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Interactions of the Novel Antipsychotic Aripiprazole (OPC-14597) with Dopamine and Serotonin Receptor Subtypes

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OPC-14597 {aripiprazole; 7-(4-(4-(2,3-dichlorophenyl)-1-piperazinyl)butyloxy)-3,4-dihydro-2(1H)-quinolinone} is a novel candidate antipsychotic that has high affinity for striatal dopamine D_2-like receptors, but causes few extrapyramidal effects. These studies characterized the molecular pharmacology of OPC-14597, DM-1451 (its major rodent metabolite), and the related quinolinone derivative OPC-4392 at each of the cloned dopamine receptors, and at serotonin $5HT_6$ and $5HT_7$ receptors. All three compounds exhibited highest affinity for D_{2L} and D_{2S} receptors relative to the other cloned receptors examined. Both OPC-4392 and OPC-14597 demonstrated dual agonist/antagonist actions at D_{2L} receptors, although the metabolite DM-1451 behaved as a pure antagonist. These data suggest that clinical atypicality can occur with drugs that exhibit selectivity for D_{2L}/D_{2S} rather than D_3 or D_4 receptors, and raise the possibility that the unusual profile of OPC-14597 in vivo (presynaptic agonist and postsynaptic antagonist) may reflect different functional consequences of this compound interacting with a single dopamine receptor subtype (D_2) in distinct cellular locales.
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Schizophrenia is a chronic psychiatric illness with two major types of symptoms—positive or psychotic symptoms, such as hallucinations and delusions, and negative or deficit symptoms, such as amotivation, apathy, and asociality. Approximately 1% of the population suffers from schizophrenia (Kaplan and Sadock 1988). The serendipitous discovery of chlorpromazine four decades ago not only provided the first efficacious therapeutic intervention, but also opened horizons into research about the etiology and therapy of this disease. It was soon hypothesized that chlorpromazine and similar drugs worked by being pharmacological antagonists of the neurotransmitter dopamine (Seeman et al. 1976; Creese et al. 1976), a hypothesis that ultimately provided the foundation for the commonly accepted division of dopamine receptors into two classes (Garau et al. 1978), now often called D_1 and D_2 (Kebabian and Calne 1979).

During the past decade, molecular cloning studies have resulted in the identification of several genes coding for dopamine receptors. There now are at least two

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D_1-like receptors [the D_{1A} (Zhou et al. 1990; Monsma et al. 1990; Sunahara et al. 1990; Dearry et al. 1990) and D_{1B} (Tiberi et al. 1991) or D_5 (Sunahara et al. 1991)], both of these linked functionally to stimulation of cAMP synthesis, and preferentially recognizing 1-phenyl-tetrahydrobenzazepines (e.g., SCH23390). The D_2-like receptors come from at least three genes and include multiple splice variants. The D_2-like receptors [D_{2S} (Bunzow et al. 1988), D_{2L} (Giros et al. 1989; Monsma et al. 1989), D_3 (Sokoloff et al. 1990), and D_4 (Van Tol et al. 1991)] sometimes are linked to inhibition of cAMP synthesis and have a different pharmacological specificity from the D_1-like receptors (i.e., having much higher affinity for spiperone or sulpiride).

The traditional view of antipsychotic drug efficacy posits a primary role for pharmacological antagonism of D_2-like receptors. Despite the demonstrable effectiveness of dopamine D_2 receptor antagonists, however, a substantial number (up to 20%) of patients are considered unresponsive to these typical antipsychotics (Kane et al. 1988). Furthermore, the typical antipsychotics have significant and serious side effects that make them less than optimal therapeutic agents (see Peacock and Gerlach 1996). For example, they cause acute drug-induced parkinsonian symptoms (Arana and Hyman 1991), they may worsen the negative symptoms associated with schizophrenia (Lader 1993), and they increase the risk of the disabling neurological condition known as tardive dyskinesia (Jeste and Caligiuri 1993). Given these limitations of the traditional antipsychotics, there has been much interest in the development of alternative pharmacotherapy for schizophrenia. There have been a variety of newer "atypical" antipsychotics that have been introduced recently or are currently in clinical trials (see Fatemi et al. 1996). These atypical compounds (e.g., clozapine, olanzapine, risperidone) exhibit antipsychotic efficacy with fewer undesirable side effects compared to typical drugs (e.g., haloperidol, fluphenazine).

At present, there is no consensus concerning the biological mechanisms that might impart clinical atypicality (see Arnt and Skarsfeldt 1998). Most atypical drugs are antagonists at D_2 receptors, yet often have other pharmacological properties, including concomitant D_1, serotonin 5-HT_2 and α_1-adrenergic receptor blockade, or selectivity for other D_2-like subtypes (Gerlach 1991). Much recent effort has been focused on producing compounds with selectivity for dopamine D_3 or D_4 receptors rather than D_2 receptors (Hartman and Civelli 1996). This strategy is based in large measure on the idea that antagonism of D_3 or D_4 receptors is necessary for antipsychotic efficacy because of the preferential distribution of these receptors in mesocorticolimbic terminal fields (Sokoloff et al. 1990; Ariano et al. 1997). Conversely, high affinity blockade of D_2 receptors is thought to be associated with extrapyramidal symptoms and is thus considered an undesirable property for an antipsychotic (see Joyce and Meador-Woodruff 1997). Recent data have suggested, however, that selective D_4 receptor blockade may not be therapeutically useful for the treatment of schizophrenia (Kramer et al. 1997). Despite this failure, there is continuing speculation that the D_2:D_4 selectivity ratio is an important predictor of atypicality (Seeman et al. 1997).

The present study involved an evaluation of a novel antipsychotic drug candidate OPC-14597 (Figure 1) that has both clinical atypicality and an unusual functional profile at dopamine receptors. This compound is derived from OPC-4392 {7-{3-[4-(2,3-dimethylphenyl)piperazinyl]propoxy}-2(1H)-quinolinone}, the dopamine (DA) autoreceptor agonist. OPC-14597 and OPC-4392 bind with high affinity to ^{3}H-spiperone-labeled dopamine D_2 receptor binding sites in rat striatum, frontal cortex, and limbic forebrain (Kikuchi et al. 1995). Both compounds inhibit reserpine- and gamma-butyrolactone (GBL)-induced increases in tyrosine hydroxylase activity in the mouse and rat brain, and these effects are completely antagonized by the dopamine D_2 receptor antagonist haloperidol (Kikuchi et al. 1995; Yasuda et al. 1988). Moreover, both OPC-14597 and OPC-4392 inhibit the activity of dopamine neurons when applied locally to the ventral tegmental area (Momiyama et al. 1990, 1996). These actions are consistent with pharmacological agonism at presynaptic dopamine D_2-like receptors.

Both compounds can be distinguished from typical D_2 agonists, however, because they do not cause behavioral signs associated with activation of either normosensitive or supersensitive postsynaptic dopamine D_2 receptors (e.g., increased locomotion in reserpinized mice or rotation in rats with unilateral 6-hydroxydopamine lesions; Kikuchi et al. 1995; Yasuda et al. 1988). Instead, both OPC-14597 and OPC-4392 exhibit clear antagonist actions at postsynaptic receptors, as evidenced by inhibition of apomorphine-induced stereotypy and locomotion in intact rats and inhibition of ipsi-

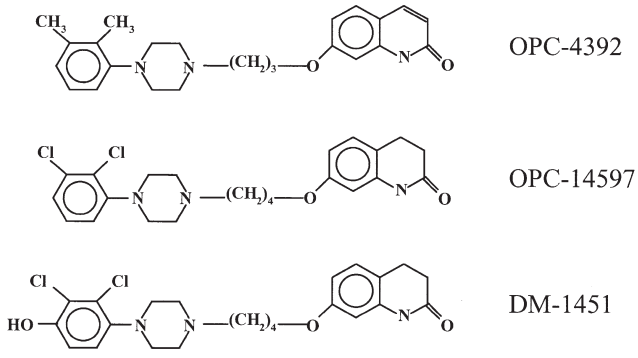

Figure 1. Structures of OPC-14597 and related compounds. Top: Structure of OPC-4392, the pharmacological ancestor of OPC-14597 (middle). The major metabolite in the rat, DM-1451, is shown at the bottom.

lateral rotation in rats with unilateral kainic acid lesions of striatum. Whereas the postsynaptic antagonist actions of OPC-4392 are observed at much higher doses than those that elicit presynaptic agonism, these opposing effects occur at equivalent doses of OPC-14597. This difference in dose ratio for pre- vs. postsynaptic effects is intriguing and may be related to the differing clinical profiles of these two compounds. OPC-4392 was shown to be effective against negative symptoms and showed no propensity to induce extrapyramidal symptoms in individuals with schizophrenia, although some exacerbation of positive symptoms was observed (unpublished observations). This latter property prompted termination of clinical testing of OPC-4392. Early clinical trials with OPC-14597, however, suggest that it is efficacious against both positive and negative symptoms, yet induces few extrapyramidal symptoms (Toru et al. 1994).

More recent preclinical data obtained with OPC-14597 have been promising as well. OPC-14597 has been reported to produce minimal increases in D_2 receptor density following chronic treatment in rat (Inoue et al. 1997). Moreover, OPC-14597 shares some characteristics of other atypical antipsychotics with respect to induction of early immediate gene expression. OPC-14597 induces c-fos protein in nucleus accumbens but not in striatum (Semba et al. 1996).

The goal of the present study was to describe the molecular pharmacology of OPC-14597, its predecessor OPC-4392, and its major rodent metabolite DM-1451 (7-(4-[4-(2,3-dichlorophenyl)-1-piperazinyl)butyloxy)-3,4-dihydro-2(1H)-quinolinone) in more detail, to provide a basis for understanding the unusual properties of this drug in the mammalian nervous system. To this end, radioreceptor and functional studies of these three compounds were conducted at each of the known molecular isoforms of the dopamine receptors in several transfection systems, as well as at novel serotonin receptor subtypes (5-HT_6 and 5-HT_7).

MATERIALS AND METHODS

Materials

[^{3}H]SCH23390 was synthesized as described by Wyrick and Mailman (1985). [^{3}H]-spiperone was purchased from Amersham, Inc. (Arlington Heights, IL). [^{3}H]methylspiperone and [^{3}H]LSD were purchased from Dupont NEN (Boston, MA). Spiperone, chlorpromazine, butaclamol, quinpirole, 7-OH-DPAT, clozapine, forskolin, and sulpiride were purchased from Research Biochemicals, Inc. (Natick, MA). Dopamine, cAMP, and isobutyl methylxanthine (IBMX) were obtained from Sigma Chemical Co (St. Louis, MO). cAMP primary antibody was obtained from Dr. Gary Brooker (George Washington University, Washington DC), and secondary antibody (rabbit antigoat IgG) covalently attached to magnetic beads was purchased from Advanced Magnetics, Inc. (Cambridge, MA).

Cell Culture

The present studies were conducted with several distinct cell lines. C-6 glioma cells stably transfected with rat D_{2L} or D_3 receptor were obtained from Dr. Kim Neve (Oregon Health Sciences University) and grown in DMEM-H medium containing 4,500 mg/l glucose, L-glutamine, 5% fetal bovine serum and 600 ng/ml G418. The CHO cells were stably transfected with cDNAs encoding the rat D_{2S}, D_{2L}, D_3, and D_4 receptors and cultured in Ham's F-12 medium (Gibco) containing 1 mM sodium pyruvate, 10% fetal bovine serum, and 20 μg/ml gentamycin (Zhang et al., 1994). The HEK cells were stably transfected with cDNAs encoding the rat 5-HT_6 and 5-HT_7 receptors and cultured as described in Monsma et al. (1993) and Shen et al. (1993). All cells were maintained in a humidified incubator at 37°C with 5% CO_2.

Membrane Preparation

C-6 cells were grown in 75-cm^2 flasks until confluent. The cells were rinsed and lysed with 10 ml of ice-cold hypo-osmotic buffer (HOB) (5 mM Hepes, 2.5 mM $MgCl_2$, 1 mM EDTA; pH 7.4) for 10 min at 4°C. Cells then were scraped from the flasks using a sterile cell scraper from Baxter (McGraw Park, IL). The combined cell suspension was homogenized (10 strokes), and spun at 43,000 × *g* (Sorvall RC-5B/SS-34, DuPont, Wilmington, DE) at 4°C for 20 minutes. The pellet was resuspended (10 strokes), and this homogenate was then spun again at 43,000 × *g* at 4°C for 20 min. The supernatant was removed, and the final pellet was resuspended (10 strokes) in ice-cold storage buffer (50 mM Tris HCl, 6 mM $MgCl_2$, 1 mM EDTA; pH 7.4) to yield a final concentration of ca. 2.0 mg of protein/ml. Aliquots of the final homogenate were stored in microcentrifuge tubes at −80°C. Prior to their use for radioligand binding or adenylate cyclase assays, protein levels for each membrane preparation were quantified using the bicinchoninic acid (BCA) protein assay reagent (Pierce, Rockford, IL) adapted for use with a microplate reader (Molecular Devices, Menlo Park, CA).

CHO cells stably transfected with dopamine receptors were prepared as described in Zhang et al. (1994). Briefly, CHO cells were harvested with 1 mM EDTA in Ca^{2+}/Mg^{2+}-free EBSS (Earle's balanced salt solution) and centrifuged at 300 × *g* for 10 min. The supernatant was removed and the cells were washed twice with EBSS (complete) then resuspended in 5 mM Tris, pH 7.4 at 4°C. The cells were homogenized using a Dounce homogenizer, and the homogenate was spun at 34,000 × *g* for 10 min. The final cell suspension was resuspended in binding buffer (50 mM Tris, pH 7.4 at 22°C, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 120 mM

NaCl) at 0.3 mg/ml. The BCA reagent was used to quantify protein concentrations.

HEK-293 cells stably transfected with 5-HT_6 or 5-HT_7 receptors were prepared as described in Monsma et al. (1993) and Shen et al. (1993), respectively. The cells were disrupted using a Dounce homogenizer in TME buffer (50 mM Tris, pH 7.4 at 37°C, 10 mM $MgSO_4$, 0.5 mM EDTA). Cell homogenates were collected after centrifugation at 43,000 × *g* and resuspended in homogenization buffer at a protein concentration of ~60 μg/ml.

Receptor Binding Assays in Rat Striatum

Frozen rat striata were homogenized by seven manual strokes in a Wheaton Teflon-glass homogenizer in 8-ml of ice-cold 50 mM HEPES buffer with 4.0 mM $MgCl_2$ (pH 7.4). Tissue was centrifuged at 27,000 × *g* for 10 min, the supernatant was discarded, and the pellet was homogenized (5 strokes) and resuspended in ice-cold buffer and centrifuged again. The final pellet was suspended at a concentration of 2.0 mg wet weight/ml. The amount of tissue added to each assay tube was 1.0 mg, in a final assay volume of 1.0 ml. D_1 receptors were labeled with [^{3}H]SCH23390 (0.30 nM); D_2 receptors were labeled with [^{3}H]spiperone (0.07 nM); these concentrations of [^{3}H]-spiperone and [^{3}H]-SCH23390 corresponded to the K_D values obtained in previous saturation binding experiments conducted in rat striatum. The estimated receptor densities were 1000 (D_1-like) and 400 (D_2-like) fmol/mg protein. Unlabeled ketanserin (50 nM) was added to mask binding to 5-HT_2 sites. Total binding was defined as radioligand bound in the absence of any competing drug. Nonspecific binding was estimated by adding unlabeled SCH23390 (1 μM) or unlabeled chlorpromazine (1 μM) for D_1 and D_2 receptor binding assays, respectively. As an internal standard, a competition curve with six concentrations of unlabeled SCH23390 (D_1 binding) or chlorpromazine (D_2 binding) was included in each assay. Triplicate determinations were made for each drug concentration. Assay tubes were incubated at 37°C for 15 min, and binding was terminated by filtering with ice-cold buffer on a Skatron 12 well cell harvester (Skatron, Inc., Sterling, VA) using glass fiber filter mats (Skatron no. 7034). Filters were allowed to dry, and 1.0 ml of Optiphase HI-SAF II scintillation fluid were added. Radioactivity was determined on an LKB Wallac 1219 RackBeta liquid scintillation counter (LKB Wallac, Gaithersburg, MD). Tissue protein levels were estimated using the BCA protein assay reagent.

Dopamine Receptor Binding Assays in Transfected Cells

Receptor binding assays were done in C-6 cells as described above for rat striatum, with the following modifications. Frozen membranes were thawed and resuspended in assay buffer (50 mM HEPES with 4 mM $MgCl_2$ and 0.001% bovine serum albumin; pH 7.4). The amount of tissue added to each tube was ca. 50 μg of protein in a final assay volume of 500 μl. Duplicate determinations were performed at each drug concentration. The final concentration of [^{3}H]-spiperone was 0.07 and 0.14 nM in assays conducted with D_{2L} and D_3 receptors, respectively. Prior to performing competition-binding studies, saturation-binding assays were conducted to determine radioligand affinity and receptor densities. These experiments demonstrated the affinity of [^{3}H]-spiperone to be 0.07 and 0.14 nM at D_{2L} and D_3 receptors, respectively. The receptor densities were estimated to be 450 (D_{2L}) and 900 (D_3) fmol/mg protein.

For the dopamine receptors transfected in CHO cells, ligand binding assays were performed as described in Monsma et al. (1990) for D_1-like receptors and as described by Zhang et al. (1994) for D_2-like receptors. The competition assays were performed in a final volume of 1 ml. Crude membranes (30 μg/assay tube) were added in triplicate to 0.2 nM [^{3}H]methlyspiperone for D_2-like receptors and 0.5 nM [^{3}H]SCH23390 for D_1-like receptors. The affinity of [^{3}H]-methylspiperone for D_{2L}, D_{2S}, D_3, and D_4 receptors in CHO cells was 0.07, 0.07, 0.5, and 0.9 nM, respectively. The corresponding receptor densities were 1600 (D_{2L}), 630 (D_{2S}), 710 (D_3), and 360 (D_4) fmol/mg protein. The affinity of [^{3}H]-SCH23390 for D_1 and D_5 receptors in CHO cells was 0.32 and 0.17 nM, respectively, with receptor densities of 1300 (D_1) and 350 (D_5) fmol/mg protein. The assay tubes were incubated for 1 h at room temperature. Nonspecific binding was determined by the addition of 1 μM (+)butaclamol for both D_2-like receptors and D_1-like receptors. The reaction was terminated by rapid filtration through GF/C filters (previously pretreated with 0.3% polyethylamine). The filters were washed with 4 ml of 50 mM Tris, pH 7.4 at 4°C five times. Scintillation counting quantified the radioactivity bound to the filters.

For the serotonin receptors transfected in HEK-293 cells, radioligand binding assays were performed as described above, except the membranes (3 μg/assay tube) were incubated with 1 nM [^{3}H]LSD for 60 min at 37°C in a total volume of 100 μl. 10 μM serotonin was used to define nonspecific binding. The affinity of [^{3}H]-LSD for $5HT_6$ and $5HT_7$ receptors was 2.8 and 6 nM, respectively. The receptor densities were 1600 ($5HT_6$) and 2400 ($5HT_7$) fmol/mg protein.

Analysis of Radioligand Binding Data

Parameter estimates (IC50 and indirect Hill slope, n_H) for competition binding data were obtained by nonlinear regression (PRISM, Graphpad, San Diego, CA) using a sigmoidal function (Motulsky and Ransnas 1987). $K_{0.5}$ values were calculated from IC50 values using the

radioligand affinity obtained in separate saturation binding studies. For each compound tested, at least three independent assays were performed, and a separate curve fit was performed for each assay. Means and standard errors of these parameter estimates were then calculated for each compound.

Measurement of cAMP Accumulation in Clonal Cell Lines

Antagonism of dopamine-mediated inhibition of cAMP accumulation in C-6 cells transfected with D_{2L} receptors was performed as follows. Cells were plated in 24 well tissue culture dishes and grown to confluence. The cells were washed in assay buffer (500 μl/well; DMEM-H, 20 mM HEPES (pH 7.4) and 500 μM IBMX) and then preincubated with 500 μl fresh assay buffer for 10 min. The assay buffer was aspirated, and new buffer containing selected test drugs was added to cells in each well (500 μl total volume per well). To facilitate the detection of a significant inhibition of cAMP accumulation induced by dopamine, cAMP synthesis was stimulated with 1 μM isoproterenol, and the ability of dopamine to inhibit this increase was measured. The ability of test compounds to antagonize the inhibition of cAMP accumulation by dopamine (10 μM) was determined using duplicate wells for each drug condition. Drug incubations proceeded for 5 min. Following this incubation, the cells were washed with assay buffer and then lysed with 500 μl of ice-cold 0.1 N HCl. After 10 min, cells were scraped and collected in 1.7-ml microcentrifuge vials. The wells were washed twice with an additional 500 μl of 0.1 N HCl that was added to the first lysate. The lysates were pelleted in a Beckman microcentrifuge (Model B), and 5 μl of the supernatant was used in the radioimmunoassay for cAMP. The cAMP determinations in duplicate wells were averaged.

Synthesis of cAMP in CHO cells was measured as described by Zhang et al. (1994). CHO cells were harvested and washed as described for radioligand binding assays, except the cells were resuspended in a final buffer containing 250 mM sucrose, 75 mM Tris, pH 7.4 at 4°C, 12.5 mM $MgCl_2$, 1.5 mM dithiothreitol, 0.2 mM sodium metabisulfite, with 100 μM of the phosphodiesterase inhibitor RO-20-1724. Cells (50 μl membrane suspension containing 250,000 cells), added to triplicate assay tubes, were preincubated for 30 min at 37°C, followed by a 5-min incubation with 10 μl of drugs or buffer. The reaction was terminated by placing the assay tubes in boiling water for 3 min. cAMP was quantified with a competitive binding assay.

Radioimmunoassay (RIA) of cAMP

The concentration of cAMP in each sample from experiments conducted in C-6 cells was determined with an RIA of acetylated cAMP, modified from that previously described (Harper and Brooker 1975). Iodination of cAMP was performed using a method described previously (Patel and Linden 1988). Assay buffer was 50-mM sodium acetate buffer with 0.1% sodium azide (pH 4.75). Standard curves of cAMP were prepared in buffer at concentrations of 2 to 500 fmol/assay tube. To improve assay sensitivity, all samples and standards were acetylated with 10 μl of a 2:1 solution of triethylamine:acetic anhydride. Samples were assayed in duplicate. Each assay tube contained 5 μl of each sample, 95 μl of buffer, 100 μl of primary antibody (sheep, anti-cAMP, 1:160,000 dilution with 1% BSA in buffer) and 100 μl of [^{125}I]-cAMP (50,000 dpm/100 μl of buffer); total assay volume was 310 μl. Tubes were vortexed and stored at 4°C overnight (~18 h). Antibody-bound radioactivity was then separated by the addition of 25 μl of BioMag rabbit, anti-goat IgG (Advanced Magnetics, Cambridge, MA), followed by vortexing and further incubation at 4°C for 1 h. To these samples, 1 ml of 12% polyethylene glycol/50 mM sodium acetate buffer (pH 6.75) was added, and all tubes were centrifuged at 1700 × *g* for 10 min. Supernatants were aspirated, and radioactivity in the resulting pellet was determined using an LKB Wallac gamma counter (Gaithersburg, MD).

Competition Binding Assay to Quantify cAMP Concentrations

The concentration of cAMP in each sample from experiments conducted in CHO cells was determined by the competition of [^{3}H]cAMP and unlabeled cAMP to a cAMP binding protein. Free [^{3}H]cAMP was removed by charcoal/bovine serum albumin treatment, and the remaining bound [^{3}H]cAMP was quantified by liquid scintillation spectroscopy. The concentration of unlabeled cAMP present in samples was determined by comparison to a standard curve (1–30 pmol cAMP/assay tube).

RESULTS

Affinity of OPC-14597 for Dopamine Receptors in Rat Striatum

The affinity of OPC-14597 for dopamine receptors in rat striatum was ascertained first. As is shown in Figure 2 and Table 1, OPC-14957 and its metabolite DM-1451 bound with high affinity to dopamine D_2-like receptors ($K_{0.5}$ ca. 4 nM). Their affinities were from four- to 20-fold lower than the reference high affinity antagonists examined (i.e., spiperone, haloperidol, eticlopride and chlorpromazine). Relative to OPC-14597, the affinity of OPC-4392 was 20-fold lower. The binding curves of OPC-14597 to D_2-like receptors in the rat striatum were steeper than the standard D_2 antagonists and this is reflected in indirect Hill slopes (n_H) that were somewhat

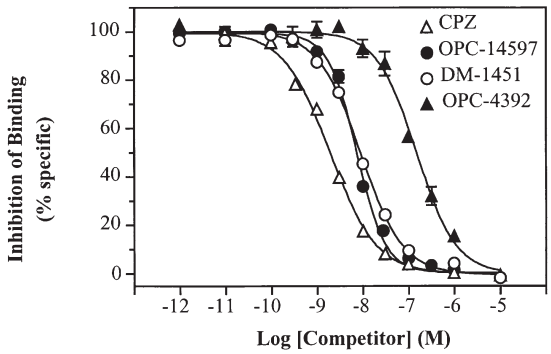

Figure 2. Affinity of OPC-14597 and selected reference compounds for dopamine D_2-like receptors in rat striatal membranes. Dopamine D_2 receptors were labeled with 0.07 nM [^{3}H]-spiperone in the presence of 50 nM ketanserin to mask 5-HT_2 sites. Nonspecific binding was defined by 1 μM chlorpromazine (CPZ). For each compound, at least three independent assays were conducted on separate days. The curves shown are from representative experiments. Means and standard errors are shown in Table 1.

greater than one. In contrast, the curves for OPC-4392 and DM-1451 were of normal steepness. The three quinolinone compounds had negligible affinity for D_1-like receptors ($K_{0.5} > 1$ μM; data not shown).

Molecular Subtype Selectivity of OPC-14597

Affinity of OPC-14597 for Cloned Dopamine Receptors in CHO Cells. The unusual functional profile reported previously for OPC-14597 in brain (Amano et al. 1995; Kikuchi et al. 1995; Momiyama et al. 1996) could involve selectivity for one of the isoforms of dopamine receptors. For this reason, the binding of OPC-14597 was examined in CHO cell lines, each transfected with a single molecular subtype of dopamine receptor. Representative data are shown in Figure 3 and summarized in Tables 2 and 3. Consistent with the studies in rat striatum, OPC-14597 had little affinity for either the D_1 or D_5 receptor ($K_{0.5}$ = 410 and 1200 nM, respectively). OPC-14597 had relatively high affinity for both the D_{2S} and D_{2L} receptors in this cell line ($K_{0.5} < 1.0$ nM) relative to that of the reference antagonist spiperone (0.07 nM). OPC-14597 had 15-fold lower affinity for the D_3 receptor ($K_{0.5}$ = 9 nM). The D_{2L}:D_3 receptor selectivity ratio of OPC-14597 was similar to that of eticlopride and greater than that of spiperone, sulpiride, or chlorpromazine. OPC-14597 bound with only moderate affinity to the D_4 receptor. The affinity of OPC-14597 for the D_4 receptor was approximately 10-fold lower than that of clozapine.

The receptor binding profile of the metabolite DM-1451 was similar to that of OPC-14597 for D_1, D_{2L}, D_3, and D_4 receptor subtypes and markedly lower for D_5. The affinity of OPC-4392 was lower than that of OPC-14597 for all dopamine receptor subtypes examined. For example, OPC-4392 had six-fold lower affinity for D_{2L}

Table 1. Affinity of OPC-14597 and Selected Reference Compounds for Dopamine D_2-like Receptors in Rat Striatal Membranes[a]

Drug	$K_{0.5}$ (nM)	n_H
OPC-14597	4.7 ± 1.1 (9)	1.27 ± 0.09
OPC-4392	88 ± 32 (6)	1.03 ± 0.12
DM-1451	4.1 ± 0.7 (7)	1.04 ± 0.12
Sulpiride	340 ± 81 (3)	0.87 ± 0.03
Chlorpromazine	1.2 ± 0.2 (13)	0.93 ± 0.06
Spiperone	0.26 ± 0.06 (7)	0.95 ± 0.07
Eticlopride	0.79 ± 0.13 (4)	0.84 ± 0.07
Haloperidol	0.41 ± 0.06 (4)	0.82 ± 0.06
Quinpirole	47 ± 15 (3)	0.74 ± 0.06
7-OH-DPAT	9.4 ± 1.5 (3)	0.69 ± 0.01

[a]Dopamine D_2 receptors were labeled with 0.07 nM [^{3}H]spiperone in the presence of 40 nM ketanserin to mask 5-HT_2 sites. Nonspecific binding was defined by 1 μM chlorpromazine. Parameter values represent means and standard errors obtained from nonlinear regression of competition curves using a sigmoid algorithm. The number of independent assays for each compound is indicated in parentheses. Representative curves are displayed in Figure 2.

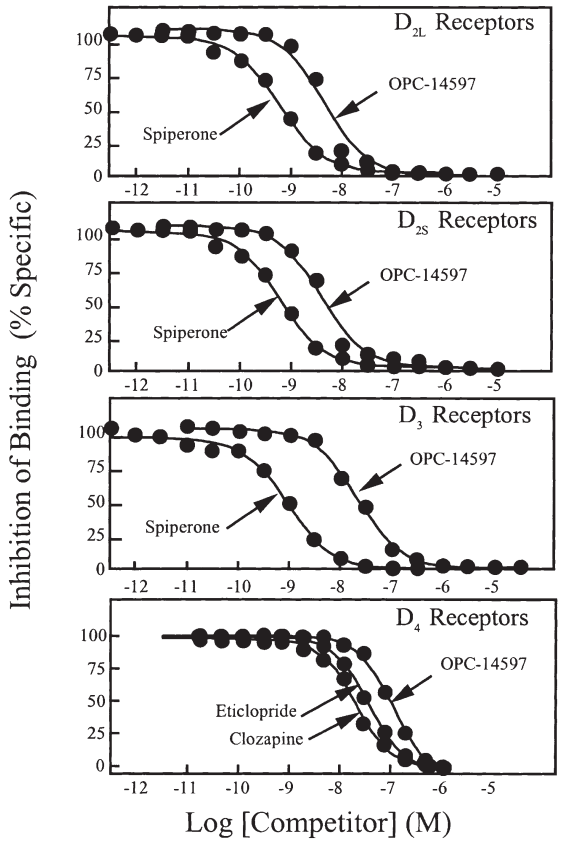

Figure 3. Affinity of OPC-14597 for cloned D_2-like dopamine receptors in CHO cells. Membranes were prepared from CHO cells stably transfected with dopamine D_{2L}, D_{2s}, D_3, or D_4 receptors. Receptors were labeled with 0.2 nM [^{3}H]-methylspiperone. Nonspecific binding was defined by 1 μM (+)-butaclamol. For each compound, at least three independent assays were conducted on separate days. The curves shown are from a single representative experiment. Means and standard errors for receptor affinities ($K_{0.5}$) are shown in Table 2.

Table 2. Affinity of OPC-14597 and Selected Reference Compounds for Dopamine D_1-like Receptors Expressed in CHO Cells[a]

Drug	CHO D_1 $K_{0.5}$ (nM)	CHO D_5 $K_{0.5}$ (nM)
OPC-14597	410 ± 10 (4)	1,200 ± 60 (3)
OPC-4392	>10 μM	>10 μM
DM-1451	460 ± 20 (3)	>10 μM
SCH23390	0.37	0.47

[a]Membranes were prepared from CHO cells stably transfected with individual dopamine receptor subtypes. Receptors were labeled with 0.5 nM [^{3}H]SCH23390 for D_{1A} and D_5 receptors. Nonspecific binding was defined by 1 μM (+) butaclamol. Values represent means and standard errors for receptor affinities ($K_{0.5}$) obtained from nonlinear regression of competition curves using a sigmoidal equation. The number of independent assays for each compound is indicated in parentheses. Representative curves are displayed in Figure 3.

receptors and at least ten-fold lower affinity for D_1 or D_5 receptors compared to OPC-14597. The affinities of OPC-14597 and OPC-4392 for D_4 receptors were similar, however. The overall differences in D_2 receptor subtype affinities observed between OPC-14597 and OPC-4392 are in agreement with the results obtained when these compounds were evaluated in competition studies using [^{3}H]-spiperone in rat striatum (Figure 2 and Table 1).

Affinity of OPC-14597 for Cloned Dopamine Receptors in C-6 Cells. Because the affinity of drugs can be influenced by the cell line in which a receptor is expressed, these compounds were studied in another cell line (C-6 glioma cells) transfected with two D_2-like isoforms for which OPC-14597 had high affinity, the D_{2L} and D_3. As shown in Table 4 and Figure 4, the affinity of OPC-14597 for D_{2L} receptors was somewhat lower in C-6 vs. CHO cells; whereas, the D_3 receptor affinities observed were comparable in the two cell lines. The result was a decreased D_2:D_3 selectivity ratio of OPC-14597 in C-6 cells (ratio of 4) vs. CHO cells (ratio of 15). The indirect Hill slope for OPC-14597 was slightly greater than one, similar to that observed in rat striatum.

The affinities of the two benzamide reference antagonists, sulpiride and eticlopride, and the aminotetralin 7-OH-DPAT at D_{2L} receptors were also somewhat lower when assessed in C-6 vs. CHO cells. In contrast, there was little effect of cell expression system on binding affinity of the reference compounds for the D_3 receptor, with the exception of sulpiride, which exhibited significantly greater D_3 receptor affinity in CHO cells.

Of all the compounds examined, the binding profiles of OPC-4392 at D_{2L} receptors displayed the most marked differences between cell lines. The affinity of OPC-4392 for D_{2L} receptors expressed in C-6 cells was 25-fold lower than was observed in CHO cells. Moreover, there was no D_{2L}:D_3 selectivity for this compound in C-6 cells, in contrast to the moderate D_{2L}:D_3 selectivity demonstrated in CHO cells. The D_{2L} receptor affinity observed for DM-1451 was also somewhat lower in C-6 cells (0.96 nM) vs. CHO cells (0.21 nM); whereas, the reverse was true for D_3 receptor affinities. As was observed for the other quinolinones, the D_{2L}:D_3 selectivity ratio of DM-1451 was markedly lower in C-6 cells (ratio of less than two) relative to CHO cells (ratio of twenty). The indirect Hill slope for DM-1451 was greater than one in C-6 D_{2L} cells, in contrast to the normal steepness exhibited for this compound in rat striatum. Despite the noted differences between cell lines, a comparison of Table 3 and Table 4 indicates that the same rank order of affinity of the three compounds (DM-1451 > OPC-14597 > OPC-4392) at D_{2L} and D_3 receptors was demonstrated in both cell lines.

Affinity of OPC-4392 for D_{2L} Receptors in the Presence and Absence of NaCl

The large difference observed in the binding affinity of OPC-4392 for D_{2L} receptors expressed in CHO vs. C-6

Table 3. Affinity of OPC-14597 and Selected Reference Compounds for Dopamine D_2-like Receptors Expressed in CHO Cells[a]

Drug	CHO-D_{2S} $K_{0.5}$ (nM)	CHO-D_{2L} $K_{0.5}$ (nM)	CHO-D_3 $K_{0.5}$ (nM)	CHO-D_4 $K_{0.5}$ (nM)
OPC-14597	0.59 ± 0.13 (3)	0.52 ± 0.16 (3)	9.1 ± 2.0 (3)	260 ± 20 (3)
OPC-4392	n.d.	3.5 ± 0.7 (3)	18 ± 0.3 (3)	280 ± 60 (3)
DM-1451	n.d.	0.21 ± 0.1 (3)	4.5 ± 1.1 (3)	120 ± 15 (3)
Spiperone	0.07	0.07	0.6	n.d.
Sulpiride	n.d.	0.21 ± 0.05 (3)	0.71 ± 0.08	n.d.
Chlorpromazine	n.d.	2.67 ± 1.64 (3)	8.94 ± 5.74 (3)	n.d.
Eticlopride	n.d.	0.076 ± 0.001 (3)	0.36 ± 0.02 (3)	64
Clozapine	n.d.	n.d.	n.d.	29
7-OH-DPAT	n.d.	69.9 ± 20.7 (3)	1.3 ± 0.7 (3)	n.d.

[a]Membranes were prepared from CHO cells stably transfected with individual dopamine receptor subtypes. Receptors were labeled with 0.2 nM [^{3}H]methylspiperone. Nonspecific binding was defined by 1 μM (+)butaclamol. Values represent means and standard errors for receptor affinities ($K_{0.5}$) obtained from nonlinear regression of competition curves using a sigmoidal equation. The number of independent assays for each compound is indicated in parentheses. Representative curves are displayed in Figure 3.

Table 4. Competition for [^{3}H]Spiperone-labeled D_3 and D_{2L} Receptors in C-6 Glioma Cell Membranes[a]

	C-6 D_{2L}		C-6 D_3	
Drug	**$K_{0.5}$ (nM)**	**n_H**	**$K_{0.5}$ (nM)**	**n_H**
OPC-14597	1.71 ± 0.32 (10)	1.23 ± 0.08	6.06 ± 1.10 (11)	1.09 ± 0.05
OPC-4392	90.9 ± 18 (8)	0.94 ± 0.08	81.68 ± 24 (8)	1.00 ± 0.07
DM-1451	0.96 ± 0.22 (8)	1.19 ± 0.13	1.63 ± 0.18 (9)	1.48 ± 0.17
Sulpiride	78.0 ± 5.0 (3)	0.90 ± 0.12	21.0 ± 5.8 (3)	0.91 ± 0.09
Chlorpromazine	4.37 ± 1.62 (3)	1.10 ± 0.09	2.51 ± 0.29 (3)	1.04 ± 0.15
Eticlopride	0.22 ± 0.01 (4)	0.85 ± 0.12	0.14 ± 0.04 (4)	1.20 ± 0.21
7-OH-DPAT	380 ± 90 (7)	0.77 ± 0.06	3.86 ± 0.37 (7)	0.80 ± 0.04

[a]Dopamine receptors were labeled with 0.07 (D_{2L}) or 0.14 (D_3) nM [^{3}H]spiperone. Nonspecific binding was defined by 1 μM chlorpromazine. Parameter estimates were obtained by nonlinear regression using a sigmoidal equation. $K_{0.5}$ and n_H values shown for each compound represent means and their standard errors. The number of independent assays performed is indicated in parentheses. Representative curves are shown in Figure 4.

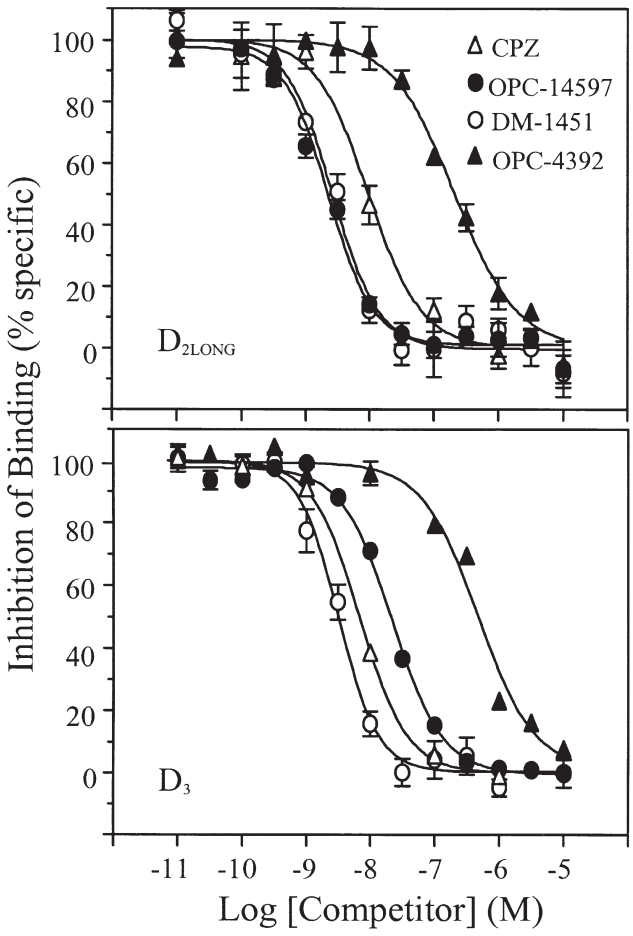

Figure 4. Affinity of OPC-14597 for dopamine D_{2L} and D_3 receptors in C-6 glioma cells. Membranes were prepared from C-6 glioma cells stably transfected with dopamine D_{2L} or D_3 receptors. Dopamine receptors were labeled with 0.07 (D_{2L}) or 0.14 (D_3) nM [^{3}H]-spiperone. Nonspecific binding was defined by 1 μM chlorpromazine. Parameter estimates were obtained by nonlinear regression using a sigmoidal equation. Mean values for $K_{0.5}$ and n_H are shown in Table 4. The curves shown are from representative experiments.

cells prompted an investigation of possible methodological considerations that may have accounted for this difference. The most obvious factor was the presence of sodium in the binding buffer for CHO but not C-6 cells, because this has been shown to modulate the affinity of dopamine D_2 receptor agonists and antagonists (see Neve 1991; Neve et al. 1990). The higher affinity of the benzamide reference compounds sulpiride and eticlopride in CHO cells vs. C-6 cells (see Tables 3 and 4) is consistent with the effect of sodium on binding affinity of this class of compounds. To assess the importance of this factor to the binding profile of OPC-4392, we conducted binding experiments with this compound in the presence or absence of 120 mM sodium in C-6 cells. As shown in Figure 5, there was no effect of

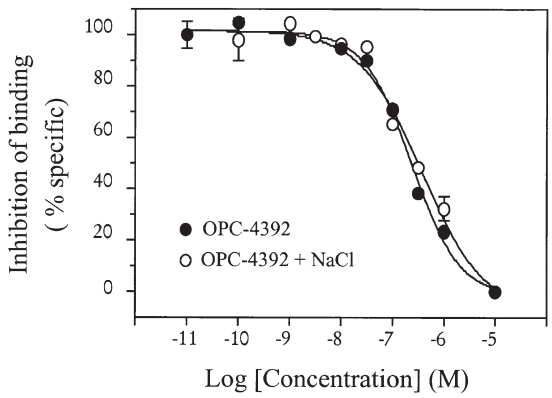

Figure 5. Affinity of OPC-4392 for D_{2L} receptors in C-6 glioma cells in the presence and absence of sodium. Dopamine receptors were labeled with 0.07 nM [^{3}H]-spiperone. The binding buffer was 50 mM Tris HCl, pH 7.4 at 22°C, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, and either 0 or 120 mM NaCl. Nonspecific binding was defined by 1 μM chlorpromazine. Curves were fit by nonlinear regression using a sigmoidal equation. The curves shown are from a representative experiment conducted in duplicate.

sodium on the binding of OPC-4392. Similar results were obtained in rat striatal tissues (data not shown).

Functional Effects of OPC-14597 at Cloned Dopamine Receptors

Previous studies have provided evidence that OPC-14597, at least in the intact nervous system, might have both agonist or antagonist properties, depending upon the function tested (Kikuchi et al. 1995). To study this further, functional studies were performed in the same cell lines in which the receptor binding affinities were evaluated.

Functional Effects of OPC-14597 in CHO Cells Expressing D_{2S} and D_{2L} Receptors. In the CHO cell line, the transfected D_{2S} and D_{2L} receptors are coupled to the inhibition of cAMP synthesis. To evaluate possible agonist or antagonist actions of OPC-14597, we measured its ability to inhibit forskolin-stimulated levels of cAMP and to block the inhibition produced by dopamine. As shown in Figure 6, a maximal concentration of OPC-14597 (10 μM) did not inhibit cAMP synthesis induced by forskolin in either D_{2L} or D_{2S} receptor transfected cells; whereas, dopamine produced a 50% inhibition. When combined with dopamine, OPC-14597 completely blocked the inhibitory effects of dopamine in both D_{2L} and D_{2S} receptor transfected cells.

Functional Effects of OPC-4392 and DM-1451 in CHO Cells Expressing D_{2L} Receptors. Additional functional studies were performed with OPC-4392 and DM-1451 in CHO cells transfected with D_{2L} receptors. In this cell line, DM-1451 behaved as an antagonist, similar to OPC-14597. That is, DM-1451 produced no inhibition of forskolin when applied alone, but fully antagonized the inhibition produced by dopamine (Figure 7). In contrast to the antagonist actions of OPC-14597 and DM-1451, the related quinolinone derivative OPC-4392 acted as a potent full agonist in CHO cells expressing D_{2L} receptors. As shown in Figure 8, OPC-4392 produced full inhibition of forskolin-stimulated cAMP synthesis, with a potency (IC50 = ca. 0.1 nM) five-fold greater than that of dopamine.

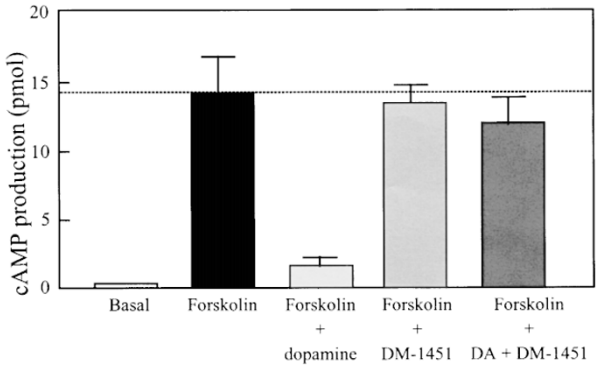

Figure 7. Functional effects of DM-1451 in CHO cells transfected with D_{2L} receptors. Whole cells were exposed to 10 μM forskolin, which produced a 15-fold increase over basal levels of cAMP. DM-1451 (10 μM) was added alone or together with 10 μM dopamine (DA), to assess possible agonist and antagonist actions. cAMP production was measured as described in Methods. Data shown represent means and standard errors from three experiments and are expressed as a percent of the stimulation produced by forskolin alone.

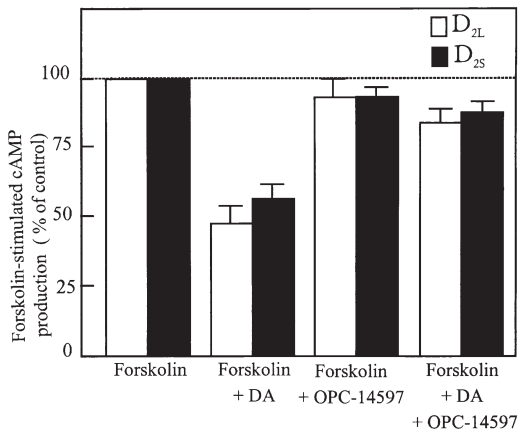

Figure 6. Functional effects of OPC-14597 in CHO cells transfected with D_{2S} or D_{2L} receptors. Intact cells were exposed to 10 μM forskolin, which produced a 10-fold increase over basal levels of cAMP. OPC-14597 (10 μM) was added alone or together with 10 μM dopamine to assess possible agonist and antagonist actions. cAMP production was measured as described in Methods. Data shown represent means and standard errors (from three experiments) and are expressed as a percent of the stimulation produced by forskolin alone to allow comparisons between the two cell lines.

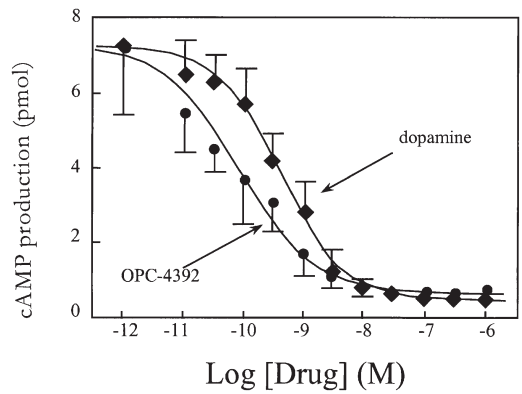

Figure 8. Functional effects of OPC-4392 in CHO cells transfected with D_{2L} receptors. Whole cells were exposed to 10 μM forskolin, which produced a seven-fold increase over basal levels of cAMP. Concentration-response data for OPC-4392 and dopamine were collected to assess the ability of these compounds to inhibit cAMP synthesis. cAMP was measured as described in Methods. Data shown represent means and standard errors obtained from three experiments. Curves were fit by nonlinear regression using a sigmoidal equation.

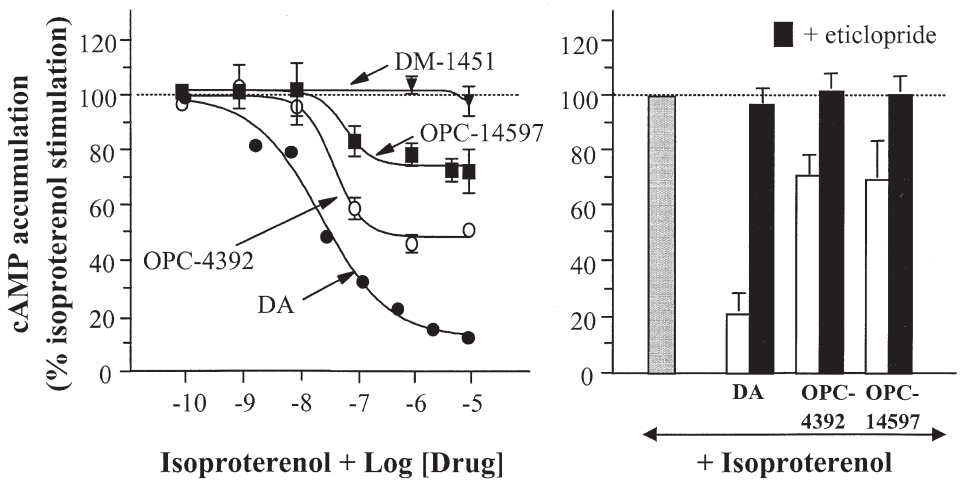

Figure 9. Partial agonist effects of OPC 14597 and derivative compounds in C-6 cells transfected with D_{2L} receptors. Cells were grown to confluence in 24-well culture plates and incubated for 10 min at 37°C with 1 μM isoproterenol, which produced a 10-fold increase over basal levels of cAMP. Under these conditions, the application of 10 μM dopamine reduced cAMP levels by more than 80%. In some assays, coincubation of test compounds (1 μM of OPC4392 or OPC-14597 or 10 μM dopamine) with 10 μM of the D_2 receptor antagonist eticlopride (right panel) was used to determine the receptor mediation of the effects observed. After the incubation period with test compounds, cells were rinsed and lysed, and intracellular cAMP was measured by RIA. Data represent means and standard errors from at least three independent experiments. Values are expressed as a percent of the stimulation produced by isoproterenol alone.

Functional Effects of OPC-14597 and Derivatives in C-6 Glioma Cells Expressing D_{2L} Receptors. We examined the functional actions of OPC-14597 and its derivatives in C-6 cells transfected with D_{2L} receptors to provide a comparison to results obtained from studies in the CHO cells. As in the CHO cells, the D_{2L} receptor expressed in C-6 cells is coupled to inhibition of cAMP synthesis. Isoproterenol was used to stimulate cAMP accumulation. Figure 9 shows that dopamine (10 μM) caused a maximal 90% inhibition of the isoproterenol-induced increase in cAMP synthesis, with a potency of ca. 30 nM. In marked contrast to the results obtained in CHO cells, both OPC-14597 and OPC-4392 produced dose-dependent partial inhibition of cAMP synthesis, with maximal inhibition of 30 and 50%, respectively; whereas, DM-1451 produced little or no inhibition even at high concentrations (see left panel of Figure 9). The functional effects of OPC-14597 and OPC-4392 were blocked by the dopamine D_2 receptor antagonist eticlopride (see right panel of Figure 9).

Next we examined the ability of OPC-14597, OPC-4392 and three reference D_2 antagonists to block the functional effects of dopamine. As shown in Figure 10, dopamine alone produced an 80% inhibition of isoproterenol-stimulated cAMP accumulation. The inhibitory actions of dopamine were blocked completely by the D_2 receptor antagonists haloperidol, chlorpromazine, and eticlopride. None of these D_2 antagonists produced inhibition when tested alone, indicating an absence of intrinsic activity. In contrast, both OPC-14597 and OPC-4392 alone produced partial inhibition of cAMP accu-

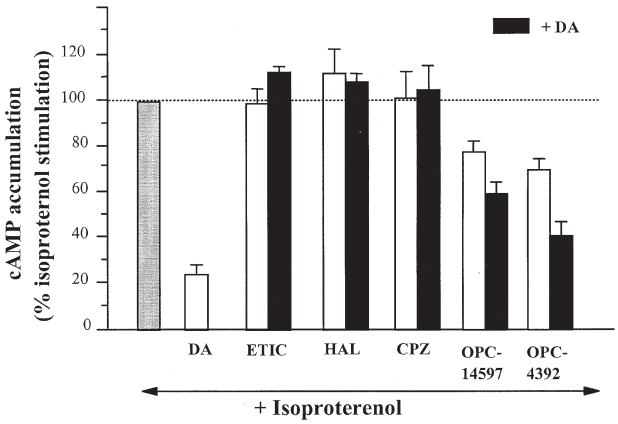

Figure 10. Comparison of functional effects of OPC 14597 and derivatives with those of known D_2 receptor antagonists in C-6 cells transfected with D_{2L} receptors. Cells were grown to confluence in 24-well culture plates and incubated for 10 minutes at 37°C with either isoproterenol (1 μM) or various test compounds, alone or in combination. Isoproterenol alone produced a 10-fold increase over basal levels of cAMP. The concentration of dopamine used was 10 μM. All other compounds were tested at 1 μM. After the incubation period with test compounds, cells were rinsed and lysed, and intracellular cAMP was measured by RIA. Data represent means and standard errors from at least three independent experiments. Values are expressed as a percent of the stimulation produced by isoproterenol alone.

Table 5. Affinity of OPC-14597 and Related Compounds for Serotonin Receptor Subtypes Expressed in HEK Cells[a]

Receptor Isoform	OPC-14597 $K_{0.5}$ (nM)	OPC-4392 $K_{0.5}$ (nM)	DM-1451 $K_{0.5}$ (nM)
5-HT_6	161 ± 17 (3)	680 ± 157 (3)	320 ± 40 (3)
5-HT_7	14.5 ± 2.5 (3)	22 ± 2.6 (3)	220 ± 40 (3)

[a]5-HT_6 and 5-HT_7 receptor subtypes were stably transfected in HEK-293 cells. Receptors were labeled with [^{3}H]-LSD (1 nM) and nonspecific binding was defined by 10 μM serotonin. Parameter estimates were obtained by nonlinear regression using a sigmoidal equation. $K_{0.5}$ values shown for each compound represent means and their standard errors. The numbers of independent assays performed is indicated in parentheses.

mulation and, when incubated with dopamine, yielded a partial reversal of dopamine's inhibitory effects, actions consistent with partial agonist characteristics.

Affinity of OPC-14597 for Cloned Serotonin Receptors

Previous studies have shown that OPC-14597 and clozapine bind with similar affinity to the 5-HT_2 receptors that are believed to be important in the actions of psychotomimetics (unpublished data). In addition, both the 5-HT_6 and 5-HT_7 receptors have been implicated as target sites for neuroleptics (Roth et al. 1994), thus the binding of OPC-14597 was tested at these sites. As shown in Table 5, OPC-14597 bound to 5-HT_7 receptors with affinity similar to what has been reported for clozapine (Boess and Martin 1994). In contrast, the affinity of OPC-14597 for 5-HT_6 receptors was moderately lower (four-fold) than that of clozapine. Functional studies performed in HEK-293 cells demonstrated that OPC-14597 exhibits antagonist activity at both the 5-HT_6 and 5-HT_7 receptors (data not shown).

DISCUSSION

The past decade has seen the development of antipsychotic drugs that seem truly atypical in terms of their clinical profile (i.e., effective antipsychotic action without neurological and possibly endocrine side effects) in comparison with typical drugs such as chlorpromazine or haloperidol. Despite this, it is unclear what molecular mechanism(s) might make a drug atypical (see Arnt and Skarsfeldt 1998). Some of the more generally accepted mechanisms include concomitant D_2/5-HT_2 receptor antagonist activity (Meltzer 1991) or selectivity for the D_3 or D_4 receptor (Sokoloff et al. 1990; Van Tol et al. 1991). In addition, it has been hypothesized that effective antipsychotic action may be caused by occupation of one of several types of noradrenergic receptors, although this is less well understood (Cohen and Lipinski 1986; Van Kammen et al. 1990). More recently, indirect actions at NMDA or purine receptors have been implicated as sites for antipsychotic actions (Deutsch et al. 1989; Kafka and Corbett 1996; Inoue 1996).

Although such molecular hypotheses abound, the use of animal models is still critical in the selection of new antipsychotic drug candidates. In fact, OPC-14597 was first selected for clinical testing because, unlike haloperidol or chlorpromazine, OPC-14597 caused inhibition of apomorphine-induced stereotyped behavior at doses far lower than those that induced catalepsy (Kikuchi et al. 1995). The initial screening of this compound also suggested that it had relatively high affinity for brain dopamine D_2-like receptors, and little for D_1 receptors. Although OPC-14597 was also found to have modest affinity for 5-HT_2 receptors and was able to antagonize the 5-HT-agonist-induced head twitch in mice, its D_2:5HT_2 receptor affinity ratio was over 15 (personal communication, T. Kikuchi), a profile different from the majority of atypical antipsychotics (Arnt and Skarsfeldt 1998). Kikuchi et al. (1995) hypothesized that OPC-14597 was an antagonist of postsynaptically located D_2-like receptors, but not at presynaptic D_2-like receptors. Because the majority of functional effects they studied are believed to be mediated by D_2 receptors, there was no ready mechanism that could explain these unusual functional effects. A broad-based receptor screening did not provide any other mechanism of action that might explain these early data (personal communication, T. Kikuchi), prompting a more detailed examination of the mechanism(s) by which OPC-14597 interacts with dopamine receptors.

Consistent with the available data (Kikuchi et al. 1995), the present studies demonstrated that OPC-14597 had little affinity for brain dopamine D_1-like receptors labeled with [^{3}H]SCH23390 or for D_1 or D_5 receptors expressed in clonal cells. Moreover, as was shown by Kikuchi et al. (1995), OPC-14597 had high affinity for D_2-like dopamine receptors in rat striatum. The affinity of the metabolite DM-1451 was likewise high, a finding indicating that its role as an active metabolite must be considered when interpreting the effects of OPC-14597 in rodents. The present study and that of Kikuchi et al. both demonstrate that OPC-14597 binds with 20-fold higher affinity to D_2-like receptors as compared to the purported autoreceptor agonist OPC-4392.

A finding of considerable interest is that, in our studies in rat striatum and C-6 glioma cells, OPC-14597 differed from traditional D_2 antagonists by exhibiting a competition curve with a steep slope ($n_H > 1.0$). The metabolite DM-1451 also exhibited an indirect Hill slope of greater than one in D_{2L}-transfected C-6 cells. Such findings are usually ascribed to such phenomena as "positive cooperativity," but it is unclear why OPC-14597 or DM-1451 causes such "steep" kinetics. Studies to determine whether this phenomenon is affected by guanine nucleotides or ions may shed light on such mechanisms. It should be noted that the existence of D_2 receptor dimers has been hypothesized (Ng et al. 1996).

Although this concept is a controversial one, it could provide a way to explain the positive cooperativity exhibited by OPC-14597.

In addition to confirming and extending the results reported previously for OPC-14597 and OPC-4392 in rat brain, the present study provided important new information concerning the molecular subtype selectivity of OPC-14597, DM-1451, and OPC-4392. This information is of special relevance for the interpretation of the unusual functional properties of these compounds in vivo. Of the D_2-like receptors examined in CHO cells, OPC-14597 and DM-1451 bound with highest affinity to D_{2L} and D_{2S} receptors and exhibited selectivity of at least 15-fold for these receptors relative to D_3 receptors expressed in this cell line. Both compounds exhibited ca. 500-fold lower affinity for D_4 receptors. The high D_2:D_4 receptor selectivity ratio for OPC-14597 is of significant interest in light of its atypical clinical profile. Although the actions of the prototypical atypical antipsychotic clozapine have been ascribed to D_4 receptor occupation (Van Tol et al. 1991; Seeman et al. 1997), the present data, coupled with the recent disappointing clinical trials with novel D_4-selective antagonists (Kramer et al. 1997), imply that atypicality may not be related to this receptor subtype.

The receptor binding profile of OPC-14597 and derivatives at D_2-like receptors differed somewhat when assessed in two distinct cell lines, although the same receptor isoform was expressed in both lines (i.e., rat D_{2L}). For OPC-14597 and DM-1451, the D_{2L} receptor affinities were somewhat higher, and the D_3 receptor affinities lower, in C-6 relative to CHO cells. This produced a marked decrease in the D_{2L}:D_3 selectivity ratio in C-6 cells. For example, the D_{2L}:D_3 receptor selectivity of OPC-14597 was 15-fold and three-fold in CHO vs. C-6 cells, respectively. For the purported autoreceptor agonist OPC-4392, fivefold D_{2L}:D_3 selectivity was demonstrated in CHO cells; whereas, no selectivity was apparent in C-6 cells. This interaction between receptor binding affinity and cell type is intriguing, but not unexpected, because there is increasing recognition that the characteristics of the local membrane environment and cellular milieu may have an impact on both receptor recognition and functional characteristics (e.g., Vallar et al. 1990; Raymond 1995; Tang et al. 1994). Although these effects, at least for G-protein-coupled receptors, are seen most often with agonists (e.g., Watts et al. 1995), they may occur with antagonists as well. For example, the selectivity of the dopamine receptor antagonist clozapine for D_4:D_{2L} or D_{2S} has been reported to vary considerably depending upon both the cell lines and the laboratories conducting the experiments (c.f. Lahti et al. 1993; Van Tol et al. 1991).

The differences in the functional properties of OPC-14597 and OPC-4392 in CHO and C-6 cells expressing D_{2L} receptors add further support to the importance of the characteristics of the expression system used in defining pharmacological properties of drugs. Although both cell lines expressed the rat form of the D_{2L} receptor, myriad other factors may be important. The D_{2L} receptor density was three-to four-fold higher in CHO than in C-6 cells (1600 vs. 450 fmol/mg protein). If we ignore other possible differences between the cell lines, this fact alone would suggest the presence of a higher receptor reserve in CHO vs. C-6 cells. It is well known that the presence of spare receptors can markedly influence assessments of functional activity, particularly with agonists of low intrinsic efficacies (Kenakin 1993; Watts et al. 1995). The greater potency of dopamine and the higher efficacy of OPC-4392 in CHO vs. C-6 cells are consistent with differences in receptor reserve. This explanation seems at odds with the profile observed for OPC-14597, however, because this compound had lower efficacy in the cell line (CHO) with the presumed greater receptor reserve. This difference in the direction of efficacy change observed for OPC-4392 and OPC-14597 in the two cell lines is indicative of factors other than, or in addition to, receptor reserve.

One factor that varied systematically between assays conducted in the two cell lines was the method of stimulating cAMP. The presence of β-adrenergic receptors in C-6 cells makes it possible to use isoproterenol as an agent for increasing cAMP levels. Unfortunately, no suitable receptor is available for this purpose in the CHO cells; thus, forskolin was used as the stimulating agent. To assess the effects of the stimulating agent on the results obtained, we have recently conducted a number of experiments with OPC-14597 and its derivatives in C-6 cells using forskolin, and our results were comparable to those obtained using isoproterenol. Thus, it seems unlikely that this aspect of our methodology can account for the differing functional profiles of the OPC compounds observed in the two cell lines. Myriad other factors may be relevant, however, including potential differences in the complement of G-protein and adenylyl cyclase isoforms in the two cell lines.

The molecular pharmacology data obtained for OPC-14597 in the present study have important implications for the interpretation of a recent study reported by Amano et al. (1995), who studied the physiological actions of OPC-14597 on neuronal activities of the nucleus accumbens using a microiontophoretic method in rats anesthetized with chloral hydrate. Spikes elicited by glutamate or by stimulation of the parafascicular nucleus of the thalamus were inhibited by iontophoretic application of dopamine, SKF38393 or quinpirole in a dose-dependent manner. Conversely, microiontophoretic application of OPC-14597 had no effect alone, yet inhibited the actions of dopamine, SKF38393, or quinpirole. Amano et al. suggested that OPC-14597 blocks dopaminergic inhibition of the accumbens neurons receiving input from the parafascicular nucleus by acting on both D_1 and D_2 receptors located on the neurons. However, our data in rat brain and in molecular expression sys-

tems clearly indicate that OPC-14597 has affinity for D_2-like receptors, but little affinity for D_1-like receptors. Although Amano et al. concluded that OPC-14597 antagonized the effects of SKF38393 via D_1 receptors, it is well known that iontophoresis cannot control concentrations. In fact, SKF38393 is only 50 to 100-fold selective for D_1 vs. D_2 receptors (e.g., Brewster et al. 1990). Thus, our data suggest that Amano et al. (1995) may have activated D_2 receptors with SKF38393, and this was the site of antagonism by OPC-14597.

Our data also bear on the question of what mechanism(s) make OPC-14597 a postsynaptic, but not presynaptic, antagonist at D_2-like receptors. There is a traditional view that relates the higher sensitivity of presynaptic receptors to a large receptor reserve, activated by low concentrations of agonists or by agonists with weak partial efficacy (Clark et al. 1985a, 1985b). The modest agonist actions of OPC-14597 observed in C-6 cells expressing D_{2L} receptors imply weak partial efficacy; thus, it might be expected to activate highly sensitive presynaptic receptors, yet to antagonize the actions of dopamine at less sensitive postsynaptic receptors. Although this idea is an appealing one, it is important to note that OPC-14597 behaves differently from other partial agonists when endogenous dopamine tone is removed by reserpine, or when postsynaptic receptors are in a supersensitive state because of permanent denervation of dopamine fibers with the neurotoxicant 6-OHDA. These conditions tend to reveal agonist properties of compounds with weak partial efficacy; however, OPC-14597 maintains a clear antagonist profile at postsynaptic receptors in these circumstances. Although it might be hypothesized that OPC-14597 has extremely low intrinsic efficacy which does not allow stimulation of postsynaptic receptors even under favorable circumstances, this explanation is somewhat unsatisfactory when we consider the robust agonism displayed by OPC-14597 at presynaptic receptors.

Inasmuch as invoking partial D_2 receptor agonism does not provide a wholly satisfactory account of the functional effects of OPC-14597, additional factors should be considered. For example, its effects at nondopamine receptors or via nonreceptor-mediated actions may be important. The actions of OPC-14597 at 5-HT receptors clearly merit consideration, although available data in mice (i.e., antagonism of 5-methoxy-N,N-dimethyltryptamine) indicate that 5-HT antagonism by OPC-14597 occurs at greater than 15-fold higher doses than its dopamine antagonist actions (personal communication, T. Kikuchi). A role for 5-HT_7 receptors cannot be excluded, however, because the present data indicate that OPC-14597 binds with high affinity to this novel 5-HT receptor isoform. It should be noted, however, that there does not seem to be a reliable association between various clinical or preclinical measures of atypicality of an antipsychotic compound and its affinity for either $5HT_6$ or $5HT_7$ receptors (Arnt and Skarsfeldt 1998).

Another general hypothesis to account for the unusual functional profile of OPC-14597 is that D_2-like receptors expressed on pre- vs. postsynaptic membranes have distinct ligand recognition characteristics for OPC-14597. For example, an exclusive localization of one dopamine molecular receptor subtype on pre- vs. postsynaptic membranes, coupled with a differential affinity of OPC-14597 for these subtypes, might be evoked to explain the unusual functional profile. Our data indicate, however, that OPC-14597 binds with highest affinity to D_{2L} or D_{2S} receptors, and it is known that this receptor type is expressed as both autoreceptors on dopamine cells and as postsynaptic receptors on target cells (Giros et al. 1989; Rao et al. 1990; Snyder et al. 1991). Although the involvement of D_3 receptors cannot be ruled out at present, they also do not seem to have an exclusive pre- or postsynaptic localization (Sokoloff et al. 1990; Diaz et al. 1995). Not enough is known of their functional properties in native tissues to allow speculation concerning their role in the unusual functional effects of OPC-14597.

In addition to these obvious hypotheses, another possibility is suggested by recent speculation concerning the ability of certain atypical ligands of G-protein coupled receptors to produce selective activation of specific effector pathways (Kenakin 1995; Leff et al. 1997; Mailman et al. 1997). Central to these ideas is the notion that different ligands acting at the same molecular receptor can either select or induce different conformational states that are differentially coupled to specific G-proteins and downstream effectors. From this point of view, the postsynaptic antagonist selectivity of OPC-14597 may be attributable not to its selectivity for a particular molecular receptor subtype, but rather to the particular conformational changes it causes when binding.

How might the ability of different compounds to induce distinct conformational changes upon binding to a given receptor subtype be related to the functional phenomena observed with OPC-14597? It is well established that dopamine D_2 receptors can couple to multiple G-proteins to achieve their diverse effects. Although typical dopamine agonists may induce a conformation capable of conferring activation to all G-protein isoforms, certain atypical drugs such as OPC-14597 may induce a conformation that is sufficient for activation of only a subset of G-proteins. This would produce an apparent functional selectivity of a ligand, in the absence of subtype selectivity for a receptor. Such functional selectivity may be expressed at any number of levels (e.g., anatomical selectivity of compounds for striatal vs. nucleus accumbens D_2 receptors, selectivity for D_2 receptors on dopamine neurons vs. postsynaptic targets of dopamine axon terminals) depending upon differences in the complement of G-proteins available to interact with the dopamine receptor. Thus, a single drug, acting on a single receptor isoform may have effects as extreme as full agonist or pure antagonist depending upon such factors as the local G-protein complement. Although this

notion is a novel one, we have demonstrated apparent functional selectivity of the dopamine agonist dihydrexidine at D_2 receptors (Mottola et al. 1992; Kilts et al. 1996, 1997; Smith et al. 1996). Moreover, the idea of ligand-induced selectivity has begun to gather support in a variety of other receptor systems (Eason et al. 1994; Gettys et al. 1994; Gurwitz et al. 1994; Spengler et al. 1993).

In summary, the present data indicate that OPC-14597 has characteristics different from other typical or atypical antipsychotic drugs. In addition to theoretical importance, the difference(s) between the dual agonist/antagonist actions of OPC-14597 and those of other partial D_2 agonists are likely to have significant clinical implications. There has been interest in the use of partial dopamine D_2 receptor agonists for the treatment of schizophrenia, based on their presumed selective agonist actions at presynaptic receptors controlling dopamine synthesis, release, and cell firing. A recent study of Lahti et al. (1998) reported modest effectiveness of the partial agonist (-)-3-PPP against both positive and negative symptoms in individuals with schizophrenia. Unfortunately, the positive clinical effects were apparent only during the first week. Lahti et al. (1998) suggest that receptor desensitization of presynaptic D_2-like receptors by the agonist actions of (-)3-PPP was responsible for the short-lived beneficial effects. It is of interest that the preliminary data with OPC-14597 indicate a sustained clinical benefit, despite the clear evidence of presynaptic agonist actions in preclinical studies (Toru et al. 1994 and unpublished data).

As importantly, the receptor binding profile of OPC-14597 suggests that clinical atypicality can occur with drugs that are selective for D_{2L} and D_{2S} rather than D_3 or D_4 receptor subtypes. The marked differences in functional effects of OPC-14597 in two different cell lines underscore other recent data indicating the importance of the local cellular environment in determining the pharmacological responses elicited by drugs acting at a single receptor isoform. One implication of the binding and functional data presented in the present study is that the unusual functional profile reported for OPC-14597 in vivo (presynaptic agonist and postsynaptic antagonist) may not involve actions at D_3 or D_4 receptors, but rather the ability of certain atypical drugs to produce different effects at the same molecular isoform of dopamine receptor when expressed in different locales. These data are certainly consistent with the atypical clinical actions of this candidate antipsychotic drug.

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REFERENCES

Amano T, Matsubayashi H, Momiyama T, Ishihara K, Todo N, Sasa M (1995): Antagonizing effects of a novel antipsychotic quinolinone derivative (OPC-14597) on dopaminergic inhibition of neuronal activities in the nucleus accumbens. Prog Neuropsychopharmacol Biol Psych 19:105–116

Arana GW, Hyman SE (1991): Handbook of Psychiatric Drug Therapy, 2nd ed. Boston, Little, Brown, and Company, pp 27–34

Ariano MA, Wang J, Noblett KL, Larson ER, Sibley DR (1997): Cellular distribution of the rat D_4 dopamine receptor protein in the CNS using antireceptor antisera. Brain Res 752:26–34

Arnt J, Skarsfeldt T (1998): Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. Neuropsychopharmacology 18:63–101

Boess FG, Martin, IL (1994): Molecular biology of 5-HT receptors. Neuropharmacology 33:275–317

Brewster WK, Nichols DE, Riggs RM, Mottola DM, Lovenberg TW, Lewis MH, Mailman RB (1990): *trans*-10,11-Dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo [*a*]-phenanthridine: A highly potent selective dopamine D_1 full agonist. J Med Chem 33:1756–1764

Bunzow J, Van Tol H, Grandy D, Albert P, Salon J, Christie M, Machida C, Neve K, Civelli O (1988): Cloning and expression of a rat D_2 dopamine receptor cDNA. Nature 336:783–787

Clark D, Hjorth S, Carlsson A (1985a): Dopamine receptor agonists: Mechanisms underlying autoreceptor selectivity. I. Review of the evidence. J Neural Transm 62:1–52

Clark D, Hjorth S, Carlsson A (1985b): Dopamine receptor agonists: Mechanisms underlying autoreceptor selectivity II. Theoretical considerations. J Neural Trans 62:171–207

Cohen BM, Lipinski JF (1986): In vivo potencies of antipsychotic drugs in blocking alpha 1 noradrenergic and dopamine D_2 receptors: Implications for drug mechanisms of action. Life Sci 39:2571–2580

Creese I, Burt DR, Snyder SH (1976): Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192:481–483

Dearry A, Gingrich JA, Falardeau P, Fremeau RT, Bates MD, Caron MG (1990): Molecular cloning and expression of the gene for a human D_1 dopamine receptor. Nature 347:72–76

Deutsch SI, Mastropaolo J, Schwartz BL, Rosse RB, Morisha JM (1989): A "glutamatergic hypothesis" of schizophrenia. Rationale for pharmacotherapy with glycine. Clin Neuropharmacol 12:1–13

Diaz J, Levesque D, Lammers CH, Griffon N, Martres MP, Schwartz JC, Sokoloff P (1995): Phenotypical characterization of neurons expressing the dopamine D_3 receptor in the rat brain. Neuroscience 65:731–745

Eason MG, Jacinto MT, Liggett SB (1994): Contribution of ligand structure to activation of α_2-adrenergic receptor subtype coupling to G_s. Mol Pharmacol 45:696–702

Fatemi HS, Meltzer HY, Roth BL (1996): Atypical antipsychotic drugs: Clinical and preclinical studies. In Csernansky JG (ed), Handbook of Experimental Pharmacology: Antipsychotics, vol. 120. New Jersey, Springer-Verlag, pp 77–115

Garau L, Govoni S, Stefanini E, Trabucchi M, Spano PF (1978): Dopamine receptors: Pharmacological and anatomical evidences indicate that two distinct dopamine receptor populations are present in rat striatum. Life Sci 23:1745–1750

Gerlach J (1991): New Antipsychotics: classification, efficacy, and adverse effects. Schizophr Bull 17:97–117

Gettys TW, Fields TA, Raymond JR (1994): Selective activation of inhibitory G-protein α subunits by partial agonists of the 5-HT_{1A} receptor. Biochem 33:4283–4290

Giros B, Sokoloff P, Martres MP, Riou JF, Emorine LJ, Schwartz JC (1989): Alternative splicing directs the expression of two D_2 dopamine receptor isoforms. Nature 342:923–926

Gurwitz D, Haring R, Heldman E, Fraser CM, Manor D, Fisher A (1994): Discrete activation of transduction pathways associated with acetylcholine m1 receptors by several muscarinic ligands. Eur J Pharmacol 267:21–31

Harper JF, Brooker G (1975): Femtomole sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2′O-acetylation by acetic anhydride in aqueous solution. J Cyclic Nucleotide Res 1:207–218

Hartman DS, Civelli O (1996): Molecular attributes of dopamine receptors: New potential for antipsychotic drug development. Ann Med 28:211–219

Inoue K, Koizumi S, Ueno S (1996): Implication of ATP receptors in brain functions. Prog Neurobiol 50:483–492

Inoue A, Miki S, Seto M, Kikuchi T, Morita S, Ueda H, Misu Y, Nakata Y (1997): Aripiprazole, a novel antipsychotic drug, inhibits quinpirole-evoked GTPase activity but does not upregulate dopamine D_2 receptor following repeated treatment in the rat striatum. Eur J Pharmacol 321:105–111

Jeste DV, Caligiuri MP (1993): Tardive dyskinesia. Schizophr Bull 19:303–315

Joyce JN, Meador-Woodruff JH (1997): Linking the family of D_2 receptors to neuronal circuits in human brain: Insights into schizophrenia. Neuropsychopharmacology 16:375–384

Kafka SH, Corbett R (1996): Selective adenosine A_{2A} receptor/dopamine D_2 receptor interactions in animal models of schizophrenia. Eur J Pharmacol 295:147–154

Kane J, Hoingfeld G, Singer J, Meltzer H, Cozaril Collaborative Study Group (1988): Clozapine for the treatment-resistant schizophrenic. Arch Gen Psychiat 45:789–796

Kaplan HI, Sadock BJ (1988): Synopsis of Psychiatry, 5th ed. Baltimore, Williams & Wilkins, 254 pp

Kebabian J, Calne DB (1979): Multiple receptors for dopamine. Nature 277:93–96

Kenakin T (1993): Pharmacological Analysis of Drug-Receptor Interactions. Raven Press, New York, pp 249–277

Kenakin T (1995): Agonist-receptor efficacy II: Agonist trafficking of receptor signals. Trends Pharmacol Sci 16:232–238

Kikuchi T, Tottori K, Uwahodo Y, Hirose T, Miwa T, Oshiro Y, Morita S (1995): 7-(4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]butyloxy)-3,4-dihydro-2(1H)-quinolinone (OPC-14597), a new putative antipsychotic drug with both presynaptic dopamine autoreceptor agonistic activity and postsynaptic D_2 receptor antagonistic activity. J Pharmacol Exp Ther 274:329–336

Kilts JD, Nichols DE, Mailman RB, Lawler CP (1997): The functionally selective agonist dihydrexidine inhibits adenylate cyclase in rat striatum via the D_2 dopamine receptor. Soc Neurosci Abstr 23:1777

Kilts JD, Lawler CP, Nichols DE, O'Malley KL, Todd RD, Mailman RB (1996): Functional selectivity: Dihydrexidine, a D_2 agonist, acts as an antagonist at dopamine release-modulating D_2 receptors. Soc Neurosci Abstr 22:1769

Kramer MS, Last B, Getson A, Reines SA (1997): The effects of a selective D_4 dopamine receptor antagonist (L-745,870) in acutely psychotic inpatients with schizophrenia. Arch Gen Psychiat 4:567–572

Lader MH (1993): Neuroleptic-induced deficit syndrome (NIDS). J Clin Psychiat 54:493–500

Lahti AC, Wiler MA, Corey PK, Lahti RA, Carlsson A, Tamminga CA (1998): Antipsychotic properties of the partial dopamine agonist (-)-3-(3-hydroxyphenyl)-N-n-propylpeperidine (preclamol) in schizophrenia. Biol Psychiat 43:2–11

Lahti RA, Evans DL, Stratman NC, Figur LM (1993): Dopamine D_4 versus D_2 receptor selectivity of dopamine receptor antagonists: Possible therapeutic implications. Eur J Pharmacol 236:483–486

Leff P, Scaramellini C, Law C, McKechnie K (1997): A three-state model of agonist action. Trends Pharmacol Sci 18:355–362

Mailman RB, Nichols DE, Lewis MM, Blake B, Lawler CP (1997): Functional effects of novel dopamine ligands: Dihydrexidine and Parkinson's disease as a first step. In P. Jenner and R. Demirdemar (eds), Dopamine Receptor Subtypes: From Basic Science to Clinic. IOS Stockton Press, Amsterdam

Meltzer HY (1991): The mechanism of action of novel antipsychotic drugs. Schizophr Bull 17:263–287

Monsma FJ, Mahan LC, McVittie LD, Gerfen CR, Sibley DR (1990): Molecular cloning and expression of a D_1 dopamine receptor linked to adenylyl cyclase activation. Proc Natl Acad Sci 87:6723–6727

Monsma FJ, McVittie LD, Gerfen CR, Mahan LC, Sibley DR (1989): Multiple D_2 dopamine receptors produced by alternative RNA splicing. Nature 342:926–929

Monsma FJ, Shen Y, Ward RP, Hamblin MW, Sibley DR (1993): Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Molec Pharmacol 43:320–327

Momiyama T, Sasa M, Takaori S (1990): D-2 receptor-mediated inhibition by a substituted quinolinone derivative, 7-[3-(4-(2,3-dimethylphenyl)piperazinyl)propoxy]-2(1H)-quinolinone (OPC-4392), of dopaminergic neurons in the ventral tegmental area. Life Sci 47:761–769

Momiyama T, Amano T, Todo N, Sasa M (1996): Inhibition by a putative antipsychotic quinolinone derivative (OPC-14597) of dopaminergic neurons in the ventral tegmental area. Eur J Pharmacol 310:1–8

Mottola DM, Brewster WK, Cook LL, Nichols DE, Mailman RB (1992): Dihydrexidine, a novel full efficacy D_1 dopamine receptor agonist. J Pharmacol Exp Ther 262:383–393

Motulsky HJ, Ransnas LA (1987): Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J 1:365–374

Neve, KA (1991): Regulation of dopamine D_2 receptors by sodium and pH. Mol Pharmacol 39:570–578

Neve KA, Henningsen RA, Kinzie JM, De Paulis T, Schmidt DE, Kessler RM, Janowsky A (1990): Sodium-dependent isomerization of dopamine D-2 receptors characterized using [^{125}I]epidepride, a high-affinity substituted benzamide ligand. J Pharmacol Exp Ther 252:1108–1116

Ng GY, O'Dowd BF, Lee SP, Chung H, Brann MR, Seeman P, George SR (1996): Dopamine D_2 receptor dimers and receptor-blocking peptides. Biochem Biophys Res Commun 227:200–204

Patel A, Linden J (1988): Purification of ^{125}I-labeled succinyl cyclic nucleotide tyrosine methyl esters by high-performance liquid chromatography. Analyt Biochem 168: 417–420

Peacock L, Gerlach J (1996): Antipsychotic-induced side effects related to receptor affinity. In JG Csernansky (ed), Handbook of Experimental Pharmacology: Antipsychotics, vol. 120. New York, Springer-Verlag, pp 359–388

Rao DD, McKelvy J, Kebabian J, MacKenzie RG (1990): Two forms of the dopamine rat D_2 receptor as revealed by polymerase chain reaction. FEBS 263:18–22

Raymond JR (1995): Multiple mechanisms of receptor-G protein signaling specificity. Am J Physiol 269:F141–158

Roth BL, Craigo SC, Choudhary MS, Uluer A, Monsma FJ, Shen Y, Meltzer HY, Sibley DR (1994): Binding of typical and atypical antipsychotic agents to 5-HT_6 and 5-HT_7 serotonin receptors. J Pharm Exp Therap 268: 1403–1410

Seeman P, Lee T, Chan-Wong M, Wong K (1976): Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 261:717–719

Seeman P, Corbett R, Van Tol HH (1997): Atypical neuroleptics have low affinity for dopamine D_2 receptors or are selective for D_4 receptors. Neuropsychopharmacology 16:93–110

Semba J, Sakai M, Miyoshi R, Mataga N, Fukamauchi F, Kito S (1996): Differential expression of c-fos mRNA in rat prefrontal cortex, sriatum, nucleus accumbens, and lateral septum after typical and atypical antipsychotics: An in situ hybridization study. Neurochem Int 29:435–442

Shen Y, Monsma FJ, Metcalf MA, Jose PA, Hamblin MW, Sibley DR (1993): Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype. J Biol Chem 268:18200–18204

Smith HP, Oxford GS, Nichols DE, Mailman RB, Lawler CP (1996): Dihydrexidine activates D_2 dopamine receptors coupled to adenylyl cyclase, but not to potassium ion channels. Soc Abstr Neurosci 22:1316

Snyder LA, Roberts JL, Sealfon SC (1991): Distribution of dopamine D_2 receptor splice variants by solution hybridization/protection assay. Neurosci Lett 122:37–40

Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC (1990): Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. Nature 347:146–151

Spengler D, Waeber C, Pantaloni C, Holsboer F, Bockaert J, Seeburg PH, Journot L (1993): Differential signal transduction by five splice variants of the PACAP receptor. Nature 365:170–175

Sunahara RK, Niznik HB, Weiner DM, Stormann TM, Brann MR, Kennedy JL, Gelernter JE, Rozmahel R, Yang YL, Israel Y, Seeman P, O'Dowd BF (1990): Human dopamine D_1 receptor encoded by an intronless gene on chromosome 5. Nature 347:80–83

Sunahara RK, Guan HC, O'Dowd BF, Seeman P, Laurier LG, Ng G, George SR, Torchia J, Van Tol HHM, Niznik HB (1991): Cloning of the gene for a human dopamine D_5 receptor with higher affinity for dopamine than D_1. Nature 250:614–619

Tang L, Todd RD, Heller A, O'Malley KL (1994): Pharmacological and functional characterization of D_2, D_3, and D_4 dopamine receptors in fibroblast and dopaminergic cell lines. J Pharmacol Exp Ther 268:495–502

Tiberi M, Jarvie KR, Silvia C, Falardeau P, Gingrich JA, Godinot N, Bertrand L, Yang-Fang TL, Fremeau RT, Caron MG (1991): Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D_1 dopamine receptor subtype: Differential expression pattern in rat brain compared with the D_{1A} receptor. Proc Natl Acad Sci 88:7491–7495

Toru M, Miura S, Kudo Y (1994): Clinical experiences of OPC-14597, a dopamine autoreceptor agonist in schizophrenic patients. Neuropsychopharmacology 10:122S

Vallar L, Muca C, Magni M, Albert P, Bunzow J, Meldolesi J, Civelli O (1990): Differential coupling of dopaminergic D_2 receptors expressed in different cell types. J Biol Chem 265:10320–10326

Van Kammen DP, Peters J, Yao J, Van Kammen WB, Neylan T, Shaw D, Linnoila M (1990): Norepinephrine in acute exacerbations of chronic schizophrenia. Negative symptoms revisited. Arch General Psychiat 47:161–168

Van Tol HH, Bunzow JR, Guan HC (1991): Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine. Nature 350:610–614

Watts VJ, Lawler CP, Gonzales AJ, Zhou QY, Civelli O, Nichols DE, Mailman RB (1995): Spare receptors and intrinsic activity: Studies with D_1 dopamine receptor agonists. Synapse 21:177–187

Wyrick S, Mailman RB (1985): Tritium labeled (±)-7-chloro-8-hydroxy-3-methyl-l-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH23390). J Labeled Compd Radiopharmac 22:189–195

Yasuda Y, Kikuchi T, Suzuki S, Tsutsui M, Yamada K, Hiyama T (1988): 7-[3-(4-[2,3-dimethylphenyl]piperazinyl)propoxy]-2(1H)-quinolinone (OPC04392), a presynaptic dopamine autoreceptor agonist and postsynaptic D_2 receptor antagonist. Life Sci 42:1941–1954

Zhang LJ, Lachowicz JE, Sibley DR (1994): The D_{2s} and D_{21} dopamine receptor isoforms are differentially regulated in Chinese hamster ovary cells. Molec Pharmacol 45:878–889

Zhou Q-Y, Grandy DK, Thambi L, Kushner JA, Van Tol HHM, Cone R, Pribnow D, Salon J, Bunzow JR, Civelli O (1990): Cloning and expression of human and rat D_1 dopamine receptors. Nature 247:76–80