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Aripiprazole, a novel antipsychotic drug, inhibits quinpirole-evoked GTPase activity but does not up-regulate dopamine D_2 receptor following repeated treatment in the rat striatum

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Abstract

Aripiprazole, a quinolinone derivative, is a new dopaminergic agent which has been recently developed and demonstrated to be clinically useful as an antipsychotic drug with reduced extrapyramidal motor side effects. Here, we found that aripiprazole competed [^{3}H]spiperone binding with a 100-fold higher affinity than [^{3}H]SCH23390 binding, and inhibited the quinpirole-induced facilitation of high-affinity GTPase activity in rat striatal membranes. The effects of chronic administration of aripiprazole and haloperidol on dopamine D_2 receptor binding and mRNA level in rat striata were examined by a [^{3}H]spiperone binding assay and a ribonuclease protection assay. Haloperidol induced a significant rise in B_{max} of [^{3}H]spiperone binding at 1 mg/kg and in the level of dopamine D_{2L} receptor mRNA at 4 mg/kg. A high dose of aripiprazole (100 mg/kg) only tended to increase the B_{max} of [^{3}H]spiperone binding non-significantly, and had no effect on the level of dopamine D_{2L} receptor mRNA. These results indicated that aripiprazole had an antagonistic activity to dopamine D_2 receptors with a high affinity, but that the potency of aripiprazole to up-regulate dopamine D_2 receptors in the striatum was much smaller than that of haloperidol. This small up-regulation may be related to the ability of aripiprazole to act without side effects including tardive dyskinesia.

Keywords: Aripiprazole; Antipsychotic drug; Dopamine receptor; Striatum, rat; Ribonuclease protection assay

1. Introduction

Antipsychotic drugs have been used effectively in ameliorating some of the symptoms of schizophrenia. However, they simultaneously induce serious extrapyramidal side effects as well as hyperprolactinemia (Levinson, 1991; Meltzer, 1991). It is now necessary to elucidate the mechanisms by which antipsychotic drugs exert their therapeutic effects and side effects, which will guide the design of the ideal drugs that will direct one disorder without affecting the other. Antipsychotic drugs can be loosely divided into two categories, i.e., typical drugs which are associated with extrapyramidal side effects, and atypical drugs which are less associated with these side effects. Buckland et al. (1993a,b) have investigated the effects of the typical and atypical antipsychotic drugs on dopaminergic systems in the rat central nervous system, and suggested that distinct dopamine receptor subtypes may be associated with the therapeutic or side effect of antipsychotic drugs.

The dopaminergic system is known to be associated with cognitive, emotive, motor and endocrine functions. A recent development in molecular biology concerning dopamine receptors indicates multiple dopamine receptor subtypes generated from at least 5 distinct dopamine receptor genes (D_1, D_2, D_3, D_4 and D_5 receptor, Sibley and Monsma, 1992; Strange, 1993; O'Dowd, 1993). The two isoforms of the D_2 receptor mRNA, called D_{2L} and D_{2S} receptor mRNA, were identified as generated by alterna-

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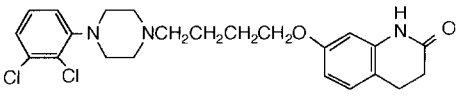

Fig. 1. Chemical structure of aripiprazole.

tive splicing (Bunzow et al., 1988; Giros et al., 1989). Binding techniques and studies of determination of their mRNA location revealed that some of them are preferentially distributed in the limbic area of the brain, which suggested that they are involved in mental illness. Indeed, dopamine receptors have been thought to be the primary targets in the medical treatment of schizophrenia.

Aripiprazole, a quinolinone derivative (OPC-14597, 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl] butoxy]-3,4-dihydro-2(1*H*)-quinolinone), is a new dopaminergic agent which has both a dopamine autoreceptor agonistic activity and a postsynaptic D_2 receptor antagonistic activity (Fig. 1, Kikuchi et al., 1995; Semba et al., 1995). It was developed as an effective antipsychotic agent without extrapyramidal side effects in consideration of the following two points: (1) the clinically effective doses of the conventional antipsychotic drugs in treating psychosis correlate remarkably well with their affinity for binding to block the postsynaptic D_2 receptor (Seeman et al., 1976) and (2) dopamine autoreceptor agonists are suggested as antipsychotic drug candidates devoid of the motor disturbances associated with the conventional antipsychotic drugs (Clark et al., 1982). Aripiprazole has recently been demonstrated to be clinically useful to alleviate both positive and negative symptoms of schizophrenia with reduced side effects (Toru et al., 1994), and in animal behavioral studies, aripiprazole showed weak cataleptogenic effects in spite of strong postsynaptic D_2 receptor antagonistic activity as compared with chlorpromazine and haloperidol (Kikuchi et al., 1995).

The ultimate aim of the present study was to understand the clinical mechanisms of aripiprazole in the treatment of schizophrenic patients without extrapyramidal side effects. We first investigated the effects of aripiprazole on the binding activity to dopamine receptors and on the high-affinity GTPase activity in the rat brain striatal membranes in vitro. Then, since the therapeutic action of antipsychotic drugs takes a much longer time to develop, the effects of chronic treatment of aripiprazole on the dopamine receptor binding activities and on the levels of D_2 receptor mRNA in the rat brain striatum were also investigated using a radioligand binding experiment and a sensitive and specific ribonuclease protection assay. The effects of a typical antipsychotic drug, haloperidol, were also investigated.

2. Materials and methods

2.1. Drugs

Aripiprazole was synthesized by Otsuka Pharmaceutical (Tokushima, Japan). Drugs were obtained from the following sources: haloperidol (Sigma, St. Louis, MO, USA), [^{3}H]SCH23390 ([*N-methyl*-^{3}H]SCH23390, 3.07 TBq/mmol, Amersham, Amersham, UK), [^{3}H]spiperone ([*benzene ring*-^{3}H]spiperone, 647.5 GBq/mmol), [γ-^{32}P]GTP (1.11 TBq/mmol) and [α-^{32}P]UTP (~ 111 TBq/mmol, DuPont-NEN, Wilmington, DE, USA), T7 polymerase (Promega, Madison, WI, USA) and restriction enzymes (New England Biolabs, Beverly, MA, USA). All other drugs were purchased from Nacalai Tesque (Kyoto, Japan) and the companies listed above.

2.2. Drug treatment of animals

Male Wistar rats (200–300 g) were housed in a 12 h light-dark cycle with free access to food and water at 25°C. They were orally administered 1, 2, or 4 mg/kg/day haloperidol, 12 or 100 mg/kg aripiprazole, or vehicle (5% arabic gum-saline) once a day for 21 days in a volume of 1 ml/kg. Rats were decapitated 3 days after the last injection, and the striatum from each rat was immediately removed and used for dopamine receptor binding experiments and the measurement of GTPase activity. For the mRNA determination, rats were decapitated on the day 6 h after the last injection.

2.3. Binding experiments for D_1- and D_2-like receptors in the rat brain striatal membranes

Membrane preparations and binding experiments were performed as described earlier (Zhang et al., 1990). The striatum was homogenized in 10 vols. of 50 mM Tris-HCl buffer (pH 7.4) and then centrifuged at $49\,000 \times g$ for 10 min. The pellet was washed once with 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ and resuspended in the same buffer to a final protein concentration of 0.5 mg/ml. [^{3}H]SCH23390 and [^{3}H]spiperone were used as radioligands for the binding experiments of D_1- and D_2-like receptors, respectively. After striatal membranes were incubated with each radioligand for 30 min at 37°C, the reaction mixture was rapidly filtered under vacuum through GF/B glass-fiber filters (Whatman Int., Maidstone, UK) with five 2-ml rinses with ice-cold 50 mM Tris-HCl buffer (pH 7.4). In both binding experiments of D_1- and D_2-like receptors, the specific binding was defined as the excess over the non-specific binding taken in the presence of (+)-butaclamol (2 μM). The protein concentration was determined according to the method of Lowry et al. (1951).

2.4. Measurement of high-affinity GTPase activity in the rat brain striatal membranes

The membrane preparations and GTPase assays were previously described by Ueda et al. (1990) with modifications. The striatum was homogenized in 10 ml of 0.32 M sucrose containing 10 mM Tris-HCl (pH 7.5) and 0.1 mM

EDTA and centrifuged at 1000 × *g* for 10 min. The supernatant was recentrifuged at 35000 × *g* for 20 min. The pellet was washed once with 10 ml of 10 mM Tris-HCl (pH 7.5), containing 0.1 mM EDTA and 5 mM dithiothreitol and resuspended in the same buffer to a protein concentration of 0.1–0.2 mg/ml. The reaction mixture (80 μl), composed of 25 mM Tris-HCl (pH 7.4), 0.1 μM EDTA, 2.5 mM dithiothreitol, 100 mM NaCl, 6 mM $MgCl_2$, 1 mM ATP, 0.5 mM 5-adenylylimidodiphosphate, 10 mM creatine phosphate, 5 units of creatine phosphokinase, 1 mM ouabain, 50 μM or 0.1 μM GTP containing approximately 70000 cpm of [γ-^{32}P]GTP, drugs to be tested and 20 μl of the membrane suspension was incubated at 37°C for 15 min. The reaction was terminated by adding 0.9 ml of 5% charcoal prewashed with 6.7 mM phosphoric acid (pH 2.3). After centrifugation at 10000 × *g* for 20 min, radioactivity of 500 μl of the supernatant was measured as the ^{32}Pi release from [γ-^{32}P]GTP indicating GTPase activity. High-affinity (low K_m) GTPase activity was calculated by subtracting GTPase activities in the presence of 50 μM GTP from that in the presence of 0.1 μM GTP.

2.5. Determination of mRNA of D_{2L} and D_{2S} receptor in the rat brain striatum

Determination of mRNA of D_{2L} and D_{2S} receptor in the rat striatum was performed as described by Inoue et al. (1994).

2.5.1. Preparation of RNA and radiolabelled antisense cRNA probe

Total RNA was isolated from the striatum according to the method of Chomczynski and Sacchi (1987). The two template cDNAs prepared by reverse transcription-polymerase chain reaction were one which contained the region corresponding to a segment extending from base 541 in the second extracellular loop to base 1227 in the third extracellular loop of the rat D_{2L} receptor (pD2(1/2)), defined a fragment of 673 bases for D_{2L} receptor and a fragment of 410 bases for D_{2S} receptor (Fig. 2, Inoue et al., 1994) and the other which contained the fourth exon of the rat β-actin (pAc), defined a fragment of 310 bases.

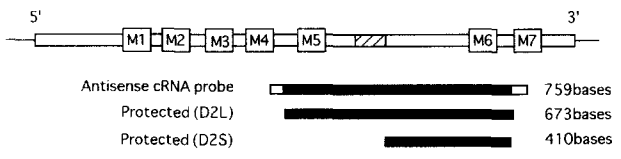

Fig. 2. Schematic illustration of D_2 receptor cDNA and the antisense cRNA probe used in this study. The boxed regions M1–M7 indicate the seven putative transmembrane domains. The hatched box represents the region which D_{2S} isoform lacks. The antisense cRNA probe is aligned under the receptor coding region, in which the filled box represents the coding region which is protected from RNase digestion and the open box represents vector sequences. Numbers on the right display the sizes of the probe and the potential protected species.

The labelled antisense RNA probes were synthesized in vitro using pD2(1/2) and pAc linearized by digestion with BamHI. After the incubation of 10 mM dithiothreitol, 20 units of RNase inhibitor, 1 μg linearized pD2(1/2) or pAc, 1.85 MBq [α-^{32}P]UTP with 12 μM UTP, 0.5 mM ATP, GTP and CTP and 20 units T7 RNA polymerase at 37°C for 60 min in a transcription buffer (Promega), 5 units of RNase-free DNase were added to the reaction mixture, and further incubation was carried out for 15 min at 37°C. Labelled RNA was precipitated after extraction with phenol/chloroform/isoamyl alcohol (25:24:1).

2.5.2. Ribonuclease protection assay

Approximately 100000 cpm and 10000 cpm of the radiolabelled antisense RNA probes for D_2 receptor and β-actin, respectively, were added to 10 μg of total RNA to be tested in the hybridization buffer composed of 40 mM PIPES (pH 6.4), 1 mM EDTA, 0.4 M NaCl and 80% formamide in a total volume of 30 μl. The hybridization was performed at 45°C for 12 h. After incubation, 40 μg/ml RNase A in 350 μl of RNase digestion buffer composed of 10 mM Tris-HCl (pH 7.4), 5 mM EDTA and 300 mM NaCl was added to the mixture. Digestion was carried out for 60 min at 30°C. 20 μl of 10% SDS and 5 μl of 10 mg/ml proteinase K were added, and further incubation was carried out for 15 min at 37°C. After extraction with phenol/chloroform/isoamyl alcohol (25:24:1), hybridized RNA with 10 μg yeast transfer RNA was precipitated with ethanol, washed with 70% ethanol once and analyzed by electrophoresis in a 4% polyacrylamide-7 M urea gel. After completion of the electrophoresis, the gel was dried and exposed to X-ray film. Alternatively, the dried gel was exposed to an Imaging Plate to analyze the intensity of each band by BAS2000 bioimaging analyzer (Fuji Film, Tokyo, Japan).

2.6. Statistics

Data are expressed as mean ± S.E.M. Effects of drug treatments in binding studies and in mRNA measurements were evaluated by one-way analysis of variance with pairwise comparison by Bonferroni method. In measuring GTPase activities, statistical comparison was carried out using the Student's *t*-test compared to the value in the presence of the corresponding dose of quinpirole. The difference was considered to be significant when the *P* value was less than 0.05.

3. Results

3.1. Displacing potency of aripiprazole in [^{3}H]SCH23390 and[^{3}H]spiperone binding in the rat brain striatal membranes

The competitive curves of aripiprazole in the binding experiments are shown in Fig. 3. Aripiprazole blocked the

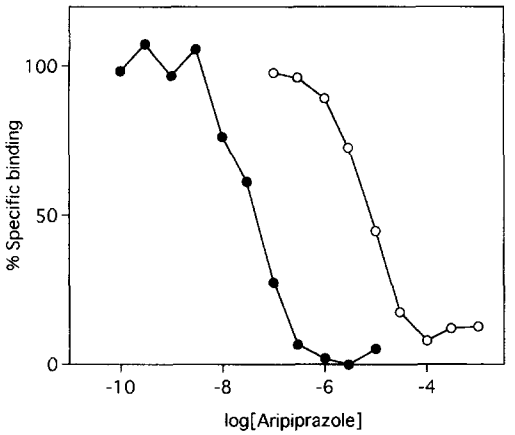

Fig. 3. Typical inhibition curves of aripiprazole in [^{3}H]SCH23390 and [^{3}H]spiperone binding in the rat brain striatal membranes. Binding experiments were performed by rat striatal membranes incubated with [^{3}H]SCH23390 (0.19 nM, ○) or [^{3}H]spiperone (0.07 nM, ●) in the presence of various concentrations of aripiprazole as described in Section 2.

[^{3}H]spiperone binding more potently than the [^{3}H]SCH23390 binding. The K_i values were $3.8 \pm 1.0 \times 10^{-8}$ M ($n = 5$) for the [^{3}H]spiperone binding and $5.1 \pm 1.1 \times 10^{-6}$ M ($n = 5$) for the [^{3}H]SCH23390 binding, indicating that aripiprazole had an approximately 100-fold higher affinity for D_2-like receptor subfamilies than for D_1-like receptor subfamilies in the rat striatum.

3.2. Effect of aripiprazole on the high-affinity GTPase activity in the rat brain striatal membranes

As shown in Fig. 4, high affinity GTPase activity was stimulated by quinpirole, an agonist of D_2-like receptors, in a concentration-dependent manner in the rat brain striatal membranes. This stimulatory effect of quinpirole was completely abolished by 1 μM (−)-sulpiride. Aripiprazole also abolished the stimulatory effects of quinpirole at the concentration of 1 μM. Aripiprazole by itself had no effect on the high-affinity GTPase activity (data not shown).

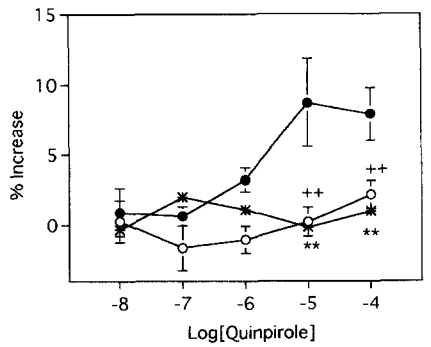

Fig. 4. Effects of aripiprazole on the quinpirole-induced high-affinity GTPase activity in the rat brain striatal membranes. Results represents the percent increase above the basal high-affinity GTPase activity induced by various concentrations of quinpirole (●) in the presence of aripiprazole (10 μM, ○) or (−)-sulpiride (10 μM, **). The basal activity was 26.2 ± 2.5 pmol/mg protein/min ($n = 7$). Data are mean ± S.E.M. (bars) values from 3–5 separate experiments. $^{++,**}$ $P < 0.01$, significantly different from the value found in the presence of the corresponding dose of quinpirole.

Table 1
[^{3}H]Spiperone binding parameters in the brain striatum from the rats chronically treated with haloperidol or aripiprazole

	$K_d \pm$S.E. (nM)	$B_{max} \pm$S.E. (fmol/mg protein)
Control (vehicle)	0.39 ± 0.06	356 ± 34
Haloperidol 1 mg/kg	0.28 ± 0.03	498 ± 39 **
Aripiprazole 12 mg/kg	0.32 ± 0.04	363 ± 33
Aripiprazole 100 mg/kg	0.33 ± 0.03	429 ± 36

Rats were given haloperidol, aripiprazole or vehicle (p.o.) for 3 weeks and killed 3 days later. [^{3}H]Spiperone binding was performed as described in Section 2. Data are mean ± S.E.M. from 5 separate experiments. ** $P < 0.05$, significantly different from the value found in the vehicle-treated rat striatal membranes (control).

3.3. Effect of chronic treatment of aripiprazole on the [^{3}H]spiperone binding parameters in the rat striatum

The effects of chronic treatment of aripiprazole on [^{3}H]spiperone binding parameters (B_{max}, K_d) were investigated in the rat brain striatal membranes. In using the striata from the drug-treated rats decapitated 24 h after the last injection, the specific binding of [^{3}H]spiperone was

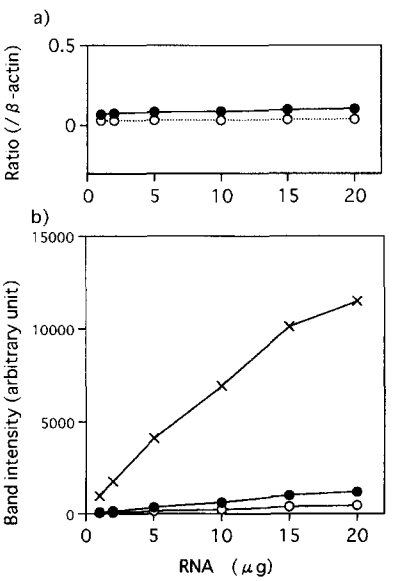

Fig. 5. The relationship between the amount of RNA and the band intensities of D_2 receptor mRNA and β-actin mRNA. RNA (1–20 μg) from rat striatum was hybridized with antisense cRNA probes, digested with RNase A and subjected to 4% PAGE. (a) The band intensities of D_{2L} (●), D_{2S} (○) receptor and β-actin (×) mRNA in each concentration of total RNA used are measured by the bioimaging analyzer BAS2000. (b) The band intensity ratio of D_{2L} (●) or D_{2S} (○) receptor mRNA ((intensity of receptors mRNA)/(intensity of β-actin mRNA)) is represented as a function of the amount of total RNA used.

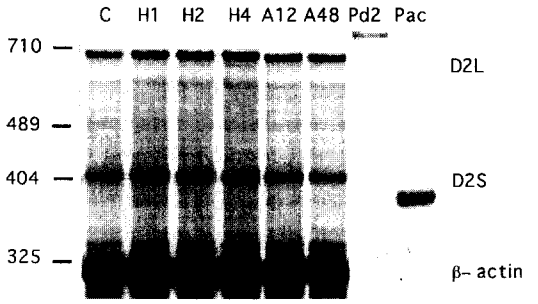

Fig. 6. Autoradiography of bands for D_{2L} and D_{2S} receptor mRNA analyzed by ribonuclease protection assay in the drug-treated rat brain striatum. Each lane represents the protected bands for mRNA from striata of the rats treated with 1, 2 and 4 mg/kg haloperidol (H1, H2 and H4) or 12 and 48 mg/kg aripiprazole (A12 and A48), or the labeled probes of D_2 receptor (Pd2, 759 bases) and β-actin (Pac, 390 bases).

small and unstable, which was caused by competition of [^{3}H]spiperone binding by residual haloperidol or aripiprazole still present in the tissue. We therefore used the striatum from the rats decapitated 3 days after the last injection. As shown in Table 1, the observed K_d values for [^{3}H]spiperone binding in the drug-treated rats were not significantly different from those in the vehicle-treated rats (control). The maximal binding capacity of [^{3}H]spiperone (B_{max}) was significantly increased by 40% in the striata of haloperidol (1 mg/kg)-treated rats compared to that of control rats. In contrast, in the rats treated with 12 mg/kg or 100 mg/kg aripiprazole, only a small, non-significant increase in B_{max} was observed.

3.4. Effect of chronic treatment of aripiprazole on the mRNA levels of D_2 receptors in the rat striatum

Fig. 6 shows the typical pattern of electrophoresis gel in the measurement of mRNA of D_2 receptors using a specific and sensitive method to identify mRNA, i.e., a ribonuclease protection assay. By using this D_2 receptor probe, which corresponds to the third intracellular loop containing the additional sequences existing in the D_{2L} isoform (Bunzow et al., 1988; Giros et al., 1989), the D_{2L} and D_{2S} isoforms can be distinctively identified in the same sample simultaneously. The band for D_{2L} migrates at the position corresponding to 673 bases, and the band of D_{2S} migrates at the position corresponding to 410 bases. The band for β-actin used as an internal standard migrates at the position corresponding to 310 bases. To assess the ability of RNase A to degrade non-hybridized RNA and the specificity of the probes, a blank assay using yeast transfer RNA in place of sample RNA was performed. We observed no protected band in the blank assay, indicating that the radiolabelled probe is specifically hybridized with respective mRNA and fully degraded by RNase A in the blank assay (data not shown). To quantify and compare the level of mRNA, the relationship of D_2 receptor mRNA signals and those of β-actin mRNA was verified. As shown in Fig. 5b, the band intensities for each mRNA were increased in proportion to the increasing amount of total RNA used, and the ratio of band intensities for D_{2L} and D_{2S} receptor mRNAs relative to those of β-actin mRNA was almost constant in the range of the amounts of total RNA used (Fig. 5a).

Fig. 6 shows the typical pattern of electrophoresis gel indicating the results of the ribonuclease protection assay of D_2 receptor mRNA in the drug-treated rat brain striatum. The level of D_{2S} mRNA was about one-third of that of D_{2L} mRNA in the rat striatum. The ratio of each band intensity for D_{2L} and D_{2S} receptor mRNA relative to that for β-actin mRNA of the same sample was calculated. The percentage values of the ratio in drug-treated rats versus that in control rats are shown in Fig. 7. Haloperidol at the dose of 1 and 2 mg/kg had no significant effects on the level of D_2 receptor mRNA. In the striatum of rats treated with 4 mg/kg haloperidol, the level of D_{2L} receptor mRNA was slightly but significantly increased compared

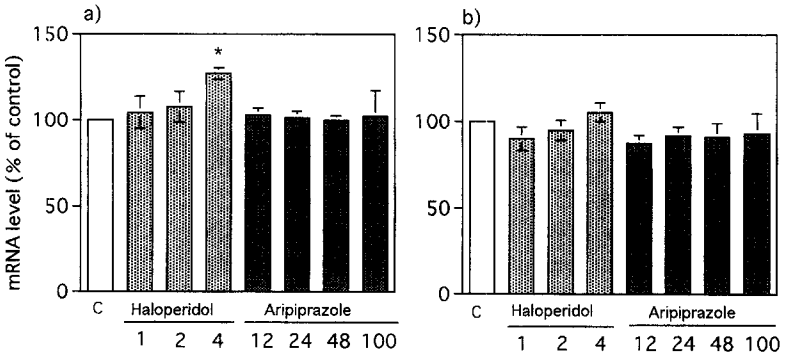

Fig. 7. The effects of chronic treatment of haloperidol and aripiprazole on the levels of D_{2L} and D_{2S} mRNA in the striatum. Relative hybridization levels for D_{2L} (a) and D_{2S} (b) receptor mRNA are expressed as the percentage of the band intensity ratio of receptor mRNA ((intensity of receptors mRNA)/(intensity of β-actin mRNA)) in the striatum from rats treated with 1, 2 and 4 mg/kg haloperidol or 12, 24, 48 and 100 mg/kg aripiprazole to that from vehicle-treated rats (C). Data are mean ± S.E.M. (bars) values from 4–10 separate experiments. * $P < 0.05$, significantly different from the value found in the control.

to that in the striatum of control rats (by 21%). In contrast, 12–100 mg/kg aripiprazole had no effect on the level of D_{2L} receptor mRNA in the rat brain striatum. The level of D_{2S} receptor mRNA was not influenced by these treatments.

4. Discussion

We demonstrated that aripiprazole (1) competed for spiperone binding sites with a high affinity, (2) had a 100-fold higher affinity for spiperone than for SCH23390 and (3) inhibited quinpirole-evoked facilitation of high-affinity GTPase activity in the rat striatum. These findings indicate that aripiprazole has a selective antagonistic activity for D_2-like receptor subfamilies over D_1-like receptors in the striatum, which is the case in conventional antipsychotic drugs (Creese et al., 1976; Seeman et al., 1976; Farde et al., 1989; Wiesel et al., 1990). Since therapeutic efficacy can be achieved after several weeks of administration of antipsychotics, we determined the effects of repeated administration of aripiprazole on D_2-like receptor activity in rats.

The chronic administration of haloperidol (1 mg/kg), a typical antipsychotic drug, significantly increased the maximal numbers of [^{3}H]spiperone binding in the rat striatum. Aripiprazole only tended to increase the B_{max} of [^{3}H]spiperone binding, even at the high dose of 100 mg/kg. Thus, the potency of up-regulation of D_2 receptors by aripiprazole seems to have been weaker than that by haloperidol in the striatum. This result is consistent with previous behavioral results in rat models. Chronic treatment with aripiprazole in rats induced supersensitization to apomorphine measured as stereotyped behaviors, but its efficacy and duration were smaller than those with chronic treatment of haloperidol (Kikuchi et al., 1995). The doses of 1 mg/kg of haloperidol and 12 mg/kg of aripiprazole are twice the ED_{50} to inhibit the stereotyped behavior induced by apomorphine, and the dose of 100 mg/kg of aripiprazole was the high dose that would evoke catalepsy in 75% of the treated rats (Kikuchi et al., 1995).

It is difficult to pharmacologically distinguish D_2-like receptors as D_2, D_3 and D_4 receptors by the binding experiment using [^{3}H]spiperone as a ligand, because spiperone has fairly high affinities for each D_2-like receptor subtype (Strange, 1993). In order to specifically determine the effect of drugs on D_2 receptors in the present study, the mRNA for this receptor was analyzed. The level of mRNA is thought to directly reflect the rate of receptor synthesis. The rate of synthesis of a receptor may be an important factor when studying the effects of a drug at the molecular level when there is a high level of receptor turnover due to high levels of a ligand. Therefore, we also investigated the effects of chronic treatment of drugs on the levels of the D_2 receptor mRNA in the rat striatum. Two isoforms are known to be generated by alternative splicing in the rat brain (D_{2L} and D_{2S}, Giros et al., 1989; Monsma et al., 1989). It is possible to determine both D_{2L} and D_{2S} mRNA simultaneously in the same sample by a sensitive and specific ribonuclease protection assay with the cRNA probe we used. Chronic treatment with haloperidol (4 mg/kg) elicited a small but significant increase (21%) in the level of D_{2L} mRNA, suggesting that the increase in the maximal binding by haloperidol was partly the result of an increase in D_{2L} mRNA. A similar up-regulation of D_2 receptor mRNA was reported by a number of groups, but the magnitude of increase differed among one another (Le Moine et al., 1990; Giros et al., 1989; Buckland et al., 1992, 1993a,b; Rogue et al., 1991; etc.). In the present study, the percent change in B_{max} (40%) was not similar to that in the level of mRNA (21%), and the dose of haloperidol (4 mg/kg) that could increase the level of D_{2L} receptor mRNA was more than that (1 mg/kg) to increase B_{max}. These results suggest that the regulation of D_2 receptors following haloperidol occurs not only by mRNA transcription but also by one or more processes such as translation, transport, insertion, internalization and degradation. Furthermore, the effect on the stability of mRNA, which is degraded soon after it is used for protein synthesis, may be involved in the change of the mRNA level. Haloperidol had no apparent effect on D_{2S} mRNA. Buckland et al. (1993a) reported that haloperidol increased both D_{2L} and D_{2S} receptor mRNA in the rat brain. Although the reason for these discrepancies is unclear, variations in experimental protocol, such as differential drug doses, time period of administration, drug administration route, differences in dissection of brain regions and so on may be causal factors. In our protocol, the change of D_{2S} receptor mRNA may be too small to detect at this time course, even by a ribonuclease protection assay. Since D_{2L} is the predominant form in the rat striatum (Giros et al., 1989), the D_{2L} receptor may have more plasticity than does the D_{2S} form to antipsychotic drugs. Rogue et al. (1991) reported rather similar magnitudes of increase in both total D_2 and D_{2L} receptor mRNAs in a Northern blot analysis.

In contrast, the chronic treatment of aripiprazole in the present study had no effect on D_{2L} and D_{2S} mRNA. Treatments with the atypical antipsychotic drugs sulpiride and clozapine were reported to up-regulate the rat brain D_3 receptor mRNA level without affecting the D_1 or D_2 receptor mRNA level, while the typical antipsychotic drugs haloperidol and loxapine up-regulated D_1, D_2 and D_3 receptor mRNA levels in the rat brain, suggesting that the up-regulation of D_3 receptor mRNA may be associated with the therapeutic action of antipsychotic drugs (Buckland et al., 1992, 1993b). Our present results also indicate that the up-regulation of D_2 receptors may be associated with the occurrence of side effects (e.g., tardive dyskinesia) but not the clinical mode of action of antipsychotics. The finding that clinically aripiprazole has reduced extrapyramidal side effects may reflect the small potency of

aripiprazole to up-regulate D_2 receptors in the striatum. Aripiprazole has been demonstrated to have fairly high affinities for both D_2 and D_3 receptors; the effects of aripiprazole on D_3 receptors and the mechanisms of its therapeutic action remain to be determined.

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