Aripiprazole, A Novel Atypical Antipsychotic Drug with a Unique and Robust Pharmacology

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Atypical antipsychotic drugs have revolutionized the treatment of schizophrenia and related disorders. The current clinically approved atypical antipsychotic drugs are characterized by having relatively low affinities for D_2-dopamine receptors and relatively high affinities for 5-HT_{2A} serotonin receptors (5-HT, 5-hydroxytryptamine (serotonin)). Aripiprazole (OPC-14597) is a novel atypical antipsychotic drug that is reported to be a high-affinity D_2-dopamine receptor partial agonist. We now provide a comprehensive pharmacological profile of aripiprazole at a large number of cloned G protein-coupled receptors, transporters, and ion channels. These data reveal a number of interesting and potentially important molecular targets for which aripiprazole has affinity. Aripiprazole has highest affinity for h5-HT_{2B}-, hD_{2L}-, and hD_3-dopamine receptors, but also has significant affinity (5–30 nM) for several other 5-HT receptors (5-HT_{1A}, 5-HT_{2A}, 5-HT_7), as well as α_{1A}-adrenergic and hH_1-histamine receptors. Aripiprazole has less affinity (30–200 nM) for other G protein-coupled receptors, including the 5-HT_{1D}, 5-HT_{2C}, α_{1B}-, α_{2A}-, α_{2B}-, α_{2C}-, β_1-, and β_2-adrenergic, and H_3-histamine receptors. Functionally, aripiprazole is an inverse agonist at 5-HT_{2B} receptors and displays partial agonist actions at 5-HT_{2A}, 5-HT_{2C}, D_3, and D_4 receptors. Interestingly, we also discovered that the functional actions of aripiprazole at cloned human D_2-dopamine receptors are cell-type selective, and that a range of actions (eg agonism, partial agonism, antagonism) at cloned D_2-dopamine receptors are possible depending upon the cell type and function examined. This mixture of functional actions at D_2-dopamine receptors is consistent with the hypothesis proposed by Lawler *et al* (1999) that aripiprazole has 'functionally selective' actions. Taken together, our results support the hypothesis that the unique actions of aripiprazole in humans are likely a combination of 'functionally selective' activation of D_2 (and possibly D_3)-dopamine receptors, coupled with important interactions with selected other biogenic amine receptors—particularly 5-HT receptor subtypes (5-HT_{1A}, 5-HT_{2A}).

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INTRODUCTION

Schizophrenia, a neuropsychiatric disorder affecting more than 1% of the world's population, is a devastating and costly disease. As of 1991, it was estimated that costs associated with schizophrenia in the United States approached $65 billion per year (Wyatt *et al*, 1995). Costs are high because schizophrenia is a chronic disease, it affects people in their youth, and treatment often requires frequent and intensive hospitalization and outpatient care. Schizophrenia is characterized by the appearance of delusions and hallucinations (positive symptoms), but may involve a variety of other symptoms including decreased social functioning and speech, lack of motivation, poor hygiene, and disorganization (all negative symptoms), as well as cognitive impairment.

Effective medication-based treatments for schizophrenia have been available for more than four decades. Early therapies were based on what are now called typical antipsychotics (eg chlorpromazine, haloperidol, and fluphenazine). These drugs were effective in reducing the positive symptoms in 70–80% of schizophrenics, but tended to cause acute extrapyramidal side effects (EPS) such as Parkinsonism and acute dystonias, a lack of improvement

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(or a worsening) of negative symptoms, elevation of serum prolactin levels, and (with chronic use) the debilitating neurological side effect tardive dyskinesia. A common feature of typical antipsychotic drugs is their affinity for D_2-dopamine receptors, typically in the low nanomolar (<2.0 nM) range (Creese *et al*, 1976)

Clozapine, the prototypical atypical antipsychotic, was reintroduced in the late 1980s following a pivotal study demonstrating its effectiveness in treatment-resistant schizophrenia (Kane *et al*, 1988). Since then, other atypical antipsychotic drugs from several chemical classes, including dibenzodiazepines (clozapine and quetiapine), thienobenzodiazepines (olanzapine), benzisothiazolyl piperazines (ziprasidone), and benzisoxazoles (risperidone), have been introduced. Unlike the typical antipsychotics, it has been difficult to find a common mechanism explaining the actions of these drugs. With the exception of quetiapine, the atypicals have 5-HT_{2A}/D_2 affinity ratios grater than 10 (Meltzer *et al*, 1989), but they also interact with other receptors from the serotonin (5-HT_{1A}, 5-HT_{2C}, 5-HT_6, 5-HT_7) (Roth *et al*, 1992, 1994, 1998), dopamine (D_3, D_4) (Roth *et al*, 1995; Sokoloff *et al*, 1990; Van Tol *et al*, 1991), muscarinic cholinergic (Stanton *et al*, 1993; Zeng *et al*, 1997), and histamine families (Kroeze *et al*, 2003; Roth *et al*, in press). In fact, the useful properties of the atypicals may depend on simultaneous effects caused by interactions with multiple G protein-coupled receptors (GPCRs) (Roth, 1994, 2000). From this perspective, it is not surprising that atypical antipsychotic drugs are effective in bipolar and schizoaffective disorders and depression (Ghaemi *et al*, 2000; Narendran *et al*, 2001; Sanger *et al*, 2001).

Recently, the FDA approved aripiprazole, a new atypical antipsychotic drug (Inoue *et al*, 1997; Inoue *et al*, 1996; Oshiro *et al*, 1998) that is proposed to differ in mechanism of action from other atypical antipsychotic drugs. Aripiprazole has high affinity for D_2- and D_3-dopamine and 5-HT_7 serotonin receptors (Lawler *et al*, 1999). There has been a long-standing hypothesis that D_2 partial agonists would be of particular utility in schizophrenia (Tamminga, 2002), and the developers of aripiprazole have proposed that the novel and improved clinical profile of aripiprazole is caused by the partial agonist properties at D_2-dopamine receptors that result in 'dopamine stabilization' (Burris *et al*, 2002; Inoue *et al*, 1996). Conversely, it has been suggested (Lawler *et al*, 1999) that aripiprazole is not simply a partial agonist, but a drug whose D_2 functional effects were dependent on the cellular location (and signaling proteins) of the targeted D_2 receptor, a phenomenon termed 'functional selectivity' (Kilts *et al*, 2002; Lawler *et al*, 1999; Mottola *et al*, 2002) or 'agonist trafficking' (Kenakin, 1995). Whatever the mechanism, aripiprazole has been shown to be effective in treating the positive and negative symptoms of schizophrenia with a low incidence of side effects including minimal short-term weight gain, low liability for inducing movement disorders, and reductions (rather than elevations) in plasma prolactin levels (Goodnick and Jerry, 2002; Goodnick *et al*, 2002; Kane *et al*, 2002).

To discover the mechanisms responsible for the favorable actions of aripiprazole in schizophrenia, we conducted a comprehensive pharmacological profiling of aripiprazole at a large number of cloned human molecular targets including GPCRs, ion channels, protein kinases and transporters. We discovered that aripiprazole has a more robust pharmacological profile than previously suspected, including moderate-to-high affinity for a large number of human cloned biogenic amine GPCRs. The current data also demonstrate that aripiprazole can have partial agonist properties at D_3-dopamine, and 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} serotonin receptors. Finally, consistent with the notion that aripiprazole is not a simple partial agonist, we show that it can appear to be a typical antagonist in many D_{2L}-dopamine functional assays, suggesting the need for further studies on the 'functional selectivity' properties of the drug.

METHODS

Radioligand Binding Assays

A large number of transiently and stably transfected cloned human cDNAs, obtained via the resources of the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP), were used for radioligand binding and functional assays as previously detailed (Rothman *et al*, 2000; Tsai *et al*, 2000). Conditions for radioligand binding assays, along with K_D values for standard compounds, are listed in Table 1. In initial screening assays, aripiprazole was tested at a concentration of 10 μM in quadruplicate at a large number of GPCRs, ion channels, and transporters. For molecular targets at which $>50\%$ inhibition was measured, K_i determinations were obtained using at least six concentrations of aripiprazole; K_i values were calculated in quadruplicate using GraphPad Prism (GraphPad Software, San Diego California, USA). [^{125}I]DOI competition assays were performed as previously described (Choudhary *et al*, 1992) with the following changes: 12 dilutions of aripiprazole spanning a range of 0.01–3000 nM were incubated with [^{125}I]DOI (0.3 nM) in total volumes of 0.25 ml at 25°C for 1 h with 5–20 μg of membrane protein in binding buffer (50 mM Tris buffer, pH 7.4, 0.5 mM EDTA, 10 mM $MgCl_2$). Membranes were harvested with a Brandel cell harvester by three ice-cold washes onto polyethyleneimine-pretreated (0.3%) Whatman GF/C filters. Radioactivity bound to filters was quantified by liquid scintillation counting.

PI Hydrolysis Assays

PI hydrolysis experiments were performed as previously described, with minor changes (Bhatnagar *et al*, 2001; Gray *et al*, 2001; Shapiro *et al*, 2000). Briefly, PO1C, GF62, or C6-glioma cells were grown in T75 flasks to 80% confluency, and plated into 24-well plates with Dulbecco's modified Eagle's medium containing 10% dialyzed fetal calf serum. After 24 h, media were aspirated and replaced with a serum-free, inositol-free basal Eagle's medium containing [^{3}H]inositol (1 μCi/ml). After 24 h, media were aspirated and replaced with 1.0 ml of a modified Krebs buffer (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 24 mM $NaHCO_3$, 11 mM dextrose, 18 mM LiCl) preincubated to 37°C and equilibrated with 5% CO_2. Seven concentrations of test compounds in triplicate were applied in a total volume of 1.0 ml per well and incubated 1 h. The solution was then quickly aspirated, replaced with 1.0 ml of

Table 1 Affinities for Aripiprazole and Reference Compounds at Various Receptors, Channels and Transporters

Receptor	Cold ligand	^{3}H-ligand	K_D (nM)	Assay conc. (nM)	Aripiprazole K_i (nM)
5-HT_{1A}	WAY 100,635	8-OH-DPAT	1	0.5	5.6 ± 0.8
5-HT_{1B}	Ergotamine	GR125743	0.3	0.3	830 ± 260
5-HT_{1D}	Ergotamine	GR125743	0.3	0.3	68 ± 11
5-HT_{1E}	5-HT	5-HT	7.2	3	8000 ± 5000
r5-HT_{2A}	Chlorpromazine	Ketanserin	0.8	0.5	22 ± 4
5-HT_{2A}	Chlorpromazine	Ketanserin	2	1	8.7 ± 2.0
5-HT_{2A}	Chlorpromazine	[125I]DOI	2.5	0.2	35 ± 4
5-HT_{2B}	Norfenfluramine	LSD	10	5	0.36 ± 0.11
r5-HT_{2C}	Chlorpromazine	Mesulergine	1.2	0.5	76 ± 8
5-HT_{2C} VGI[a]	Chlorpromazine	Mesulergine	1.7	1	180 ± 37
5-HT_{2C} INI[a]	Chlorpromazine	Mesulergine	1.7	1	75 ± 14
5-HT_{2C} VGI[a]	Chlorpromazine	[125I]DOI	3	0.2	97 ± 100.0
5-HT_{2C} INI[a]	Chlorpromazine	[125I]DOI	3	0.2	22 ± 11
r5-HT_3	LY-278,584	Zacopride	0.3	0.3	630 ± 110
5-HT_{5A}	Ergotamine	LSD	1.6	1	1240 ± 280
5-HT_6	Chlorpromazine	LSD	1.5	1	570 ± 95
5-HT_7	Chlorpromazine	LSD	2	1	10.3 ± 3.7
D_1	SKF38393/Fluphenazine	SCH23390	0.35	0.2	1960 ± 670
D_{2L}	Haloperidol	*N*-methylspiperone	0.5	0.2	0.74 ± 0.09
D_2	Haloperidol	*N*-methylspiperone	0.4	0.2	3.3 ± 1.1
D_3	Chlorpromazine	*N*-methylspiperone	0.4	0.2	9.7 ± 5.4
D_3	Chlorpromazine	*N*-methylspiperone	0.4	0.2	1.0 ± 0.40
rD_4	Chlorpromazine	*N*-methylspiperone	0.5	0.2	510 ± 93
D_5	SKF38393/Olanzapine	SCH23390	0.3	0.2	2590 ± 1350
MOR	Naloxone	Diprenorphine	0.2	0.2	> 10000
DOR	Naltrindole	Diprenorphine	0.2	0.2	> 10000
KOR	Naloxone	Bremazocine	4	2	> 10000
SERT	Fluoxetine	Citalopram	0.8	0.5	1080 ± 180
NET	Nortriptyline/imipramine	Nisoxetine	1.2	0.5	2090 ± 750
DAT	4′,4′-Diflouro-3a (diphenyl-methoxy) tropane HCl	GBR12935	1	0.5	3220 ± 660
H_1	Chlorpheniramine	Pyrilamine	3.6	1	25.1 ± 2.6
H_2	Me-histamine	Tiotidine	10	0.5	> 10000
gpH_3	Histamine	Me-histamine	1	0.5	224 ± 164
H_4	Clozapine	Histamine	10	5	> 10000
rBZP	Diazepam	RO 15-1788	0.8	0.4	> 10000
rGABA-A	GABA	Muscimol		3	> 10000
rGABA-B	GABA	Baclofen		15	> 10000
α-PKC	PMA	PDBU	1	0.5	> 10000
γ-PKC	PMA	PDBu	1	0.5	8000 ± 2600
rNMDA (PCP site)	PCP/ketamine	TCP	1	0.5	4001 ± 2177
α1A	Urapidil	Prazosin	0.2	0.2	25.7 ± 5.0
α1B	Corynanthine	Prazosin	0.2	0.2	34.8 ± 5.8
α2A	Oxymetazoline	Clonidine	2	2	74.3 ± 11.7
α2B	Prazosin	Clonidine	2.0	2.0	103 ± 10.6
α2C	Prazosin	Clonidine	2.0	2.0	37.9 ± 3.3
hβ1	Atenolol	Pindolol	0.1	0.1	141 ± 4.2
hβ2	ICI-118,551	Pindolol	0.1	0.1	163 ± 15.0
M1	Pirenzepine	QNB	0.2	0.5	6780 ± 570
M2	Methoctramine	QNB	0.2	0.5	3510 ± 620
M3	4-DAMP	QNB	0.2	0.5	4680 ± 440
M4	Tropicamine	QNB	0.2	0.5	1520 ± 230
M5	Pirenzepine	QNB	0.2	0.5	2330 ± 383
Prostaglandin Ep3	PGE2	PGE2	2	1	> 10000
Prostaglandin Ep4	PGE2	PGE2	2	1	> 10000

Experiments were performed as described in Methods using the radioligands and unlabeled reference ligands listed above. Data represent mean ± SEM of at least four separate experiments. All studies were performed with human cloned cDNAs expect where specified; r = rat; gp = guinea-pig.
[a]Refers to mRNA editing isoform.

a stop solution (formic acid/dH_2O 1 : 100). After 20 min, the stop solution containing the accumulated inositol phosphates (PIs) was added to columns containing 1 ml of anion exchange resin (formate form) and washed with 12 ml of water followed by 10 ml of 5 mM sodium borate/50 mM sodium formate (Roth *et al*, 1986). Total PIs were eluted with 10 ml of 0.1 M formic acid/0.2 M ammonium formate into vials containing 3a70B liquid scintillation cocktail (Research Products International, Elk Grove Village, IL), and radioactivity was measure by liquid scintillation counting.

Effects of Aripiprazole on cAMP Production

Inhibition of forskolin-stimulated cAMP production. Inhibition of forskolin-stimulated 3′,5′-cyclic adenosine monophosphate (cAMP) production in stable D4 and 5-HT_{1A} receptor expressing cell lines was measured as previously reported (Lawler *et al*, 1999; Zhang *et al*, 1994). In brief, cells were grown in 24-well plates and growth media were replaced with fresh F12 medium containing 100 μM IBMX and 100 μM forskolin (all on ice) just prior to experimentation. Serial dilutions (10-fold) of aripiprazole ranging from 0.1 to 10.000 nM were added to the cells, which were then incubated 20 min at 37°C and 5% CO_2. The reaction was terminated by aspiration and the addition of 0.5 ml of ice-cold 3% trichloroacetic acid. Plates were chilled for 1 h at 4°C and spun at 1000 *g* for 15 min. cAMP was quantified using a competitive binding assay adapted with minor modifications (Nordstedt and Fredholm, 1990). For measurement of cAMP content, trichloroacetic acid extracts (40 μl) were added to reaction tubes containing cAMP assay buffer (100 mM Tris-HCl, pH 7.4, 100 mM NaCl, 5 mM EDTA). [^{3}H]cAMP (1 nM final concentration) was added to each tube, followed by cAMP-binding proteins (approximately 100 μg of crude extract from bovine adrenal cortex in 500 μl of cAMP buffer). The reaction tubes were incubated on ice for 2 h, then harvested with a Brandel cell harvester onto Whatman GF/C filters soaked in water. Filters were allowed to dry, and bound radioactivity was quantified by liquid scintillation counting. The concentration of cAMP in each sample was estimated from a standard curve ranging from 0.1 to 100 pmol of cAMP/assay.

Stimulation of cAMP production. Studies of the effects of serotonin and aripiprazole at 5-HT_6 and 5-HT_7 receptors were carried out in stable transfectants using methods previously described (Max *et al*, 1995; Monsma *et al*, 1993; Shen *et al*, 1993).

Electrophysiological Studies of D_{2L}, D_3, and D_4 Receptors

Electrophysiology experiments were performed on MES-23.5 cells stably transfected with D_{2L}, D_3, and D_4 receptor DNA, and drugs were administered in the bath as previously described (Liu *et al*, 1999). D_{2L}, D_3, and D_4 receptor expression levels were 325, 360, and 470 fmol/mg respectively, as originally reported by Liu *et al* (1999). In brief, cover slips with attached MES-23.5 cell were transferred from 24-well plates into a recording chamber, where cells were perfused continuously at a flow rate of 1–2 ml/min by a solution containing (in mM): NaCl, 135; KCl, 5.4; $CaCl_2$, 1.8; glucose, 20; HEPES, 5; plus 0.5 μM tetrodotoxin, adjusted to pH 7.3 with Tris base, osmolarity adjusted to 320–330 mOsmol. The dish was placed on the stage of an inverted microscope (Zeiss ICM 405) and visualized using Hoffman differential interference contrast optics at × 400. The recording headstage and electrode assembly were held on an articulated arm of a three-position joystick-controlled micromanipulator (MM-8000F, Activational System Inc., Warren, MI) during recording. The voltage-clamp in the whole-cell configuration used an EPC-7/list patch clamp amplifier (Adams-List Associates, Ltd., Westbury, NY). Patch electrodes were fabricated from 1.5 to 1.6 OD MicroHematocrit capillary tubes (VWR Scientific) pulled in a two-stage process on a Narishigie vertical puller (model PA-81) and polished on a Narishigie microforge (model MF-83). The holding potential and all voltage steps were controlled by PCLAMP software and hardware (Axon Instruments, Foster City, CA) using the CLAMPEX program. The patch electrode solution contained the following (in mM): KCl, 140; $MgCl_2$, 2; $CaCl_2$, 1; HEPES, 10; ATP, 2; cAMP, 0.25; and BAPTA, 0.5; adjusted to pH 7.3 with KOH, osmolarity adjusted to 320–330 mOsmol. When tetraethylammonium (TEA)-Cl (30 mM) was added to the bathing medium, an equimolar concentration of KCl was removed. All solutions were filtered through a 0.45 μM membrane filter. The patch electrode resistance ranged between 2 and 4 MΩ. The seal resistances were greater than 5 GΩ and after membrane rupture the series resistance was between 4.3 and 10.0 MΩ (mean ± SEM: 7.1 ± 1.8 megohms; $n = 100$) and the level of capacitance compensation ranged between 10 and 22 pF (mean ± SEM and 15.9 ± 6.5 pF; $n = 100$). All recordings were carried out at room temperature (18–22°C). To activate steady-state outward currents, the cells were clamped at a holding potential of −60 mV and stepped to membrane potentials between −80 mV and +90 mV. All drugs were dissolved in the bath medium applied by a perfusion system.

GTPγS Binding Studies

The effects of drugs on [^{35}S]-GTPγ-S binding were determined in the presence and absence of agonist. Nonspecific binding was defined as binding in the presence of 10 μM cold GTPγ-S. Assay tubes contained 150–200 pM [^{35}S]-GTPγ-S, binding buffer (50 mM HEPES, 100 mM NaCl, 4 mM $MgCl_2$, 1 mM EDTA, 0.1% BSA, 0.1% ascorbic acid, pH 7.4 with NaOH), 10 μM GDP and varying concentrations of compounds. CHO hD_{2L} cell membranes (from cells expressing 4.6 pmol/mg receptor) were incubated with test compounds for 15 min at 30°C before addition of [^{35}S]-GTPγ-S. Assay tubes continued to incubate for 30 min. The assay was terminated by filtration with ice-cold wash buffer (50 mM HEPES, 4 mM $MgCl_2$, pH 7.4 with KCl). Filters were allowed to dry, and 20 μl of Packard MicroScint 20 scintillation cocktail was added to each well. Radioactivity in each sample was determined on a Packard Top Count NXT.

Data Analyses

Dose–response curves were analyzed by nonlinear regression using Prism 3.0 software (GraphPad, Inc., San Diego, CA). Other data analysis used InStat 2.0 × (GraphPad, Inc., San Diego, CA) or Prism 3.0 ×. Statistical significance ($\alpha = 0.05$) was determined either by ANOVA followed by the listed *post hoc* test, or by two-tailed Student's *t*-test. Some receptor data were analyzed using LIGAND (Munson and Rodbard, 1980).

RESULTS

Affinities of Aripiprazole for Dopamine, Serotonin, and Other Receptors

The affinity of aripiprazole for a variety of receptors was measured by competition radioligand binding assays.

Table 1 summarizes the affinity values, and also lists the radioligands and reference compounds used for each assay. The affinities for human 5-HT_{2A} and 5-HT_{2C} receptors were determined for both the inhibition of antagonist ([^{3}H]ketanserin) and agonist ([^{125}I]DOI) radioligands.

Of interest are the relatively high affinities (K_i's 10 nM or less) of aripiprazole for the hD_2, and rD_3-dopamine, and h5-HT_{1A}−, h5-HT_{2B}−, and h5-HT_7 serotonin receptors (Table 1). In addition, aripiprazole had moderate affinity for the hH_1 (25.1 nM) and gpH_3-histamine (224 nM) receptors, but low affinity ($K_i > 10\,000$ nM) for the hH_2- and hH_4-histamine receptors (Table 1). At all of the adrenergic receptors tested, aripiprazole displayed moderate affinity, highest at the α_{1A} ($K_i = 25.7$ nM) and lowest at α_{2B} ($K_i = 103$ nM). Low affinities were observed at the m1–m5 muscarinic receptors ($K_i > 1\,500$ nM).

Of *particular* interest was the observation that aripiprazole's affinity at D_2 receptors was profoundly affected by assay conditions. Thus, when rat D_2-receptors were expressed in HEK293 cells using an assay buffer containing NaCl (50 mM Tris-HCl, pH = 7.4, 50 mM NaCl, 5 mM $MgCl_2$, 0.5 mM EDTA) affinities were 6.8-fold lower than that reported in rat striatal membranes, but a striking 62-fold lower than those values obtained using stably transfected CHO cells assayed in a Na-free buffer (Lawler *et al*, 1999). The large effect of sodium ions on the measured affinity of aripiprazole is likely due to the fact that sodium ions decrease agonist affinities for D_2-family receptors (Neve, 1991; Neve *et al*, 1991, 2001).

Functional Effects at Serotonin Receptors

The functional characteristics of aripiprazole were assessed in several different cell systems, including some that were also used for receptor screening, as well as others chosen because of their functional utility. The first system studied was the 5-HT_{1A} receptor stably expressed in Chinese Hamster Ovary cells. As shown in Figure 1, serotonin had an EC_{50} of 4.5 nM, whereas aripiprazole was a low potency ($EC_{50} = 329$ nM) partial agonist at inhibiting forskolin-stimulated cAMP production. At the 5-HT_{2A} receptor (Figure 2), aripiprazole was of even lower intrinsic activity. Thus, in C6-glioma cells that express 5-HT_{2A} receptors without receptor reserve, aripiprazole caused increases in PI hydrolysis only at the highest concentration tested (10 μM). In GF62 cells, a cell line expressing the rat 5-HT_{2A} receptor with substantial receptor reserve, aripiprazole was a partial agonist with an EC_{50} of 48 nM and intrinsic activity 12.7% that of the full agonist 5-HT.

Next, aripiprazole was studied at 5-HT2B and 5-HT2C receptors. In HEK-293 cells transiently expressing 5-HT_{2B} receptors 5-HT-stimulated PI hydrolysis with an EC_{50} of 41 nM (Figure 3). At 5-HT_{2B} receptors, aripiprazole was an inverse agonist that decreased PI hydrolysis to approximately half of basal levels, with an EC_{50} of 11 nM. In contrast, aripiprazole was a partial agonist, albeit with relatively high intrinsic activity, at serotonin 5-HT_{2C} receptors expressed in PO1C cells (r5-$HT_{2c\text{-}VGV}$). As shown in Figure 4, aripiprazole had an EC_{50} of 26 nM, and an intrinsic activity 82% that of serotonin. Using COS-7 cells transiently transfected with 5-HT_{2C} INI receptor, aripiprazole was a weak full agonist, with an EC_{50} of 4625 ± 2991 nM and efficacy matching that of the serotonin control (data not shown). The effects of aripiprazole were completely blocked by 100 μM ketanserin (a 5-$HT_{2A/2C}$ antagonist), showing that the effects were indeed 5-HT_{2C} mediated.

The diverse effects caused by aripiprazole were seen again in studies of serotonin 5-HT_6 and 5-HT_7 receptors. At the 5-HT_6 receptor (Figure5, left), serotonin-stimulated cAMP production in 5-HT_6 stable transfectants. Aripiprazole (10 μM) had not only no intrinsic activity of its own, but also it blocked essentially all of the effects of serotonin. At

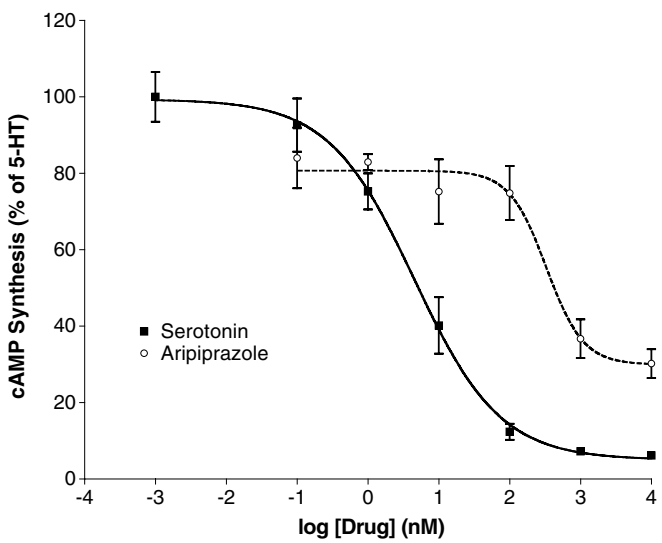

Figure 1 Aripiprazole is a low potency partial agonist at the 5-HT_{1A} receptor stably expressed in 1ACHO cells. Inhibition of forskolin-stimulated cAMP production was compared for serotonin and aripiprazole. Data represent mean ± SEM from three separate experiments.

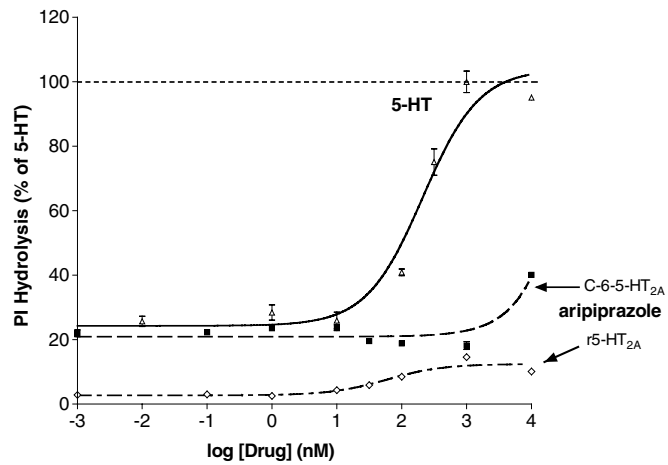

Figure 2 Effect of aripiprazole on serotonin 5-HT_{2A} receptors. Functional effects of aripiprazole on PI hydrolysis were studied in the C-6 glioma line expressing an endogenous rat 5-HT_{2A} receptor (C-6-5-HT_{2A}) and in GF62 cells transfected with the rat 5-HT_{2A} (r5-HT_{2A}). Response data are plotted relative to maximal percentage stimulation by 5-HT in each cell line, with the 5-HT curve being shown in the C-6 line for the sake of clarity. Serotonin-stimulated PI hydrolysis in C-6-glioma cells with an $EC_{50} = 211$ nM, whereas aripiprazole caused partial stimulation only at the highest tested concentration (10 μM). Similarly, in the GF62 cells, aripiprazole was a partial agonist of extremely low intrinsic activity. Data represent mean ± SEM of triplicate determinations from three (C-6) or two (GF62) separate experiments.

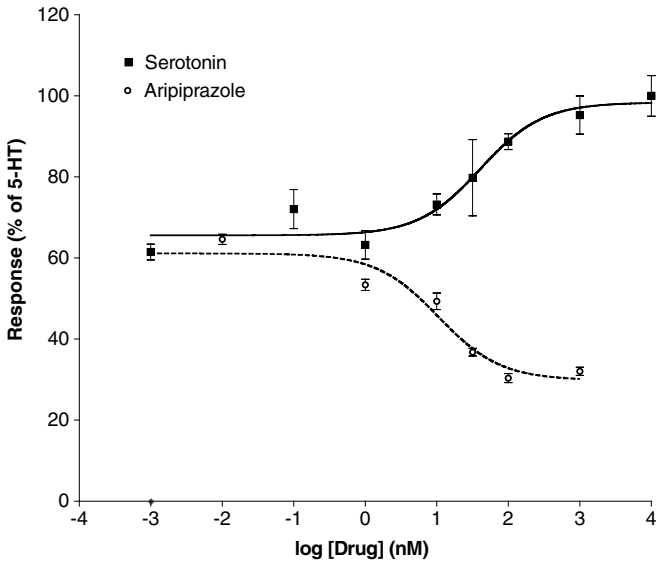

Figure 3 Aripiprazole is an inverse agonist at the 5-HT_{2B} receptor in HEK-293 cells. Serotonin-stimulated PI hydrolysis in HEK 293 cells transiently transfected with 5-HT_{2B} receptors with an EC_{50} of 41 nM. Aripiprazole decreased PI hydrolysis from a basal level of 61% down to a low of 30% at 1000 nM, with an EC_{50} of 11 nM. Data represent means ± SEM from three separate experiments.

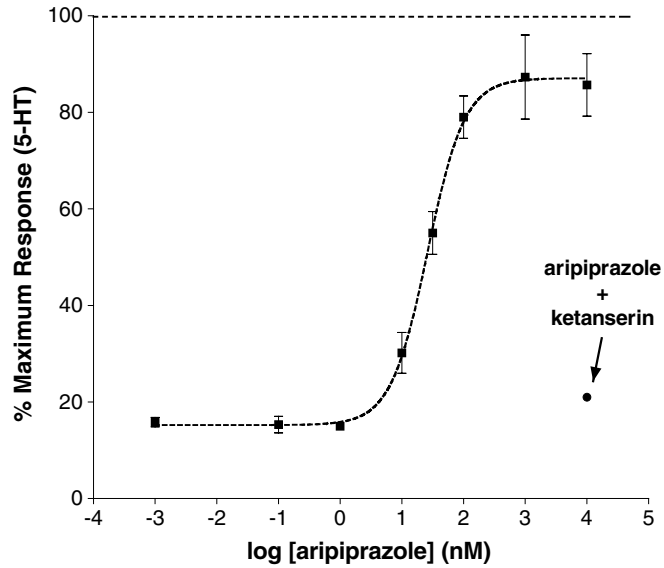

Figure 4 Effect of aripiprazole on serotonin 5-HT_{2C} receptors in PO1C cells. Data represent means and standard errors of triplicate determinations. The single point demonstrates that 100 μM ketanserin can completely block the effects of 10 μM aripiprazole.

the 5-HT_7 receptor (Figure 5, right), serotonin, as expected, stimulated cAMP production in 5-HT_7 stable transfectants. Here, aripiprazole (10 μM) had barely measurable intrinsic activity, and consistent with this conclusion, the same concentration was able to reduce the effects of serotonin to a level equal to aripiprazole alone. Thus, aripiprazole was an antagonist at 5-HT_6 receptors and a weak partial agonist at 5-HT_7 receptors.

Functional Effects at Dopamine Receptors

Some prior studies have suggested that aripiprazole is a partial agonist at D_2 receptors (Burris *et al*, 2002), while others have proposed that the drug is 'functionally selective' at D_2 receptors (Lawler *et al*, 1999). The present experiments thus tested aripiprazole in several different systems, including many not previously studied. The first system studied (Figure 6a) was the MES-23.5 cell line that had stably transfected D_{2L} receptors. As expected, quinpirole caused a 75% increase in outward K^+-currents, whereas 10 μM aripiprazole had no effect whatsoever, despite its known affinity for this receptor. A similar pattern was seen when GTPγS binding studies were carried out in D_{2L}-transfected CHO cells (Figure 6b). GTPγS binding was increased markedly by both dopamine and quinpirole, and the D_2 antagonist domperidone could block completely the effects of either dopamine or quinpirole. Conversely, aripiprazole alone caused no significant effect, and was equally effective as domperidone at blocking the actions of either dopamine or quinpirole. Finally, in two different cell lines stably expressing the D_{2L} receptor, it was not possible to inhibit forskolin-stimulated cAMP production at any concentration of aripiprazole tested (data not shown). Thus, aripiprazole was a pure antagonist in three different systems expressing the D_{2L} receptor.

On the other hand, we replicated the data reported by Burris *et al* (2002) who found that aripiprazole was a relatively efficacious partial agonist in HEK-293 cells expressing the D_{2L} receptor. As shown in Figure 7, aripiprazole displayed an EC_{50} of 0.15 nM ($pEC_{50} = 9.82 \pm 0.12$) and an intrinsic activity of ca 75% compared to 0.10 nM (10.0 ± 0.05) and 90% for the reference compound bromocriptine. In CHO cells expressing the D_{2L} receptor, aripiprazole had clear agonist activity, although it was less potent ($EC_{50} = 18.3$ nM; $pEC_{50} = 7.7 \pm 1.2$) than bromocriptine ($EC_{50} = 0.12$ nM; $pEC_{50} = 9.9 \pm 0.15$).

MES-23.5 cells were used for studies of both the D_3 and D_4 receptors. As shown in Figure 8, and in contrast to the effects at D_{2L} receptors in this cell line, both quinpirole (10 μM) and aripiprazole (10 μM) activated D_3 receptors. Moreover, the effects of aripiprazole were completely blocked by the D_2-like antagonist sulpiride (20 μM). When this same MES-23.5 cell line is transfected with D_4 receptors (Figure 9, left), K^+ currents were inhibited, rather than stimulated by quinpirole. Aripiprazole (10 μM) caused the same magnitude of effect (ca 30% decrease) as did quinpirole (10 μM). In the same cell line (Figure 9, right), aripiprazole was a partial agonist ($EC_{50} = 16$ nM) at the D_4 receptor *vs* inhibition of forskolin-stimulated cAMP production. Dopamine also caused a dose-dependent inhibition with an $EC_{50} = 363$ nM.

DISCUSSION

Receptor Profile and Mechanism of Action

While it was known from prior studies that aripiprazole interacted with dopamine and some serotonin receptors (Lawler *et al*, 1999), the present data show that aripiprazole has a novel and unexpectedly robust pharmacology,

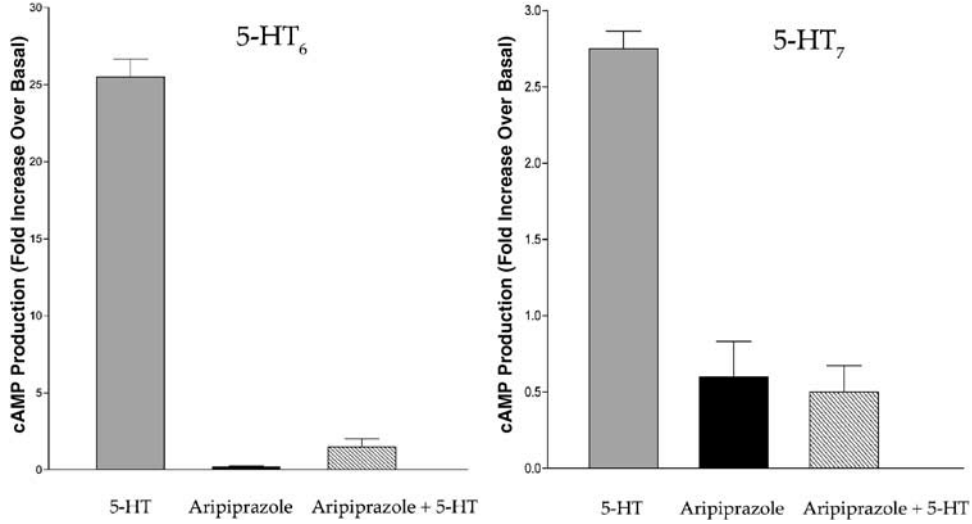

Figure 5 Antagonist effects of aripiprazole at 5-HT_6 and 5-HT_7 receptors in stable transfectants. (Left) 5-HT-stimulated cAMP production in 5-HT_6 stable transfectants. Aripiprazole (10 μM) blocked essentially all of the effects of serotonin at the 5-HT_6 receptor, and had no intrinsic activity of its own. (Right) 5-HT-stimulated cAMP production via 5-HT_7 mechanisms, whereas aripiprazole (10 μM) had low intrinsic activity at the 5-HT_7 receptor, and effectively blocked the effects of serotonin.

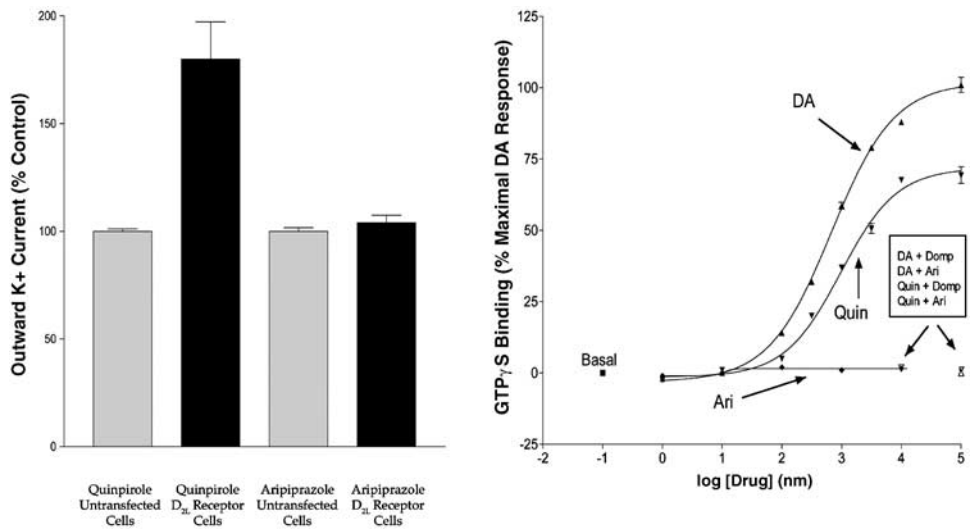

Figure 6 Functional effects of aripiprazole at D_{2L} receptors. (a) Quinpirole caused a 75% increase in outward K^+-currents in MES-23.5 cells stably transfected with D_{2L} receptors. Conversely, 10 μM aripiprazole had no effect. (b) In D_{2L}-transfected CHO cells, GTPγS binding was increased by both dopamine and quinpirole. Conversely, aripiprazole caused no significant effect on its own, and like the D_2 antagonist domperidone, could block the effects of either dopamine or quinpirole. Data represents one of two experiments with identical results.

displaying functionally significant interactions at a large number of biogenic amine GPCRs including D_2-, D_3-, and D_4-dopamine receptors, and 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_6 and 5-HT_7 serotonin receptors. The current work clearly demonstrates that aripiprazole displays a receptor binding profile substantially distinct from all other approved atypical antipsychotics (ie clozapine, olanzapine, quetiapine, risperidone, and ziprasidone).

Clozapine is now regarded as the prototypical atypical antipsychotic drug, and is the treatment of choice for treatment-resistant and treatment-intolerant schizophrenia and related disorders because of its efficacy and relative lack of EPS. Clozapine treatment also substantially decreases suicidality among patients with schizophrenia, and it may improve negative symptoms, cognition, and tardive dyskinesia (Kane *et al*, 1988; Meltzer *et al*, 1989; Meltzer and McGurk, 1999). The molecular mechanism(s) responsible for the actions of clozapine and other related atypical antipsychotics (eg olanzapine, risperidone, quetiapine and ziprasidone) are not proven, but the common view is that it depends on a balanced occupancy of 5-HT_{2A} serotonin and D_2-dopamine receptors (Meltzer *et al*, 1989).

All atypical antipsychotic drugs have a relatively complex in vitro pharmacology with high affinities for a number of serotonin receptors (eg 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6 and 5-HT_7 (Milan *et al*, 1992; Roth *et al*, 1992, 1994). At 5-HT_{2A} and 5-HT_{2C} receptors, most atypical antipsychotic drugs are inverse agonists (Rauser *et al*, 2001). Atypical antipsychotic

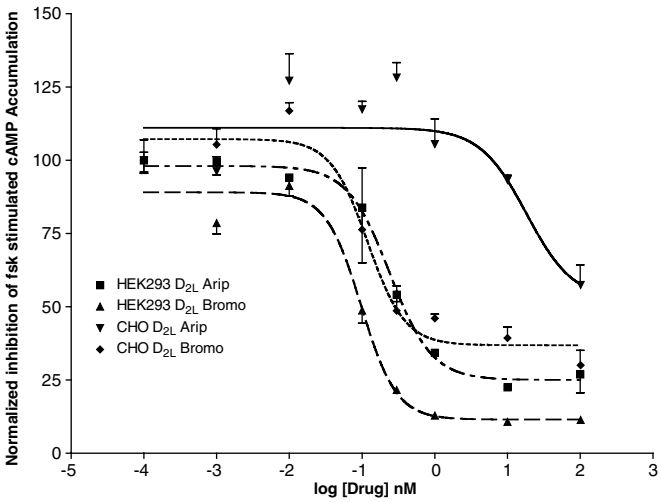

Figure 7 Aripiprazole is a partial agonist in cells stably expressing high levels of the dopamine D_{2L} receptor. HEK 293 and CHO cells stably expressing the dopamine D_2 receptor (Burris *et al*, 2002) were used to assess the agonist actions of aripiprazole. Activities of aripiprazole and the partial agonist bromocriptine were measured using inhibition of forskolin (fsk)-stimulated cAMP production. Values along ordinate represent the cAMP measured at each inhibitor concentration relative to uninhibited, forskolin-stimulated assay wells, normalized to 100%. Data represent mean ± SEM of triplicate determinations from one of three replicate experiments having similar results.

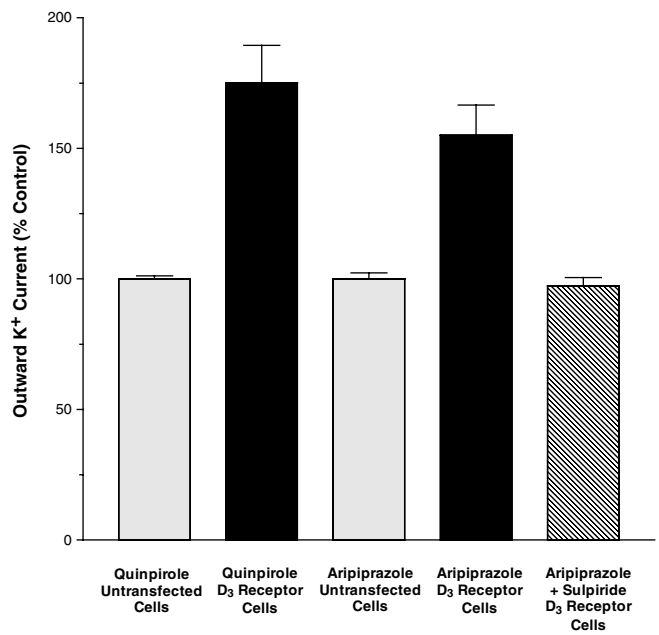

Figure 8 Strong agonist activity of aripiprazole at D_3-linked K^+-currents in MES-23.5 cells. Quinpirole (10 μM) or aripiprazole (10 μM) both activated D_3 receptors, and the effects of aripiprazole were completely blocked by sulpiride.

drugs also interact, to a greater or lesser extent, with various dopamine receptors including D_3-dopamine (Sokoloff *et al*, 1990) and D_4-dopamine (Roth *et al*, 1995; Van Tol *et al*, 1991) receptors. At present, it is unclear to what extent binding to receptors other than D_2-dopamine and 5-HT_{2A} serotonin receptors contributes to the actions of the atypical antipsychotic drugs. In this regard, it is important to note that aripiprazole has much higher affinity for the human dopamine D_2 receptor (0.74 nM) than other atypical antipsychotic drugs, although its affinity is markedly attenuated by sodium ($K_i = 32.1$ nM in the presence of 150 mM NaCl). Of the atypical antipsychotics, only risperidone is reported (in some systems) to show an affinity for the human D_2 receptor (0.3–4 nM) approximating that of aripiprazole, while the other atypical antipsychotic drugs have affinities for both rat and human D_2 receptors in the range of 3–800 nM (see on-line database at: http://kidb.bioc.cwru.edu/pdsp.php and Arnt and Skarsfeldt, 1998). This is of particular clinical relevance, since blockade of mesolimbic D_2 receptors is believed to be related to efficacy against the positive symptoms of schizophrenia (Worrel *et al*, 2000).

Aripiprazole displayed variable binding affinities for the tested 5-HT receptors, ranging from 0.36 nM (5-HT_{2B}) to 8 μM (5-HT_{1E}) (Table 1). With a $K_i = 22.4$ nM for human 5-HT_{2A} receptors labeled with [^{3}H]ketanserin, aripiprazole had 10–100-fold lower affinity for these receptors than all other tested atypical antipsychotic drugs except quetiapine (31 nM; see on-line database at: http://kidb.bioc.cwru.edu/pdsp.php). Aripiprazole also had low affinity at 5-HT_{2C} receptors labeled with the antagonist [^{3}H]mesulergine ($K_i = 428$ nM), yet much higher affinity for agonist labeled 5-HT_{2A} and 5-HT_{2C} receptors (Table 1). Higher affinity for agonist *vs* antagonist-labeled 5-HT_2- receptors is characteristic of agonists and partial agonists (Egan *et al*, 2000). This may explain the higher potency aripiprazole has for activating 5-HT_{2C} receptors (23 nM) relative to its inhibition of [^{3}H]mesulergine binding (76 nM).

One of the greatest concerns associated with the use of atypical antipsychotics is the propensity of the drugs to induce weight gain in up to 40% of treated patients (Richelson, 1999; Worrel *et al*, 2000). Long-term health problems associated with the weight gain (including cardiovascular disease, diabetes, and increased risk of cancer) may be so severe as to contraindicate treatment in some patients. Furthermore, the weight gain may be an important factor in noncompliance. Retrospective studies have concluded that clozapine and olanzapine treatments offer the greatest risk of weight gain, although only clozapine-induced weight gain appeared to be long term and not easily controllable through intervention such as diet and exercise. Of the atypical antipsychotic drugs currently approved for use, risperidone presents an intermediate risk, followed by sertindole and ziprasidone (Wirshing *et al*, 1999). The greatest single correlate for weight gain has been antagonism of the H_1-histamine receptor (Wirshing *et al*, 1999; Kroeze *et al*, 2003). The moderate binding affinity of aripiprazole for the human H_1-histamine receptor (25.1 nM) predicts that aripiprazole will exhibit a minimal propensity to induce short-term weight gain (Kroeze *et al*, 2003). The results of ongoing clinical trials, however, should answer this question conclusively.

Functional Effects at Target Receptors and the 'Functional Selectivity Hypothesis'

D_2-receptors. The functional findings in the current work are of particular importance, since the actions of

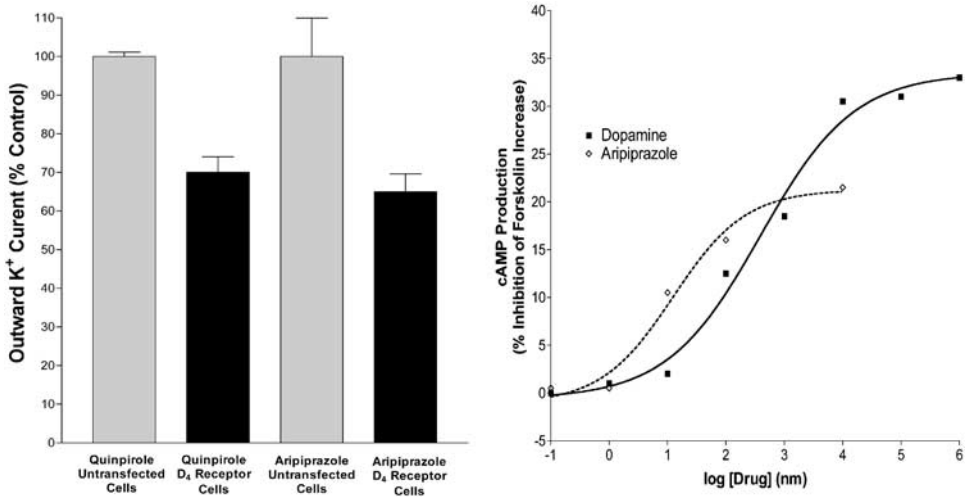

Figure 9 Differential functional effects of aripiprazole at D_4-linked functions. (Left) Aripiprazole (10 μM) and quinpirole (10 μM) caused similar (30–35%) decreases in K^+ currents in MES-23.5 cells stably transfected with D_4 receptors. (Right) Dopamine and aripiprazole both increased cAMP production in this cell line, although aripiprazole ($EC_{50} = 16$ nM) was of lower intrinsic activity than dopamine ($EC_{50} = 363$ nM). All data represent mean ± SEM from three separate experiments.

aripiprazole differ markedly across receptor systems. Thus, in the present studies, aripiprazole was sometimes an antagonist (eg at 5-HT_6 and D_{2L}), sometimes an inverse agonist (eg 5-HT_{2B}), sometimes a partial agonist (eg D_{2L}), and sometimes a full agonist (D_3, D_4). Aripiprazole was frequently found to be a partial agonist, with an intrinsic activity that could be low (D_{2L}, 5-HT_{2A}, 5-HT_7), intermediate (5-HT_{1A}), or high (D_4, 5-HT_{2C}). We recognize (*vide infra*) that *in vitro* assay systems may not accurately reflect the actions of the drug *in vivo*, yet we feel the present data do provide important clues as to the mechanism of action of aripiprazole. This mixture of agonist actions at D_2-dopamine receptors is consistent with the hypothesis proposed by Lawler *et al* (1999) that aripiprazole has 'functionally selective' actions. The 'functional-selectivity' hypothesis proposes that, depending upon the cellular milieu (eg receptor and G protein complement and concentration), a mixture of agonist/partial agonist/antagonist actions are likely (Kilts *et al*, 2002; Lawler *et al*, 1999; Mottola *et al*, 2002). According to this hypothesis, agonists may induce structural changes in receptor conformations that are differentially 'sensed' by the local complement of G proteins to induce a variety of functional actions depending upon the precise cellular milieu. The diverse actions of aripiprazole at D_2-dopamine receptors are clearly cell-type specific (eg agonism, antagonism, partial agonism), and are most parsimoniously explained by the 'functional selectivity' hypothesis.

Indeed, the functional effects of aripiprazole have been extensively studied at dopamine D_2 receptors. Prior studies have supported the notion that the unique clinical actions of aripiprazole are the result of its partial agonist properties at the D_{2L} receptor (Burris *et al*, 2002; Inoue *et al*, 1996; Tamminga, 2002). Burris *et al* (2002) have shown that in CHO cells transfected with hD_{2L} (500–1000 fmol/mg; see Filtz *et al*, 1993), aripiprazole behaves as a classical partial agonist. In this study, we were able to replicate the Burris *et al* (2002) finding that aripiprazole has partial agonist actions when using the identical cell lines. Conversely, others (Lawler *et al*, 1999) concluded that the functional properties of aripiprazole are affected markedly by cell type and other variables to a much greater degree than by other drugs. Thus, Lawler *et al* (1999) hypothesized that a mechanism called 'functional selectivity'/'agonist trafficking' might be critical to the actions at the D_{2L} receptor in brain. Specifically, they suggested that the functional characteristics of aripiprazole at a single receptor isoform (eg the D_{2L}) are highly dependent on the location of the receptor (ie influenced by the signaling partners such as G proteins). The current studies report data from a variety of systems in which aripiprazole has not only no detectable intrinsic activity, but in one of the systems is a complete antagonist of both dopamine and quinpirole. It is unlikely that the differential actions of aripiprazole as an agonist, antagonist, or partial agonist were entirely due to differences in relative D2 receptor expression since aripiprazole was an antagonist in cells with the highest level of expression 4.6 pmol/mg) and a partial agonist in cells with an intermediate level of expression (0.5–1 pmol/mg). Instead, the current data are most parsimoniously explained by the 'functional selectivity' hypothesis of Lawler *et al* (1999).

5-$HT_{2A/B/C}$ receptors. Since 5-HT_{2C} receptors have been implicated in the control of depression, OCD, and appetite, agonism at the 5-HT_{2C} receptor might be associated with therapeutic potential in obsessive compulsive disorder, obesity, and depression (Martin *et al*, 1998). 5-HT_{2C} agonism has been demonstrated to induce anorexia via enhancement of serotonergic neurotransmission via activation of 5-HT_{2C} receptors (Vickers *et al*, 1999); it is conceivable that the 5-HT_{2C} agonist actions of aripiprazole may, thus, be partly responsible for the minimal weight gain associated with this compound in clinical trials. In terms of potential action as an antiobsessional agent, it is worthwhile noting that a variety of 5-HT_{2A}/5-HT_{2C} agonists have shown promise as antiobsessional agents, yet many of these compounds are hallucinogenic, presumably due to 5-HT_{2A}

activation (Roth *et al*, 1999). Based on data obtained using C6-glioma cells, which express 5-HT_{2A} receptors with minimal receptor reserve, we conclude that aripiprazole will function as a 5-HT_{2A} antagonist *in vivo*. Thus, aripiprazole has a favorable pharmacological profile in being a 5-HT_{2A} antagonist and a 5-HT_{2C} partial agonist. Based on this profile, one can predict that aripiprazole may have antiobsessional and anorectic actions in humans.

The other member of the 5-HT2-receptor family that aripiprazole has high affinity for is the 5-HT_{2B} receptor. Since agonism at 5-HT_{2B} receptors is associated with vavlular heart disease (Fitzgerald *et al*, 2000; Rothman *et al*, 2000), it is important to determine whether aripiprazole was an agonist or antagonist at 5-HT_{2B} receptors. The current data demonstrate that aripiprazole is an inverse agonist at 5-HT_{2B} receptors, and is thus unlikely to induce valvular heart disease. On the other hand, 5-HT_{2B} antagonism has been promoted as a pharmacologic approach for treating migraine headadches (Hamel, 1999; Johnson *et al*, 1998). This is because of the observation that, during a migraine attack, activation of 5-HT_{2B} receptors has been reported to accompany a sudden rise in serotonin, potentially resulting in activation of sensory neurons and the onset of pain. It will be interesting to see whether the potent antagonism (inverse agonism) of aripiprazole at the 5-HT_{2B} receptor will translate into an effective antimigraine therapy.

The prefrontal cortex contains large densities of both 5-HT_{1A} and 5-HT_{2A} receptors, located primarily on pyramidal neurons. As cortical 5-HT_{2A} receptors are excitatory, and cortical 5-HT_{1A} receptors are inhibitory, it has been suggested recently that blockade of cortical 5-HT_{2A} receptors and/or functional 5-HT_{1A} receptor agonism may mediate the physiological balance between excitatory and inhibitory inputs onto prefrontal pyramidal neurons (Martin-Ruiz *et al*, 2001). In preclinical trials, 5-HT_{1A} selective agonists were capable of antagonizing (attenuating) neuroleptic-induced EPS in animal models (Ellenbroek *et al*, 1994; Liebman *et al*, 1989). Based on this finding, Feenstra *et al* (2001) examined the structure/affinity relationship of a large panel of 5-HT_{1A} receptor binding an analogs. At least one of these analogs shows promise as an antipsychotic drug in rodents and is being developed for pharmacological study. Of the current atypical antipsychotic drugs, ziprasidone is the only drug that binds with similar affinity to aripiprazole at the 5-HT_{1A} receptor. Thus, 5-HT_{1A} partial agonism is not a common pharmacological characteristic of atypical antipsychotic drugs. It remains to be seen, then, whether 5-HT_{1A} selective agonism, alone or in combination with effective 5-HT_{2A} antagonism, will be efficacious in treating the various symptoms of schizophrenia.

It is nonetheless clear that aripiprazole is an atypical antipsychotic drug with a unexpectedly robust pharmacology, and, thus, shows promise as the first in a new generation of atypical antipsychotic drugs. The pharmacological profile of aripiprazole is consistent with a favorable *in vivo* pharmacology in humans including a relatively low propensity to induce either weight gain or EPS. It is also possible that aripiprazole may prove to be effective in other disorders based on the unusually broad pharmacologic profile uncovered in this survey. The current data suggest that the new class of drugs represented by aripiprazole may work by being either *dopamine-serotonin partial agonists* and/or by working through the mechanism of 'functional selectivity'/'agonist trafficking' (eg at the D_2 and possibly some 5-HT receptors). Our research clearly points to the need for further studies on both multiple receptor targeting and 'functional selectivity'/'agonist trafficking.' Such mechanisms also open the door for other therapeutic indications for aripiprazole, including its use as a treatment for migraine, obesity, obsessive-compulsive disorder and other psychiatric conditions.

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