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Short communication

The antipsychotic aripiprazole is a potent, partial agonist at the human 5-HT$_{1A}$ receptor

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Abstract

Aripiprazole, 7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy}-3,4-dihydro-2(1*H*)-quinolinone, a novel antipsychotic with partial agonist activity at dopamine D2 receptors, bound with high affinity to recombinant human 5-HT$_{1A}$ receptors (h5-HT$_{1A}$) in Chinese hamster ovary cell membranes and displayed potent, partial agonism at 5-HT$_{1A}$ receptors in a guanosine-5′-*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS)-binding assay that was blocked completely by a selective 5-HT$_{1A}$ receptor antagonist. An interaction with 5-HT$_{1A}$ receptors may contribute to the overall efficacy of aripiprazole against symptoms of schizophrenia, including anxiety, depression, cognitive and negative symptoms, and to its favorable side-effect profile. Combined with previous studies demonstrating the potent partial agonism of aripiprazole at dopamine D2 receptors, this study suggests aripiprazole is the first dopamine–serotonin system stabilizer.

Keywords: Aripiprazole (OPC-14597); Partial agonist; Schizophrenia; 5-HT (5-hydroxytryptomine, seretonin); Dopamine

1. Introduction

Aripiprazole, 7-{4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy}-3,4-dihydro-2(1*H*)-quinolinone, is a novel antipsychotic with a mechanism of action that differs from all currently marketed typical and atypical antipsychotics. Biochemically, aripiprazole is a partial agonist at members of the D2 family of dopamine receptors (Inoue et al., 1996; Burris et al., 2000). In vivo, aripiprazole displays dopamine D2 receptor antagonist effects in models of dopaminergic hyperactivity (e.g. blockade of apomorphine-induced stereotypy) and dopamine D2 receptor agonist activity in a model of dopaminergic hypoactivity (blockade of increased dopamine synthesis in reserpine-treated rats) (Kikuchi et al., 1995). Limited preclinical evidence indicates that aripiprazole has activity at several serotonin receptors of clinical relevance to schizophrenia including antagonism at 5-HT$_{2A}$ (Kikuchi et al., unpublished observations). Aripiprazole is an efficacious treatment for the positive and negative symptoms of schizophrenia; however, it does not induce significant extrapyramidal symptoms or elevate serum prolactin, and has a low propensity to produce weight gain, sedation or prolongation of QT$_c$ interval on electrocardiogram (Carson et al., 2000; Kane and Ingenito, 2000).

While it has long been established that antipsychotic drug activity is a direct correlate of dopamine D2 receptor-binding affinity (Seeman et al., 1976), recent attention has focused on the 5-HT$_{1A}$ receptor as a therapeutic target for the development of improved antipsychotic drugs (Meltzer, 1999; Millan, 2000). Several clinically effective antipsychotics bind in vitro with moderate to high affinity to cloned human 5-HT$_{1A}$ receptors (Richelson and Souder, 2000), and ziprasidone and clozapine behave as partial agonists at cloned human 5-HT$_{1A}$ receptors (Newman-Tancredi et al., 1998). In the present study, a guanosine-5′ -*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS)-binding assay was used to determine the potency and relative intrinsic activity of aripiprazole at the human 5-HT$_{1A}$ receptor (h5-HT$_{1A}$) expressed stably in recombinant Chinese hamster ovary (CHO) cell membranes. A competitive radioligand-binding assay was

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also performed with hydrogen-3 8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT) to evaluate the binding affinity of aripiprazole at this recombinant h5-HT$_{1A}$ receptor.

2. Materials and methods

[^{35}S]GTPγS (1200 Ci/mmol), [^{3}H]8-OH-DPAT (124.9 Ci/mmol) and CHO cell membranes stably expressing the h5-HT$_{1A}$ receptor (CHO-h5-HT$_{1A}$, receptor expression (B_{max}) = 1.0 pmol/mg membrane protein) were purchased from NEN Life Science Products (Boston, MA). Aripiprazole and ziprasidone were synthesized by Otsuka Pharmaceutical (Tokushima, Japan). Risperidone and GDP were purchased from Sigma (St. Louis, MO) and all other reference compounds were obtained from RBI (Natick, MA).

Estimates of potency (pEC$_{50}$) and relative intrinsic activity (E_{max}, maximal drug effect on basal [^{35}S]GTPγS binding to CHO-h5-HT$_{1A}$ membranes expressed as a percentage of the effect of 10 μM serotonin (5-HT)) were obtained for aripiprazole, 5-HT, (+)-8-OH-DPAT, (−)-8-OH-DPAT, ziprasidone, buspirone, risperidone, clozapine and WAY-100635 (*N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide) by incubating each drug at 10 different concentrations for 60 min at 22 °C with CHO-h5-HT$_{1A}$ membranes (10 μg protein), mixed with buffer (25 mM Tris–HCl, 50 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EGTA, pH = 7.4) containing GDP (1 μM) and [^{35}S]GTPγS (0.1 nM). The same [^{35}S]GTPγS-binding assay was also used to determine the inhibitory potency (pIC$_{50}$) of the selective 5-HT$_{1A}$ antagonist WAY-100635, tested at 0.01, 0.1, 1, 10, 50, 100, 500, 1000, 5000 and 10,000 nM concentrations, against a 10 μM concentration each of aripiprazole, 5-HT, ziprasidone and clozapine.

All incubations were performed in triplicate, proceeded for 60 min at room temperature, and were terminated by rapid filtration through Whatman GF/B filter paper presoaked in 50 mM Tris–HCl, pH = 7.4, using a Brandel harvester and 4 × 3 ml ice-cold washes with the same buffer. Radioactive counts were detected by liquid scintillation counting (Clingamma, LKB/Wallach).

The h5-HT$_{1A}$ receptor-binding affinity of aripiprazole was determined against the 5-HT$_{1A}$-selective radioligand, [^{3}H]8-OH-DPAT. The h5-HT$_{1A}$ membranes (15–20 μg protein) were incubated for 60 min at 20 °C in a buffer (50 mM Tris–HCl, 10 mM $MgSO_4$, 0.5 mM EDTA, 0.1% ascorbic acid, pH = 7.4) containing 1 nM [^{3}H]8-OH,-DPAT and vehicle or aripiprazole at the same concentrations used in the [^{35}S]GTPγS assays. The binding assays were terminated by rapid filtration through Whatman GF/B filter paper presoaked in 50 mM Tris–HCl, pH = 7.4, and washed once in 4 ml of the same buffer. Nonspecific binding was defined in the presence of 10 μM (+)-8-OH-DPAT. The binding affinity of aripiprazole for h5-HT$_{1A}$ receptors (K_i) was calculated by the equation, $K_i = (IC_{50})/(1+([[^3H]8\text{-OH-DPAT}]/K_d)$, where the K_d for [^{3}H]8-OH-DPAT at h5-HT$_{1A}$ = 0.69 nM (NEN Life Science Products) using nonlinear regression analysis.

All estimates of h5-HT$_{1A}$-binding affinity, potency, relative intrinsic activity and inhibitory potency were determined by nonlinear regression analysis of each binding isotherm using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA).

3. Results

Fig. 1 shows the effects of aripiprazole and reference drugs on basal [^{35}S]GTPγS binding to the h5-HT$_{1A}$ receptor expressed in CHO cell membranes. Aripiprazole stimulated [^{35}S]GTPγS binding to the h5-HT$_{1A}$ receptor with a potent (pEC$_{50}$ = 8.67 ± 0.16), partial agonist (E_{max} = 68.1% of the

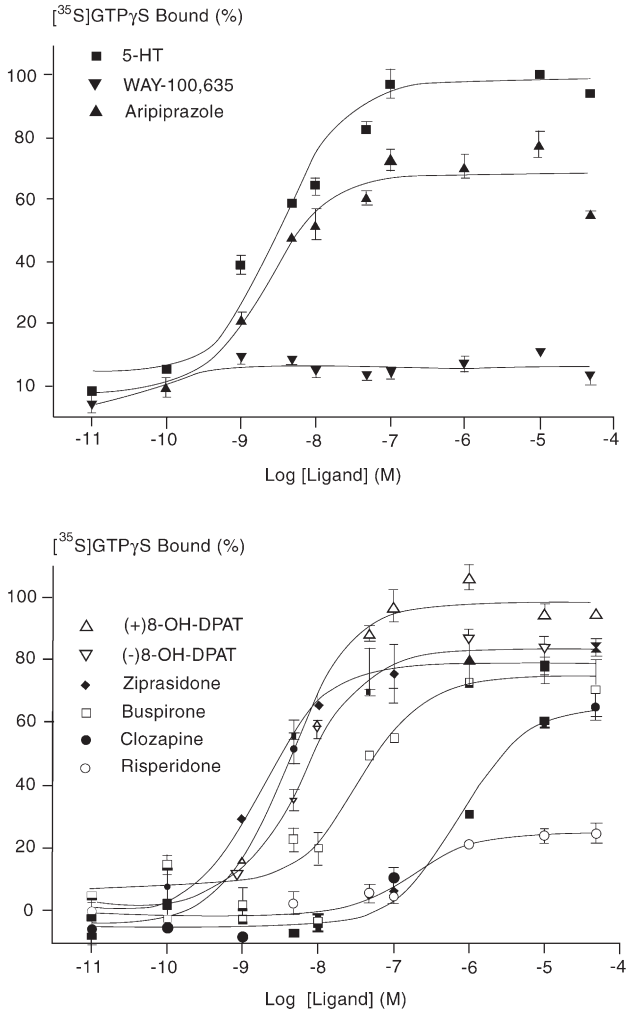

Fig. 1. Drug effects upon basal [^{35}S]GTPγS binding to CHO cell membranes expressing the h5-HT$_{1A}$ receptor. All data points are means ± S.E.M. of triplicate determinations from a single representative experiment and are expressed as a percentage of the stimulatory effect of 10 μM 5-HT on basal [^{35}S]GTPγS binding.

Table 1
Functional parameter estimates for aripiprazole and reference drugs in a [^{35}S]GTPγS-binding assay using CHO cell membranes expressing the h5-HT$_{1A}$ receptor

Agonist	pEC$_{50}$ ± SEM	E_{max}(%)	R^2
5-HT	8.43 ± 0.16	98.4	0.99
(+)-8-OH-DPAT	8.37 ± 0.08	98.5	0.99
(−)-8-OH-DPAT	8.21 ± 0.06	82.9	0.99
Ziprasidone	8.70 ± 0.08	79.0	0.99
Buspirone	7.48 ± 0.13	74.2	0.99
Aripiprazole	8.67 ± 0.16	68.1	0.99
Clozapine	6.14 ± 0.13	64.7	0.98
Risperidone	6.66 ± 0.16	25.1	0.99
WAY-100635 inhibition	pIC$_{50}$ ± SEM		R^2
10 μM 5-HT	6.66 ± 0.10		0.99
10 μM Aripiprazole	6.41 ± 0.10		0.99
10 μM Ziprasidone	6.75 ± 0.09		0.99
10 μM Clozapine	8.47 ± 0.29		0.87

Agonist potency (pEC$_{50}$) and relative intrinsic activity (E_{max}, maximal drug effect on basal [^{35}S]GTPγS binding expressed as a percentage of that produced by 10 μM 5-HT) were estimated by nonlinear regression analysis of the data shown in Fig. 1. Nonlinear regression was also used to estimate the inhibitory potency (pIC$_{50}$) of WAY-100635, tested at 0.01, 0.1, 1, 10, 50, 100, 500, 1000, 5000 and 10,000 nM, against 10 μM concentrations each of 5-HT, aripiprazole, ziprasidone and clozapine. R^2 represents the goodness of fit between observed concentration effect data points and nonlinear functions derived for each drug or drug combination studied.

effect of 10 μM 5-HT on basal [^{35}S]GTPγS binding) profile (Table 1). In comparison, potent, full agonist profiles were detected for 5-HT and (+)-8-OH-DPAT, while (−)-8-OH-DPAT and ziprasidone behaved as potent, partial agonists. Less potent, partial h5-HT$_{1A}$ agonist profiles were displayed by buspirone, risperidone and clozapine, whereas WAY-100635 failed to stimulate basal [^{35}S]GTPγS binding on its own. However, WAY-100635 blocked the stimulatory effects of 10 μM concentrations of 5-HT, aripiprazole, ziprasidone and clozapine on [^{35}S]GTPγS binding to CHO-h5-HT$_{1A}$ cell membranes, in a concentration-dependent manner in each case (Table 1). In this respect, WAY-100635 was 100-fold more potent as an inhibitor of clozapine than of either aripiprazole or ziprasidone. Aripiprazole bound with high affinity (K_i = 1.65 nM, 95% confidence interval = 1.09 to 2.48 nM; IC$_{50}$ = 4.03 nM, 95% confidence interval = 2.68 to 6.08 nM) to the h5-HT$_{1A}$ receptor in a competition-binding assay using [^{3}H]8-OH-DPAT.

4. Discussion

The main finding of the present study is that aripiprazole displayed a potent, partial agonist profile in a h5-HT$_{1A}$ [^{35}S]GTPγS-binding assay using recombinant CHO cell membranes. These data are consistent with the observation that aripiprazole binds with high affinity to the h5-HT$_{1A}$ receptor. The potency and relative intrinsic activity of ziprasidone and all other reference drugs tested in the current h5-HT$_{1A}$ [^{35}S]GTPγS-binding assay are similar to previously published estimates for these drugs (Lejeune et al., 1997; Newman-Tancredi et al., 1996, 1998; Pauwels et al., 1997). Thus, the present h5-HT$_{1A}$ [^{35}S]GTPγS-binding assay was able to identify correctly drugs with varying degrees of intrinsic agonist efficacy and potency. The h5-HT$_{1A}$ selective nature of the present assay was further demonstrated by the ability of the selective 5-HT$_{1A}$ receptor antagonist WAY-100635 to inhibit fully 5-HT, aripiprazole, ziprasidone and clozapine-induced increases in [^{35}S]GTPγS binding. In this respect, WAY-100635 was a 50- to 100-fold more potent inhibitor of clozapine than either 5-HT, aripiprazole or ziprasidone. A likely explanation for this is that 5-HT, aripiprazole and ziprasidone were far more potent in stimulating the h5-HT$_{1A}$ receptor in the present system than was clozapine. Thus, higher concentrations of WAY-100635 were required to block 10 μM concentrations of the more potent drugs, than the less potent clozapine.

Most neuropharmacological evidence is consistent with aripiprazole being a potent, partial agonist at the dopamine D2 receptor (Inoue et al., 1996; Burris et al., 2000). The present data, as well as the ability of aripiprazole to stimulate [^{35}S]GTPγS binding in rat hippocampal membranes (S. Jordan, unpublished observations) and suppress 5-HT metabolism in vivo (Jordan et al., 2001), provide the first evidence that aripiprazole is also a potent, partial agonist at 5-HT$_{1A}$ receptors. It is interesting to note that the h5-HT$_{1A}$ receptor agonist potency of aripiprazole, ziprasidone and clozapine in the present [^{35}S]GTPγS-binding assay are similar to their respective published binding affinities for h5-HT$_{1A}$ receptors (Schotte et al., 1996; Richelson and Souder, 2000).

A variety of preclinical data has suggested that the 5-HT$_{1A}$ receptor is a therapeutic target for the development of improved antipsychotic drugs (Meltzer, 1999; Millan, 2000). A putative association has been postulated between partial agonist activity at 5HT$_{1A}$ receptors and improvements in anxiety, depression, cognitive and negative symptoms, and decreased extrapyramidal symptom liability (Millan, 2000). In animal studies, 5-HT$_{1A}$ receptor agonists can diminish catalepsy, a model of extrapyramidal symptoms in humans, induced by dopamine D2 receptor antagonists (McMillen et al., 1988; Andersen and Kilpatrick, 1996; Prinssen et al., 1999), whereas elevations in serum prolactin produced by dopamine D2 receptor antagonists can be attenuated by partial 5HT$_{1A}$ receptor agonists (Nash and Meltzer, 1989).

The present study demonstrated that aripiprazole is a high affinity, potent, partial agonist at the h5-HT$_{1A}$ receptor expressed in CHO cell membranes. In the context of previous studies demonstrating that aripiprazole is a partial agonist at dopamine D2 receptors (Inoue et al., 1996; Burris et al., 2000) and an antagonist at 5-HT$_{2A}$ receptors (Kikuchi et al., unpublished observations), the results of the present study suggest that aripiprazole may best be described as a dopamine–serotonin system stabilizer. The preclinical pro-

file of aripiprazole is supportive of clinical efficacy in the treatment of schizophrenia with an excellent safety and tolerability profile.

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