The effects of antipsychotics on the density of cannabinoid receptors in the dorsal vagal complex of rats: implications for olanzapine-induced weight gain

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Abstract

Some atypical antipsychotics clinically used to treat schizophrenia induce weight gain by unknown mechanisms. The dorsal vagal complex (DVC) of the brainstem and the endogenous cannabinoid system are implicated in the regulation of appetite signalling and food intake. We investigated whether antipsychotic drugs alter cannabinoid receptor-binding density in the DVC. Female Sprague–Dawley rats were treated with olanzapine, haloperidol, aripiprazole or vehicle for 1 wk (short-term) or 12 wk (chronic). Quantitative autoradiographic methods were employed to investigate the binding density of cannabinoid receptors in the DVC using a highly sensitive Beta Imager. Short-term olanzapine induced a significant 39% decrease in cannabinoid receptor binding compared to controls, whilst short-term aripiprazole and haloperidol had no significant effect. Chronic olanzapine treatment induced a significant 46% decrease in cannabinoid receptor binding compared to controls, aripiprazole slightly decreased cannabinoid receptor binding (12%), whilst haloperidol had no effect. Consistent with binding changes, short-term and chronic olanzapine treatment induced significant weight gain, but not aripiprazole or haloperidol. Cannabinoid receptor binding was negatively correlated to weight gain following chronic olanzapine treatment only ($r = 0.83$, $p = 0.01$). In addition, only chronic olanzapine treatment increased food intake. These results show that olanzapine, an antipsychotic with a high risk of weight gain as a side-effect, significantly decreased cannabinoid receptor binding in the DVC, whilst aripiprazole and haloperidol, antipsychotics with a low risk of weight gain had little or no effect on binding. These results suggest that a mechanism for antipsychotic-induced weight gain may be through the modulation of cannabinoid receptors in the DVC.

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Introduction

Antipsychotic drugs such as olanzapine, aripiprazole and haloperidol play a key role in the therapeutic treatment of schizophrenia. The clinical efficacies of the atypical antipsychotics olanzapine and aripiprazole are considered to be superior to conventional antipsychotics such as haloperidol in treating schizophrenia patients that are unresponsive to conventional drug therapy, with reduced risk of extrapyramidal symptoms (Keefe et al., 2006; Naber and Lambert, 2004). However, a significant side-effect of olanzapine is weight gain, which is of social and clinical importance as it may lead to further complications such as cardiovascular disease, diabetes and non-compliance to medication (Chagnon et al., 2004). Although the weight gain side-effect of aripiprazole is controversial, studies have shown that aripiprazole has less risk than olanzapine, but poses a higher risk than haloperidol (Chrzanowski et al., 2006; Naber and Lambert, 2004). Many suggestions have been made to explain the possible mechanisms underlying weight gained during antipsychotic drug-use (Atmaca et al., 2007; Baptista, 1999; Chagnon et al., 2004), however, this question appears to remain largely unanswered.
The dorsal vagal complex (DVC) is involved in gastrointestinal function and responds to hunger and satiety signals influencing energy homeostasis and appetite signalling; imbalances in this system can result in metabolic disturbances such as obesity and metabolic disorders (Blessing, 1997; Orr and Davy, 2005). The DVC comprises the dorsal motor nucleus of the vagus (DMN), nucleus tractus solitarius (NTS) and the area postrema (AP). The NTS and the hypothalamic arcuate nucleus (Arc) receive information about energy status via afferent signals from peripheral gut peptides such as ghrelin and leptin (Orr and Davy, 2005). This signalling results in alterations of neuronal activity in the brainstem and hypothalamus, which stimulate or suppress appetite and feeding accordingly (Orr and Davy, 2005).

The appetite-increasing effect of Cannabis sativa (marijuana) has been observed since AD 300. Administration of the endogenous cannabinoid, anandamide induces overeating in rats (Williams and Kirkham, 1999) and delta(9)-tetrahydrocannabinol (Δ⁹-THC), the main psychoactive component of marijuana, increases food consumption in mice (Wiley et al., 2005). Endogenous cannabinoids exert their effects through G-protein-coupled receptors, termed cannabinoid receptors CB₁ and CB₂, CB₃ receptors and mRNA are widely distributed in the brain, including the DVC of rats and humans (Derbenev et al., 2004; Glass et al., 1997). A study by Miller et al. (2004) found that injections of CP-55940, a cannabinoid receptor agonist, into the hindbrain influenced feeding behaviour by increasing the intake of palatable food in rats. In addition, clinical studies have shown that the CB₁ receptor antagonist, SR141716A (rimonabant hydrochloride; Sanofi Aventis, Paris, France), decreases appetite and body weight (Cota et al., 2003) and CB₁ receptor knockout mice consume less food than their wild-type littermates, even after fasting (Di Marzo et al., 2001).

Despite the awareness of the influence of cannabinoid neurotransmission on appetite signalling and food intake, and the localisation of these receptors in the region of the brainstem involved in appetite signalling, the physiological effects of antipsychotics on cannabinoid neurotransmission in the DVC have not previously been explored. In addition, rimonabant (Acomplia®) has been approved by the European Medicines Agency (EMEA) for the treatment of obesity in patients with risk for Type 2 diabetes or heart disease, and may be a candidate for the treatment of antipsychotic-induced weight gain. However, it is unclear whether an interaction exists between antipsychotics and cannabinoid receptors in the brain centres involved in the regulation of food intake and body weight. Therefore, in the present study, we examined the binding of [³H]CP-55940 in the DVC of rats treated with olanzapine, aripiprazole, haloperidol or vehicle. We hypothesized that the antipsychotic known to clinically induce the most weight gain would induce the most significant change in cannabinoid receptor-binding density in the DVC, whilst the antipsychotic known to induce the lowest weight gain would cause little or no effect on receptor binding density.

**Methods**

**Animals, diet and experimental procedures**

Female Sprague–Dawley rats (226–250 g) were obtained from the Animal Resources Centre (Perth, WA, Australia). They were housed at 22 °C, on a 12-h light–dark cycle (lights on: 06:00 hours), and allowed ad libitum access to water and standard laboratory chow diet (3.9 kcal/g; 10% fat, 74% carbohydrate, 16% protein) throughout the study. Rats were randomly assigned to one of the following treatments: 0.3 mg/kg.d haloperidol (Sigma Aldrich, St Louis, MO, USA; n = 26), 1.5 mg/kg.d olanzapine (Eli Lilly, Indianapolis, IN, USA; n = 25), 2.25 mg/kg.d aripiprazole (Bristol–Myers Squibb, New York, NY, USA; n = 25), or vehicle (control, n = 25). Clinical studies have shown that haloperidol reaches a therapeutic level in the treatment of schizophrenia when 65–80% of D₂ dopamine receptors are occupied in the brain, a level achieved by a dosage 0.3 mg/kg.d in rats (Kapur et al., 2000). In this study, dosages of olanzapine and aripiprazole were determined by comparing their clinical dose to that of haloperidol. Antipsychotics were orally administered via premixed drug-chocolate pellets, three times daily, as previously described by Deng et al. (2007). Weight gain and food intake were measured once weekly. After 7 d treatment, 12 rats from each treatment were sacrificed using carbon dioxide asphyxiation, 48 h after the last drug treatment, to examine short-term treatment effects. Treatment of the remaining rats (n = 13–14 for each treatment) was continued for a total of 12 wk, after which time they were sacrificed to examine chronic treatment effects. Therefore, there were eight treatment groups (4 drug treatments × 2 time-points). Six to eight rats per treatment group were used to examine [³H]CP-55940 binding in the DVC. All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.
Histological procedures

Brain tissue was immediately removed after death and frozen using liquid nitrogen, and then stored at \(-80^\circ C\). Coronal sections were cryostatically cut (14 \(\mu m\)) at \(-18^\circ C\) from the level of Bregma \(-11.00 \text{ mm to } -14.60 \text{ mm}\) (Paxinos and Watson, 1998), thaw-mounted onto Polysine\textsuperscript{TM} Microscope Slides (Menzel GmbH & Co. KG, Braunschweig, Germany) and stored at \(-20^\circ C\).

Cannabinoid receptor binding using \([\text{^3}H]\text{CP-55940}\)

Cannabinoid receptor binding was performed as previously described by our group (Newell et al., 2006). Briefly, slides were air-dried then pre-incubated for 30 min in 50 mM Tris–HCl buffer (pH 7.4) containing 5% bovine serum albumin (BSA) at room temperature. Sections were incubated for 120 min with 10 nM \([\text{^3}H]\text{CP-55940}\) (168 Ci/mmol, PerkinElmer, Boston, MA, USA) in 50 mM Tris–HCl buffer (pH 7.4) containing 5% BSA to determine total binding, and non-specific binding was determined by incubating subsequent sections in 10 nM \([\text{^3}H]\text{CP-55940}\) in the presence of 10 \(\mu M\) CP-55940. Sections were then washed in 50 mM Tris–HCl buffer for 5 min at 4 °C, dipped in cold milliQ H\(_2\)O to remove buffer salts and gently dried in a stream of cool air.

Autoradiography and quantification of cannabinoid receptor binding

Cannabinoid receptor autoradiographic images were captured using a beta image camera (BioSpace, Paris, France), as previously described by Newell et al. (2006). Briefly, the level of radioactivity bound to the brain sections was counted directly from the amount of \(\beta\)-particles emitted from the tissue. Exposure time was 3.5 h at a high-resolution setting. Sections containing known amounts of tritium \([\text{^3}H]\) ligand were used to construct a standard curve. Quantitative analysis of images was performed using \(\beta\)-Image Plus software (version 4, BioSpace). Cannabinoid receptor-binding densities in the DVC and cerebellum were analysed by averaging samples from four Bregma levels: \(-13.24 \text{ mm}, -13.30 \text{ mm}, -13.68 \text{ mm}, -14.60 \text{ mm}\) (Figure 1) (Paxinos and Watson, 1998). Measurements of radioligand binding were converted to fmol/mg tissue equivalent using the aforementioned standards. Specific binding was obtained by subtracting non-specific binding values from total binding values. Anatomical positioning of the DVC was obtained by comparison with a set of corresponding Cresyl Violet-stained slides, assisted by the use of a standard rat brain atlas (Paxinos and Watson, 1998).

Quantitative data were statistically analysed using SPSS (version 13, SPSS, Chicago, IL, USA). Two-way ANOVAs (drug \(\times\) treatment duration) were employed to examine cannabinoid receptor-binding density in the DVC and cerebellum. Multiple comparisons were performed using post-hoc Tukey tests. Pearson’s correlations were used to determine the relationship between cannabinoid receptor binding and food intake and weight gain in overall groups. Spearman’s correlations were employed to examine correlations between specific treatment groups and food intake and weight gain due to the smaller sample size.

Figure 1. \([\text{^3}H]\text{CP-55940}\) binding in the dorsal vagal complex (DVC) of rats treated with vehicle [control (a, b), or olanzapine (c, d)] for 1 wk. (a, c) At the level of Bregma \(-14.60 \text{ mm}\), (b) at the level of Bregma \(-13.30 \text{ mm}\), and (d) at the level of Bregma \(-13.68 \text{ mm}\) (adapted from Paxinos and Watson, 1998).
Results

Figure 1 illustrates examples of $[^3]$H][CP-55940 binding in the brainstem. There was a significant effect of treatment ($F_{3,49}=64.94$, $p=0.00$) but not duration ($F_{1,49}=2.48$, $p=0.12$) on $[^3]$H][CP-55940 binding density in the DVC. In addition, there was no significant interaction between the two factors ($F_{3,48}=2.48$, $p=0.07$). Compared to controls short-term olanzapine treatment induced a significant $-39\%$ decrease ($p=0.00$, Figure 2a) in $[^3]$H][CP-55940 binding density in the DVC, whilst no significant change in $[^3]$H][CP-55940 binding was observed following short-term aripiprazole ($p=0.27$, Figure 2a), or haloperidol treatment.

Figure 2. (a, b) $[^3]$H][CP-55940 binding density (fmol/mg tissue; mean ± S.E.M.) in the dorsal vagal complex of rats following 1 wk (a) or 12 wk (b) treatment with aripiprazole, haloperidol, olanzapine or vehicle (control). (c, d) $[^3]$H][CP-55940 binding density (fmol/mg tissue; mean ± S.E.M.) in the cerebellum of rats following 1 wk (c) or 12 wk (d) treatment with antipsychotic drugs. (e, f) Body weight gain (g, mean ± S.E.M.) in rats following 1 wk (e) or 12 wk (f) treatment with antipsychotic drugs. (g, h) Accumulative food intake (g, mean ± S.E.M.) in rats following 1 wk (g) or 12 wk (h) treatment with antipsychotic drugs. * $0.01 < p < 0.05$ vs. control; ** $p \leq 0.01$ vs. control.
(p=0.31, Figure 2a). Chronic olanzapine treatment induced a significant 46% decrease (p=0.00, Figure 2b) in [\textsuperscript{3}H\textsuperscript{]}CP-55940 binding density in the DVC compared to controls, chronic aripiprazole treatment significantly decreased binding but to a lesser extent than olanzapine (−12%, p=0.01, Figure 2b), whilst chronic haloperidol treatment had no effect on [\textsuperscript{3}H\textsuperscript{]}CP-55940 binding density (p=0.37, Figure 2b). However, there was no significant effect of antipsychotic treatment on [\textsuperscript{3}H\textsuperscript{]}CP-55940 binding in the cerebellum (F\textsubscript{1,48}=0.241, p=0.867) after both short-term and chronic treatment (Figure 2c, d).

A significant effect of antipsychotic treatment on weight gain was observed after 1 wk (F\textsubscript{1,44}=4.07, p=0.01). Consistent with changes in [\textsuperscript{3}H\textsuperscript{]}CP-55940 binding density, short-term olanzapine treatment induced significant weight gain (p=0.01, Figure 2e), but not short-term aripiprazole (p=0.73, Figure 2e) or haloperidol (p=0.59, Figure 2e) treatment compared to controls. However, short-term antipsychotic treatment had no significant effect on food intake compared to controls (all p > 0.05, Figure 2g). There was a significant effect of chronic drug treatment on weight gain (F\textsubscript{3,48}=3.172, p=0.03). Chronic olanzapine treatment induced significant weight gain (p=0.03, Figure 2f), whereas rats treated with chronic aripiprazole or haloperidol showed no significant weight gain compared to controls (aripiprazole vs. control: p=0.87; haloperidol vs. control: p=0.90; Figure 2f). Accordingly, there was a significant negative correlation between weight gain and [\textsuperscript{3}H\textsuperscript{]}CP-55940 binding density in the DVC following chronic olanzapine treatment only (r = −0.83, p=0.01) (Figure 3a). In addition, there was a significant effect of chronic drug treatment on food intake (F\textsubscript{3,48}=8.63, p=0.00). Chronic olanzapine induced a significant increase in food intake in rats (p=0.04, Figure 2h), however, rats chronically treated with aripiprazole or haloperidol did not exhibit increased food intake compared to controls (aripiprazole vs. control: p=0.99; haloperidol vs. control: p=0.09; Figure 2h). There was a significant negative correlation between accumulated food intake and [\textsuperscript{3}H\textsuperscript{]}CP-55940 binding density in the DVC (r = −0.41, p=0.03) following chronic antipsychotic treatment (Figure 3b).

**Discussion**

We investigated the effects of olanzapine, aripiprazole and haloperidol on the binding of [\textsuperscript{3}H\textsuperscript{]}CP-55940 in the DVC of rats, food intake and weight gain. A significant decrease in [\textsuperscript{3}H\textsuperscript{]}CP-55940 binding was observed following short-term and chronic olanzapine treatment compared to controls, whilst only a slight decrease in binding was noted following chronic aripiprazole treatment, and no significant change was apparent following treatment with haloperidol. In addition, although there was a high binding density of [\textsuperscript{3}H\textsuperscript{]}CP-55940 in the cerebellum, binding in this region was not affected by antipsychotic treatment. This result suggests that antipsychotic-induced alterations of cannabinoid receptor binding in the DVC is region-specific. In a similar trend, short-term olanzapine treatment significantly increased weight gain, and chronic olanzapine treatment increased weight gain and food intake. Whilst acute and chronic aripiprazole or haloperidol treatment had no effect on weight gain and food intake compared to controls. These results coincide with the clinical setting, in which patients treated with olanzapine exhibit higher weight gain.
than aripiprazole (Naber and Lambert, 2004) and haloperidol (Zipursky et al., 2005). For example, over an 8-wk period patients treated with olanzapine had an increase in body weight of 20% compared to aripiprazole (Naber and Lambert, 2004), and a 2-yr study by Zipursky et al. (2005) reported that olanzapine treatment induced significantly higher weight gain than haloperidol. Zipursky et al. (2005) also identified that patients treated with olanzapine had a five times greater risk for developing clinically significant weight gain than those treated with haloperidol.

In contrast to the high expression of cannabinoid CB$_2$ receptors in the DVC (Glass et al., 1997), the distribution of CB$_2$ receptors in the brainstem is controversial. Derbenev et al. (2004) reported that CB$_2$ receptor protein was not present in the rat DVC following examinations using both reverse transcription–polymerase chain reaction (RT–PCR) and immunohistochemical methods, however, Van Sickle et al. (2005) identified CB$_2$ receptor mRNA expression and immunoreactivity in the DMN, although at a much lower level. Since [H]$\text{CP-55940}$ has a similar affinity for both CB$_2$ and CB$_3$ receptors (Pertwee, 1999), CB$_2$ receptors cannot be completely excluded in the present study. However, due to the low abundance of CB$_2$ receptors in the DVC, it is probable that [H]$\text{CP-55940}$ binding observed in this study was mostly to the CB$_3$ receptor.

To our knowledge, the present study is the first to observe the effects of antipsychotic drugs on the binding density of cannabinoid receptors in the brainstem and its relationship with weight gain. Previous studies have examined the effects of olanzapine and haloperidol on cannabinoid receptor-binding density in other regions of the brain; the outcome dependent on the region studied. For example, olanzapine and haloperidol had no effect on [H]$\text{CP-55940}$ binding density in the hippocampus, frontal cortex, striatum and nucleus accumbens (Sundram et al., 2005), whilst haloperidol increased cannabinoid receptor mRNA levels in the caudate putamen (Maileux and Vanderhaeghen, 1993), and [H]$\text{CP-55940}$ binding density in the substantia nigra and striatum, but not the globus pallidus (Andersson et al., 2005). However, the DVC differs from these regions as it forms a direct component of the brain–gut axis, receiving signals from important peripheral satiety hormones such as ghrelin, leptin, peptide YY (3–36) and cholecystokinin (Orr and Davy, 2005). The absence of a blood–brain barrier on the medial and lateral aspects of the commissural NTS and the AP of the DVC allows greater access to circulating peptides and expedites appetite-signalling effects (Gross et al., 1990). In addition, neurons of the NTS send projections that converge with neurons derived from the Arc, which is traditionally thought to be the primary site for the regulation of food intake (Sainsbury et al., 2002).

As discussed previously, the appetite-enhancing effects of cannabinoid receptor agonists such as anandamide and $\Delta^9$-THC are well-documented (Miller et al., 2004; Wiley et al., 2005; Williams and Kirkham, 1999). In addition, previous studies show that an increase in endogenous cannabinoids decreases CB$_1$ receptor mRNA (Rubino et al., 1994) and G-protein expression (Rubino et al., 1997) in the rat brain. In the present study, olanzapine treatment significantly induced weight gain and decreased [H]$\text{CP-55940}$ binding. It is possible that antipsychotic treatment induces an increase in endogenous cannabinoid release, which leads to a compensatory decrease in cannabinoid receptor-binding density; the latter of which may be a result of decreased receptor expression and a possible functional decrease in G-protein activation.

Although the antipsychotics used in this study may not possess a profile to act directly on the cannabinoid system (Naber and Lambert, 2004; Richelson and Souder, 2000), they act on multiple neurotransmitter systems and may influence other pathways that can indirectly affect CB$_1$ receptors. For example cannabinoids can presynaptically inhibit glutamatergic and GABAergic neurotransmission to anorexigenic pro-opiomelanocortin (POMC) neurons of the Arc (Nguyen and Wagner, 2007), which may result in positive energy balance. In fact, Ho and colleagues (2007) found a strong correlation between behavioural feeding changes induced by CB$_1$ agonists (such as increasing meal frequency, duration and amount of food consumed) and the inhibition of glutamatergic neurotransmission presynaptically on anorexigenic POMC neurons of the Arc. However, studies by Corchero and colleagues (Corchero et al., 1997, 1999) found that administration of $\Delta^9$-THC increased POMC mRNA expression in the Arc of the rat, and that gonadal steroids could alter the effects of $\Delta^9$-THC on POMC regulation (Corchero et al., 2001). These studies suggest that the cannabinoid system can regulate Arc POMC neuron activation, which influences food intake and weight gain, however, further studies are required to reveal the exact mechanisms.

It is interesting that the NTS is the only nucleus besides the Arc to express POMC (Schwartz et al., 2000), however, the role of POMC neurons in the DVC is unclear. A study by Derbenev and colleagues (2004) found that cannabinoids act on CB$_3$ receptors located on presynaptic terminals to inhibit GABAergic and
binoid and muscarinic $M_2$ and it is possible that an interaction between canna-
binoids may be via the suppression of inhibitory synapses from the NTS on neurons of the DMN, induced by the release of endocannabinoids from the active DMN. Further studies are required to confirm this mechanism.

The regulation of appetite signalling and energy homeostasis is highly complex and involves inter-
actions between a variety of neurotransmitter systems. We previously found that olanzapine treatment decreases the binding density of muscarinic $M_2$ receptors in the DVC (Deng et al., 2007). Muscarinic $M_2$ and cannabinoid $CB_1$ receptors are co-localized in the DVC and it is possible that an interaction may exist between the muscarinic and endocannabinoid system in the regulation of energy balance. In the present study, aripiprazole induced a slight decrease in $[^3H]CP-55940$ binding but had no significant effect on weight gain or food intake, despite results indicating a slight elevation compared to controls. Although there was a significant change in $[^3H]CP-55940$ binding in the DVC, it may not have been sufficient to induce a physiological alteration in body weight and food intake. Contrary to olanzapine, aripiprazole does not act on muscarinic receptors (Naber and Lambert, 2004) and it is possible that an interaction between canna-
binoids and muscarinic $M_2$ receptors is necessary to facilitate weight gain. In addition, olanzapine treatment decreases serotonin receptor mRNA in the rat hypothalamus (Huang et al., 2006) and chronic ad-
ministration of the $CB_1$ receptor antagonist SR141716A results in increased central serotonin release (Darmani et al., 2003). Moreover, serotonin receptors are local-
ized in the DVC (Browning and Travaglì, 2001). Thus, another possible mechanism by which olanzapine may induce weight gain is through the combined modulation of cannabinoid and serotonergic neuro-
transmission systems, whereby these antipsychotics may decrease cannabinoid receptor-binding density in the DVC, resulting in a decrease in serotonin and an increase in food intake.

In conclusion, these results indicate that olanzapine, an antipsychotic with a high risk of weight gain as a side-effect decreased cannabinoid receptors in the DVC, whilst aripiprazole and haloperidol, i.e. drugs with less risk of weight gain had little or no effect on cannabinoid receptors in the DVC. This study supports a role for brainstem cannabinoid receptors in the mechanisms of antipsychotic-induced weight gain.

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Statement of Interest

None.

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