As discussed in “Introduction and Literature Review”, olanzapine has the antagonist property of histamine H₁ receptor, and accumulated evidence suggests that the antagonistic properties of this drug at H₁ receptors may contribute to its metabolic side effects. The aim of this study was to investigate whether a combined treatment of H₁ receptor agonist betahistine and antagonist olanzapine could improve/reduce the olanzapine-induced body weight gain/obesity and hormonal deregulation in a rat model.

Specific aims:
1. To determine the effects whether betahistine on improving olanzapine-induced weight gain.
2. To examine whether olanzapine and betahistine could influence H₁ receptor binding density in the brain regions involved in food intake and body weight regulation.
3. To study the effects of olanzapine and betahistine on appetite hormone levels and their relationships with body weight gain and food intake.
1.11 Hypotheses

Since olanzapine is an H₁ receptor antagonist that can increase weight gain/obesity, we hypothesized that:

1. H₁ receptor activation by an agonist could reduce body weight gain caused by olanzapine.

2. Co-treatment of H₁ receptor agonist betahistine and olanzapine may reduce the side effects of olanzapine.
CHAPTER 2
GENERAL METHODS

2.1 Animals and housing

Forty-nine female Sprague–Dawley rats (201-225g) were obtained from the Animal Resources Centre (Perth, WA, Australia). In order to reduce potential stress caused by transportation, rats were housed in pairs for 1-week prior to the start of the studies to allow adaptation to the new environment. They were allowed *ad-libitum* access to water and standard laboratory chow diet (3.9 kcal/g; 10% fat, 74% carbohydrate and 16% protein) throughout the whole experiment. During the experiment, they were housed in individual cages under environmentally controlled conditions (22°C, light cycle from 07:00 to 19:00 and dark cycle from 19:00 to 07:00). Rats were randomly assigned to one control group and three treatment groups, as detailed in Table 2.1. The cages were changed for cleaning once a week, in order to reduce interference with animals and to provide a tidy living environment for the rats.

2.2 Experiment design

As shown in Table 2.1, this study was a 2×2 factorial design experiment to investigate the effects of olanzapine and/or betahistine treatment on body weight gain, food intake and other parameters (see below for details). Therefore, there were four groups in this study, namely, control, olanzapine, betahistine and co-treatment olanzapine with betahistine (n=12/group).
Table 2.1 Outline of the experiment design.

<table>
<thead>
<tr>
<th>Factor 1</th>
<th>Non-olanzapine</th>
<th>Olanzapine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor 2</td>
<td>Non-betahistine</td>
<td>Control ( (n=12) )</td>
</tr>
<tr>
<td></td>
<td><strong>Betahistine</strong></td>
<td>Betaistine ( (n=12) )</td>
</tr>
</tbody>
</table>

*: One rat in the olanzapine group was removed from the experiment, due to a failure to take medication.

2.3 **Drug preparation and treatment**

Prior to drug treatment, rats were trained for drug treatment procedures by feeding them cookie dough without drugs for one-week. In brief, the cookie dough containing cornstarch (30.9%), sucrose (30.9%), gelatine (6.3%), casein (15.5%), fibre (6.4%), minerals (8.4%) and vitamins (1.6%) was prepared. A dry powder of the dough was made before administration. After the one-week training period, animals were administered olanzapine (3 mg/kg/day; Eli Lilly, USA) and/or betahistine (8 mg/kg/day; Manus Aktteva, India) orally by mixing the drugs with cookie-dough pellets (0.3g) for two weeks (Deng et al., 2007; Han et al., 2008; Weston-Green et al., 2008). Controls received an equivalent pellet without the drug. The dosage of olanzapine was based on a previous experiment showing a dose response of weight gain in our laboratory (Weston-Green et al., 2010). Olanzapine of this dosage can significantly increase body weight gain in female rats. The dosage of betahistine was based on a previous study in rats (Szelag et al., 2001). At this dosage, betahistine can effectively decrease body weight gain in obese rats (Szelag et al., 2001) (See more details in Chapter 1).
The 0.3g dry cookie dough containing the planned dosage of olanzapine and/or beta-histidine was achieved by adding a small amount of water and given to rats by a metal spoon 3 times per day (07:00h, 14:00h, and 22:00h; with 8±1 hour interval) for two weeks. Rats were observed daily, making sure there was complete consumption of the medication pellet, as well as no missing water and lab chow. Body Weight, food intake and water intake were measured every two days. An intra-peritoneal glucose challenge test was employed on treatment day 7 (see Chapter 3 for more details). Open-field testing was performed on treatment day 12 (see Chapter 3 for details). All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia (2009).

2.4 Tissue sampling

All rats underwent a drug wash-out period of 24-26 hours and 12-14 hours fasting after the two weeks drug treatment. They were then sacrificed by carbon dioxide asphyxiation. Blood was drawn immediately from the left ventricle of the rats, and was centrifuged. Plasma samples were stored at -20°C for further analysis (see Chapter 4). In order to detect the effects of drug treatments on adiposity, post-mortem white adipose tissue (WAT) and sub-scapular brown adipose tissue (BAT) were dissected and individually weighed (g) using the standard procedures established in our laboratory (Huang et al., 2006; Han et al., 2008) (see Chapter 3). Brain samples were removed and frozen in liquid nitrogen, then stored at -80 °C for autoradiographic analysis (see details in Chapter 5).
2.5 Statistical analysis

A power analysis (at a power of 80%) for determining the animal numbers per group (n=12 rats/group) was completed before the experiment, based on previous experimental data in our laboratory. Statistical analysis was performed using SPSS (Windows version 17.0, SPSS Inc., Chicago). The Kolmogorov-Smirnov test was used to examine the distribution of data from all experiments. Two–way ANOVAs (OLANZAPINE × BETAHISTINE) were used to examine behaviour, fat, receptor binding density and hormone levels. Three-way repeated ANOVAs (OLANZAPINE × BETAHISTINE × DAYS) were applied to examine accumulated weight gain, food and water intake data. Multiple comparisons were performed using post-hoc Dunnett-T tests. Pearson’s correlation test was used to assess the relationships of non-parametric data. All data are expressed as mean ± SEM, and statistical significance was accepted when $p<0.05$. Radioactive ligand binding signals were expressed as fmoles/mg of tissue equivalent (see Chapter 5).
CHAPTER 3

REDUCING OLANZAPINE-INDUCED SIDE EFFECTS USING BETAHISTINE

3.1 Introduction

As reviewed in Chapter 1, in controlled studies atypical antipsychotics have demonstrated comparable or even slightly better global clinical efficacy in the acute treatment of schizophrenia compared to haloperidol, while they perform significantly better than a placebo in reducing positive symptoms (Lieberman et al., 2005; Patel et al., 2009). Olanzapine as an atypical antipsychotic drug has demonstrated an improvement in the domains of positive and negative symptoms secondary to psychosis and certain cognitive symptoms such as attention, executive functioning and working memory of schizophrenia (Lieberman et al., 2005). However, it leads to a common side effect of body weight gain/obesity, which can result in more hazardous complications such as cardiovascular disease and metabolic disorders (Nasrallah, 2003). However, there is currently no effective way to reduce the antipsychotic-induced weight gain/obesity side effect. Olanzapine is a potent histamine H₁ receptor antagonist, and high H₁ receptor affinity is a predictor for the development of the antipsychotic-induced weight gain/obesity side effect. On the other hand, the H₁ receptor agonist, betahistine, acts as a modulator of the histaminergic system and has both H₁-agonistic and H₃-antagonistic activity in the brain (Yoshida et al., 2000; Fossati et al., 2001), which has been shown to be effective in reducing body weight gain in obese rats (Machidori et al., 1992). In this chapter, I examined the effects of adjunctive treatment of betahistine on reducing the
olanzapine-induced body weight gain side effect. It has been suggested that enhanced energy intake and reduced energy expenditure may contribute to a positive energy balance in schizophrenia patients (Eder et al., 2001). Therefore, body weight, food intake and locomotor activity of rats were measured in this experiment.

3.2 Materials and methods

3.2.1 Animals and drug treatment

The procedures of animal housing, drug treatment and histology have been described in Chapter 2. In brief, female Sprague Dawley rats (n=12-13/group, see the reason in Chapter 2) were administered orally with vehicle (control), olanzapine (3 mg/kg/day), betahistine (8 mg/kg/day), or olanzapine combined with betahistine (O+B) three times per day for two weeks.

3.2.2 Body weight, food and water intake

Body weight, food intake and water intake of the rats were measured and recorded once every two days. Feeding efficiency of the rats (body weight gain/food consumed) was also calculated and analysed.

3.2.3 Intra-peritoneal glucose challenge test

The intra-peritoneal glucose challenges were performed on day 7 of the treatments. After 10-12 hours fasting, rats were given an intra-peritoneal glucose injection (1g/kg body weight). Blood samples were collected immediately before glucose injection (0 minute) and at 30, 60, 90 and 120-minutes post-injection for glucose challenge test. The 30-minute time interval was selected according to a previous
report by Albaugh and colleagues (2006), who found a significant difference in glucose only after 30 minutes in rats under similar conditions (Albaugh et al., 2006).

**Method for Blood Sampling:** blood was collected from a wound at the tip of the rat tail for glucose challenge test. Bleeding was stopped using a bandage until the next blood sample was taken (Albaugh et al., 2006). Blood glucose was measured at different time points using Accu-Chek blood glucose monitor (Roche Diagnostics Group, Mannheim, Germany).

### 3.2.4 Open field test

The open field test was performed on day 12 of drug treatment in order to determine whether olanzapine and/or betahistine could influence the locomotor activity of rats, which is related to energy expenditure. A rat was placed in the centre of a black rectangular arena (60×60cm², 40 cm high), which was exposed to an average lighting of 25 lux. A video camera was used to record the behaviour of the rats for 30 minutes from the top of the arena. The locomotor activity of the rats was analysed using EthoVision Color-Pro software (Noldus Information Technology, Wageningen, the Netherlands) (du Bois et al., 2008) (Figure 3.1A). The arena was divided into central and peripheral zones. moved distance (total, central and peripheral; cm), velocity (total, central and peripheral; cm/s), latency to leave the centre of the arena, duration (second), in-zone frequency and rearing frequency were measured in the central and peripheral zones separately (Figure 3.1B). In nine rats (five from sole olanzapine and four from O+B groups), behavioural data were not recorded due to video camera
failure. Therefore, only some of the data from the sole olanzapine \((n=7)\) and \(O+B\) co-treatment \((n=8)\) groups was able to be analysed.

**Figure 3.1** A: Examples of locomotor activities from rats in the four treatment groups. The locomotor activities in the open field test were traced using the Ethovision software. \((O+B):\) co-treatment of olanzapine and betahistine. B: Central and peripheral zones of arena were defined for analysing locomotor activity in the open field test.
3.2.5 Measurements of adipose tissue, body and femur length

After the rats were sacrificed, white adipose tissue (WAT) including perirenal, periovary and inguinal fat, as well as sub-scapular brown adipose tissue (BAT) were dissected and individually weighed (g). Body length and femur length were also measured and recorded to examine the effect of body growth on body weight of rats.

3.2.6 Statistical analysis

Details of statistical analyses are outlined in Chapter 2. In brief, Three way repeated ANOVAs (OLANZAPINE × BETAHISTINE × DAYS as repeated measures) were applied to analyse accumulated body weight gain, food and water intake data. Two-way ANOVAs (OLANZAPINE × BETAHISTINE) was used to analyse glucose level, behaviour and fat data. Multiple comparisons were performed using post-hoc Dunnett-T tests. Pearson’s correlation test was used to examine the relationships among the measurements. All data are expressed as mean ± SEM, and statistical significance was accepted when \( p<0.05 \).

3.3 Results

3.3.1 Body weight

Figure 3.2A presents the development of body weight gain during the two weeks treatment. A three-way repeated ANOVAs (OLANZAPINE × BETAHISTINE × DAYS as repeated measures) observed significant main effects of DAYS \((F_{7,308}=145.679, \ p=0.000)\), OLANZAPINE factor \((F_{1,44}=31.887, \ p=0.000)\), and a tendency to be significant with the BETAHISTINE factor \((F_{1,44}=3.167, \ p=0.082)\) on accumulated body weight gain. There was significant interaction between the
OLANZAPINE factor and DAYS ($F_{7,308}=19.489$, $p=0.000$), between the OLANZAPINE and BETAHISTINE factors ($F_{1,44}=3.958$, $p=0.053$), as well as among all three factors ($F_{7,308}=2.987$, $p=0.005$) (Figure 3.2A).

Post-hoc tests showed that body weight gain during the two week period was significantly greater in the sole olanzapine group ($p=0.000$) compared to controls (Figure 2A). Further analyses revealed that sole olanzapine treatment significantly increased body weight gain, occurring after four days of treatment with olanzapine and lasting for the rest of the treatment period (all $p=0.000$). On the other hand, O+B co-treatment groups had lower body weight gain than the sole olanzapine group (-33%) ($p=0.015$). It is important that, compared to sole olanzapine group, O+B co-treatment significantly decreased body weight gain on treatment day 10 ($p=0.042$) and day 14 ($p=0.015$), and it tended to be significant in reducing body weight gain on day six ($p=0.052$), day nine ($p=0.060$) and day twelve ($p=0.071$) (Figure 3.2A).

The figure 3.2B shows the total body weight gain of the four groups of rats at the end of the drug treatment. It showed a significant increase in total body weight following sole olanzapine treatment (60% increase; $p=0.000$), and a trend to be significant in the O+B co-treatment ($p=0.055$), compared to controls. It also illustrates that the rats in the O+B co-treatment group had a significantly lower body weight gain compared to the sole olanzapine treatment group (33% decrease; $p=0.015$) (Figure 3.2B) (Table 3.1). No significant difference was demonstrated between the controls and sole betahistine-treated rats. Therefore, co-treatment of betahistine was effective to reduce the body weight gain side effect induced by olanzapine.
Figure 3.2 A: Cumulative Body Weight Gain (mean±SEM, g) of female Sprague Dawley rats treated with olanzapine (3 mg/kg/day), betahistine (8 mg/kg/day), co-treatment (O+B) or control (vehicle) for 14 days. B: Total Body Weight Gain (mean±SEM, g) after 14 days of treatment. **p<0.01 vs. control, #p<0.05 vs. olanzapine.
3.3.2 Food intake

When the accumulated food intake was compared, the three-way repeated ANOVAs (OLANZAPINE × BETAHISTINE × DAYS as repeated measures) demonstrated significant main effects of the OLANZAPINE factor ($F_{1,42}=22.668, p=0.000$) and DAYS ($F_{6,252}=4106.666, p=0.001$), as well as a significant interaction between DAYS and the OLANZAPINE factor ($F_{6,252}=21.149, p=0.000$), and between DAYS and the BETAHISTINE factor ($F_{6,252}=2.588, p=0.019$) (Figure 3.3A).

Similar to the accumulated body weight gain, post-hoc analysis revealed that the accumulated food intake of rats significantly increased in the sole olanzapine treatment group ($p=0.001$), compared to controls (Figure 3.3A). Further analysis indicated significantly greater accumulated food intake after four days of sole olanzapine treatment ($p<0.05$).

There was a significant effect of the OLANZAPINE factor on total accumulated food intake ($F_{3,42}=9.244, p=0.000$) (Figure 3.3B). Post-hoc analysis revealed a significant increase in accumulated food intake following sole olanzapine treatment only (+15% increase; $p=0.001$) compared to control (Figure 3.3B), and the O+B co-treatment group tended to be significant ($p=0.085$) (Figure 3.3B) (Table 3.1). Although the rats with O+B co-treatment tended to have lower food intake than the sole olanzapine-treated rats during this period of drug treatment (Figure 3.3A), the differences were not significant.
Figure 3.3 A: Cumulative Food Intake (mean±SEM, g) of female Sprague Dawley rats treated with olanzapine (3 mg/kg/day), betahistine (8 mg/kg/day), co-treatment (O+B) or control (vehicle) for 14 days. B: Total Food Intake (mean±SEM, g) after 14 days of treatment. **p<0.01 vs. control, *p<0.05 vs. control.
3.3.3 Feeding efficiency

There were significant effects of the OLANZAPINE factor \((F_{1,42}=25.379, p=0.000)\) and interaction between the OLANZAPINE and BETAHISTINE factors \((F_{1,42}=8.767, p=0.005)\) on feeding efficiency (grams of accumulated body weight gain/grams of accumulated food intake) (Figure 3.4). Feeding efficiency of rats was significantly increased in the sole olanzapine treated group \((p=0.000)\) and O+B co-treatment group \((p=0.043)\), but not the sole betahistine treatment group \((p>0.05)\), compared to the control group (Figure 3.4). It is important that, compared to the sole olanzapine treatment group, O+B co-treatment significantly reduced feeding efficiency (31% decrease; \(p=0.032\)). In addition, there was a significant difference in the sole olanzapine and sole betahistine treatment groups \((p=0.000)\) (Figure 3.4). Therefore, co-treatment of betahistine was effective in decreasing feeding efficiency compared to the sole olanzapine treatment.

![Figure 3.4](image)

**Figure 3.4** Feeding Efficiency (weight gain/food intake±SEM, g) of female Sprague Dawley rats treated with olanzapine (3 mg/kg/day), betahistine (8 mg/kg/day), co-treatment (O+B) or control (vehicle) for 14 days. **\(p<0.01\) vs. control, #\(p<0.05\) vs. olanzapine.
3.3.4 Water intake

A three-way repeated ANOVAs (OLANZAPINE × BETAHISTINE × DAYS as repeated measures) of accumulated water intake only revealed a significant main effect of DAYS ($F_{6,264}=1415.886$, $p=0.000$), but not the OLANZAPINE factor ($F_{1,44}=0.189$, $p=0.666$) and BETAHISTINE factor ($F_{1,44}=0.562$, $p=0.457$) ($p>0.05$). There was also no interaction between the OLANZAPINE and BETAHISTINE factors ($F_{1,44}=1.547$, $p=0.220$), and among all three factors ($F_{6,264}=1415.886$, $p=0.099$) (Figure 3.5) (Table 3.1). In conclusion, no water intake difference was found among the groups in the two weeks experiment.

![Cumulative Water Intake](image)

**Figure 3.5** Cumulative Water Intake (mean±SEM, ml) of female Sprague Dawley rats treated with olanzapine (3 mg/kg/day), betahistine (8 mg/kg/day), co-treatment (O+B) or control (vehicle) for 14 days.
Table 3.1 Mean body weight, food intake, water intake, fat pad mass (mean ± SEM) in female Sprague Dawley rats treated with olanzapine (3 mg/kg/day) and/or betahistine (8 mg/kg/day) or control (vehicle) for 14 days.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Olanzapine</th>
<th>Betahistine</th>
<th>O+B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBW</td>
<td>219.24 ± 3.43</td>
<td>220.80 ± 3.31</td>
<td>217.51 ± 3.52</td>
<td>218.96 ± 3.42</td>
</tr>
<tr>
<td>FBW</td>
<td>231.52 ± 4.99</td>
<td><strong>250.63 ± 4.29</strong></td>
<td>231.28 ± 3.52</td>
<td>239.13 ± 4.72</td>
</tr>
<tr>
<td>BWG</td>
<td>12.28 ± 2.46</td>
<td><strong>29.82 ± 2.14</strong></td>
<td>13.78 ± 1.47</td>
<td><strong>20.17 ± 2.99</strong></td>
</tr>
<tr>
<td><strong>AFI (g)</strong></td>
<td>286.83 ± 7.29</td>
<td><strong>337.17 ± 7.60</strong></td>
<td>280.15 ± 7.26</td>
<td>314.66 ± 12.30</td>
</tr>
<tr>
<td><strong>Fat Pad Mass (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perirenal</td>
<td>2.26 ± 0.22</td>
<td>3.07 ± 0.32</td>
<td>2.11 ± 0.25</td>
<td>2.90 ± 0.30</td>
</tr>
<tr>
<td>Periovary</td>
<td>2.95 ± 0.33</td>
<td>3.66 ± 0.39</td>
<td>2.44 ± 0.18</td>
<td>3.23 ± 0.26</td>
</tr>
<tr>
<td>Inguinal</td>
<td>2.36 ± 0.17</td>
<td><strong>3.30 ± 0.39</strong></td>
<td>2.05 ± 0.15</td>
<td><strong>3.35 ± 0.29</strong></td>
</tr>
<tr>
<td>Total Intra-abdominal Fat</td>
<td>5.22 ± 0.51</td>
<td>6.73 ± 0.67</td>
<td>4.54 ± 0.40</td>
<td>6.14 ± 0.45</td>
</tr>
<tr>
<td>Total White Fat</td>
<td>7.58 ± 0.61</td>
<td>10.02 ± 1.01</td>
<td>6.60 ± 0.5</td>
<td>9.49 ± 0.70</td>
</tr>
<tr>
<td>Brown Fat</td>
<td>0.22 ± 0.03</td>
<td>0.24 ± 0.02</td>
<td>0.20 ± 0.02</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>Total White Fat / FBW (%)</td>
<td>3.26 ± 0.23</td>
<td>3.99 ± 0.38</td>
<td>2.85 ± 0.21</td>
<td>3.96 ± 0.26</td>
</tr>
<tr>
<td><strong>Body Length (cm)</strong></td>
<td>19.29 ± 0.23</td>
<td>19.16 ± 0.21</td>
<td>19.28 ± 0.20</td>
<td>19.42 ± 0.21</td>
</tr>
<tr>
<td><strong>Femur Length (cm)</strong></td>
<td>4.81 ± 0.06</td>
<td>4.84 ± 0.05</td>
<td>4.86 ± 0.05</td>
<td>4.96 ± 0.07</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 vs. Control, #p<0.05 vs. olanzapine. IBW: initial body weight, FBW: final body weight, BWG: body weight gain, AFI: accumulated food intake, AWI: accumulated water intake.
3.3.5 Fat deposits

3.3.5.1 Individual fat pad mass

The two-way ANOVAs (OLANZAPINE × BETAHISTINE) showed that there were significant effects of the OLANZAPINE factor on the perirenal ($F_{1,44}=8.495$, $p=0.006$), periovary ($F_{1,44}=6.285$, $p=0.016$) and inguinal ($F_{1,44}=17.481$, $p=0.000$) fat, but no difference was found in sub-scapular brown fat mass. However, there was no significant effect of BETAHISTINE on any fat pad mass (Figure 3.6A, B, C & D) (Table 3.1).

Post-hoc analysis revealed a significantly higher inguinal fat mass following sole olanzapine treatment ($p=0.046$) and O+B co-treatment groups ($p=0.031$) compared to control. However, the sole betahistine group was no different from the control group (Figure 3.6C). A significant difference was found between the co-treatment group and sole betahistine treatment rats ($p=0.004$) (Figure 3.6C). With the perirenal and periovary fat, sole olanzapine treatment tended to increase the fat mass of rats more than in the control group (Figure 3.6A & B). O+B co-treatment had less fat than the sole olanzapine group, but this was not significant (Figure 3.6A & B). However, there was no significant difference in sub-scapula brown fat mass between treatment groups and control (Figure 3.6D) (Table 3.1).
Figure 3.6 Fat Mass (mean±SEM, g) of female Sprague Dawley rats treated with olanzapine (3 mg/kg/day), beta histine (8 mg/kg/day), co-treatment (O+B) or control (vehicle) for 14 days of treatment. A: Perirenal Fat, B: Periovary Fat, C: Inguinal Fat, D: Sub-scapular Fat. * $p<0.05$ vs. control.
3.3.5.2 Combined white fat mass

In terms of intra-abdominal fat (the sum of perirenal and periovary) and total white fat mass (the sum of periovary, perirenal and inguinal fat mass), there were significant effects of the OLANZAPINE factor on total intra-abdominal fat ($F_{1,44}=8.944, p=0.005$) and total white fat mass ($F_{1,44}=13.378, p=0.001$) (Figure 3.7A & B) (Table 3.1).

Post-hoc analyses observed that the sole olanzapine group tended to have more total white fat mass than the control group ($p=0.058$). However, the O+B co-treatment group rats had less fat than the sole olanzapine group, but this was not significant (Figure 3.7B). In terms of intra-abdominal fat, the sole olanzapine group had a larger amount of fat than the control group. Similarly, the co-treatment group had a smaller amount of fat than the sole olanzapine group. However, both of them were not significantly different (Figure 3.7A) (Table 3.1).

As a result, both the sole olanzapine and O+B co-treatment rats had more fat than the other two groups. Although the co-treatment group had less fat than the sole olanzapine group, no significance was found.
Figure 3.7 Fat Mass (mean±SEM, g) of female Sprague Dawley rats treated with olanzapine (3 mg/kg/day), beta histine (8 mg/kg/day), co-treatment (O+B) or control (vehicle) for 14 days. A: Intra-abdominal Fat Mass, B: White Fat Mass.
3.3.5.3 Body and femur length

Figure 3.8 illustrates the body length and femur length data of all treatment groups. There were no effects of olanzapine and/or betahistine on the body or femur length ($p=0.893$ and $0.233$, respectively) (Figure 3.8A & B) (Table 3.1). Therefore, there was not any difference in the body development between the groups.

**Figure 3.8** Body and Femur length (mean±SEM, cm) of female Sprague Dawley rats treated with olanzapine (3 mg/kg/day), betahistine (8 mg/kg/day), co-treatment (O+B) or control (vehicle) for 14 days. A: Body Length, B: Femur Length.
3.3.6 Intra-peritoneal glucose tolerance test

The three-way ANOVAs (OLANZAPINE × BETAHISTINE × TIME as repeated measures) showed significant main effects of testing TIME ($F_{4,144}=83.505$, $p=0.000$), and an interaction between testing TIME and OLANZAPINE factor ($F_{2,88}=4.368$, $p=0.002$) on the glucose levels, but no effect of BETAHISTINE and no interaction between BETAHISTINE and OLANZAPINE factors. As presented in the Figure 3.9, there was no significant difference in the fasting glucose (all at ~6 fmole/ml) between groups. The glucose levels reached the maximum at 30 minutes after glucose injection (1g/kg body weight). The glucose levels declined to 6.5 fmole/ml at 120 minutes in all the groups.

Further analysis of the glucose area under curve (AUC) by a two-way ANOVA (OLANZAPINE × BETAHISTINE) showed that there was a tendency towards a significant effect of OLANZAPINE on the AUC ($F_{1,36}=3.611$, $p=0.065$). Since there was no significant effect of BETAHISTINE and no significant interaction between the two factors, data from the two groups with olanzapine treatment (sole olanzapine and O+B co-treatment) were combined to compare with the combined data of the two non-olanzapine (control and sole betahistine) groups. It showed that olanzapine tended to have a significantly lower AUC (848.2±16.9 fmol/ml) than non-olanzapine groups (899.2±20.1 fmole/ml; $t=1.942$, df=38, $p=0.060$) (Figure 3.9).
Figure 3.9 Blood glucose levels (fmole/ml±SEM) of female Sprague Dawley rats treated with olanzapine (3 mg/kg/day), betahistine (8 mg/kg/day), co-treatment (O+B) or control (vehicle) for 14 days.
3.3.7 Open field test

3.3.7.1 Distance moved and velocity

Two-way ANOVAs (OLANZAPINE × BETAHISTINE) exhibited significant effects of the OLANZAPINE factor on distance moved (total: $F_{1,34}=23.284$, $p=0.000$; central: $F_{1,34}=10.037$, $p=0.003$; peripheral: $F_{1,34}=24.383$, $p=0.000$), but no effect of the BETAHISTINE factor. Also, there was a significant interaction between the two factors on distance moved in the peripheral zone ($F_{1,34}=4.582$, $p=0.040$), and a trend for significance on total distance moved ($F_{1,34}=3.636$, $p=0.065$) (Figure 3.10A) (Table 3.2). In terms of the velocity, a significant decrease was found in the OLANZAPINE factor (total: $F_{1,34}=26.032$, $p=0.000$; central: $F_{1,34}=4.462$, $p=0.042$; peripheral: $F_{1,34}=31.010$, $p=0.000$) (Figure 3.10B). In addition, the interactions of the two factors were significant for total and peripheral velocity (total: $F_{1,34}=3.190$, $p=0.083$; peripheral: $F_{1,34}=3.239$, $p=0.081$) (Figure 3.10B) (Table 3.2).

The results showed that the distance moved significantly decreased in the co-treatment group compared to the control (total: -33%, $p=0.003$; central: -55%, $p=0.039$; peripheral: -28%, $p=0.003$). Although the distance moved was decreased in the olanzapine group (total: -19%, central: -39%, peripheral: -16%) compared to the control, there was no significance (Figure 3.10A). Post-hoc analysis displayed significant changes in rats with O+B co-treatment compared to the control group for peripheral velocity (total: -33%, $p=0.002$; peripheral: -33%, $p=0.001$), but there was no significant difference in velocity in the central zone 13% ($p=0.550$) (Figure 3.10B). In terms of the olanzapine group compared to the control group, peripheral velocity was significantly decreased ($p=0.038$), and total velocity tended to be
significant \((p=0.076)\) (Figure 3.10B). Therefore, both the olanzapine and O+B co-
treatment groups displayed less movement and velocity compared to the control, but
no significant difference was found between the olanzapine and control groups.

Figure 3.10 Data of open field test of female Sprague Dawley rats treated with
olanzapine (3 mg/kg/day), beta histidine (8 mg/kg/day), co-treatment (O+B) or control
(vehicle) for 14 days. A: Distance Moved (Total, Central, Peripheral, cm), B:
Velocity (Total, Central, Peripheral, cm/s). *\(p<0.05\) vs. control, **\(p<0.01\) vs. control.
3.3.7.2 In-zone frequency and rearing frequency

OLANZAPINE differed in its effects on the central and peripheral in-zone frequency (central: $F_{1,34}=14.081, p=0.001$; peripheral: $F_{1,34}=14.056, p=0.001$) (Figure 3.11A), and rearing frequency (central: $F_{1,34}=17.382, p=0.000$; peripheral: $F_{1,34}=17.888, p=0.000$) (Figure 3.11B), but there was no effect of BETAHISTINE. Furthermore, no significant interaction was found between the two factors (Figure 3.11) (Table 3.1).

Post-hoc analysis revealed a significant decrease in the rats with O+B co-treatment compared to the control group in in-zone frequency (central: -54%, $p=0.016$; peripheral: -53%, $p=0.016$) and rearing frequency (central: -62%, $p=0.006$; peripheral: -51%, $p=0.019$) (Figure 3.11A & B), and there was no significant difference found between the sole olanzapine and control groups, although sole olanzapine appeared to be lower in Figure 3.11. There was no difference between sole beta-histidine treatment and control (Figure 3.11). Therefore, both olanzapine and O+B co-treatment groups displayed less in-zone and rearing frequency compared to control, but no significance was found between the olanzapine and control groups.
Figure 3.11 Data of open field test of female Sprague Dawley rats treated with olanzapine (3 mg/kg/day), beta histine (8 mg/kg/day), co-treatment (O+B) or control (vehicle) for 14 days. A: In-zone Frequency (Central, Peripheral), B: Rearing Frequency (Central, Peripheral). *p<0.05 vs. control, **p<0.01 vs. control.
Table 3.2  Open field test in female Sprague Dawley rats treated with olanzapine (3 mg/kg/day) and/or betahistine (8 mg/kg/day) or control (vehicle) for 14 days.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Olanzapine</th>
<th>Betahistine</th>
<th>O+B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Distance Moved (cm)</td>
<td>10264.1±645.7</td>
<td>8330.6±721.7</td>
<td>11360.3±573.9</td>
<td>6900.4±651.8**</td>
</tr>
<tr>
<td>Central Distance Moved (cm)</td>
<td>1801.7±329.5</td>
<td>1192.7±255.0</td>
<td>1933.4±215.1</td>
<td><strong>825.0±180.4</strong>*</td>
</tr>
<tr>
<td>Peripheral Distance Moved (cm)</td>
<td>8462.4±367.1</td>
<td>7137.8±536.7</td>
<td>9426.9±413.6</td>
<td><strong>6075.4±589.6</strong>*</td>
</tr>
<tr>
<td>Total Velocity (cm/s)</td>
<td>5.7±0.4</td>
<td>4.5±0.4</td>
<td>6.3±0.3</td>
<td><strong>3.8±0.4</strong></td>
</tr>
<tr>
<td>Central Velocity (cm/s)</td>
<td>10.3±0.9</td>
<td>12.6±0.8</td>
<td>10.3±0.6</td>
<td><strong>11.7±1.1</strong></td>
</tr>
<tr>
<td>Peripheral Velocity (cm/s)</td>
<td>5.3±0.3</td>
<td><strong>4.1±0.3</strong>*</td>
<td>5.9±0.3</td>
<td><strong>3.6±0.4</strong></td>
</tr>
<tr>
<td>Latency of first occurrence</td>
<td>26.3±5.9</td>
<td>18.9±6.3</td>
<td>22.4±6.6</td>
<td><strong>14.7±3.8</strong></td>
</tr>
<tr>
<td>Central Duration (s)</td>
<td>189.0±36.0</td>
<td>99.1±22.7</td>
<td>191.2±19.7</td>
<td><strong>79.3±23.1</strong></td>
</tr>
<tr>
<td>Peripheral Duration (s)</td>
<td>1611.0±36.0</td>
<td>1701.0±22.7</td>
<td>1608.8±19.7</td>
<td><strong>1720.7±23.1</strong></td>
</tr>
<tr>
<td>Central In-zone Frequency</td>
<td>76.6±12.6</td>
<td>54.1±10.6</td>
<td>88.1±6.8</td>
<td><strong>35.1±7.2</strong></td>
</tr>
<tr>
<td>Peripheral In-zone Frequency</td>
<td>77.5±12.5</td>
<td>55.1±10.6</td>
<td>89.0±6.9</td>
<td><strong>36.0±7.2</strong></td>
</tr>
<tr>
<td>Central Rearing Frequency</td>
<td>72.2±12.0</td>
<td>45.9±8.1</td>
<td>81.3±7.8</td>
<td><strong>27.4±5.9</strong></td>
</tr>
<tr>
<td>Peripheral Rearing Frequency</td>
<td>105.3±13.4</td>
<td>70.3±10.7</td>
<td>130.0±14.2</td>
<td><strong>51.3±8.9</strong></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. *p<0.05 compared to control.
3.3.8 Correlations

3.3.8.1 Relationships among body weight, food intake and fat

There was a highly significant correlation between total body weight gain and total food intake ($r=0.702$, $p=0.000$, Figure 3.12A) and feeding efficiency ($r=0.948$, $p=0.000$, Figure 3.12B). Furthermore, intra-abdominal and total fat mass were also significantly correlated with total body weight gain ($r=0.319$, $p=0.027$; $r=0.329$, $p=0.022$, Figure 3.12C & D).
Figure 3.12 The correlations between Total Body Weight Gain (g) and A: Total Food Intake (g), B: Feeding Efficiency, C: Intra-abdominal Fat Mass (g), D: White Fat Mass (g).
3.3.8.2 Correlations among behaviours data and body weight, food intake and fat

In terms of open field testing, total distance moved was negatively correlated with total body weight gain ($r=-0.360$, $p=0.027$) (Figure 3.13A), and total white fat mass ($r=-0.346$, $p=0.024$, Figure 3.13B). Furthermore, velocity was negatively correlated with total body weight gain ($r=-0.383$, $p=0.018$) (Figure 3.13C).
Figure 3.13 The correlations between Total Distance Moved (cm) and A: Total Body Weight Gain (g), B: White Fat Mass (g), C: Correlation between Total Body Weight Gain (g) and Total Velocity (cm/s).
3.4 Discussion

In summary, the results from this chapter have shown that rats treated solely with olanzapine gained three times more body weight than controls. On the other hand, sole betahistine treatment had no effect. Olanzapine-treated rats had higher food intake than controls. The co-treatment of olanzapine and betahistine had lower feeding efficiency than sole olanzapine treatment. The co-treatment of olanzapine and betahistine reduced the body weight gain induced by olanzapine treatment by 50%. Therefore, co-treatment of olanzapine with betahistine can partially reduce the weight gain side effect induced by olanzapine. Rats in both the sole olanzapine and co-treatment groups were less active than rats treated with betahistine and control, which suggests that the lower activity levels contributed partially to the weight-gain induced by olanzapine, however betahistine treatment did not affect locomotor activity.

3.4.1 Body weight gain

Our results show that the body weight of the rats was substantially greater in the sole olanzapine group compared to controls since 4 days after treatment. This result is consistent with previous studies on the olanzapine induced body weight gain side effect in rats and humans (Allison et al., 1999; Goudie et al., 2002; Fell et al., 2004; Cooper et al., 2005; Kalinichev et al., 2005; Albaugh et al., 2006; Huang et al., 2006; Choi et al., 2007; Han et al., 2008; Nasrallah, 2008). A recent study from Han and colleagues showed that one week of olanzapine treatment (1.5mg/kg/day) in female rats led to a threefold increase in body weight, and this significance was maintained up to eight weeks of treatment (Han et al., 2008). In addition, the body weight gain
results in the present study also in agreement with a recent report from our laboratory (Weston-Green et al., 2010), showing that after four days of olanzapine treatment, there is a significant increase in body weight in rats treated with various dosages (1.5, 3, 6mg/kg/day), but not at a low dosage of 0.75 mg/kg/day. In the present study, it was clearly shown that the sole olanzapine group gained three times more body weight compared to the control after two weeks of treatment.

Betahistine has been used to reduce weight gain in obese rats (Szelag et al., 2001). However, in the present study, sole betahistine administration to female rats did not have any effect on body weight during the two weeks of experiments. This is consistent with a study on humans showing that sole betahistine treatment did not increase patients’ body weight while olanzapine did (Poyurovsky et al., 2005). The reasons for this discrepancy are unknown.

This is the first study in rats to show that betahistine plus olanzapine treatment can significantly decrease olanzapine-induced body weight gain from the sixth day of the treatment. Very interestingly, the co-treatment group didn’t completely prevent the body weight gain induced by olanzapine treatment. Compared to the sole olanzapine group, O+B co-treatment significantly decreased body weight, but there was still more weight gain than the control and sole betahistine groups. The results revealed that the combined betahistine and olanzapine treatment can only partially reduce the body weight gain side effect induced by olanzapine. Furthermore, other parameters such as food intake could be used to further explain the above body weight findings. A clinical trial performed by Poyurovsky showed that schizophrenia patients co-
administered with olanzapine (10mg/day) and betahistine (48mg t.i.d.) for six weeks gained less weight than those only treated with olanzapine in schizophrenia patients (Poyurovsky et al., 2005).

There was no difference in body and femur length between the groups in this experiment, demonstrating that none of the drug treatments, including olanzapine and/or betahistine or control, had an influence on animal growth.

### 3.4.2 Food intake and feeding efficiency

Olanzapine treatment increased total food intake after two weeks’ administration in rats. Similar results were observed in human schizophrenia patients (Eder et al., 2001; Meltzer et al., 2003). In rats, olanzapine treatment has been found previously to induce a significant increase in food intake in female rats (Thornton-Jones et al., 2002; Arjona et al., 2004; Albaugh et al., 2006; Huang et al., 2006; Choi et al., 2007; Davoodi et al., 2009; Deng et al., 2010). In this study, a significantly positive correlation of body weight gain and food intake has been found. In terms of feeding efficiency (amount of weight gain divided by the amount of food intake), there was a significant increase in the sole olanzapine group compared with controls. Therefore, data from this study experiment and previous studies all suggest that increased food intake contributed largely to the olanzapine-induced body weight gain.

No significant difference in food intake and feeding efficiency has been observed in the sole betahistine group compared to the control in this study. It is interesting that, similar to the body weight gain data, the amount of food intake in the co-treatment
group was always lower than the sole olanzapine-treated group during the experimental period, but higher than the control and sole beta histine groups. Also of interest is the fact that the feeding efficiency of the co-treatment group was higher than the control group, but significantly lower than the olanzapine group. These results suggest that the ability of beta histine to decrease olanzapine-induced body weight gain is at least partially attributed to reduced food intake and reduced feeding efficiency.

3.4.3 Water intake
Polydipsia (excessive thirst) is a serious complication for schizophrenia patients. Compared with typical antipsychotics, atypical antipsychotics could more or less relieve this syndrome in schizophrenia (Littrell et al., 1997; Kruse et al., 2001; Bersani et al., 2007). However, there was no significant difference in water intake between the groups in the present experiment. This result is consistent with previous studies by our laboratory (Han et al., 2008; Weston-Green et al., 2010) and other laboratories (Fell et al., 2004; Stefanidis et al., 2008).

3.4.4 Intra-peritoneal glucose tolerance test
As discussed in Literature Review, antipsychotic treatments may cause a series of metabolic side effects, such as type 2 diabetes (Newcomer, 2004; Lieberman et al., 2005). The results showed that olanzapine and beta histine treatment did not affect overnight (10-12 hours) fasting glucose levels after seven days treatments. Previously, it has been reported that two weeks treatment of olanzapine reduced the glucose level (Weston-Green et al., 2010). It is possible that a longer treatment
 (>seven days) is needed to have an effect on fasting glucose levels. However, one acute study also showed that one day treatment of olanzapine reduced glucose levels after five hours’ fasting (Albaugh et al., 2006). Therefore the fasting period is also important in detecting changes in fasting glucose. In glucose tolerance tests, olanzapine-treated rats tend to have a lower blood glucose level at 30 and 60 minutes after glucose change, but returned to a normal level at 90 minutes. Taken together, olanzapine treated rats tend to have a lower AUC (Area Under Curve). This result was similar with Albaugh and colleagues’ study which found the glucose levels of olanzapine treated (both acute and two weeks) rats were lower than control animals at all time points (Albaugh et al., 2006). They also found that rats with two weeks’ treatment of olanzapine showed decreased insulin sensitivity, but rats with acute treatment had no changes in insulin sensitivity (Albaugh et al., 2006). Further study is required to examine the time course of insulin sensitivity changes after olanzapine treatment.

3.4.5 Fat pad mass, body and femur length

In order to detect the effects of drug treatment, adiposity, white adipose tissue, including inguinal, perirenal and periovary as well as sub-scapular brown adipose tissue, were collected and individually weighed from rats. The sole olanzapine group was found to have more fat mass than the control and sole betahistine groups. In addition, in the sole olanzapine group, the fat mass increase was observed only in the intra-abdominal white fat (periovary plus perirenal fat) and subcutaneous white fat. These results were consistent with previous studies (Fell et al., 2004; Cooper et al., 2005). Furthermore, an increase in white adiposity was also seen in rodents treated
with other atypical antipsychotic drugs such as clozapine (Fell et al., 2007; Cooper et al., 2008). In the present study, there was a significant positive correlation between total body weight gain and total white fat mass. This suggests that the increased rat fat pad mass was highly associated with the olanzapine-induced body weight gain side effect.

In the present study, sole betahistine treatment had no effect on white fat mass. The co-treatment group had slightly decreased total white fat, but this was not statistically significant, which suggests that betahistine possibly does not have much effect on white fat mass.

Furthermore, olanzapine was found to have no effect on sub-scapular fat mass (brown adipose tissue, BAT). This result is consistent with a previous study in our laboratory (Weston-Green et al., 2010). In contrast to white adipose tissue (WAT), BAT is primarily used for thermo-regulation. Stefanidis and colleagues found that olanzapine-treated female rats continued to increase food intake and body weight, which has a partial relationship with the function of BAT (Stefanidis et al., 2008). Using biotelemetry devices, Stefanidis et al. measured the temperature of BAT, and found that BAT temperature was decreased after the onset of olanzapine treatment in female rats. Following chronic olanzapine treatment, the temperature continually decreased due to reduced physical activity, and therefore UCP1 (Uncoupling protein 1) expression in inter-scapular BAT also declined. The authors argued that decreased BAT temperature may have partially contributed to olanzapine induced body weight gain in rats (Stefanidis et al., 2008). In the present study, only the fat mass, but not
the function/temperature, was measured. It would be interesting to measure the temperature of BAT in further studies. Nevertheless, this is the first study to examine the effect of betahistine on the BAT mass, and the results showed that betahistine did not affect BAT mass.

3.4.6 Open field test

By tracing the locomotor activity of rats in the open field test arena, this study showed that olanzapine but not betahistine reduced animal activities. Compared to the control group, olanzapine treatment exhibited a trend of diminished locomotor activity in rats. Olanzapine reduced both distance moved and velocity. Previous studies revealed a similar result of decreased locomotor activity in animals treated with olanzapine (Arjona et al., 2004; Chintoh et al., 2008b). Recently, colleagues from our laboratory tested the dose dependent effect of olanzapine (using a dosage range of 0.75-6 mg/kg/day) on locomotor activity, and found that the higher the dosage was the stronger the effects on locomotor activity of rats. They found that 3 mg/kg/day significantly decreased activity of the rats (Weston-Green et al., 2010). In the present study, there was only a trend, but no significant decrease in activity in the sole olanzapine group, which was possibly due to a technical reason failure that made some data in the olanzapine treatment group lost. During the open field test, the animal behaviours of nine rats (five from the sole olanzapine group and four from the O+B co-treatment group) were not recorded due to camera failure. Therefore, we only had behavioural data from seven rats in the sole olanzapine group. The sample size may not enough to detect the significance in the sole olanzapine group. The O+B co-treatment group showed significantly decreased activity of rats compared to the
controls, but not the sole olanzapine group. The ANOVA detected an effect of olanzapine treatment, but not betahistine, and no interaction between the two factors. This suggests that the decreased activity in the co-treatment group is due to olanzapine treatment.

It is interesting that there was a negative correlation among locomotor activity, body weight and white fat mass, which suggests that the weight gain/obesity induced by olanzapine treatment is at least partially attributable to a decrease in energy expenditure. In fact, in the clinic, schizophrenia patients treated with olanzapine would lead to less vigorous physical exercise (Suzanne et al., 2007; Treuer et al., 2009) and affect energy expenditure (Allison et al., 1999), which contributed to a high risk of body weight gain/obesity and its sequelae such as metabolic side effects (Green et al., 2000).

In the present study, betahistine had no effect on any of the parameters measured in the open field testing, which suggests that betahistine had no effect on the locomotor activity of rats. However, a previous study revealed that betahistine treatment increased the locomotor activity of rats at a high dose (10 or 25 mg/kg) (Alvarez et al., 1993). Further experiments are needed to reveal what dosage of betahistine is necessary for influencing locomotor activity.

Co-treatment of olanzapine with betahistine significantly decreased the activity of rats (33%) in this study, but co-treatment with betahistine was no different from sole olanzapine treatment. This suggests that reduced locomotor activity by olanzapine
treatment cannot be improved by betahistine. In fact, the co-treatment group had slightly less activity. These results may explain why betahistine can only partially improve olanzapine-induced body weight gain, because it does not affect on locomotor activity, and active energy expenditure.

### 3.5 Summary

In summary, this chapter has shown that olanzapine-treated rats have much higher body weight gain, food intake, and white fat mass, but less locomotor activity than controls. However, sole betahistine treatment has no influence on these parameters. Co-treatment with olanzapine and betahistine partially improved body weight gain side effects. Compared to the sole olanzapine treatment, co-treatment of olanzapine with betahistine (at a dose of 8 mg/kg/day) partially reduced feeding efficiency, but had no effect on locomotor activity, which may explain its partial effect in reducing olanzapine-induced weight gain. Further studies may be conducted to examine whether a long-term experiment (for example five weeks) or a higher dose of betahistine could have a stronger effect on reducing the olanzapine-induced weight gain side effect.
CHAPTER 4

EFFECTS OF OLANZAPINE AND BETAHISTINE TREATMENTS ON PLASMA INSULIN AND PYY IN THE RAT

4.1 Introduction

As reviewed in Chapter 1, appetite hormones such as peptide YY (PYY) and insulin are involved in the regulation of food intake and obesity (Meyer, 2002). Previous studies have shown that antipsychotic drugs (including olanzapine) could influence these hormone levels in schizophrenia patients and in animal models (Lieberman et al., 2005; Jin et al., 2008). For example, although there is evidence that olanzapine induces alterations in PYY binding in regions of the brain such as the medial amygdaloid nucleus and medial geniculate nucleus (Wang and Huang, 2008), the effect of olanzapine on plasma PYY level remains unknown. The reports on the effect of olanzapine on plasma insulin appear inconsistent: chronic olanzapine treatment increased insulin levels, reduced insulin sensitivity and led to insulin resistance (Perez-Iglesias et al., 2008; Wu et al., 2008), however acute treatment of olanzapine decreased plasma insulin levels in rats (Chintoh et al., 2008b). To date, there are no studies investigating the effects of betahistine on hormone levels, and the effect of co-treatment of betahistine with olanzapine on hormonal levels remains unknown. Therefore, the aim of this chapter is to examine the effects of olanzapine and/or betahistine on circulating insulin and PYY.
4.2 Materials and methods

4.2.1 Animals and drug treatment

Details relating to animal housing, drug administration and experimental design have been described in Chapter 2. Briefly, forty-nine female Sprague Dawley rats (n=12-13/group, see the details in Chapter 2) were administered with olanzapine (3 mg/kg/day), betahistine (8 mg/kg/day), co-treatment olanzapine with betahistine (at same dosages as first 2 groups) or vehicle (control), orally 3 times per day for two weeks.

4.2.2 Blood sampling

Rats were sacrificed on day 15 of treatments using CO₂ asphyxiation after 10-12 hours fasting. Blood samples were immediately collected from the left ventricle of the heart. Blood samples were collected in tubes containing EDTA (an anticoagulant), and centrifuged at 3000 × g for 5 min at 4 ºC. Plasma was aliquoted, and stored in -20 ºC freezer for plasma hormonal measurements.

4.2.3 Plasma hormonal measurements

Fasting plasma insulin and PYY levels were measured by immunoassay using the Rat Gut Hormone Panel kit (Milliplex™ Map) from Luminex® (Millipore, Billerica, MA, USA). 50 µl of plasma samples were analyzed using antibody-immobilized beads specific for the hormones insulin and PYY. The hormonal quantification was performed using the Luminex® (Luminex, Austin, TX). The results were based on fluorescent reporter signals. The median fluorescent intensity (MFI) data was
obtained from Luminex® and analyzed using a weighted 5 parameter logistic or spine curve-fitting method for calculating analyte concentrations in samples.

4.2.4 Statistical analysis

The details of statistical analyses are outlined in Chapter 2. In brief, two-way ANOVAs (OLANZAPINE × BETAHISTINE) were used to analyse the hormonal data. Multiple comparisons were examined using independent T tests. Data and figures are presented as mean ± SEM. The $p<0.05$ was accepted as significant value.

4.3 Results

For the fasting plasma insulin level, the two-way ANOVAs (OLANZAPINE × BETAHISTINE) displayed that there was a significant interaction between the OLANZAPINE and BETAHISTINE factors ($F=5.725$, $p=0.022$), but no significant effects of OLANZAPINE and BETAHISTINE factors themselves (both $p>0.05$) (Figure 4.1A). Sole olanzapine tended to decrease the plasma insulin level (olanzapine: 214.40±32.70 pg/ml vs. control: 318.62±42.12 pg/ml; $t=1.929$, df=18, $p=0.070$), however sole betahistine and O+B co-treatment groups showed no significant difference from the control group. Instead, the O+B co-treatment group had significantly higher insulin levels than the sole olanzapine group (O+B: 375.56±67.94 pg/ml vs. olanzapine: 214.40±32.70 pg/ml; $t=-2.161$, df=19, $p=0.044$) (Figure 4.1A).

There were no significant effects of OLANZAPINE ($F_{1,38}=3.055$, $p=0.226$), and no significant interaction between the OLANZAPINE and BETAHISTINE factors
(F_{1,38}=0.078, \ p=0.781). Although there was a tendency for an effect of the BETAHISTINE factor (F_{1,38}=3.055, \ p=0.089), multiple comparisons showed that there was no significant difference between any of these treatments and control groups (Figure 4.1B).

**Figure 4.1** The fasting plasma hormone levels (pg/ml±SEM): A: Plasma Insulin, B: Plasma PYY in female Sprague Dawley rats treated with olanzapine (3 mg/kg/day), betahistine (8 mg/kg/day), O+B co-treatment and control (vehicle) for 14 days. #p<0.05 vs. olanzapine.
4.4 Discussion

As reviewed in Chapter 1, appetite hormones such as peptide YY (PYY) and insulin are associated with obesity, and other metabolic disorders, which could be induced by antipsychotic treatments (Meyer, 2002; Jin et al., 2008). It is suggested that olanzapine could influence these hormonal levels in schizophrenia patients and animals (Lieberman et al., 2005; Jin et al., 2008). However, the effects of betahistine and co-treatment of olanzapine with betahistine on hormonal levels remain unknown.

As the glucose regulation hormone, the level of insulin could be increased after eating. Fasting plasma insulin levels have been shown to be increased in human obesity (Batterham et al., 2003). In the present study, it was revealed that two weeks’ treatment of olanzapine decreased fasting plasma insulin levels compared to the control. This result was similar to another study by colleagues in our laboratory, that found two weeks’ treatment of olanzapine at a dosage ranging from 0.75-6 mg/kg/day decreased fasting insulin levels (Weston-Green et al., 2010). Furthermore, recent human studies also reported that insulin secretion of schizophrenic patients significantly decreased after olanzapine treatment for two weeks (Chiu et al., 2006; Chiu et al., 2010). However, chronic olanzapine treatment increased insulin levels, reduced insulin sensitivity and led to insulin resistance (Perez-Iglesias et al., 2008; Wu et al., 2008). It has been suggested that the effects of olanzapine on insulin secretion is time-dependent (Chiu et al. 2010). Further study is needed to reveal the mechanisms underlying the time-course of insulin changes induced by olanzapine. It is interesting that the O+B co-treatment had a normal insulin level which suggested that betahistine might possibly improve insulin changes caused by olanzapine.
treatment. This finding may be explained by the binding profiles of these drugs, as olanzapine and betahistine are a histamine $H_1$ receptor antagonist and agonist, respectively (Allison et al., 1999; Yoshida et al., 2000), however further studies are necessary.

PYY appears to fulfil the function of inhibiting gastric motility, reducing appetite and suppressing pancreatic secretion (Batterham and Bloom, 2003). The plasma levels of PYY could be increased after food intake or decreased after fasting (Jin et al., 2008). The body weight gain of rodents could be reduced by long-term administration of PYY (Batterham et al., 2003). The PYY is secreted less in obese humans than normal weight humans (Batterham et al., 2003). PYY acts through binding to NPY receptors in the central nervous system to regulate food intake (Schwartz and Moran, 2002; Brain and Cox, 2006). Wang and Huang (2008) reported that PYY receptor binding density in various regions of the rat brain was changed after 36 days’ olanzapine treatments. However, the effect of olanzapine on plasma PYY remains unknown. The present study didn’t show any difference in drug treatments on the level of circulating PYY, which is consistent with a recent report (Weston-Green et al., 2010). A recent clinical study also showed that a one week treatment of olanzapine did not affect the PYY levels in schizophrenia patients (Vidarsdottir et al., 2010). These results suggested that short-term treatment does not influence blood PYY levels, however further studies should investigate whether chronic treatment of olanzapine affects blood PYY levels.
4.5 Summary

In conclusion, this chapter has shown that olanzapine (3 mg/kg/day) tended to decrease fasting plasma insulin level in rats, and that co-treatment with betahistine can improve the insulin level reduced by olanzapine. Olanzapine and/or betahistine treatment had no effect in fasting plasma PYY levels. Further studies are required to investigate the time course of changes in insulin levels induced by olanzapine, as well as the effects of chronic treatment of olanzapine on PYY levels.
CHAPTER 5

EFFECTS OF OLANZAPINE AND BETAHISTINE TREATMENT ON HISTAMINE H₁ RECEPTOR BINDING IN THE RAT BRAIN

5.1 Introduction

As discussed in Chapter 1, histamine H₁ receptors are extensively expressed in the brain including the hypothalamus, which plays a significant role in energy homeostasis. Several meta-analyses have revealed that an antipsychotic’s affinity to the histamine H₁ receptor positively correlated with its ability to cause body weight gain (Kroeze et al., 2003; Matsui-Sakata et al., 2005). Olanzapine is a potent H₁ receptor antagonist, and its high affinity for the H₁ receptor is thought to contribute to its body weight gain side effect (Allison et al., 1999; Han et al., 2008).

It is now clear that the regulation of body weight relates to both hormonal and neural mechanisms, controlling both energy intake and expenditure (see more details in Chapters 1 and 4). The neural mechanisms for the regulation of body weight and energy homeostasis involve the hypothalamus, brainstem and higher brain centres (Schwartz et al., 2000). Hypothalamic nuclei such as the arcuate hypothalamic nucleus (Arc) and the ventromedial hypothalamic nucleus (VMH) play key roles in the regulation of appetite, energy expenditure and body weight (Schwartz et al., 2000; Wynne et al., 2005). Alterations in hypothalamic function can result in metabolic disturbances such as obesity and anorexia (Almeras et al., 2004). For example, olanzapine has been reported to reduce H₁ receptor mRNA expression in the Arc and VMH which correlated with its weight gain side effect (Han et al., 2008). In addition,
the dorsal vagal complex (DVC) of the brainstem plays a role in controlling food intake (Schwartz et al., 2000). Previous studies have reported that olanzapine treatment can modulate the neural transmission of muscarinic M₂ and cannabinoid CB₁ receptors in the DVC (Deng et al., 2007; Weston-Green et al., 2008), however it is not clear whether H₁ receptors in the DVC are affected by olanzapine.

The present study has shown that co-treatment with betahistine can partially reduce olanzapine-induced weight gain (see Chapter 3). As a histamine H₁ agonist, betahistine may compete against olanzapine through H₁ receptors in the hypothalamus and DVC to diminish its weight gain side effect. Therefore, in this chapter, the effects of olanzapine and/or betahistine treatment on H₁ receptor binding in the hypothalamus and DVC, as well as their relationship with weight gain, were investigated.

5.2 Materials and methods

5.2.1 Animals and drug treatment

The details of animal housing, drug administration and experimental design have been presented in Chapter 2. In brief, forty-nine female Sprague Dawley rats (n=12-13/group, see details in Chapter 2) were administered with olanzapine, (3 mg/kg/day), betahistine (8 mg/kg/day), co-treatment olanzapine with betahistine (at same dosages as first two groups) or vehicle (control), orally three times per day for two weeks.
5.2.2 Histology

Brain tissue (collected from the experiment in Chapter 3) was cut at -18 °C into 14 μm coronal sections using a cryostat (Leica CM1850, Leica Microsystems, Germany). The hypothalamus and DVC of the brainstem were obtained based on a standard rat brain atlas (Paxinos and Watson, 1998) (Figure 5.1). These brain regions were selected based on their involvement in the regulation of food intake and body weight. Brain sections were thaw-mounted onto Polysine™ Microscope Slides (Menzel GmbH & Co. KG, Braunschweig, Germany) and stored at -20 °C (Figure 5.1).

![Figure 5.1](image)

**Figure 5.1** The location of brain areas sectioned in this study (Adapted from Paxinos and Watson, 1998).
5.2.3 Brain \( H_1 \) receptor autoradiography

The following \[ ^3H \]pyrilamine autoradiography procedure has been successfully performed in our laboratory and others (Ryu et al., 1995; Han et al., 2008; Hu et al., In press). Brain sections containing the hypothalamus and brainstem were thawed at room temperature, then incubated with 10nM \[ ^3H \]pyrilamine (Specific activity: 25.8 Ci/mMol; PerkinElmer, Boston, MA) in 50 mM sodium potassium phosphate buffer (pH 7.4) for 1-hour at room temperature. Non-specific binding was determined by incubating sequential sections with 10nM \[ ^3H \]pyrilamine incubation buffer, with the addition of 2 \( \mu \)M triprolidine (Sigma Pharmaceuticals, Australia). Slides were washed four times for 2 minutes in ice-cold buffer, dipped in ice-cold distilled water, and then dried under a stream of cool air.

5.2.4 Quantification

Sections were placed in the detection chamber of a Beta-imager™ camera (Version 4, BioSpace, Paris, France) and scanned for 3.5 hours at a high-resolution setting. One slide with a known amount of radioactivity was used as a standard. The level of radioactivity in the brain sections was determined by directly counting the number of \( \beta \)-particles emitted from the tissue, which was performed by analysis of activity in the brain regions of interest using a Beta Vision Plus program (BioSpace). The radioligand binding signal was converted from counts per minute per square millimetre (cpm/mm²) to nanocuries per milligram (nCi/mg) of tissue equivalents (nCi/mg TE) using the previously mentioned standards (Deng et al., 2007). Specific binding was calculated by subtracting non-specific binding from total binding. A set of sections from each animal was stained with 0.5% cresyl violet solution (Nissl
staining) and used for confirmation of anatomical structures. Specific brain regions including the Arc, VMH and DVC were identified by reference to the Nissl-stained sections and a standard rat brain atlas (Paxinos and Watson, 1998). Examples of receptor binding images captured by the Beta-imager™ camera in this study are presented in Figure 5.2.

![Figure 5.2 Examples of histamine H1 receptor binding using a tritium labelled ligand [³H]pyrilamine in the VMH (Ventromedial hypothalamic nucleus) and Arc (Arcuate hypothalamic nucleus) of the hypothalamus and the DVC (Dorsal vagal complex) of the brain stem in the rat brain.](image)

5.2.5 Statistical analysis

The data were analysed using the SPSS program (Version 17.0, SPSS Inc., Chicago). Two-way ANOVAs (OLANZAPINE × BETAHISTINE) were used to analyse histamine H1 receptor binding density in relevant brain regions, including the Arc, VMH and DVC of the rat. The post-hoc Dunnett-T test followed when data was identified as significant. Data are expressed as mean ± SEM. Significance was accepted when $p<0.05$. 

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5.3 Results

VMH Two-way ANOVAs (OLANZAPINE × BETAHISTINE) showed that there was a tendency for BETAHISTINE (F_{1,19}=3.130, p=0.093) to have a significant effect on H₁ receptor binding density in the VMH. Betahistine tended to increase H₁ binding density in this nucleus (Figure 5.3A). However, there was no significant effect of OLANZAPINE (F_{1,19}=0.724, p=0.405) on H₁ receptor binding density in the VMH, and no significant interaction between the two factors (F_{1,19}=0.012, p=0.914). Since there was no significant effect of OLANZAPINE and no significant interaction between the two factors, data from the two groups with betahistine treatment (sole betahistine and O+B co-treatment) were combined to compare with the combined data of the two non-betahistine (control and sole olanzapine) groups. It showed that betahistine tended to have a significantly increase of H₁ binding density in VMH than non-betahistine groups (betahistine: 3.22±0.16 nCi/mg vs. non-betahistine: 2.84±0.12 nCi/mg; t=-1.874, df=21, p=0.075).

ARC No significant effect of any factor in the Arc was found in this experiment (Figure 5.3B).

DVC In the DVC, there was a significant main effect of BETAHISTINE on H₁ receptor binding density (F_{1,17}=4.757, p=0.044) and a significant interaction between BETAHISTINE and OLANZAPINE (F_{1,17}=7.382, p=0.015), however there was no significant effect of OLANZAPINE (F_{1,17}=0.635, p=0.437) on H₁ receptor binding in this region (Figure 5.3C). Post-hoc analysis showed that sole betahistine significantly decreased H₁ receptor binding density in the DVC compared to the controls (p=0.005)
(Figure 5.3C). However, sole olanzapine and the O+B co-treatment showed no significant difference from the control group (both $p>0.05$) (Figure 5.3C).
Figure 5.3 The levels of H₁ receptor binding density (nCi/mg tissue; mean±SEM) in the A: Ventromedial Hypothalamic Nucleus (VMH), B: Arcuate Hypothalamic Nucleus (Arc), and C: Dorsal Vagal Complex (DVC) of rats treated with olanzapine (3 mg/kg/day), betahistine (8 mg/kg/day), co-treatment (O+B) or control (vehicle) for 14 days. * p<0.05 vs. control.
5.4 Discussion

The present study showed that sole betahistine treatment significantly decreases H\textsubscript{1} receptor binding density in the DVC, with a tendency to increase histamine H\textsubscript{1} receptor binding density in the VMH, but with no effect in the Arc. To this author’s knowledge, this is the first study to investigate the effects of betahistine treatment on H\textsubscript{1} receptor binding density in the rat brain. These results suggest a regional difference exists in the effects of betahistine on H\textsubscript{1} receptors in the brain. Brain regional differential effects have also been observed in studies of the effects of other drugs on H\textsubscript{1} receptor binding density. For example, recently, Hu et al. found that 1mg/kg/day simvastatin (a hypolipidemic drug) significantly decreased H\textsubscript{1} receptor binding in the primary motor cortex, VMH, caudate putamen, accumbens core and prefrontal cortex. Simvastatin (10 mg/kg/day) increased H\textsubscript{1} receptor density in the medial amygdaloid nucleus, but had no significant effect in other examined regions (Hu et al., In press). In the present study, a single dosage of 8 mg/kg/day betahistine was used and an important subject of future study would be to identify the regional effect of dosage on H\textsubscript{1} receptor binding density in the brain.

Interestingly, betahistine has both H\textsubscript{1} agonist and H\textsubscript{3} receptor antagonist binding profiles (Yoshida et al., 2000; Fossati et al., 2001). Therefore, it is possible that interactions between H\textsubscript{1} and H\textsubscript{3} receptors differ in various brain regions, which may contribute to the regional difference observed in the present study. Further study is needed to investigate the mechanisms and possible functional relevance regarding the regional differences of betahistine on H\textsubscript{1} receptors.
Unexpectedly, sole olanzapine treatment had no effect on H₁ receptor binding density in the VMH and Arc. Previously, Han et al. (2008) reported that olanzapine treatment for 1 and 12 weeks significantly decreased H₁ receptor mRNA expression in the VMH and Arc, and H₁ receptor binding in the VMH. It is interesting that the current study was not able to replicate the previous report by Han et al (2008). One major difference between this study and that of Han et al. is that the rats were fasted overnight in the present study but not in Han’s experiment. Moreover, the drug wash out time in this study was 24 hours, rather than the 48 hours in Han’s study. The VMH and Arc are brain regions that play key roles in the regulation of food intake (Schwartz et al., 2000). It is possible that overnight fasting induces a stronger hunger signal, which may stimulate H₁ receptor expression in these hypothalamic nuclei, and may therefore make the olanzapine-induced decrease in H₁ receptor binding undetectable. Previously, reports have shown that short-term and chronic treatment of antipsychotic drugs has different effects on other receptor systems, such as serotonin 5-HT₁A and acetylcholine muscarinic receptors (Terry Jr et al., 2006; Han et al., 2008; Han et al., 2009). Therefore, it is also important to examine the effects of olanzapine and/or betahistine on H₁ receptors after chronic treatment.

Further studies are important in order to examine histamine H₁ receptor mRNA expression. Moreover, it may be of benefit to omit overnight fasting prior to obtaining brain tissue which limited suitability of correlative analysis. This alteration of the method used in the present study would demonstrate the effects of olanzapine and betahistine on H₁ receptor expression in a non-fasting state.
5.5 Summary

In summary, the present study identified a trend for betahistine to increase $H_1$ receptor binding density in the VMH and significantly decrease $H_1$ binding in the DVC, which are important regulators of food intake and weight gain. However, this study showed that sole olanzapine treatment had no effect on $H_1$ receptor binding density in the VMH, Arc and DVC, compared to controls. Examination of histamine $H_1$ receptor mRNA expression in the brain regions following betahistine and olanzapine treatment is important for future studies. In addition, a chronic treatment period, non-fasting conditions and different drug dosage could be applied to further investigate the mechanism of betahistine and olanzapine on $H_1$ receptors.
CHAPTER 6
CONCLUSIONS, OVERALL DISCUSSION AND FUTURE DIRECTIONS

6.1 Overall conclusion

Second-generation antipsychotic drugs have improved the treatment of schizophrenia symptoms with fewer extra-pyramidal side effects. However, body weight gain/obesity is a notable side effect of some atypical antipsychotics such as clozapine and olanzapine (Allison et al., 1999; Allison and Casey, 2001). Furthermore, more serious implications such as metabolic cardiovascular disease, patient compliance and morbidity could follow this weight gain/obesity side effect. Although the exact mechanism of atypical antipsychotic drugs, including olanzapine, in inducing body weight gain side effects remains unknown, recent evidence indicates that the binding affinities of these drugs to various neurotransmitter receptors, particularly histamine H₁ receptors, correlate with the weight gain/obesity side effect, and may even be used to predict the likelihood of weight gain (Reynolds, 1997; Kroeze et al., 2003; Matsui-Sakata et al., 2005). A series of experimental studies in animals and clinical trials including behavioural and pharmacological treatments have been applied to treat antipsychotic-induced weight gain/obesity side effects (Greenberg et al., 1999; Allison and Casey, 2001; Khazaal et al., 2007). These trials were not based on H₁ receptor antagonistic binding affinity as a predictor of antipsychotic-induced weight gain/obesity, but were conducted largely according to empirical experience in the clinic.
• **We investigated the effect of betahistine in reducing olanzapine-induced weight gain.** This study investigated the effect of betahistine (an H\textsubscript{1} receptor agonist) in reducing olanzapine (an H\textsubscript{1} receptor antagonist) -induced weight gain in a rat model. Consistent with previous reports from both our laboratory and other laboratories, olanzapine treatment substantially increases the body weight of rats (+60% increase). Although betahistine treatment did not influence body weight or food intake, co-treatment of betahistine with olanzapine partially decreased olanzapine-induced body weight gain (-50% decrease) (see more details in Chapter 3). Furthermore, food intake and feeding efficiency were increased in rats treated with olanzapine, but feeding efficiency was reduced by co-treatment. These results illustrate that olanzapine can induce body weight gain, while co-treatment of olanzapine and betahistine may be used to reduce the olanzapine-induced weight gain.

• **The role of locomotor activity proved important.** Behavioural experiments revealed that locomotor activity was decreased in both sole olanzapine and co-treatment groups. In contrast, sole betahistine treatment had no effect on locomotor activity. There was also no interaction between olanzapine and betahistine. These results suggest that reduced activity partially contributed to the weight gain side effect induced by olanzapine. Co-treatment with betahistine did not improve locomotor activities decreased by olanzapine. This finding may be one possible explanation of why betahistine can only partially improve olanzapine-induced weight gain.
• **The study showed that olanzapine can decrease insulin levels and O+B co-treatment induced higher insulin levels than olanzapine.** This hormone measurement experiment has shown that olanzapine tended to decrease fasting plasma insulin levels in rats. Interestingly, the O+B co-treatment of olanzapine with betahistine can reverse the insulin level decreased by olanzapine. No plasma PYY level change was detected among the different drug groups in this study. Olanzapine and/or betahistine treatment had no effect on fasting plasma PYY levels.

• **The first study to investigate the effects of betahistine treatment on histamine H\textsubscript{1} receptor binding density.** This is the first study to investigate the effects of betahistine treatment on histamine H\textsubscript{1} receptor binding density in the brain. Interestingly, we identified a brain regional response to betahistine treatment. In other words, betahistine tended to increase H\textsubscript{1} receptor binding density in the VMH, while significantly decreasing H\textsubscript{1} receptor binding in the DVC, but had no effect in the Arc. As these regions play significant roles in the regulation of food intake (Schwartz et al., 2000), a regional difference in response to betahistine treatment may be related to the regulation of food intake, although further experiments are necessary to reveal exact functional relevance. It was unexpected that olanzapine lacked effect on H\textsubscript{1} receptor binding density in the regions analysed. As discussed in Chapter 5, compared to a previous report (Han et al. 2008), overnight fasting may affect H\textsubscript{1} receptor expression. Another possible explanation is that olanzapine may affect the binding affinity of H\textsubscript{1} receptors, however this could
not be revealed in an assay of a single concentration (10nM) of 
\[^{3}H\]pyrilamine (Tran et al., 1978). In further experiments to examine the 
effects of olanzapine and/or betahistine on H\(_1\) receptors, an assay using 
multiple concentrations of \[^{3}H\]pyrilamine assay will be important.

### 6.2 General discussion and recommendations for future research

Based on the findings presented in this thesis, recommendations for further research 
are listed below.

- **Need for clinical trials indicated by results in rat model.** The finding that co-
treatment of olanzapine with betahistine can significantly reduce olanzapine-
induced weight gain in the rat model provides an important clinical implication 
for controlling the antipsychotic-induced weight/gain obesity side effect. Based 
on our results, a clinical trial is recommended to test whether co-treatment of 
olanzapine with betahistine is effective in controlling olanzapine-induced weight 
gain in schizophrenia.

- **Extension of co-treatment to other antipsychotics.** Once proven effective in 
olanzapine-treated schizophrenia patients, co-treatment of betahistine could be 
extended to other antipsychotics with notable likelihood of weight gain/obesity. 
As reviewed in Chapter 1, like olanzapine, clozapine, risperidone, and quetiapine 
have strong or moderate weight gain side effects (Patel et al. 2009), which have 
been correlated to their H\(_1\) receptor antagonist affinities (Kroeze et al., 2003; 
Correll, 2008; Lian et al., 2010b). Therefore, it is valuable to trial how effectively
the drug-induced weight gain side effect can be controlled through co-treatment of olanzapine and betahistine.

- **Need for increased physical activity in schizophrenia patients.** Consistent with previous reports in rats (Arjona et al., 2004; Weston-Green et al., 2010), this study showed that reduced behavioural activity partially contributed to olanzapine-induced weight gain. Several clinical studies have found that few schizophrenia patients engage in vigorous physical exercise, particularly those patients under olanzapine administration (Suzanne et al., 2007; Treuer et al., 2009). This reduced activity of schizophrenia patients affects their energy expenditure and contributes to high risks of body weight gain/obesity and other metabolic side effects (Allison et al., 1999; Green et al., 2000). In this study, betahistine did not improve locomotor activity reduced by olanzapine treatment, which may account for the fact that it was only partially effective in improving olanzapine-induced weight gain side effects. These results suggest that additional physical activities are valuable when combining olanzapine therapy with betahistine for a more effective control of weight gain/obesity side effects.

- **Investigation of the time course of changes in insulin levels induced by olanzapine, as well as the effects of chronic treatment of olanzapine on PYY levels, needs to be undertaken.** The result in this experiment was similar to the acute olanzapine treatment studies in which using the same dosage in humans and animals at dosages ranging from 0.75-6 mg/kg/day decreased fasting insulin levels (Chiu et al., 2006; Chiu et al., 2010; Weston-Green et al., 2010).
However, chronic olanzapine treatment increased plasma insulin levels (Perez-Iglesias et al., 2008; Wu et al., 2008). Therefore, further study is necessary to discover the mechanisms of the time-course of insulin changes induced by olanzapine. The O+B co-treatment of olanzapine and betahistine can reverse the insulin decrease induced by olanzapine. Further binding experiments are necessary to detect the cause. Although short-term treatment didn’t show any difference in blood PYY levels, chronic olanzapine treatment requires further investigation.

- **Study of the effects of betahistine on different brain regions needed.** Results of this study have revealed that betahistine affects H₁ receptor binding in a regional difference pattern in the nuclei (DVC, VMH and Arc) relating to the regulation of food intake and body weight. Reports have suggested the involvement of the histamine H₁ receptor in the pathophysiology of schizophrenia (Nakai et al., 1991; Poyurovsky et al., 2005). Therefore, it is also important to investigate whether betahistine has different effects in other brain regions, particularly the areas related to the pathology of schizophrenia, such as the prefrontal cortex and hippocampus (Brown et al., 2001).

- **Other effects of betahistine need to be investigated.** Although this study found that betahistine could partially reduce olanzapine-induced weight gain, the mechanisms underlying this action are not clear, for example, how betahistine interacts with olanzapine in the hypothalamic and brainstem nuclei to regulate food intake. In addition to H₁ receptor binding, we should examine how
betahistine affects mRNA expression of the H₁ receptor. Furthermore, as olanzapine can reduce thermogenesis in rats, contributing to body weight gain (Stefanidis et al., 2008), it is also important to investigate whether betahistine affects thermogenesis.

- **Investigation of different dosages and testing periods may be of value.** In this study, only one dosage of olanzapine (3 mg/kg/day) and betahistine (8 mg/kg/day) were administered over the two weeks experimental period. Since different dosages of drugs or testing periods may induce various effects, further studies involving, other dosages and long-term drug treatment may be of significant value.

- **Injection of FMPH in hypothalamus of rats.** Betahistine exhibits both H₁ agonism and H₃ antagonism properties. However, only its H₁ agonistic effect was studied in this project because no other highly selective and orally deliverable H₁ agonist was available in the Australian market. Another selective H₁ receptor agonist is 2-(3-trifluoromethylphenyl)histamine (FMPH), which is unable to cross the blood brain barrier. Injecting FMPH into the hypothalamus of rats may prove necessary to detect the effect of the H₁ receptor on the key brain region.

**Conclusion**

In conclusion, this project has shown that olanzapine can induce body weight gain in rats, resulting from increased food intake and feeding efficiency, and decreased locomotor activity. Co-treatment of betahistine with olanzapine can partially improve
olanzapine-induced weight gain side effect through reduced feed efficiency and food intake. Although the pharmacological mechanisms still require further investigation, this study confirmed our hypothesis that the use of a H₁ receptor agonist can improve antipsychotic-induced weight gain/obesity side effects. These findings have important implications for clinical trials using betahistine to improve olanzapine induced weight gain/obesity side effects obesity.
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