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Abstract

Increasing evidence suggests that 5-HT1A receptors are involved in the pathophysiology and treatment of schizophrenia. This paper investigated 5-HT1A receptor mRNA expression and binding density in female rats treated with aripiprazole (2.25 mg/kg/day), olanzapine (1.5 mg/kg/day), haloperidol (0.3 mg/kg/day) or vehicle (control) orally three times/day for 1 or 12 weeks. Animals were sacrificed 48 h after the last administration. Aripiprazole significantly increased 5-HT1A receptor binding density by 33% in the CA1 region of the hippocampus and by 21% in the medial posterodorsal nuclei of posterior amygdala (MeP) compared to the control group after 1 week of treatment. Olanzapine significantly decreased 5-HT1A receptor binding density by 17–22% in Layers I–IV of the cingulate cortex after 1 week of treatment. Neither of these antipsychotic drugs affected 5-HT1A receptor binding density after 12 weeks drug treatment. As expected, haloperidol treatment did not have any significant effect on 5-HT1A binding density after 1 or 12 weeks of treatment. 5-HT1A receptor mRNA expression was not altered by antipsychotic treatment in any brain region. The results indicate that aripiprazole and olanzapine have differential effects on 5-HT1A receptor expression, which may contribute to their distinct profiles in improving negative symptoms and cognitive deficits in schizophrenia. Aripiprazole and olanzapine may produce adaptation and desensitization of 5-HT1A receptor expression after long term treatment.

Disciplines

Arts and Humanities | Life Sciences | Medicine and Health Sciences | Social and Behavioral Sciences

The effects of antipsychotic drugs administration on 5-HT1A receptor expression in the limbic system of the rat brain

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Abbreviations:

5-HT, serotonin

ANOVA, analysis if variance

ARP, aripiprazole

Cg, cingulate cortex

CA1, hippocampus CA1

HPD, haloperidol

 MeP, medial posterodorsal nuclei of posterior amygdala

OLZ, olanzapine

CONT, control

Abstract

Increasing evidence suggests that 5-HT1A receptors are involved in the pathophysiology and treatment of schizophrenia. This paper investigated 5-HT1A receptor mRNA expression and binding density in female rats treated with aripiprazole (2.25 mg/kg/day), olanzapine (1.5 mg/kg/day), haloperidol (0.3 mg/kg/day) or vehicle (control) orally 3 times/day for 1 or 12 weeks. Animals were sacrificed 48 hours after the last administration. Aripiprazole significantly increased 5-HT1A receptor binding density by 33% in the CA1 region of the hippocampus and by 21% in the medial posterodorsal nuclei of posterior amygdala (MeP) compared to the control group after 1 week of treatment. Olanzapine significantly decreased 5-HT1A receptor binding density by 17-22% in Layers I-IV of the cingulate cortex after 1 week of treatment. Neither of these antipsychotic drugs affected 5-HT1A receptor binding density after 12 weeks drug treatment. As expected, haloperidol treatment did not have any significant effect on 5-HT1A binding density after 1 or 12 weeks of treatment. 5-HT1A receptor mRNA expression was not altered by antipsychotic treatment in any brain region. The results indicate that aripiprazole and olanzapine have differential effects on 5-HT1A expression, which may contribute to their distinct profiles in improving negative symptoms and cognitive deficits in schizophrenia. Aripiprazole and olanzapine may produce adaptation and desensitisation of 5-HT1A receptor expression after long term treatment.

INTRODUCTION

Compared to typical antipsychotics, atypical antipsychotic drugs such as olanzapine and aripiprazole are superior in ameliorating negative symptoms and cognitive function deficits in schizophrenia patients (Kapur and Mamo, 2003, Keefe et al., 2006). It has been proposed that the atypical properties of antipsychotics are mainly achieved through high serotonin 5-HT2A receptor and weak D2 receptor blockade (Meltzer, 1999). However, 5-HT2A antagonism alone does not appear to be effective, as the 5-HT2A antagonist ritanserin showed no effect on symptom improvement of schizophrenia (Bantick et al., 2001). Research into the mechanisms of atypical antipsychotic drugs has previously focused on the serotonin 5-HT2A subtype, however, increasing attention is now also being shifted to other serotonin receptor subtypes, such as 5-HT1A receptors. In fact, newly approved antipsychotic drug aripiprozale has a 5-HT1A receptor partial agonist property.

The 5-HT1A receptor belongs to the family of G-protein-coupled receptors. 5-HT1A receptors are expressed with a high density in the raphe nuclei, and in cortical and limbic areas, especially in the cerebral cortex and hippocampus (Barnes and Sharp, 1999). Lower levels of 5-HT1A receptors are found in brain regions such as the amygdala and striatum (Hall et al., 1997). In the raphe nuclei, 5-HT1A receptors are located on the serotoninergic cell body and dendrites and act as presynaptic autoreceptors. Studies have shown that 5-HT1A agonists inhibit serotonin release in the hippocampus through acting on the 5-HT1A autoreceptors (Sprouse and Aghajanian, 1988, Barnes and Sharp, 1999). They also act as postsynaptic receptors on terminals of serotoninergic neurons. It is well known that the hippocampus is related to cognition, emotion, learning and memory (Vogt et al., 1992, Vizi and Kiss,

1998). Previous studies have demonstrated that 5-HT1A receptors are involved in mood, cognition, attention and memory (Barnes and Sharp, 1999, Sumiyoshi et al., 2000, Sumiyoshi et al., 2007); therefore, 5-HT1A receptors may be an important target of atypical antipsychotic drugs.

Most of post-mortem studies have indicated that 5-HT1A receptor binding densities are increased in several neocortical areas, such as the prefrontal, and anterior and posterior cingulate cortices of schizophrenia patients (Hashimoto et al., 1991, Joyce et al., 1993, Burnet et al., 1996b, Sumiyoshi et al., 1996b, Joyce and Gurevich, 1997). However, a PET study showed that 5-HT1A receptor density is decreased in the amygdala of schizophrenia (Yasuno et al., 2004). It should be noted that despite the seemingly discrepant results as to whether there is an increase or decrease in the number of 5-HT1A receptors in schizophrenia, an intensive study into the high affinity sites (or "G-protein coupled and functional" sites) of 5-HT1A receptors in the post-mortem prefrontal cortex showed that these high affinity state receptors are, in fact, increased by up to 80% (Sumiyoshi et al., 1996b). These authors used several concentrations of radioactive ligands to labele 5-HT1A receptors, while other post-mortem (Dean et al., 1999) and PET (Frankle et al., 2006) studies used only one concentration of radiolabeled ligands, which might not be sensitive enough to replicate the 5-HT1A receptor up-regulation in these brain regions, such as prefrontal areas. Furthermore, clozapine has been reported to down-regulate 5-HT1A receptors in most cortical areas (Ase et al., 1999). This combined evidence suggests that 5-HT1A receptors are involved in schizophrenia and antipsychotic drug treatments, especially for functions involved with the neocortical areas. Further studies examining the effects of antipsychotic drugs on 5-HT1A receptors in animals are warranted to

help clarify the role of 5-HT1A receptors in the etiology and treatment of schizophrenia.

Studies have shown that the atypical antipsychotic drugs aripiprazole, clozapine and olanzapine may increase dopamine release in the prefrontal cortex and the hippocampus, an effect which was significantly inhibited by WAY-100635, a selective 5-HT1A receptor antagonist (Kapur and Remington, 1996, Ichikawa et al., 2001, Li et al., 2004). Another study found that atypical antipsychotics olanzapine, clozapine, and ziprasidone (but not typical antipsychotic drug haloperidol) enhanced dopamine release in the medial prefrontal cortex, however this effect was abolished in 5-HT1A knock-out mice (Diaz-Mataix et al., 2005). These findings provide strong evidence to support the role of 5-HT1A receptors in the action of atypical antipsychotic drugs including those that do not show a direct *in vitro* affinity for these receptors (i.e. olanzapine), at least partly via increasing dopamine release in the prefrontal cortex and the hippocampus. These effects may partly explain how atypical antipsychotic drugs improve negative symptoms and cognitive deficits. However, there is evidence that aripiprazole and olanzapine may have differential effects on cognitive function in schizophrenia patients. For example, aripiprazole has a greater efficacy to improve verbal memory compared to olanzapine (Kern et al., 2006). Therefore, it is important to examine whether there are differential effects between the two atypical antipsychotics on 5-HT1A receptor expression, especially in brain regions related to cognitive function such as the hippocampus.

Previous studies have demonstrated that antipsychotic drugs have different affinities for serotonin and dopamine receptors. Olanzapine has a high affinity for 5-HT2A

receptors (Ki=4nM) and D2 receptors (Ki=11nM), but no significant affinity for 5-HT1A receptors (Ki>7100nM) (DeLeon et al., 2004), but it still increased dopamine release in the prefrontal cortex and hippocampus. On the other hand, aripiprazole is a 5-HT1A receptor partial agonist, D2 receptor partial agonist and 5-HT2A receptor antagonist (Li et al., 2004). It has a high affinity for 5-HT2A receptors (Ki=3.4nM), 5-HT1A receptors (Ki=4.4nM) and D2 receptors (Ki=0.45nM) (DeLeon et al., 2004). The typical antipsychotic drug, haloperidol, mainly possesses D2 receptor antagonist properties and has high affinity with D2 receptor (Ki=0.7nM). It has no significant affinity for 5HT1A or 5-HT2A receptors (DeLeon et al., 2004) and has no significant role in the improvement of negative symptoms and cognitive function. In this study, we aimed to determine (1) whether the level of 5-HT1A receptor mRNA and/or binding density would be altered after antipsychotic drug treatment; (2) if any observed 5-HT1A receptor changes are brain region specific.

EXPERIMENTAL PROCEDURES

Animals and antipsychotic treatment

Female Sprague Dawley rats weighing 220-250 grams were obtained from the Animal Resource Centre (Perth, WA, Australia). After arrival, in order to accurately measuring food and water intake, rats were housed 1 per cage under environmentally controlled conditions (temperature 22°C, light cycle from 0700 to 1900h and dark cycle from 1900 to 0700h), with *ad libitum* access to water and standard laboratory chow. To minimize the possible stress caused by individual housing, the rat cages were put placed next to each other in the same room, and therefore they can smell each other. Rats could also see each other through the cage cover. In addition, a PVC tube was placed in the cage for rat playing. After a 1-week familiarization period, they

were treated with aripiprazole (2.25mg/kg/day, Otsuka, Japan), olanzapine (1.5mg/kg/day, Eli Lilly, USA), haloperidol (0.3mg/kg/day, Sigma, Australia), or vehicle. The daily dosage was divided into 3 equal amounts and all rats were treated 3 times a day (06:00, 14:00, 22:00 hours) orally with a specially prepared drug pellet as described previously (Huang et al., 2006a, Han et al., 2008c). Dosages used in this study are based on our previous study and literature, and have been shown to be pharmacologically and behaviourally effective (Arjona et al., 2004, Huang et al., 2006a). These selected doses all share a D2 occupancy of approximately 70-80% in rats (Kapur et al., 2003, Natesan et al., 2006). We have shown previously that the doses of the drugs and treatment (at 3 times a day) used in this study affects central receptor systems relative to their pharmacological profiles (Huang et al., 2006b, Han et al., 2008a, Han et al., 2008b, Han et al., 2009) indicating the effectiveness of these treatments. The minimum number of rats per group was 12. All rats were sacrificed forty-eight hours after the last drug treatment. Five rat brains from each group were used to measure 5-HT1A receptor mRNA and protein expression. All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia, and complied with the NHMRC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition, 2004).

Histology

All rats were sacrificed using carbon dioxide asphyxiation between 0700 and 0900h, in order to minimize the circadian-induced variation of mRNA expression. Brains were immediately removed after death and frozen in liquid nitrogen and then stored at -80°C until sectioning. Coronal brain sections (14 μm) were cut at -17°C with a cryostat, and thaw-mounted onto polysine-coated slides. Sections for *in situ*

hybridization were fixed in ice-cold phosphate-buffer containing 4% paraformaldehyde. Acetylation was carried out in 0.25% acetic anhydride in 0.1M triethanolamine buffer (pH 8.0) for 10 min. Sections were then dehydrated in ethanol and stored at -20°C until use.

In situ hybridization

The specific antisense hybridization probes for the 5-HT1A - receptor were 5'-AGAGAGGTGATCACTTGGTAGCTGAAGGTCACGTCGGAGAT-3' (NM012585.1, encoding bases 82-122), 5'-CACGCAGGCATTGCCGAGCACCGCGCAGAAAATGAGAGTACCCAGCAAC-3' (NM012585.1, encoding bases 123-171) and 5'-TGACTCGCTGGGCAGAGGAAGGTGCTCTTTGGAGTTGCCCACTCGGTGC-3' (NM012585.1, encoding bases 885-933). No sequences bearing significant homology to the designed probes were found in the Gene Bank (NCBA). All oligonucleotide probes were terminally labeled with a 10-fold molar excess of / dATP (specific activity: 1000 Ci/mmol, Amersham, Buckinghamshire, UK) and terminal transferase (Promega, Madison, WI), and purified over a MicroSpin G-50 column (Amersham, UK). The probe concentration was 10^7 pcm of [^{35}S] labeled probes in 750 µl hybridization solution. Hybridization was carried out by incubating sections in the hybridization buffer (50% deionized formamide, 4 × SSC, 10% dextran sulfate, 1 × Denhardt's solution, 0.2% sheared salmon sperm DNA, 0.1% long-chain polyadenylic acid, 0.012% heparin, 20 mM sodium phosphate, pH7.0, 10^6/75 µl of labeled probe and 5% DTT) at 37°C for 16 hours. Non-specific hybridization was determined by including 100-fold molar excess of non-labeled probes in the respective hybridization solution. After hybridization, sections were washed in 1 × SSC buffer at 55°C three

times for 20 minutes each followed by two washes in $1 \times$ SSC at room temperature for 1 hour each. Finally, sections were dipped sequentially in Milli-Q water, 70% ethanol and 95% ethanol before air-drying and exposure to Hyper-ß-max film (Amersham, UK). After 4 weeks of exposure, films were developed using standard procedures. The sections containing positive signals were dipped in emulsion solution (Amersham, UK) and then exposed for 6 weeks. This allowed a further examination of positive signals at the cellular level and confirmation of the results from the film.

Receptor autoradiography

5-HT1A receptor binding was performed based on that described previously (Khawaja, 1995, Burnet et al., 1997). Brain sections were warmed to room-temperature and pre-incubated in 50nM Tris-HCl buffer (pH 7.4) for 30 min. The sections were then incubated with 5nM [^{3}H]WAY-100635 (specific activity 83.0Ci/mmol, Amersham Biosciences UK Limited) at room temperature for 2.5h in 50mM Tris-HCl (pH7.4) containing10µM pargyline (Sigma). Non-specific binding was determined by incubating consecutive sections exposed to 10µM 5-HT. All sections were washed for 2 min and then 3 min in ice-cold 50mM Tris-HCl buffer. After a brief rinse in ice-cold distilled water, the slides were rapidly dried under a stream of cold air and exposed to Kodak BioMax MR films for 15 weeks. Films were then developed using standard procedures.

Quantification

As in our previous work (Huang et al., 2006a), all films was analyzed by using a computer-assisted image analysis system, Multi-Analysis, connected to a GS-690 Imaging Densitometer (Bio-Rad, USA). The 5-HT1A receptor mRNA expressions

and binding densities were quantified bilaterally in the brain areas from two Bregma levels: 1.20mm, -2.76mm (as indicated in Figure 1), which identification of neuroanatomical structures was according to a standard rat brain atlas (Paxinos and Watson, 1997). The values of 5-HT1A mRNA expression levels were then compared against a [^{14}C]-labeled autoradiographic standards (Amersham, UK). The optical measurements of 5-HT1A receptor binding densities were converted into fmoles [^{3}H]ligand per mg tissue equivalent, according to the calibration curve obtained from the tritium standards. The average data from both hemispheres were used for further analysis. The specific binding values were obtained by subtracting non-specific binding values from the total binding values.

Statistical analysis

The data were analysed statistically using the SPSS 15.0 program (SPSS, Chicago, IL). 5-HT1A receptor mRNA expression and binding density for each brain area were analysed by two-way ANOVA (drug × treatment duration) followed by a *post-hoc* Tukey-Kramer-HSD test.

RESULTS

5-HT1A receptor binding

5-HT1A receptor binding was widely distributed in the rat brain. High levels of 5-HT1A receptor binding density were observed in the lateral septal nucleus, cingulate cortex, and hippocampus. 5-HT1A receptor binding was also observed in the amygdala and ventromedial hypothalamic nucleus, but at lower levels (Fig 1).

Cingulate cortex

5-HT1A binding sites showed a laminar distribution pattern in the cingulate cortex and two binding bands were observed (Fig 1). The upper band corresponded to cortical layers I-II and the deeper band corresponded to cortical layers III-IV.

In the upper binding band of the cingulate cortex, there was a significant effect of drug [$F(3,32) = 5.0$, $P =0.006$] and treatment period [$F (1,32) = 15.2$, $P =0.000$] on 5-HT1A receptor binding density. There was also a significant interaction between the two factors [$F(3,32) = 3.1$, $P =0.039$]. Compared with the control group, olanzapine decreased the binding density of 5-HT1A receptors after 1 week of treatment (-22%, $P =0.003$), although there was no significant difference after 12 weeks of treatment (Fig 2). Aripiprazole and haloperidol did not significantly affect 5-HT1A receptor binding in the upper binding band of the cingulate cortex after 1 week or 12 weeks of treatment.

In the deeper 5-HT1A binding band of the cingulate cortex, there were also significant effects of drug [$F(3,32) = 2.8$, $P =0.052$] and treatment period [$F(1,32) =12.6$, $P =0.001$], but no significant interaction between the two factors [$F(3,32) = 1.9$, $P=0.152$]. As in the upper binding band, olanzapine significantly decreased 5-HT1A receptor binding density compared to controls in the deeper band after 1 week of treatment (-17%, $P =0.038$), but not after 12 weeks of treatment (Fig 2). Aripiprazole and haloperidol had no significant effect on 5-HT1A receptor binding in the deeper band.

Hippocampus

There was a significant effect of drug treatment on 5-HT1A binding density in the CA1 region of the hippocampus [F(3,32) = 3.7, P =0.021], but no significant effect of treatment period [F(1,32) = 1.0, P=0.330] or interaction between the two factors [F(3,32) = 1.9, P =0.151]. Compared with the controls, aripiprazole significantly increased 5-HT1A receptor binding density after 1 week of treatment (+33%, P =0.004), but there was no significant change after 12 weeks of treatment (Fig 2). Olanzapine and haloperidol treatment did not affect 5-HT1A receptor bindings in the CA1 area. There was no effect of antipsychotic treatment on 5-HT1A receptor binding density in the CA2 [F(1,32)=0.4, p=0.727] and CA3 [F(1,32)=1.9, p=0.157] regions of the hippocampus.

Medial posterodorsal nuclei of posterior amygdala (MeP)

Significant effects of drug treatment on 5-HT1A binding density were also observed in the MeP [F(3,32) = 3.1, P =0.043]. However, there was no effect of treatment period [F(1,32) = 1.1, P=0.314] or interaction between the two factors [F(3,32) = 2.7, P =0.064]. Compared with the control group, 5-HT1A receptor binding density was significantly decreased following 1 week of aripiprazole treatment (+21%, P =0.022), although no difference was observed after 12 weeks of treatment (Fig 2). Olanzapine and haloperidol did not significantly affect 5-HT1A binding density in this brain region.

High levels of 5-HT1A binding density were found in the lateral septal nucleus. However, no significant differences of 5-HT1A binding density were found between the antipsychotic and control groups (Table 1 and 2).

5-HT1A receptor mRNA expressions

High levels of 5-HT1A receptor mRNA expression were observed in the lateral septal nucleus and hippocampus, and lower levels of 5-HT1A mRNA expression were found in the amygdala. Unlike 5-HT1A receptor binding density, 5-HT1A mRNA expression did not present a clear laminar distribution in the cingulate cortex. Also, a high level of expression of 5-HT1A mRNA was found in the ventromedial hypothalamic nucleus, while low level expression was observed in the cingulate cortex (Fig 3). As shown in Table 3 and Table 4, compared to the control group, 5-HT1A receptor mRNA expression was not significantly affected by any treatment of the three antipsychotic drugs.

DISCUSSION

This study examined 5-HT1A receptor binding density and mRNA expression in the rat brain following short and long-term administration of aripiprazole, olanzapine and haloperidol. These antipsychotic drugs differed in their effects on 5-HT1A receptor binding density. After 1 week of treatment, aripiprazole upregulated 5-HT1A receptor binding density in the CA1 region of hippocampus and MeP. Conversely, olanzapine downregulated 5-HT1A receptor binding density in the cingulate cortex. The changes in 5-HT1A receptor binding density produced by aripiprazole and olanzapine were absent after 12 weeks of treatment. Haloperidol had no effect on 5HT1A receptor binding after 1 or 12 weeks. Moreover, none of the antipsychotic drugs affected 5HT1A mRNA expression.

Aripiprazole is a 5-HT1A partial agonist and has high affinity with 5-HT1A receptors (DeLeon et al., 2004). The 5-HT1A agonist properties of aripiprazole may have contributed to the upregulation of 5-HT1A receptor binding density in the present study. Previous studies have shown that 5-HT1A agonists target 5-HT1A autoreceptors on the raphe nuclei, which might inhibit serotonin production and then reduce release on terminal projections. This might lead to an upregulation of postsynaptic 5-HT1A receptors (Sprouse and Aghajanian, 1988, Barnes and Sharp, 1999). Therefore, aripiprazole might target at 5-HT1A autoreceptors in the raphe nuclei to inhibit serotonin production and in the hippocampus (CA1) and MeP to reduce serotonin release, which are the areas that showed changes in 5-HT1A binding density in this study.

It is not clear why aripiprazole altered 5-HT1A receptor binding density after short term (1 week) treatment only, but not long term (12 weeks) treatment. It may be the result of adaptation and desensitization of 5-HT1A receptors to aripiprazole. Previous studies have suggested that chronic administration of 5-HT1A receptor agonists results in desensitization of receptors in the median raphe nucleus (Kreiss and Lucki, 1997, Blier and Ward, 2003).

The hippocampus and amygdala have been involved in the neuropathology of schizophrenia (Rajarethinam et al., 2001). For example, magnetic resonance imaging (MRI) studies have shown changes of hippocampal and amygdala size and shape in schizophrenia patients (Nelson et al., 1998, Wright et al., 2000, Csernansky et al., 2002). Furthermore, a study indicated that the volumes of the hippocampus and amygdala had a significant inverse correlation with positive, and negative symptoms,

and with thought disorder in schizophrenia (Rajarethinam et al., 2001). A PET scan suggested lower 5-HT1A receptor binding density in amygdala in schizophrenia (Yasuno et al., 2004). This study has shown that aripiprazole upregulates 5-HT1A receptor binding density in the hippocampus (CA1) and MeP (Fig1, Table 1 and Table 2), which may be how aripoprazole improves the cognitive dysfunction and negative symptoms of schizophrenia. As discussed in the Introduction, aripiprazole is more effective than olanzapine in improving verbal memory (Kern et al., 2006). This may be relevant to the present results showing that aripiprazole, but not olanzapine, upregulated 5-HT1A binding density in the hippocampus.

Olanzapine differed in its effects on 5-HT1A binding density compared with aripiprazole. Olanzapine has only a weak affinity for the 5-HT1A receptor (DeLeon et al., 2004). Therefore, the decrease in 5-HT1A receptor binding density in the cingulate cortex following 1 week of olanzapine treatment may be due to indirect pharmacological effects of olanzapine with the 5-HT1A receptor. Rather, it may be a downstream effect of changes in other receptors influenced by olanzapine. Olanzapine has multireceptor antagonist properties, including at 5-HT2A, M1, H1 and D2 receptors (DeLeon et al., 2004). Previously we have found that olanzapine treatment significantly increases 5-HT2A receptor mRNA expression after 36 days of drug treatment (Huang et al., 2006a). Furthermore, studies have suggested that the 5-HT1A receptor has close interactions with the 5-HT2A receptor (Ichikawa et al., 2001, Li et al., 2004). Studies have also found neuropathalogical changes in the cingulate cortex in schizophrenia (Knable et al., 2002, Zavitsanou et al., 2004). Postmortem studies have shown a significant increase in 5-HT1A binding density in the cingulate cortex in schizophrenia (Gurevich and Joyce, 1997). In the present study olanzapine reduced

5-HT1A receptor binding density in the cingulate cortex after 1 week of treatment, which may contribute to efficacy of olanzapine in improving cognitive deficits, at least after acute treatment. In fact, olanzapine has been reported to be associated with improvement in higher cognitive functions related to the frontal lobe, such as memory organization (Sumiyoshi et al., 2006).

In this study, haloperidol had no affect on 5-HT1A receptor binding density or mRNA expression. This is not surprising, given that haloperidol has no significant affinity for 5-HT1A or 5-HT2A receptors (DeLeon et al., 2004). This is consistent with the therapeutic profile of haloperidol, in which it mainly controls positive symptoms of schizophrenia through dopamine D2 receptor antagonism (Kapur and Mamo, 2003). In consistent to our results, a previous study found that 2-weeks treatment of haloperidol did not affect the expression of 5-HT1A receptor mRNA (Burnet et al., 1996a). However, another study reported that three weeks treatment of haloperidol increased (+25%) 5-HT1A receptor density in the inner layers of the cingulate cortex but decreased (-15%) in the entorhinal region of male rats (Ase et al., 1999). It was noticed that, differing from our study, Ase et al. study had a longer drug withdraw period after last drug treatment (72 *vs* 48 hours), higher dosage in different treatment methods (1mg/kg, once daily, i.p. *vs* 0.3mg/kg, three time per day, oral), and used male rats (Ase et al., 1999). These methodological differences may explain why they have obtained different results.

Since the present study used female rats only, the estrous cycle could be a factor which influences 5-HT1A receptor levels. However, pre-experiments in our laboratory have shown that, under the rearing conditions used in this study, the

estrous cycles of all female rats are synchronized (unpublished data). Our observation was supported by an early report that female rats can synchronise their estrous cycles when housed together (McClintock, 1978), although another study has reported that female rats do not synchronise their estrous cycles (Schank, 2001). However, even if there is asynchrony of the estrous cycles, the control and antipsychotic treatment groups would be considered to have had a similar proportion of rats at various stages of the estrous cycle randomly distributed within them. Furthermore, the standard deviation of the data within each group was quite small, showing there is little variation in 5-HT1A receptor expressions between the rats in the same groups. Therefore, the estrous cycle has minimal effects on 5-HT1Areceptor expressions in this study.

Previous studies showed that serotonin system such as 5-HT1A, 5-HT2A receptors have been involved in food intake and body weight gain (Dryden et al., 1996, Park et al., 1999, López-Alonso et al., 2007). These studies suggested that 5-HT1A agonist affected food intake in a function selective way. 5-HT1A agonist may cause hypophagic or hyperphagic effects by targeting 5-HT1A receptor in the dorsal and median raphe (as presynaptic receptor) or hypothalamus (as postsynaptic receptor) (Park et al., 1999, López-Alonso et al., 2007).

Among the three antipsychotics used in this study, only aripiprazole has significant affinity to 5-HT1A receptors (DeLeon et al., 2004). However, our previous study using the same set of animals has found that aripiprazole did not affect significantly food intake and body weight gains compared to vehicle (Han et al., 2008). In this study, the expression of 5-HT1A receptors was detected only in the VMH, but not in

the PVN and Arc. In consistent, there is no significant difference in 5-HT1A receptor expression in the VMH between aripiprazole and vehicle groups in this study. In addition, combined with data from previous report (Han et al., 2008), we were able to analyse the relationships between the 5-HT1A receptor expression in the VMH and body weight/food intake, but we found no significantly correlation with food intake (all $r<0.315$, $P>0.10$) and body weight gain (all $r<0.2$, $P>0.40$). These results suggest that the property of 5-HT1A partial agonist of aripiprazole may not contribute to body weight gain and food intake changes, which is consistent with clinical observation that aripiprazole dose not lead significant body weight gain and increase of food intake (Han et al., 2008, Nasrallah, 2008).

In addition, our previous studies showed olanzapine can lead significant body weight gain and increase of food intake (Han et al., 2008), although olanzapine has no significant affinity to 5-HT1A receptors. Our previous studies have suggested that the olanzapine-induced weight gain might be produced by its targeting at H1 and 5-HT2A receptors in the specific nuclei of hypothalamus, as well as striatum and limbic nuclei relevant to the regulation of energy and reward (Huang et al., 2006, Han et al., 2008). Although aripiprazole also has high affinity with 5-HT2A receptors (DeLeon et al., 2004), it does not cause body weight gain and food intake increase (Han et al., 2008). It is interested to further investigate whether aripiprazole affects expressions of 5-HT2A receptors.

It is worth noting that the antipsychotic drugs used in this study have a different half-life in the brain. For example, olanzapine has a half-life of 5.1 hours in the rat brain (Aravagiri et al., 1999). Since rats were sacrificed 48 hours after the last drug

treatment, we could not rule out that the olanzapine-induced changes in 5-HT1A receptors is a possible drug withdrawal response observed 2 days after the last treatment in this study. However it is unlikely that this is the case for the observed changes in the aripiprazole group. It has been shown that aripiprazole has a long elimination half-life (60-70 hours) and exerts its effects on D2/D3 receptors for almost 1 week after the last dose in humans (Grunder et al., 2008). Unfortunately, there is no data available on its half-life in rats. It is understandable that aripiprazole may have a different half-life in rats from that in humans, however, even assuming a 4-6 times faster half-life of aripiprazole in rats, it may still have effects one and half days after the last treatment in rats. Therefore, the changes in 5-HT1A receptor density observed in this study are most likely an aripiprazole treatment effect.

In conclusion, atypical antipsychotic drugs such as aripiprazole and olanzapine may achieve some of their acute effects via directly and indirectly targeting on 5-HT1A receptors. However chronic use may result in adaptation and densensitization of 5-HT1A, which suggests that long term administration of these antipsychotic drugs is not associated with alterations in receptor binding or mRNA expression, indicating secondary compensation (either at the level of second messenger systems or changes in neurotransmitter levels). It may also be achieved through compensation within other neurotransmitter systems such as dopaminergic and muscarinic systems.

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FIGURE LEGENDS

Figure 1. Autoradiographic images show the examples of [^{3}H] WAY-100635 bindings on 5-HT1A receptors. (A) The schematic diagram is adapted from standard rat brain atlas (Paxinos and Watson, 1997), indicating the level (Bregma 1.2mm) where the images (B and C) were taken. (D) The schematic diagram indicates the level (Bregma -2.76mm) where the images (E and F) were taken. B and E are examples of [^{3}H] WAY-100635 total bindings in the rat brain. C and F present examples of non-specific bindings of [^{3}H] WAY-100635. *: indicates the sampling areas for quantifications. Abbreviations: Cg, cingulate cortex; CA1, hippocampus CA1; MeP, medial posterodorsal nuclei of posterior amygdala. Scale bar =1mm.

Figure 2. The binding densities of 5-HT1A receptors (A, B, C and D) in rats treated with aripiprazole (ARP), olanzapine (OLZ), haloperidole (HPD) and controls (CONT) in the cingulate cortex I-II (Cg1); cingulate cortex II-IV(Cg2); hippocampus CA1 (CA1) and medial posterodorsal nuclei of posterior amygdala (MeP). * vs. control, $p<0.05$. ** vs. control, $p<0.01$. White bars: 1 week treatment. Black bars: 12 weeks treatment.

Figure 3. Photographs show the examples of 5-HT1A receptor mRNA expressions in the rat brain (A and B). Scale bar =1mm.