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Abstract

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Keywords

Aripiprazole, differentially, affects, mesolimbic, nigrostriatal, dopaminergic, transmission, implications, for, long, term, drug, efficacy, low, extrapyramidal, side, effects

Disciplines

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

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Abstract

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

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Key words: Antipsychotics, dopamine D₂ receptor, dopamine transporter, tyrosine hydroxylase, ventral tegmental area.

Introduction

Aripiprazole is a newly introduced antipsychotic drug, with clinical efficacy to control both positive and negative symptoms, which has been recommended as a first-line therapy for the treatment of schizophrenia (Cassano *et al.* 2007; DeLeon *et al.* 2004). An advantage of aripiprazole over existing antipsychotics is a favourable safety and tolerability profile, including a low incidence of extrapyramidal symptoms (EPS) compared with typical antipsychotics (such as haloperidol), and a low incidence of weight gain and other

metabolic syndromes compared with other atypical antipsychotics (such as olanzapine) (DeLeon *et al.* 2004). However, the mechanisms underlying its clinical efficacy and improved safety and tolerability are not well understood. A unique pharmacology of aripiprazole has been proposed to explain its favourable clinical profile: unlike other antipsychotics, aripiprazole has both partial agonist and antagonist activity at the dopamine D₂ receptor (Hirose *et al.* 2004; Shapiro *et al.* 2003). It is also a partial agonist to 5-HT_{1A} receptors and a partial antagonist to 5-HT_{2A} receptors, a so-called dopamine and 5-HT stabilizer. However, there is evidence that at therapeutic doses, aripiprazole exhibits low levels of 5-HT_{1A} and 5-HT_{2A} receptor occupancy and activity (Wolff *et al.* 2003; Wood & Reavill, 2007), and acts predominantly on

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dopamine D₂ receptors (Urban *et al.* 2007; Wood & Reavill, 2007).

Dopaminergic neurons have two major projections which are derived from the mesencephalon: the mesolimbic pathway, in which the ventral tegmental area (VTA) projects to nucleus accumbens (NAc), and the nigrostriatal pathway, in which the substantia nigra (SN) projects to caudate putamen (CPu). Blockade of dopamine D₂ receptor activity in the mesolimbic pathway is the main mechanism of antipsychotic drug action (Kapur & Mamo, 2003). On the other hand, the EPS side-effects of typical antipsychotics (such as haloperidol) relates to blockade of D₂ receptors in the SN-striatal pathway (Stephen & Stahl, 2003). Whether aripiprazole exerts its effects solely through partial agonism on D₂ receptors (Burris *et al.* 2002; Wood & Reavill, 2007) or due to functionally selective activity at D₂ receptors (Lawler *et al.* 1999; Mailman, 2007; Shapiro *et al.* 2003; Urban *et al.* 2007) is controversial. Aripiprazole is a partial agonist at human D₂L receptors coupled to the inhibition of forskolin-stimulated cAMP accumulation (Burris *et al.* 2002). It has been suggested that aripiprazole is not simply a partial agonist, but a drug with functional selectivity, exerting effects differentially depending on the cellular location of the targeted receptor (Shapiro *et al.* 2003). For example, it acts as an agonist on pre-synaptic D₂ autoreceptors, whereas it also acts as an antagonist at post-synaptic D₂ receptors (Kikuchi, 1995; Shapiro *et al.* 2003). Recently aripiprazole has been found to act as a potent partial agonist at D₂ receptor-mediated signaling responses, such as the potentiation of arachidonic acid release, and as a weak partial agonist using MAPK (mitogen-activated protein kinase) phosphorylation, but lacked agonist activity on receptor internalization (Urban *et al.* 2007). These results support aripiprazole as being a functionally selective D₂ receptor ligand rather than a simple partial agonist (Mailman, 2007).

As mentioned above, aripiprazole has less EPS side-effects, which could be attributed to its partial agonism at D₂ receptors, in which some signals to post-synapses can be persistently generated although the post-synaptic D₂ receptors are fully occupied (Hirose *et al.* 2004). However, it is possible that aripiprazole is not only limited to straightforward receptor-drug interaction but, may also have differential effects on the mesolimbic and nigrostriatal dopaminergic pathways.

In-vivo microdialysis studies have found that acute aripiprazole treatment increases dopamine release in the medial prefrontal cortex and hippocampus (Bortolozzi *et al.* 2003; Zocchi *et al.* 2005) and elevates

3,4-dihydroxyphenylacetic acid (the major metabolite of dopamine) in the medial prefrontal cortex and striatum (Jordan *et al.* 2004). The dopamine transporter (DAT) regulates the re-uptake of free dopamine from the intra-synaptic cleft. It is the primary mechanism for inactivation of dopamine following its release into the synapse (Iversen, 1971). DAT binding density may therefore reflect levels of free dopamine in the intra-synaptic cleft. However, it is unclear whether aripiprazole, particularly chronic treatment, affects DAT binding density. Furthermore, although aripiprazole has been reported to reduce dopamine synthesis and tyrosine hydroxylase activity (TH; a rate-limiting enzyme for the synthesis of dopamine) in reserpine-treated rodents through action at pre-synaptic dopamine autoreceptors (Kikuchi, 1995), it is unclear whether aripiprazole affects TH mRNA expression. Therefore, in the present study, we examined the selective effects of short-term (1-wk) and chronic (12-wk) aripiprazole treatment on D₂ receptor and TH mRNA expression, and binding densities of D₂ receptor and DAT in the mesolimbic and nigrostriatal dopaminergic pathways. For comparison, the effects of haloperidol and olanzapine treatment were also examined.

Methods

Animals and experimental procedures

Sprague-Dawley rats (220–250 g) were obtained from the Animal Resource Center (Perth, Australia). Upon arrival, rats were housed individually in environmentally controlled conditions (temperature 22 °C, 12-h light/dark cycle, lights on 07:00 hours), and had *ad-libitum* access to water and standard laboratory chow diet. After 1 wk adaptation to the new environment, they were treated with aripiprazole (2.25 mg/kg.d; Otsuka, Japan), olanzapine (1.5 mg/kg.d; Eli Lilly, USA), haloperidol (0.3 mg/kg.d, Sigma, Australia), or vehicle (control) (Han *et al.* 2008a). The daily dosage was divided into three equal amounts and all rats were treated three times a day (06:00, 14:00, 22:00 hours) orally by specially prepared drug pellets as described previously (Han *et al.* 2008b; Huang *et al.* 2006). Each drug group was randomly subdivided into short-term (1-wk, *n* = 5) and chronic (12-wk, *n* = 5) treatment groups. Rats were sacrificed 48 h after the last drug treatment. 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 Animal for Scientific Purposes.

Histology

All rats were sacrificed by carbon dioxide asphyxiation between 07:00 and 09:00 hours, in order to minimize the variation of circadian mRNA expression. Brains were immediately removed and frozen in liquid nitrogen. Coronal brain sections (14 μ m) were cut at -17°C a cryostat and thaw-mounted onto polysine-coated slides. For *in-situ* hybridization, sections were immediately fixed in ice-cold phosphate buffer containing 4% paraformaldehyde. Acetylation was carried out in 0.25% acetic anhydride in 0.1 M triethanolamine buffer (pH 8.0) for 10 min. Sections were then dehydrated in ethanol and stored at -20°C until use. For autoradiography, the sections were stored at -20°C after cutting until use without fixation. Identification of neuroanatomical structures was according to a standard rat brain atlas (Paxinos & Watson, 1997).

In-situ hybridization

The protocol followed that previously described (Huang *et al.* 2006). Briefly, the specific antisense hybridization probes for the D_2 receptor were:

5'-cat gat aac ggt gca gag ttt cat gtc ctc agg gtg ggt aca gtt gcc-3'

(NM010077, encoding bases 853–900),

5'-gac cca ttg aag ggc cgg ctc cag ttc tgc ctc tcc aga tca tca tc-3'

(NM010077, encoding bases 157–203),

and for TH:

5'-tgg gtc agg gtg tgc agc tca tcc tgg acc ccc tct aag gag cg-3'

(NM009377, encoding bases 1437–1480).

No sequences bearing significant homology to the designed probes were found in the Gene Bank (NCBA). All oligonucleotide probes were terminally labelled with 10-fold molar excess of [^{35}S]dATP (specific activity 1000 Ci/mmol, Amersham, UK) and terminal transferase (Promega, USA), and purified over a MicroSpin G-50 column (Amersham). The probe concentration was 10^7 cpm of ^{35}S -labelled probes in 750 μl hybridization solution. Hybridization was performed by incubating sections in hybridization buffer [50% deionized formamide, 4 \times SSC, 10% dextran sulfate, 1 \times Denhardt's solution, 0.2% sheared salmon sperm DNA, 0.1% long-chain polyadenylic acid, 0.012% heparin, 20 mM sodium phosphate (pH 7.0), $10^6/75$ μl labelled probe and 5% DTT] at 37°C for 16 h. Non-specific hybridization was determined by including 100-fold molar excess of non-labelled probes in the

respective hybridization solution. After hybridization, sections were washed in 1 \times SSC buffer at 55°C for 3 \times 20 min followed by 1 h incubation in 1 \times SSC buffer at room temperature. Finally, sections were dipped sequentially in Milli-Q water, 70% ethanol and 95% ethanol before air-drying and exposure to Hyper- β -max film (Amersham). After exposure (4 wk for D_2 receptor and 2 wk for TH) *in-situ* hybridization films were developed using standard procedures. The sections containing positive signals were dipped in the emulsion solution (Amersham) and exposed (6 wk for D_2 receptor and 4 wk for TH). This allows further examination of positive signals at cellular level and confirmation of the results from the film. As in our previous work (Huang *et al.* 2006), all films were analysed using a computer-assisted image analysis system, Multi-Analyst, connected to a GS-690 Imaging Densitometer (Bio-Rad, USA). Quantification of mRNA expression levels in various brain regions were performed by measuring the average density of each region. Values were then compared against a ^{14}C -labelled autoradiographic standard (Amersham).

D_2 receptor binding

Sections were preincubated in 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 0.1% ascorbic acid buffer (pH 7.4) for 30 min at room temperature to remove endogenous ligands. Sections were then incubated at room temperature for 1 h in the same buffer containing 5 nM [^3H]raclopride (specific activity 60.1 Ci/mmol; PerkinElmer, USA). Non-specific binding was determined in the presence of 10 μM butaclamol. The sections were then washed 2 \times 5 min in ice-cold buffer and rinsed briefly in ice-cold distilled water. Sections were then dried under a stream of cold air.

DAT binding

Sections were preincubated in 50 mM Tris-HCl containing 120 mM NaCl and 0.1% BSA (pH 7.4) for 20 min at 4°C and then incubated for 2 h in the same buffer containing 15 nM [^3H]WIN 35,428 (specific activity 85.6 Ci/mmol; PerkinElmer). Non-specific binding was determined in the presence of 50 μM benzotropine. The sections were washed 2 \times 1 min in ice-cold buffer. After a brief rinse in ice-cold distilled water, the slides were rapidly dried under a stream of cold air.

Autoradiographic images were produced using a Beta image camera (BioSpace, France) as previously described (Deng & Huang, 2005). The exposure time was 3.5 h at a high-resolution setting. A series of

sections with a known amount of ligand was used as a standard in all scans. Quantitative analysis of these images was performed with β -Image Plus (version 4; BioSpace). The density of the binding signal was first expressed in counts per minute per square millimetre (cpm/mm^2) of area selected and was then converted into femtomoles of radioligand bound per milligram tissue equivalent ($\text{fmol}/\text{mg TE}$) by comparing with the standard.

Statistical analysis

The data was analysed statistically using SPSS version 15.0 software (SPSS Inc., USA). Data for each brain area were analysed by two-way ANOVA (drug treatment \times treatment duration), followed by Tukey–Kramer–HSD *post-hoc* analyses. Pearson's correlations were used to assess the relationships between D_2 receptor mRNA expression, TH mRNA expression, D_2 receptor binding and DAT binding.

Results

D_2 receptor mRNA expression

Differential expression of D_2 receptor mRNA was observed in NAc core (NAcC), NAc shell (NAcS), CPu, SN and VTA. Examples of D_2 receptor mRNA expression are presented in Fig. 1.

CPu. There were significant effects of drug treatment ($F_{3,32} = 5.712$, $p = 0.003$) and treatment duration ($F_{1,39} = 7.066$, $p = 0.012$) on D_2 mRNA expression in CPu, but no significant interaction between the two factors ($F_{3,32} = 0.697$, $p = 0.561$). After 1-wk treatment, only the aripiprazole group (237.9 ± 2.5 nCi/g TE) had significantly higher D_2 receptor mRNA expression compared to the control group (206.7 ± 10.7 , $p = 0.031$; Fig. 2d). However, after 12-wk treatment, no significant difference in D_2 receptor mRNA expression was observed between the aripiprazole and control groups ($p = 0.471$; Fig. 2d). Olanzapine and haloperidol groups also did not significantly differ from controls in D_2 receptor mRNA expression (all $p > 0.300$; Fig. 2d).

VTA. There were significant effects of drug treatment ($F_{3,32} = 11.243$, $p = 0.000$) and treatment duration ($F_{1,39} = 4.770$, $p = 0.036$) on D_2 mRNA expression in VTA. However, there was no significant interaction between the two factors ($F_{3,32} = 2.350$, $p = 0.091$). Compared to the control group, aripiprazole treatment significantly increased the level of D_2 receptor mRNA expression in the 1-wk (aripiprazole 256.8 ± 6.3 vs. control 203.1 ± 10.6 , $p = 0.008$; Fig. 1b–d and Fig. 2b)

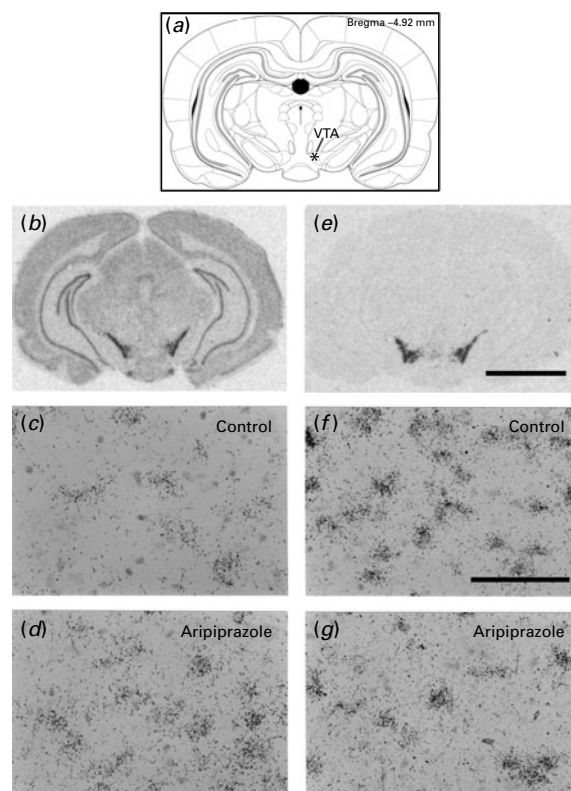


Fig. 1. (b–d) D_2 receptor mRNA expression and (e–g) tyrosine hydroxylase (TH) mRNA expression in the ventral tegmental area (VTA) after 1-wk aripiprazole treatment. (a) The schematic diagram is adapted from a rat brain atlas (Paxinos & Watson, 1997). (b) Low magnification films of D_2 receptor mRNA expression. (c, d) High magnification emulsion autoradiographs of D_2 receptor mRNA expression in VTA. (e) Low magnification films of TH expression. (f, g) High magnification emulsion autoradiographs of TH mRNA expression in VTA. * Indicates where high magnification autoradiographs were taken. Scale bars: (b, e), 2 mm; (c, d, f, g), 50 mm.

and 12-wk (aripiprazole 254.2 ± 10.2 vs. control 205.1 ± 5.1 , $p = 0.002$; Fig. 2b) treatment groups. However, compared to controls, olanzapine and haloperidol had significantly increased D_2 receptor mRNA expression only in the 12-wk treatment groups (olanzapine 246.7 ± 5.7 vs. control 205.1 ± 5.1 , $p = 0.008$; haloperidol 244.4 ± 9.1 vs. control, $p = 0.013$; Fig. 2b) but not in the 1-wk treatment groups (all $p > 0.299$; Fig. 2b). There were no significant effects of drug treatment on D_2 receptor mRNA expression in NAcC (Fig. 2a), NAcS, and SN (Fig. 2e).

TH mRNA expression in VTA and SN

There was a significant effect of drug treatment on the level of TH mRNA expression in VTA ($F_{3,32} = 14.5$,

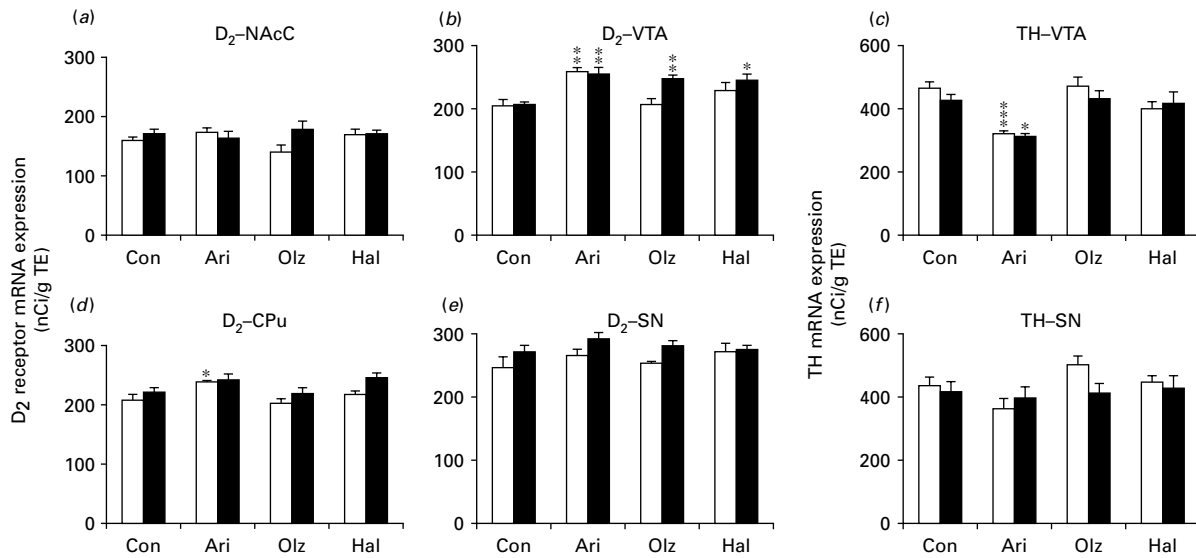


Fig. 2. Expression of (a, b, d, e) D₂ receptor mRNA and (c, f) tyrosine hydroxylase (TH) mRNA in rats treated with either aripiprazole (Ari), olanzapine (Olz), haloperidol (Hal), or vehicle (Con). NAcC, Nucleus accumbens core; CPu, caudate putamen; SN, substantia nigra; VTA, ventral tegmental area. Compared to control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. □, 1-wk treatment; ■, 12-wk treatment.

$p = 0.000$), but no significant effect of treatment period ($F_{1,39} = 1.2$, $p = 0.287$). There was also no interaction between the two factors ($F_{3,32} = 0.7$, $p = 0.554$). Compared to the control group, TH mRNA expression levels were significantly decreased in the aripiprazole group after 1-wk (aripiprazole 319.6 ± 8.4 vs. control 460.3 ± 22.0 , $p = 0.001$; Figs 1e–g, 2c) and 12-wk (aripiprazole 312.1 ± 8.4 vs. control 422.2 ± 19.9 , $p = 0.024$; Fig. 2c) treatment. However, olanzapine and haloperidol treatment did not differ from controls in TH mRNA expression levels in VTA (Fig. 2c). There was no significant effect of drug treatment on TH mRNA expression in SN ($F_{3,32} = 2.3$, $p = 0.092$; Fig. 2f). It is interesting that in the VTA, D₂ receptor mRNA expression was negatively correlated with TH mRNA expression ($r = -0.449$, $p = 0.004$; Fig. 3a).

D₂ receptor binding

Examples of D₂ receptor binding are presented in Fig. 4c.

NAcC and NAcS. There were significant effects of drug treatment on D₂ receptor binding densities in NAcC ($F_{3,32} = 16.827$, $p = 0.000$) and NAcS ($F_{3,32} = 12.572$, $p = 0.000$); however, there were no effects of treatment duration in NAcC ($F_{1,39} = 0.054$, $p = 0.817$) and NAcS ($F_{1,39} = 0.062$, $p = 0.805$). There was also no significant

interaction between the two factors in the NAcC ($F_{3,32} = 1.199$, $p = 0.326$) and NAcS ($F_{3,32} = 2.226$, $p = 0.104$). Compared to the control group, only haloperidol treatment significantly increased binding densities in these brain areas after 1- and 12-wk treatment in these brain regions (NAcC, 1 wk: haloperidol 89.4 ± 4.1 fmol/mg TE vs. control 69.4 ± 2.1 , $p = 0.003$; 12 wk: haloperidol 85.5 ± 4.7 vs. control 68.2 ± 1.9 , $p = 0.005$; Fig. 5a. NAcS, 1 wk: haloperidol 79.9 ± 2.5 vs. control 65.2 ± 3.7 , $p = 0.030$; 12 wk: haloperidol 79.1 ± 1.8 vs. control 58.9 ± 2.1 , $p = 0.002$).

CPu. There was a significant effect of drug treatment on D₂ receptor binding density in CPu ($F_{3,32} = 8.318$, $p = 0.000$), but no effect on treatment duration ($F_{1,39} = 1.622$, $p = 0.212$). There was also no significant interaction between the two factors in CPu ($F_{3,32} = 0.195$, $p = 0.899$). Compared to the control group, only haloperidol treatment significantly increased binding densities after 1- and 12-wk treatment (1 wk: haloperidol 146.9 ± 5.0 vs. control 120.4 ± 4.6 , $p = 0.008$; 12 wk: haloperidol 143.9 ± 8.1 vs. control 109.2 ± 4.6 , $p = 0.020$; Fig. 5b).

However, haloperidol treatment had no effect on D₂ receptor binding density in SN or VTA (Fig. 5c). Furthermore, aripiprazole and olanzapine treatments did not affect D₂ receptor binding in any of these brain regions examined (Fig. 5a–c).

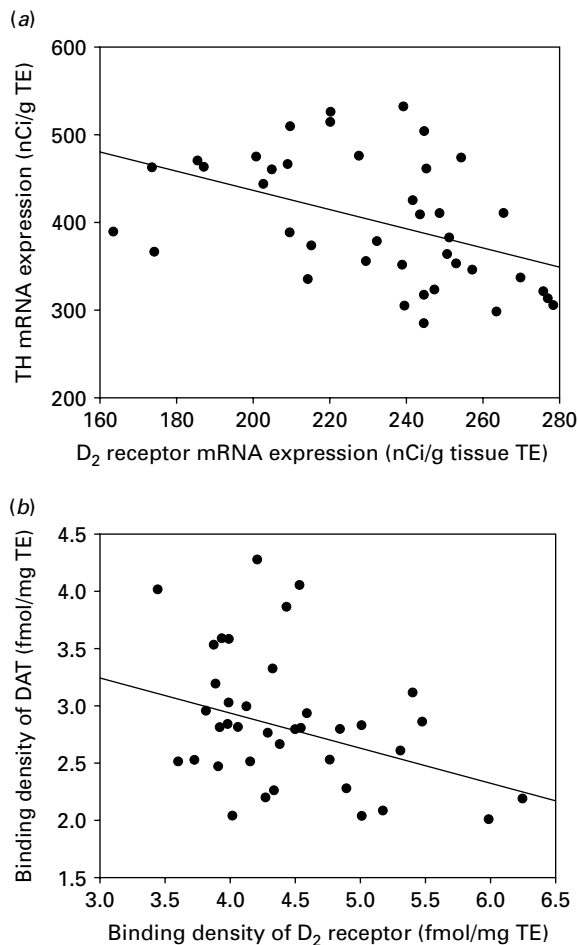


Fig. 3. (a) A significant negative correlation was found between D_2 receptor and tyrosine hydroxylase (TH) mRNA expression in the ventral tegmental area. (b) A significant negative correlation was found between the binding densities of D_2 receptor and dopamine transporter in the nucleus accumbens core.

DAT binding

Examples of DAT binding are presented in Fig. 4d, e.

NAC. There was a significant effect of drug treatment ($F_{3,32}=12.057$, $p=0.000$), but no significant effect of treatment period ($F_{1,39}=1.578$, $p=0.218$) on DAT binding in NAC. There was also no significant interaction between the two factors ($F_{3,32}=0.789$, $p=0.509$). Compared to the control group, DAT binding density was significantly decreased in the 1- and 12-wk aripiprazole and haloperidol groups (Fig. 5d). However, in the olanzapine group, a significant difference was observed only after 1-wk treatment (olanzapine 29.7 ± 3.7 vs. control 41.2 ± 7.1 , $p=0.018$), although

there was a tendency for binding to be decreased after 12-wk olanzapine treatment (olanzapine 30.3 ± 2.7 vs. control 36.0 ± 3.8 , $p=0.109$; Fig. 5d).

CPu. There was a significant effect of drug treatment ($F_{3,32}=5.338$, $p=0.004$), but no effect of treatment duration ($F_{1,39}=0.258$, $p=0.615$) on DAT binding in CPu. There was also no significant interaction between the two factors ($F_{3,32}=1.335$, $p=0.280$). As shown in Fig. 5e, compared to the controls, haloperidol significantly decreased DAT binding density after 1- and 12-wk treatment (Fig. 5e). However, aripiprazole and olanzapine treatments did not affect DAT binding in CPu (Fig. 5e).

VTA. There were significant effects of drug treatment on DAT binding in VTA ($F_{3,32}=4.214$, $p=0.013$) and no effect of treatment period ($F_{1,39}=0.044$, $p=0.836$). However, a significant interaction between drug treatment and treatment period was observed ($F_{3,32}=5.731$, $p=0.003$). After 1-wk treatment, the aripiprazole group (10.1 ± 0.6 fmol/mg TE) had significantly lower DAT binding density compared to the control group (13.7 ± 0.7 fmol/mg TE, $p=0.03$; Fig. 5f), but was not significantly different from the other groups. After 12-wk drug treatment only the haloperidol (9.7 ± 0.7 fmol/mg TE) group had significantly lower DAT binding density than controls (12.5 ± 0.5 , $p=0.048$) (Fig. 5f). However, there were no effects of drug treatment and treatment period on DAT binding in NAcS and SN.

Discussion

This study has showed that aripiprazole has selective effects on mesolimbic vs. nigrostriatal dopaminergic pathways. Aripiprazole significantly increased D_2 receptor mRNA expression in VTA and CPu compared to controls after 1- and 12-wk treatment, but not in SN and NAc. Aripiprazole also significantly down-regulated TH mRNA expression in the VTA, but not in SN, compared to controls. It is interesting that D_2 receptor mRNA expression is negatively correlated with TH mRNA expression in the VTA. Aripiprazole also decreased DAT binding sites in NAc (both 1- and 12-wk treatment) and VTA (1-wk treatment). In contrast, haloperidol had a significant influence on mesolimbic and nigrostriatal dopaminergic pathways. Haloperidol treatment increased D_2 receptor binding in CPu and NAc after 1- and 12-wk treatment, but decreased DAT binding in CPu and NAc (1- and 12-wk treatment), as well as VTA (12-wk treatment). Olanzapine had less widespread effects compared to the other two

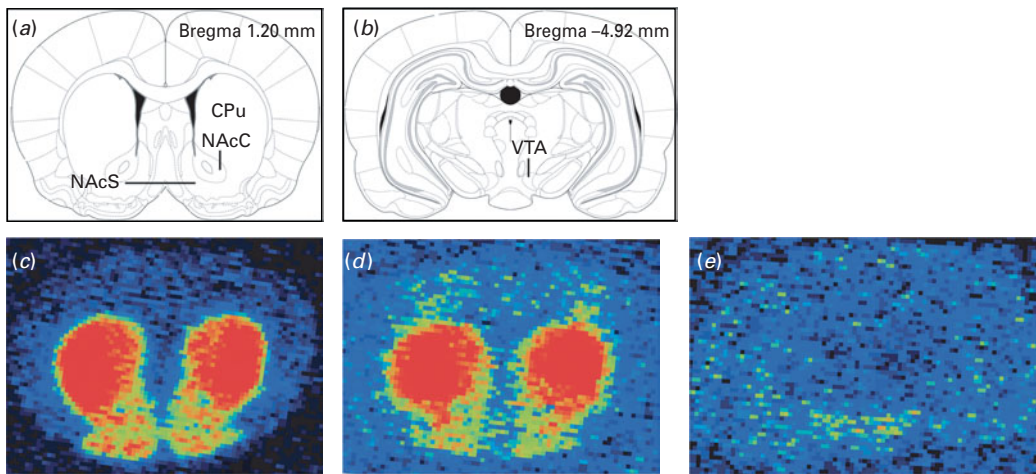


Fig. 4. Autoradiographic images taken using a Beta image camera (BioSpace, Paris) showing the high binding density of (c) D_2 receptor and (d, e) dopamine transporter in the nucleus accumbens core (NAcC), nucleus accumbens shell (NAcS), caudate putamen (CPu) and ventral tegmental area (VTA) in the rat brain. Maps (a) and (b) are adapted from a standard rat brain atlas (Paxinos & Watson, 1997).

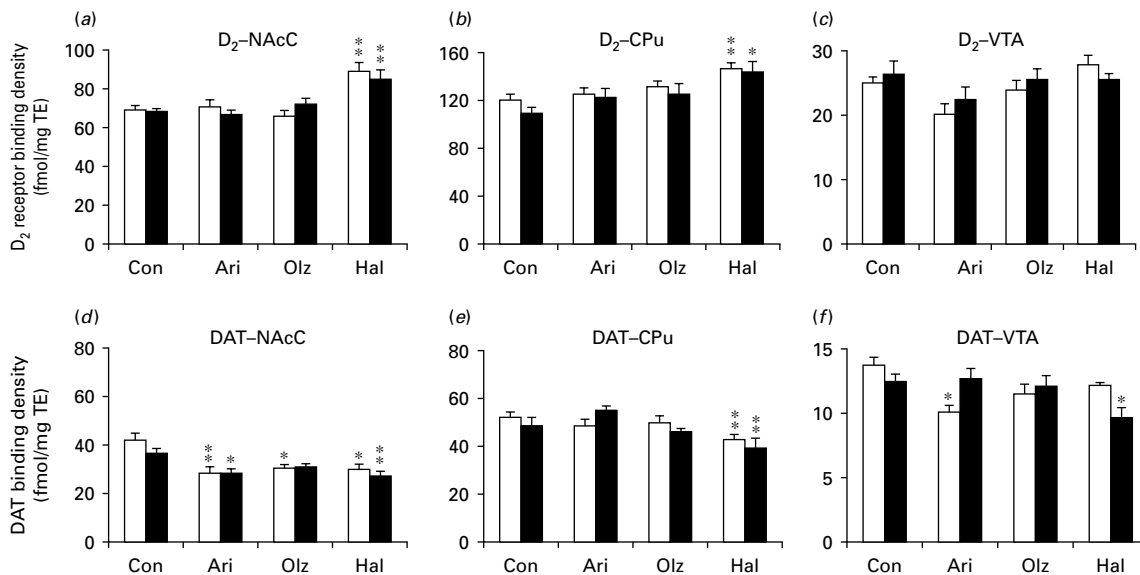


Fig. 5. The binding densities of (a–c) D_2 receptor and (d–f) dopamine transporter (DAT) in rats treated with aripiprazole (Ari), olanzapine (Olz), haloperidol (Hal) and vehicle (Con) in the nucleus accumbens core (NAcC), caudate putamen (CPu) and ventral tegmental area (VTA). Compared to control: * $p < 0.05$, ** $p < 0.01$. □, 1-wk treatment; ■, 12-wk treatment.

treatments, namely an increase in DAT binding in NAcC (1-wk treatment only) and D_2 receptor mRNA expression in VTA (12-wk treatment only).

A series of short-term (4–6 wk) and longer term (26 or 52 wk) clinical trials have revealed that aripiprazole not only has significant short- but also long-term efficacy in the treatment of positive and negative symptoms of schizophrenia (Cassano *et al.* 2007; DeLeon

et al. 2004; Kane *et al.* 2007; Kasper *et al.* 2003; Travis *et al.* 2005). Compared with placebo, aripiprazole improved symptoms of schizophrenia as early as 1 wk after treatment (Travis *et al.* 2005), and this efficacy lasted up to 52 wk (Kasper *et al.* 2003). It has been suggested that the partial agonism of dopamine D_2 receptors is one of the mechanisms underlying the therapeutic effects of aripiprazole (Wood & Reavill,

2007). However, it is worth noting that another D₂ receptor partial agonist (preclamol) also decreases positive and negative symptoms of schizophrenia, but its effects only last for 1 wk (Lahti *et al.* 1998). Therefore, the partial agonism of dopamine D₂ receptors cannot completely explain the long-term efficacy of aripiprazole. TH mRNA expression in VTA was decreased in the aripiprazole group in the present study. Since TH is the rate-limiting enzyme for the synthesis of dopamine, this indicates a reduction of dopamine synthesis in this brain region. The selective effects of aripiprazole on reducing dopamine production found in the present study may provide a mechanism to explain its long-term efficacy.

A reduction in dopamine synthesis may be mediated by D₂ autoreceptors (Wolf & Roth, 1990). Previously, *in-vivo* studies have found that aripiprazole has potent agonist activities at dopamine autoreceptors (Kikuchi, 1995). Aripiprazole may act on these D₂ autoreceptors in the VTA to reduce chronic dopamine synthesis, and continual treatment of aripiprazole would reduce dopamine release. Although a single low-dose (0.3 mg/kg) administration of aripiprazole increases dopamine release (Zocchi *et al.* 2005), a higher dose (2–40 mg/kg) decreases or has no effect on extracellular levels of dopamine in the rat cortex and striatum (Jordan *et al.* 2004; Zocchi *et al.* 2005). It follows that in our experiment, aripiprazole treatment (2.5 mg/kg.d) for 1 or 12 wk should decrease dopamine release. As a compensatory mechanism, D₂ autoreceptor synthesis in the VTA may be increased in response to the decrease of dopamine synthesis and release caused by aripiprazole treatment. Consistent with this, an increase in D₂ receptor mRNA expression was observed in VTA of the aripiprazole group. In fact, a negative correlation between D₂ receptor mRNA and TH mRNA expressions was found in this brain region. This suggests that aripiprazole may achieve its pharmacological effects by reducing dopamine production in VTA. Moreover, since the effects of aripiprazole on D₂ receptor mRNA and TH mRNA expression have been observed after 1- and 12-wk treatment, our theory may well explain its fast effects (1 wk) on improving symptoms and its long-term efficacy maintenance. It is interesting that aripiprazole had no effect on D₂ receptor mRNA expression and D₂ receptor binding in NAc. It is possible that aripiprazole reaches its long-term efficacy by reducing dopamine levels in the VTA–NAc pathway, but not directly through blocking post-synaptic D₂ receptors in the NAc.

In this study, like haloperidol, olanzapine increased D₂ receptor mRNA expression in VTA after 12-wk

treatment, but it did not change in TH mRNA expression. Since olanzapine has relatively low affinity for D₂ receptors but high affinity for 5-HT_{2A}, 5-HT_{2C} and M₁ receptors (DeLeon *et al.* 2004), it has been suggested to achieve its pharmacological effects through its actions on these neurotransmission systems (Tyson *et al.* 2004). In fact, we have previously found that chronic olanzapine treatment down-regulated 5-HT_{2A} receptor mRNA expression in NAc (Huang *et al.* 2006) and up-regulated muscarinic M₁ receptor mRNA expression in the hippocampus (Han *et al.* 2008b).

It is an exciting finding that aripiprazole has differential effects on the VTA–NAc pathway compared to the SN–CPu pathway. Aripiprazole did not affect the expression of D₂ receptor mRNA or TH mRNA in SN. This result suggests that there are no changes in dopamine synthesis in SN, which may be due to the fact that D₂ autoreceptors in VTA are about 10-fold more sensitive to dopamine and D₂ receptor agonists than those in SN (Roth, 1979). Although 1-wk aripiprazole treatment slightly increased D₂ receptor mRNA expression in CPu compared with controls, this difference disappeared after 12-wk treatment. It is also interesting that 1- and 12-wk aripiprazole treatment did not affect D₂ receptor binding in CPu in this study. Therefore, the absence of changes to dopaminergic transmission in the SN–CPu pathway following chronic treatment of aripiprazole may partially explain why aripiprazole does not cause serious EPS after long-term treatment in the clinic. However, a recent human PET study using [¹⁸F]Fallypride has shown that aripiprazole occupies a very high percentage of the D₂/D₃ receptor in all brain regions investigated (including the putamen, caudate nucleus, thalamus, amygdala, and interior temporal cortex) (Grunder *et al.* 2008). Therefore, the more robust actions in mesolimbic dopamine neurons may be possibly due to the fact that the same amount of receptor binding of aripiprazole (a partial agonist) may cause different functional consequences in the nigrostriatal system.

It is important to note that a series of studies have shown aripiprazole is a functionally selective D₂ receptor ligand (Lawler *et al.* 1999; Mailman, 2007; Shapiro *et al.* 2003; Urban *et al.* 2007). The selective effects of aripiprazole on the mesolimbic and nigrostriatal systems might be due to its properties of functional selectivity. For example, as discussed in the Introduction, aripiprazole can act as a potent partial agonist at D₂ receptor-mediated signalling responses, such as the potentiation of arachidonic acid release, and as a weak partial agonist at MAPK

phosphorylation, but lacks agonist activity on receptor internalization (Urban *et al.* 2007). It is possible that aripiprazole may act as a potent agonist at dopamine D₂ autoreceptors in VTA to reduce dopamine synthesis, but as a weak agonist (or even short of agonist activity) in SN, therefore it modulates dopaminergic transmission mainly in the VTA–NAc pathway but not the SN–CPu pathway.

In contrast, haloperidol affected the mesolimbic and nigrostriatal pathways by blocking D₂ receptors. Haloperidol significantly increased D₂ receptor binding density in NAc and CPu after 1- and 12-wk treatment, which is in agreement with previous reports (Gross *et al.* 1991; Prosser *et al.* 1988; Sawa & Snyder, 2002; Stephen & Stahl, 2003). Some studies have also found that haloperidol (at a dose of 1.5–4 mg/kg) increases D₂ receptor mRNA expression in the striatum (Damask *et al.* 1996; Rogue *et al.* 1991); however, the present study did not observe any changes in D₂ receptor mRNA expression in CPu. Although haloperidol increased D₂ receptor mRNA expression in VTA after 12-wk treatment, it did not cause any change in TH mRNA expression. These results confirm previous findings that haloperidol improves schizophrenia symptoms by blocking D₂ receptors in NAc, while causing EPS side-effects by blocking D₂ receptors in CPu after chronic treatment (Kapur *et al.* 2000; Vohora, 2007). In this study, olanzapine did not affect D₂ receptor binding or expression of D₂ mRNA and TH mRNA in the CPu, which is consistent with a PET study showing that olanzapine had lower D₂ receptor blockade compared to haloperidol in the striatum (Xiberas *et al.* 2001). One study observed that olanzapine increased D₂ receptor density in the striatum of rats; however, much higher doses of olanzapine (5.0 mg/kg.d compared to 1.5 mg/kg.d in this study) were used (Tarazi *et al.* 2001). These results supported the observation that olanzapine does not cause EPS at common clinical dosages (Vohora, 2007).

Consistent with the changes of D₂ receptor and TH mRNA expression, selective effects of aripiprazole treatment on DAT binding densities were also observed in mesolimbic and nigrostriatal pathways. Since DAT mediates the re-uptake of free dopamine from the synaptic cleft (Iversen, 1971), decreased DAT density in NAc could be explained by decreased D₂ synthesis in this nucleus as discussed above. In contrast, haloperidol treatment reduced DAT binding densities in NAc and CPu. Although aripiprazole and haloperidol reduced DAT binding density, they might act through different mechanisms. Haloperidol has previously been reported to reduce the reuptake transport of dopamine in the striatum (McElvan &

Schenk, 1992; Meiergerd *et al.* 1993). In addition, a negative correlation between D₂ receptor binding density and DAT binding density was found in NAcC in this study (Fig. 3b). These results further suggest that haloperidol may control schizophrenia symptoms by D₂ receptor blockade in NAc, and produce EPS by D₂ receptor blockade and prolonged free dopamine reuptake in CPu. Olanzapine decreased DAT density in NAc only, which suggests that olanzapine might prolong dopamine activity in NAc.

DAT binding densities in VTA were decreased only after 1-wk aripiprazole treatment, but returned to a normal level after 12-wk treatment. It is interesting why only short-term treatment of aripiprazole affected DAT binding density. In contrast haloperidol had long-term effects on DAT binding in VTA. One possible explanation is the specific profile of aripiprazole as a selective D₂ receptor partial agonist. It was reported that D₂ receptor agonists may increase the reuptake of dopamine via stimulating D₂ autoreceptors, but this effect could be reversed by D₂ receptor antagonists (Meiergerd *et al.* 1993; Parsons *et al.* 1993). Since aripiprazole has dual D₂ partial agonist and antagonist properties, it is possible that its dual effects may reach a balance after long-term treatment. However, haloperidol only has D₂ antagonist properties and needs long-term action to reduce DAT binding density in VTA.

In the literature, the dosages of aripiprazole, haloperidol, and olanzapine vary significantly in the animal studies. Similar doses to that used in this study have been used previously in the literature and have been shown to be pharmacologically and behaviourally effective. For example, aripiprazole has been used at doses ranging from 2 to 3 mg/kg (Kalinichev *et al.* 2005; Li *et al.* 2005; Schwabe & Koch, 2007). Haloperidol has frequently been utilized at a dose of 0.3 mg/kg (Pouzet *et al.* 2003; Wiley, 2008), while olanzapine treatments ranging from 1.2 mg/kg (Arjona *et al.* 2004; Huang *et al.* 2006) to 2.0 mg/kg (Cooper *et al.* 2005) have been used in rats. These selected doses all share a D₂ occupancy of ~70–80% in rats (Kapur *et al.* 2003; Natesan *et al.* 2006). Furthermore, antipsychotics were administered orally three times a day in this study. We have shown previously that the doses of the drugs and treatment (at three times a day) used in this study affected central receptor systems relative to their pharmacological profiles (Han *et al.* 2008a,b; Huang *et al.* 2006), indicating the effectiveness of these treatments. Since rats were sacrificed 48 h after the last drug treatment, we could not completely rule out a possible drug withdrawal response observed 2 d after the last aripiprazole

treatment; however, it is unlikely. It has been shown that aripiprazole has a long elimination half-life (60–70 h) and exerts its effects on D₂/D₃ receptors for almost 1 wk 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- to 6-fold faster half-life of aripiprazole in rats, it may still have effects 1.5 d after the last treatment in rats. Further studies are necessary to measure the half-life of aripiprazole in rats (particularly in the brain) and to investigate changes of TH and D₂ receptor mRNA expression a short period (e.g. a few hours) after the last aripiprazole treatment.

In conclusion, the present results suggest that aripiprazole, unlike other antipsychotics, has selective effects on dopaminergic pathways, in which both short- and long-term treatment predominantly modulates the dopaminergic neurotransmission in the mesolimbic but not nigrostriatal pathways. Selectively reducing dopamine synthesis in VTA (but not SN) is the possible therapeutic mechanism for long-term efficacy of aripiprazole in controlling schizophrenia symptoms with less extrapyramidal side-effects.

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Statement of Interest

None.

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