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Distinct functional profiles of aripiprazole and olanzapine at RNA edited human 5-HT$_{2C}$ receptor isoforms

Jean Y. Zhang *, *Dianne M. Kowal, Stanley P. Nawoschik, Zhuangwei Lou, John Dunlop*

Neuroscience Discovery Research, Wyeth Research, CN 8000, Princeton, NJ 08543, USA

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ABSTRACT

In this study we have functionally characterized aripiprazole (OPC-14597; 7-(4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butyloxy-3,4-dihydro-2-(1H)-quinolinone), the prototype of a new generation antipsychotic drug termed dopamine–serotonin-system stabilizer, in cells expressing 5-hydroxytryptamine2 (5-HT$_2$) receptor subtypes in comparison with olanzapine. In Chinese hamster ovary (CHO) cells stably expressing 5-HT$_2$ receptors, aripiprazole displayed a dual agonist/antagonist profile for 5-HT$_{2C}$ receptor (VNI isoform) mediated calcium signaling (EC$_{50}$ 1070 nM, IC$_{50}$ 281 nM). It exhibited no appreciable 5-HT$_{2A}$ or 5-HT$_{2B}$ agonism, whereas it antagonized 5-HT-stimulated calcium increase at either 5-HT$_{2A}$ or 5-HT$_{2B}$ receptor expressed in CHO cells (IC$_{50}$s of 369 and 0.46 nM, respectively). In comparison, olanzapine was devoid of agonism but was an antagonist at all three subtypes, with a potency rank order of 5-HT$_{2A}$ (IC$_{50}$, 2.5 nM) > 5-HT$_{2B}$ (47 nM) > 5-HT$_{2C}$ (69 nM). In human embryonic kidney (HEK) cells transiently expressing 5-HT$_{2C}$ receptor isoforms, aripiprazole exhibited full agonism at the unedited INI, but partial agonism at the partially edited VNI and fully edited VSV isoforms (EC$_{50}$s of 571, 1086 and 2099 nM, respectively). A partial antagonism was also observed for aripiprazole at the two edited isoforms (IC$_{50}$s of 1138 and 1000 nM, respectively). In contrast, while lacking agonist activity at the VNI and VSV, olanzapine showed inverse agonism at the INI isoform (IC$_{50}$ 594 nM), reaching a maximal attenuation of 20%. In addition, olanzapine was a full antagonist at all three isoforms, with a rank order of potency of VNI (IC$_{50}$, 79 nM) > VSV (101 nM) > INI (3856 nM). The modest 5-HT$_{2A}$ antagonism and 5-HT$_{2C}$ partial agonism, along with reported D$_2$ and 5-HT$_{1A}$ partial agonism, may allow aripiprazole to stabilize the disturbed dopamine–serotonin interplay in schizophrenia with a moderate yet adequate pharmacological intervention. 5-HT$_{2C}$ agonism may also underlie the minimal weight gain seen with aripiprazole.

1. Introduction

Serotonin (5-HT)$_2$-receptor-based mechanisms have been proposed to play a critical role in the pathophysiology of schizophrenia and in the action of atypical antipsychotic drugs [1,2]. Studies indicate that compared with typical antipsychotics (e.g. chlorpromazine and haloperidol), atypical antipsychotics (e.g. clozapine and olanzapine) display improved efficacy and tolerability for some aspects of schizophrenia partly due to their effects on 5-HT$_2$ receptors [3]. Typical antipsychotics, generally potent dopamine (DA) D$_2$ receptor antagonists, are effective in reducing the positive symptoms of schizophrenia, but have a high liability for extrapyramidal side effects (EPS) as a result of striatal D$_2$

* *Corresponding author*. Tel.: +1 732 274 4077; fax: +1 732 274 4292.
E-mail address: zhangj@wyeth.com (J.Y. Zhang).
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receptor blockade, and have no impact on the negative symptoms and cognitive function. Atypical antipsychotics, many with more potent 5-HT_{2A}than D_2 antagonism, are effective in schizophrenia resistant to classical treatment, and are proposed to be beneficial in the treatment of negative symptoms and cognitive deficits. Most importantly, atypicals have a low propensity for EPS at doses with demonstrated antipsychotic activity. It has been suggested that potent 5-HT_{2A} receptor antagonism together with weak D_2 receptor antagonism are the principal pharmacological features that differentiate atypical from typical antipsychotic drugs [3]. Nevertheless, atypical antipsychotics are not without their problems. Some of the troublesome side effects associated with atypicals are weight gain, electrocardiogram abnormalities, hyperprolactinaemia, and impaired glucose tolerance [4]. These adverse effects do, to varying degrees, limit the clinical use of atypicals.

Aripiprazole (OPC-14597; 7-(4-[4-(2,3-dichlorophenyl)-l-piperazinyl]butyloxy-3,4-dihydro-2-(1H)-quinolinone), the most recently approved agent for the treatment of schizophrenia, has a mechanism of action that differs from both typical and atypical antipsychotics now on the market. In contrast to typical antipsychotics, aripiprazole exhibits characteristics of atypicals with respect to its stronger blockade of 5-HT_{2A} compared to D_2 receptors. Aripiprazole is reported to be an efficacious treatment for the positive and negative symptoms as well as cognitive deficits of schizophrenia [5]. There is a low clinical occurrence of EPS associated with aripiprazole treatment [6,7]. Unlike the current atypicals that are dopamine–serotonin antagonists, aripiprazole is considered the prototype of a new class of atypical antipsychotics termed 'dopamine–serotonin-system stabilizer', attributed largely to its distinct feature of D_2 partial agonism [8] and 5-HT_{1A} receptor partial agonist activity [9,10]. It is postulated in the pathophysiological mechanism of schizophrenia that dopaminergic hyperactivity in the mesolimbic pathway is correlated with the positive symptoms, whereas DA hypoactivity in the prefrontal cortex is associated with the negative symptoms and cognitive dysfunction [8,11]. Thus, the development of D_2 partial agonists is a logical strategy for the treatment of schizophrenia, for their actions are dependent upon the intensity of dopamine transmission in the particular regions of the brain. As a partial agonist, aripiprazole binds to the D_2 receptor and prevents the occupancy by endogenous dopamine, and at the same time stimulates D_2 receptors to a lesser degree. Aripiprazole can, therefore, act as a functional antagonist in areas of high levels of dopamine, but not in areas of low dopamine levels; rather, aripiprazole will show functional agonist activity in such an area [12]. As a D_2 receptor antagonist, aripiprazole would also reduce DA activity in the nigrostriatal region, which produces EPS. However, as aripiprazole retains some intrinsic activity, it may therefore adequately balance the dopamine level in nigrostriatal area, making antagonism-related adverse effects less likely [13]. D_2 partial agonist property also favors a lower risk for hyperprolactinaemia [14]. It is important to emphasize that as a new addition to the family of antipsychotics, aripiprazole has had limited opportunities to be included in meta-analysis of antipsychotic efficacy and the ultimate benefit over other antipsychotic treatment is yet to be determined by a broader clinical exposure. Nevertheless, clinical studies have provided evidence that aripiprazole is not associated with significant weight gain [15], cardiac rhythm disturbance [16], or impaired glucose tolerance [17].

Although D_2 partial agonism is a prominent pharmacological characteristic of aripiprazole, it may not be the sole contributor of the therapeutic and safety profile of the compound. Initial studies with pure dopamine partial agonists did not show the expected advantages over conventional antipsychotics [8]. Activities of aripiprazole at other receptors may also contribute to its improved antipsychotic profile. For example, aripiprazole is a partial agonist at 5-HT_{1A} receptors, an action that may protect against D_2 antagonism associated EPS and provide anxiolytic activity [10]. Recent attention has focused particularly on 5-HT_2 receptors as they are implicated in modulating DA imbalance and stabilizing dopamine neurons of schizophrenia patients [2]. More comprehensive studies have revealed a broad range of CNS receptors for which aripiprazole has affinity [18]. In addition to 5-HT_{2A} receptors, the structurally related 5-HT_{2C} and 5-HT_{2B} receptors are among the major serotonin receptors that aripiprazole binds with moderate to high affinity. All three receptor subtypes belong to the 5-HT_2 receptor family that is coupled via G-protein (G_q) to phospholipase C leading to an increase of inositol phosphates and intracellular calcium. 5-HT_2 receptors have been reported to play a major role in a range of central nervous system functions including anxiety, depression, obsessive compulsive disorder (OCD), migraine, sleep, satiety and schizophrenia [19]. The involvement of 5-HT_{2A} and 5-HT_{2C} receptors in the control of mesolimbic and cortical dopamine transmission is well established, and they are reported to exert opposite influence on central DA function. 5-HT_{2A} receptors have been shown to facilitate stimulated but not basal DA release, while 5-HT_{2C} receptors have demonstrated inhibitory control on both basal and stimulated DA release [20,21]. Like 5-HT_{2A} receptors, 5-HT_{2C} receptors have been proposed to represent a pharmacologically important site of action of atypical antipsychotics, for which most atypicals exhibit potent interactions [22] and many demonstrate inverse agonism [23]. RNA editing of the 5-HT_{2C} receptor, a post-transcriptional modification that produces several edited receptor variants, has been observed to be reduced in the prefrontal cortex in schizophrenia patients, resulting in increased expression of the unedited 5-HT_{2C}-INI isoform and decreased expression of the edited 5-HT_{2C}-VSV isoform [24]. Since agonism at 5-HT_{2B} receptors is possibly associated with such conditions as migraine pathogenesis [25], pulmonary hypertension [26] and irritable bowel syndrome [27], it is of interest to determine whether aripiprazole, which possesses sub-nanomolar affinity for the 5-HT_{2B} receptor, is an agonist or antagonist at this subtype.

Aripiprazole has been characterized extensively in radioligand binding studies and its functional actions at several 5-HT and dopamine receptors have also been reported [16]; however, few studies have directly compared its functional effects at all three 5-HT_2 receptor subtypes. Further pharmacological profiling of aripiprazole at these receptors may offer more insight into the mechanisms that underlie any potential benefit in schizophrenia and point the way for future development of antipsychotics with more optimal therapeutic profiles. In the present study, we have performed a functional

characterization of aripiprazole at the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors stably expressed in Chinese hamster ovary (CHO) cells. In addition, the effects of aripiprazole on three isoforms of the 5-HT_{2C} receptor, the unedited INI, partially edited VNI and fully edited VSV isoforms, transiently transfected in human embryonic kidney (HEK-293) cells were examined. A direct comparison with the prototypical atypical antipsychotic drug, olanzapine, was also conducted.

2. Materials and methods

2.1. Materials

5-HT and probenecid were purchased from Sigma. Olanzapine and aripiprazole are commercially available. Fluorescence dye Calcium 3 was from Molecular Devices Corp. (Sunnylvale, CA). Cell culture and assay reagents were purchased from Gibco and Sigma–Aldrich. Cell culture plastic-ware was purchased from Falcon or Corning Costar. Robbins tips used on fluorometric imaging plate reader (FLIPR) were from Molecular Bioproducts. Transfection reagents were obtained from Invitrogen.

2.2. Cell culture and transfection

Human 5-HT_{2C}-VNI, 5-HT_{2A} and 5-HT_{2B} receptors were expressed in stably transfected Chinese hamster ovary cell lines. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, non-essential amino acids, penicillin/streptomycin, and relevant selection markers. Cells were maintained and passaged upon reaching approximately 80% confluence.

The human 5-HT_{2C}-VNI isoform was cloned by PCR using published sequence data into pcDNA3.1 and subsequently mutagenized by PCR to the VSV and INI isoforms. For transient transfections of the three isoforms, human embryonic kidney (HEK-293) cells were plated at a density of 12×10^6 cells onto 150 mm plates in DMEM media containing 10% fetal bovine serum and incubated at 37 °C, 5% CO_2 for 24 h. The three isoforms (5 μg of pcDNA3.1 plus 5 μg of h5-HT_{2C} isoform) were then transfected into the cells using Lipofectamine-Plus Reagent according to manufacturer's instructions.

2.3. Calcium mobilization

Functional studies were performed by measuring the stimulation of Ca^{2+} mobilization using the fluorometric imaging plate reader. CHO cells stably transfected with 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors were plated at a density of 50,000 cells per well into 96-well black wall clear bottom plates 24 h prior to the experiment. HEK-293 cells transiently transfected with 5-HT_{2C} receptor isoforms were reseeded into 96-well poly-D-lysine Biocoat black wall clear bottom plates, at a density of 80,000 cells per well. Hank's buffered saline solution (HBSS) supplemented with 20 mM HEPES and 2.5 mM probenecid was prepared fresh on the day of assay and was used as FLIPR buffer. Drugs were dissolved in DMSO and diluted further in FLIPR buffer. The final concentration of DMSO exposed to the cells was kept below 0.1% in order to prevent non-specific calcium flux in the cells. For dye loading, the growth medium was removed and the cells were loaded with Calcium 3 fluorescent dye (Molecular Devices, Sunnyvale, CA) prepared in FLIPR buffer for 1 h at 37 °C. The dye-loaded cells were then transferred to FLIPR to monitor fluorescence (λ_{ex} = 488 nM, λ_{em} = 515 nM). A signal test was taken and the laser intensity was adjusted to obtain a basal level of approximately 10,000 fluorescence units. Agonist additions were made 10 s after baseline measurements by the FLIPR-equipped 96-well pipettor loaded with black tips to obtain a 1:10 dilution from a 10× compound plate. Generally, 20 μl of compound was added to each well containing 180 μl of Calcium 3 solution. Fluorescence readings were recorded once every second for the first 55 s and once every 6 s for the next 30 s. For evaluation of antagonist activities, compounds were included during the second half hour of the dye-loading procedure, followed by the addition of an EC_{80} concentration of 5-HT by FLIPR. Cells were exposed to the antagonist during the pre-incubation time period and throughout agonist activation.

2.4. Data analysis

Agonist stimulated calcium responses were determined as peak minus basal fluorescence intensities and were expressed as a percentage of a maximal 5-HT (10 μM) response. Concentration–effect data were analyzed by non-linear regression fitted to a four-parameter logistic equation to derive half-maximal effective concentrations (EC_{50} or IC_{50}) and the relative intrinsic activities (E_{max} or I_{max}, maximal drug effect) for the test compounds.

3. Results

3.1. Agonism by aripiprazole and olanzapine in 5-HT_2 receptor stably transfected $CHOK_1$ cells

In each cell line, 5-HT displayed potent and efficacious agonist activity, with EC_{50} values of 6.7 ± 1.9, 0.24 ± 0.03 and 0.3 ± 0.08 nM at 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, respectively. Neither aripiprazole nor olanzapine was able to evoke calcium responses in 5-HT_{2A} receptor stably transfected $CHOK_1$ cells ($CHOK_1$/5-HT_{2A}) (Fig. 1(A)), and similarly both drugs elicited negligible responses at the $CHOK_1$/5-HT_{2B} cell line (Fig. 1(B)). The 5-HT_{2C}-VNI isoform was used to establish the $CHOK_1$/5-HT_{2C} stable cell line. Aripiprazole caused a dose-dependent increase in intracellular calcium in these cells, with an EC_{50} value of 1070 ± 234 nM and an efficacy relative to 5-HT of 45% (Fig. 1C), while olanzapine was inactive. Other 5-HT_2 receptor agonists, WAY-161503, RO 60-0175, WAY-163909, alpha-methyl-5HT, DOI, BW723C86 and mCPP were used as reference compounds for assay validation purposes. They exhibited a range of potencies and relative efficacies (Fig. 1 and Table 1), which was consistent with the established pharmacology in our assay systems.

3.2. Antagonism by aripiprazole and olanzapine in 5-HT_2 receptor stably transfected $CHOK_1$ cells

In antagonist studies, both aripiprazole (Fig. 2(A)) and olanzapine (Fig. 2(B)) produced concentration-dependent

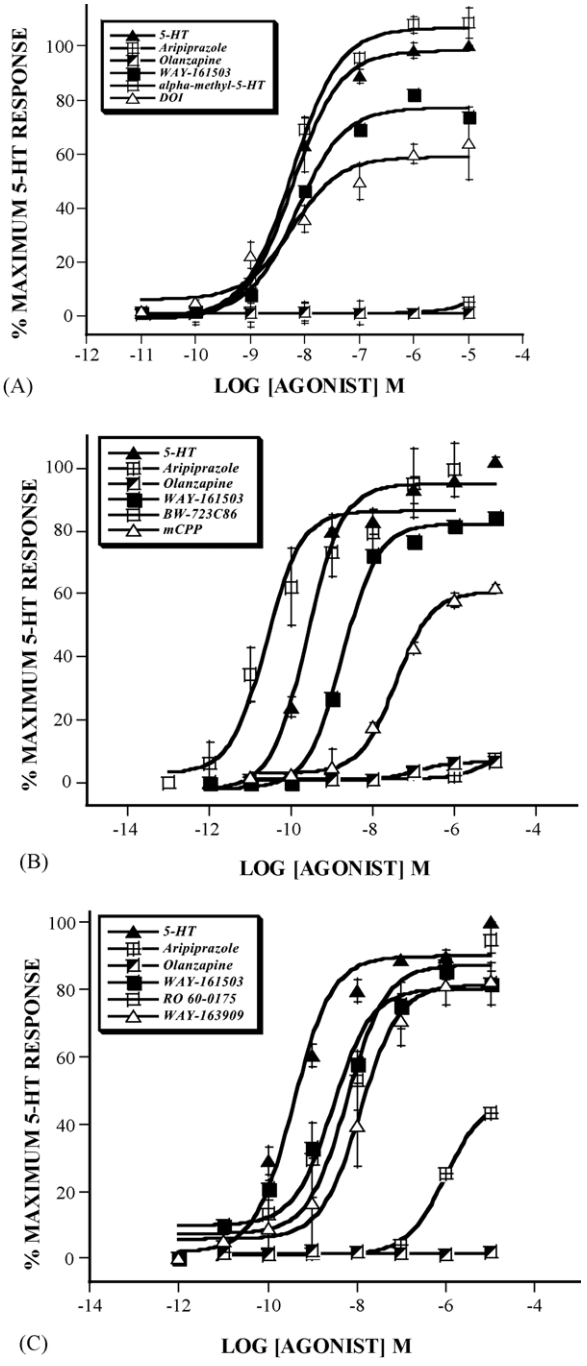

Fig. 1 – Effects of aripiprazole, olanzapine, 5-HT, and select reference agonists on intracellular calcium in CHO cells stably expressing 5-HT_{2A} (A), 5-HT_{2B} (B) and 5-HT_{2C} (C) receptors. Agonist-induced calcium increases were tested in cells loaded with the fluorescence calcium indicator dye Calcium 3. Data are expressed as percent of 10 μM 5-HT response and represent the mean ± S.E.M. from three independent experiments.

inhibition of 5-HT-stimulated calcium increases at all three 5-HT_2 receptor subtypes (Table 2). Aripiprazole potently antagonized the 5-HT_{2B} receptor with an IC_{50} of 0.46 ± 0.46 nM and was 1000-fold more selective for 5-HT_{2B} receptors than for 5-HT_{2A} and 5-HT_{2C} receptors (IC_{50} values of 369 ± 153 and 281 ± 82 nM, respectively). Olanzapine, on the other hand, was found to be a more potent 5-HT_{2A} receptor antagonist, with an IC_{50} of 2.5 ± 0.76 nM, which was about 20-fold and 30-fold more selective for 5-HT_{2A} over 5-HT_{2B} (IC_{50} of 47 ± 11 nM) and 5-HT_{2C} receptors (IC_{50} of 69 ± 23 nM), respectively. In comparison with olanzapine, aripiprazole exhibited 100-fold higher potency for 5-HT_{2B} receptors, but approximately 100-fold and 4-fold lower potency for 5-HT_{2A} and 5-HT_{2C} receptors, respectively.

3.3. *Agonism by aripiprazole and olanzapine in HEK cells transiently expressing 5-HT_{2C} receptor isoforms*

In HEK cells transiently expressing 5-HT_{2C} receptor isoforms, the unedited 5-HT_{2C}-INI and edited 5-HT_{2C}-VNI and 5-HT_{2C}-VSV, a potent agonist activity of 5-HT was observed with all three isoforms (Fig. 3(A)). Its effect at the INI isoform was more potent (EC50 of 0.05 ± 0.02 nM) than was observed at the VNI and VSV isoforms (EC_{50} of 0.48 ± 0.04 and 0.43 ± 0.17 nM, respectively) (Table 3). Aripiprazole acted as a full agonist at the INI isoform, with an EC_{50} of 571 ± 144 nM (Fig. 3(B) and Table 3); and was a partial agonist at the VNI and VSV isoforms (efficacy relative to 5-HT of 35 and 45%, respectively), with similar potencies (1086 ± 228 and 2099 ± 481 nM, respectively). While olanzapine failed to activate calcium responses at the VNI and VSV isoforms, it concentration-dependently attenuated the calcium signal below the baseline level at the INI isoform, suggesting inverse agonist activity at this isoform. Its derived IC_{50} value and the maximal inhibition were 605 ± 238 nM and 20%, respectively (Fig. 3(C) and Table 3). In addition to olanzapine and aripiprazole, we also tested haloperidol, a typical antipsychotic drug, and risperidone, another atypical antipsychotic agent, at the three 5-HT_{2C} receptor isoforms to further explore the ability of compounds to produce inverse agonist activity (Fig. 4). Like olanzapine, risperidone exhibited no agonism at the VNI and VSV isoforms (data not shown), whereas it behaved as an inverse agonist at the INI isoform and decreased the basal calcium signal in a dose-dependent manner, reaching a maximum of 20% (IC_{50} of 439 ± 58 nM). By contrast, haloperidol was inactive at all the three isoforms, and specifically exhibited neither intrinsic or inverse agonist activity at the INI isoform (Fig. 4).

3.4. *Antagonism by aripiprazole and olanzapine in 5-HT_{2C} receptor isoforms transiently transfected HEK cells*

In 5-HT_{2C}-VNI and 5-HT_{2C}-VSV isoform transiently transfected HEK cells, aripiprazole dose-dependently reduced 5-HT-stimulated calcium responses with similar potency, and achieved as much as 70% maximal inhibition at the highest concentration of 10 μM (Fig. 5(A)), consistent with the intrinsic activity reported in agonist activity determinations. While olanzapine fully antagonized 5-HT response at all the three isoforms, its inhibition at the VNI and VSV isoforms was about 40-fold more potent than at the INI isoform (Fig. 5(B)). Derived IC_{50} parameters are summarized in Table 4. Comparing aripiprazole and olanzapine, the latter was a 415- and 10-fold more potent antagonist at the VNI and VSV isoforms, respectively.

Table 1 – Agonist potencies and relative efficacies of compounds at 5-HT$_{2C}$, 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors stably expressed in CHO cells

Compound	5-HT$_{2C}$		5-HT$_{2A}$		5-HT$_{2B}$	
	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
5-HT	0.31 ± 0.08	100	6.7 ± 1.9	100	0.24 ± 0.03	100
Aripiprazole	1070 ± 234	45 ± 3	NE		NE	
Olanzapine	NE		NE		NE	
WAY-161503	7 ± 3	85 ± 5	12 ± 5.5	90 ± 2	1.8 ± 0.3	85 ± 1
RO 60–0175	14 ± 9	95 ± 4	NT		NT	
WAY-163909	14 ± 3	80 ± 3	NT		NT	
alpha-methyl-5HT	NT		6 ± 0.3	100 ± 3	NT	
DOI	NT		7.9 ± 3	65 ± 5	NT	
BW723C86	NT		NT		0.01 ± 0.002	97 ± 3
mCPP	NT		NT		37 ± 2.7	60 ± 2

Efficacy (E_{max}) values are the maximal responses observed expressed as a percentage of a maximum concentration of 5-HT. Data are mean ± S.E.M. from three independent experiments. NE: no effect at concentrations up to 10 μM. NT: not tested.

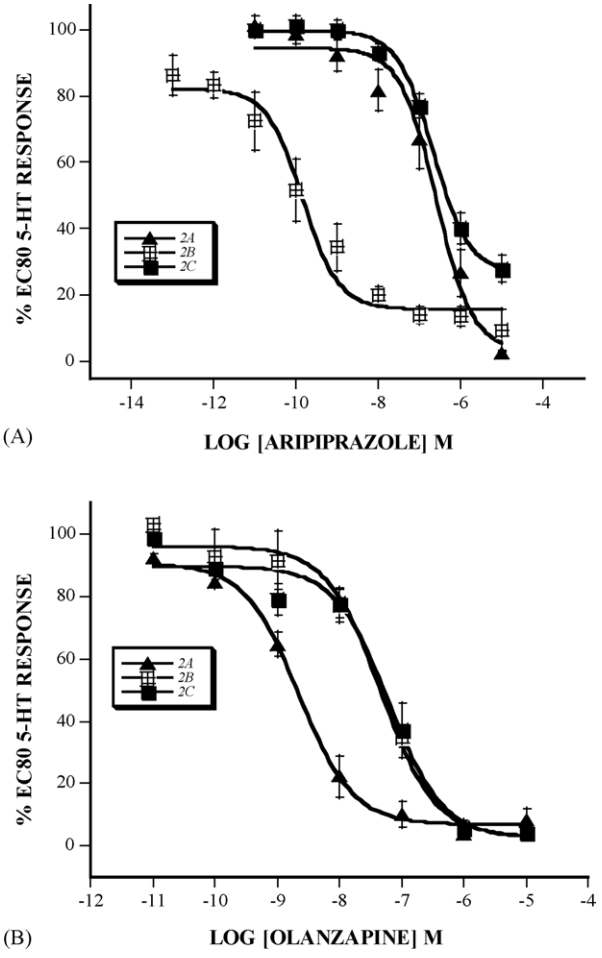

Fig. 2 – Inhibition of 5-HT induced stimulation of intracellular calcium by aripiprazole (A) and olanzapine (B) in CHO cells stably expressing 5-HT$_{2A}$, 5-HT$_{2B}$ or 5-HT$_{2C}$ receptors. 5-HT-stimulated calcium response was measured following 30 min pre-incubation with aripiprazole or olanzapine. 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptor transfected cells were challenged with 5-HT of 100, 1 and 10 nM, respectively. Data are expressed as percent of 5-HT response measured in cells without drug pre-treatment and represent the mean ± S.E.M. from three independent experiments.

4. Discussion

In an attempt to uncover molecular mechanisms of potential significance to the properties of aripiprazole, much interest has revolved around the contribution of its interactions with 5-HT$_2$ receptors as they have been the targets for the development of improved antipsychotic drugs. Extensive receptor binding studies have revealed a fundamentally different affinity profile of aripiprazole at the 5-HT$_2$ receptors compared with other atypical antipsychotics [18]. Interestingly, at 5-HT$_{2C}$ receptors, aripiprazole showed a lower affinity at 5-HT$_{2C}$ receptors labeled with an antagonist radioligand than most atypicals, yet a much higher affinity for agonist labeled 5-HT$_{2C}$ receptors, characteristic of agonist/partial agonist activity.

In the present study we have functionally characterized aripiprazole in detail at the three 5-HT$_2$ receptor subtypes, in comparison with the prototype atypical agent, olanzapine. Our main finding is that aripiprazole is a partial agonist/antagonist at the edited 5-HT$_{2C}$-VNI and 5-HT$_{2C}$-VSV isoforms, while a full agonist at the unedited 5-HT$_{2C}$-INI isoform, and is an antagonist of both 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors. Olanzapine, on the other hand, is an antagonist at all three 5-HT$_2$ receptor subtypes, and an inverse agonist at the 5-HT$_{2C}$-INI isoform. Our results are in agreement with the reported binding studies, with the current investigation demonstrating that aripiprazole is 100-fold less potent than olanzapine in antagonizing 5-HT induced calcium signaling at the 5-HT$_{2A}$ receptor [18], has the highest antagonist potency (IC_{50} of 0.46 nM) at the 5-HT$_{2B}$ receptor, and is an agonist at 5-HT$_{2C}$ receptors.

Noticeable functional differences between aripiprazole and olanzapine are their actions at the 5-HT$_{2C}$ receptors. 5-HT$_{2C}$

Table 2 – Antagonist potencies of compounds in CHO cells stably expressing 5-HT$_2$ receptor subtypes

Compound	IC_{50} (nM)		
	5-HT$_{2C}$	5-HT$_{2A}$	5-HT$_{2B}$
Aripiprazole	281 ± 82	369 ± 153	0.46 ± 0.46
Olanzapine	69 ± 23	2.5 ± 0.76	47 ± 11

Data are mean ± S.E.M. from three independent experiments.

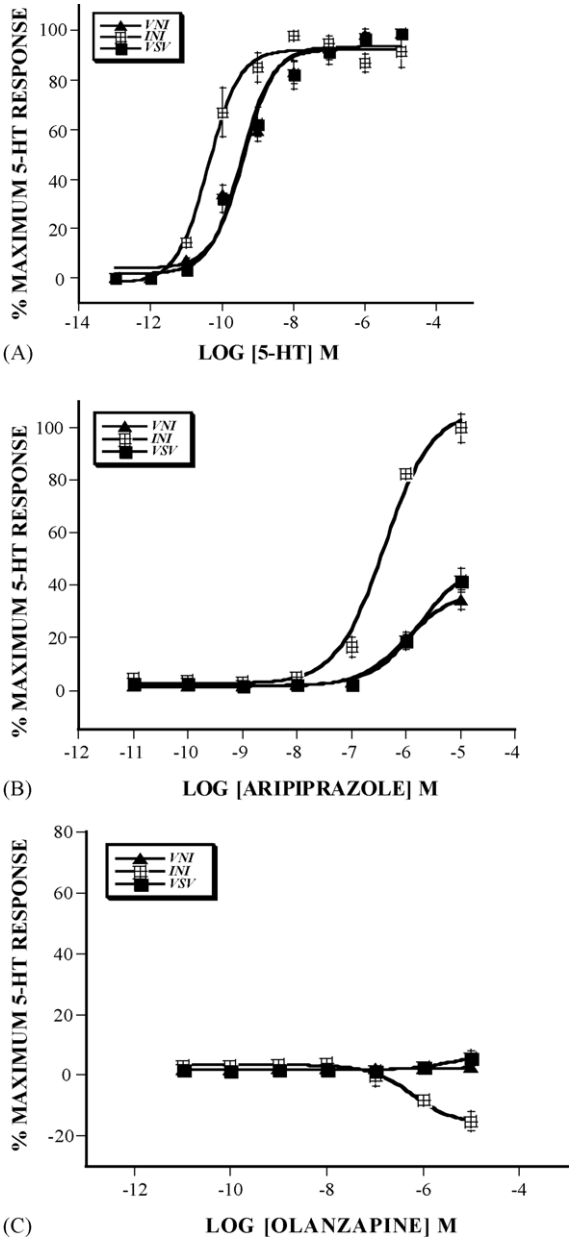

Fig. 3 – Effects of 5-HT (A), aripiprazole (B) and olanzapine (C) on calcium response in HEK cells transiently expressing 5-HT_{2C}-VNI, INI and VSV receptor isoforms. Data are expressed as percent of 10 μM 5-HT response and are mean ± S.E.M. from three independent experiments.

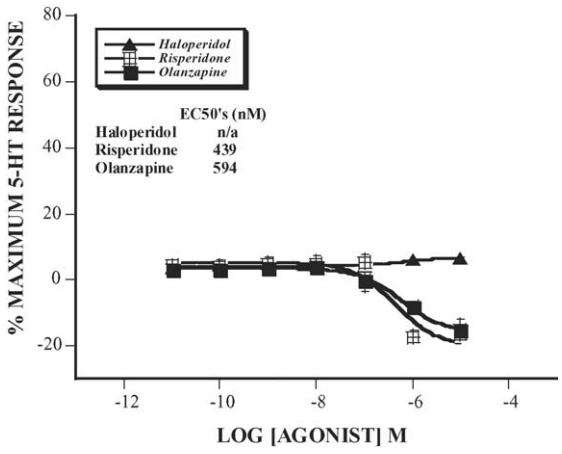

Fig. 4 – Effects of haloperidol, risperidone and olanzapine on calcium signaling in HEK cells transiently expressing 5-HT_{2C}-INI receptor isoform. Data are expressed as percent of 10 μM 5-HT response and represent mean ± S.E.M. from three independent experiments.

receptors have been implicated in a number of conditions such as depression, obsessive compulsive disorder, and regulation of appetite. One of the greatest concerns associated with the use of atypical antipsychotics is the liability of the drugs to induce weight gain in up to 40% of treated patients [28]. Profound clinical effects can result from being overweight, such as coronary artery or heart disease, hypertension, type 2 diabetes, and noncompliance with treatment [17]. 5-HT_{2C} agonism has been observed to induce anorectic effects, and consequently cause a substantial decrease in food intake [29,30], which can be reversed by 5-HT_{2C} receptor antagonists. Thus, it is conceivable that the 5-HT_{2C} agonist effect of aripiprazole may be partly accountable for the minimal weight gain associated with this compound. In contrast, 5-HT_{2C} antagonism may be a contributing factor in weight gain associated with olanzapine treatment. Studies have concluded that clozapine and olanzapine treatments offered the greatest risk of weight gain among atypicals [31]. Indeed, in the present study, aripiprazole displayed much lower potency at 5-HT_{2C} receptors compared with 5-HT. But it also exhibited significantly less 5-HT_{2C} antagonism compared with olanzapine. It might be speculated that this unique feature of partial agonism allows aripiprazole to fine tune serotonin tone resulting in minimized weight gain.

Table 3 – Agonist potencies and relative efficacies of compounds in HEK cells transiently expressing 5-HT_{2C} receptor isoforms

Compound	VNI		INI		VSV	
	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
5-HT	0.48 ± 0.04	100	0.05 ± 0.02	100	0.43 ± 0.17	100
Aripiprazole	1086 ± 228	35 ± 2	571 ± 144	100 ± 2	2099 ± 481	45 ± 1
Olanzapine	NE		605 ± 238	−20 ± 2*	NE	

Data are mean ± S.E.M. from three independent experiments. NE: no effect at concentrations up to 10 μM.
* Denotes inverse agonist activity.

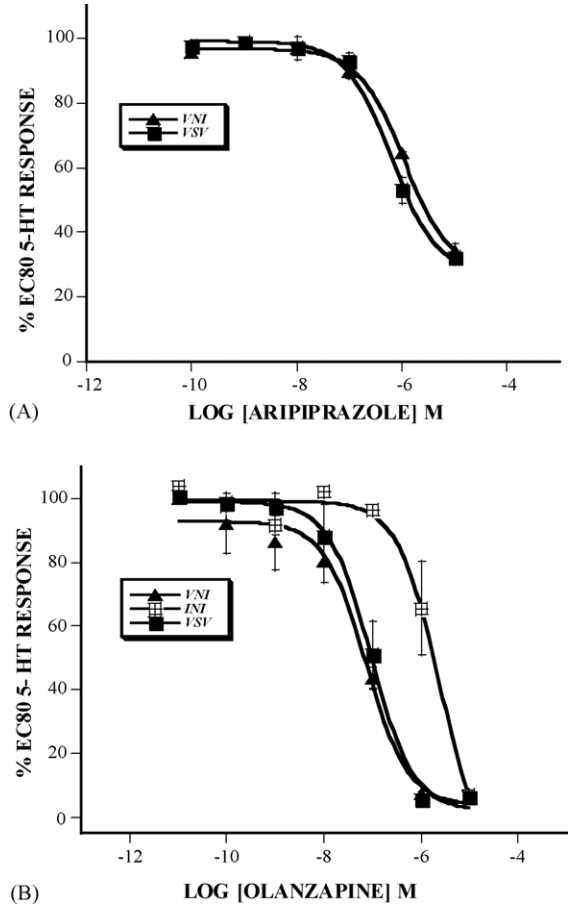

Fig. 5 – Inhibition of 5-HT-stimulated calcium increase by aripiprazole (A) and olanzapine (B) in HEK cells transiently transfected with 5-HT_{2C}-VNI, INI and VSV isoforms. 5-HT activated calcium response was measured following 30 min pre-treatment with aripiprazole or olanzapine. EC_{80} 5-HT concentrations of 10, 1 and 10 nM were used for the VNI, INI and VSV isoforms, respectively. Data are normalized to the EC_{80} 5-HT response in the absence of drug pre-treatment and are mean ± S.E.M. from three independent experiments.

5-HT_{2C} receptors are also implicated in modulation of dopamine transmission, specifically a greater inhibition of dopamine release is exerted by 5-HT_{2C} receptors in the mesolimbic than in the nigrostriatal system [20,32]. Like its actions at the D_2 receptor, whether aripiprazole acts as an agonist, a partial agonist or an antagonist at 5-HT_{2C} receptors may depend on the prevailing intensity of serotonin function in the region; it may behave like an agonist in circumstances of low 5-HT_{2C} stimulation, whereas act as an antagonist in conditions of high stimulation, and therefore stabilize 5-HT_{2C} receptor-mediated serotonin control over dopaminergic function.

Table 4 – Antagonist potencies in HEK cells transiently expressing 5-HT_{2C} receptor isoforms

	IC_{50} (nM)		
Compound	VNI	INI	VSV
Aripiprazole	1138 ± 929	–	1000 ± 469
Olanzapine	79 ± 9	3856 ± 2190	101 ± 44

Data are mean ± S.E.M. from three independent experiments.

The 5-HT_{2C}-INI isoform is the unedited, naturally occurring, constitutively active isoform of the 5-HT_{2C} receptor. The most abundant isoform in normal human brain has been found to be the VSV isoform [24,33], whereas the unedited INI variant is rare. However, one study has reported that reduced RNA editing of the 5-HT_{2C} receptor was detected in the prefrontal cortex of schizophrenia patients, leading to increased expression of the unedited INI isoform and decreased expression of the VSV isoform [24]. As a result, 5-HT_{2C} receptor-mediated effects may be enhanced in schizophrenia patients due to the greater receptor-G-protein coupling efficiency of the INI isoform. This may contribute to the prefrontal cortex dysfunction observed in the disorder. Whether this receptor alteration occurs widely in the schizophrenia population is uncertain until a growing body of evidence comes to light. It is also unclear how full agonism of aripiprazole at the INI isoform, as demonstrated in the present study, would impact its clinical profile.

Many atypical antipsychotics have been found to be inverse agonists at the 5-HT_{2C}-INI isoform, leading some to propose that inverse agonism at the 5-HT_{2C}-INI is essential for the atypical features and might therefore be used to predict atypicality [22]. Others argue that this inverse agonism at the INI isoform is not central to the beneficial actions of atypicals as it is not their unique attribute, but has also been exhibited by several typical antipsychotics as well as agents without significant clinical antipsychotic actions, such as MDL 100907 and ketanserin [23]. Therefore, inverse agonism at 5-HT_{2C}-INI receptors does not, by itself, reliably distinguish between typical and atypical antipsychotic drugs. Our study has not yet assessed a large number of typical and atypical antipsychotic drugs, rather, we randomly included haloperidol and risperidone in parallel to aripiprazole and olanzapine. Indeed, an inverse agonism was observed with risperidone and olanzapine, but not with haloperidol. On the other hand, aripiprazole, the first of a new generation of atypical antipsychotic drugs, is devoid of such inverse agonism at 5-HT_{2C}-INI receptors in the assay condition used in this study. In this respect, our data would agree that inverse agonism at 5-HT_{2C}-INI may not be a reliable predictor for atypicality.

High affinity at 5-HT_{2A} receptors has been considered an important feature of atypical antipsychotic drugs. Yet, aripiprazole appears to be unusual, displaying significantly lower binding affinity at 5-HT_{2A} receptors than most of the atypicals. Correlating with binding studies, the current investigation reveals that aripiprazole is 100-fold less potent than olanzapine in inhibiting 5-HT_{2A} receptor-mediated responses. Besides, binding studies have also shown that aripiprazole has a much higher affinity for the D_2 receptor than classical atypical antipsychotic drugs. Then, the 5-HT_{2A}/D_2 affinity ratio would be much lower for aripiprazole than for the majority of the atypicals. A relatively high 5-HT_{2A}/D_2 affinity ratio has been proposed to be an important characteristic to predict atypical properties [34], and aripiprazole

seems to deviate from this notion. However, unlike conventional atypical antipsychotic drugs, aripiprazole is a low-efficacy D_2 agonist rather than D_2 antagonist. Thus, while the 5-HT_{2A}/D_2 affinity ratio might be a key determinant for atypicality, aripiprazole highlights the importance of also taking receptor intrinsic activity into consideration. What aripiprazole has in common with other atypicals is the stronger action at 5-HT_{2A} over D_2 receptors, which may contribute to a therapeutic benefit of both positive and negative symptoms with reduced risk of EPS.

In summary, the results of the present study reveal that aripiprazole is functionally a partial agonist at 5-HT_{2C}-VNI and 5-HT_{2C}-VSV receptors, but a full agonist at 5-HT_{2C}-INI receptors and an antagonist at 5-HT_{2A} and 5-HT_{2B} receptors. The modest 5-HT_{2A} antagonism along with 5-HT_{2C} partial agonism allows aripiprazole to alter serotonin function in moderation, and is likely to contribute to its characteristic as a dopamine–serotonin-system stabilizer. 5-HT_{2C} agonism may also underlie the minimal weight gain associated with aripiprazole treatment.

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