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Pharmacological Characterization of PD 152255, a Novel Dimeric Benzimidazole Dopamine D_3 Antagonist

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Dopamine D_3 receptors Dopamine D_3 antagonist Locomotor activity Antipsychotic

BLOCKADE of dopamine (DA) receptors in the limbic forebrain is believed to be the mechanism by which antipsychotic drugs produce their therapeutic effects (8,12). However, antipsychotic drugs with prominent DA D_2 antagonist effects cause extrapyramidal side effects, presumably through blockade of striatal DA receptors (3). DA D_3 receptors are among the five subtypes of identified DA receptors (24, 30). The amino acid sequence of the DA D_3 receptor subtype has been shown to have significant homology to that of the DA D_2 receptor (24); however, the D_3 receptor messenger RNA distribution in brain is much less abundant than D_2 receptor mRNA (31). Because DA D_3 receptor mRNA appears to be more abundant in the ventral vs. dorsal striatal regions of the brain (5,15,19), including the nucleus accumbens, it has been proposed that D_3 antagonists may provide antipsychotic efficacy without neurological side effects (31).

DA D_3 receptors have been proposed to be involved in the control of locomotor activity (11,33,35), brain dopamine synthesis (20), cocaine self-administration (6), and body temperature (22). Several DA D_3-preferring agonists have been identified and characterized, such as PD 128907 (10,25), and (+) 7-OH-DPAT (1,11,12,33). However, the possible contribution of D_2 receptor effects in the profiles of these agents cannot be easily excluded. Certain DA D_3-preferring antagonists have also been described, including (+)-AJ 76 and (+)-UH 232 (32), U99194A (35), (+)-S-14297 (13,22) and nafadotride (26).

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Because these agents have only about 5–20-fold selectivity for D_3 vs. D_2 receptors, it has been difficult to attribute effects of these drugs unequivocally to blockade of D_3 sites. The present study reports the pharmacological effects of PD 152255, a new D_3 antagonist (36) (Fig. 1). PD 152255 appears to be 28- to 45-fold more potent at D_3 receptors than at D_2 receptors, as assessed by in vitro receptor binding and functional assays. The present report demonstrates that although PD 152255 can be distinguished from D_2 antagonists in behavioral and neurochemical tests, certain preclinical effects suggest antipsychotic utility.

METHOD

Animals

Male Sprague–Dawley rats (180–250 g) and Long–Evans hooded rats (180–250 g) from Harlan Labs Inc., Indianapolis, IN, were used for the behavioral and biochemistry studies, respectively. Male CD-1 mice from Charles River (20–30 g) were used for locomotor activity studies. Animals were maintained on a 12 L:12 D cycle (lights on between 0600 and 1800 hs) and housed in groups of 5 rats or 10 mice per cage, in accordance with guidelines established by the National Institutes of Health and the American Association of the Accreditation of Laboratory Animal Care. Animals had free access to rat chow and water until the moment of dosing, except that animals used for oral dosing were food deprived for 16 h prior to dosing.

Materials

PD 152255 was dissolved in 0.9% saline and administered orally (by gavage), IP or SC in 5 ml/kg body weight in rats or 10 ml/kg body weight in mice. *d*-Amphetamine (Sigma Chemical Co., St. Louis, MO), (+)-AJ-76 and (+)-UH-232 (synthesized at Parke-Davis) were dissolved in 0.9% saline and administered either IP in a volume of 5 ml/kg, or SC in a volume of 2 ml/kg. When necessary, 1 N HCl was used to facilitate solubilizing compounds, and the pH was titrated to 5.5–7.0 with 1 N NaOH. The surfactant, Emulphor, was also added in small amounts to facilitate dissolution. Doses represent the active moiety. Quinpirole (Eli Lilly and Co., Indianapolis, IN) and NSD 1015 (3-hydroxybenzylhydrazine; Sigma Chemical Co., St. Louis, MO) were used in the [^{3}H]-thymidine uptake and biogenic amine studies, respectively. Haloperidol was purchased from Research Biochemicals International (Natick, MA). Tritiated spiperone (107 Ci/mol) and N-0437 (103 Ci/mmol) were obtained from Amersham Corp. (Arlington Heights, IL). SCH 23390 was purchased from Dupont NEN Research Products (Boston, MA).

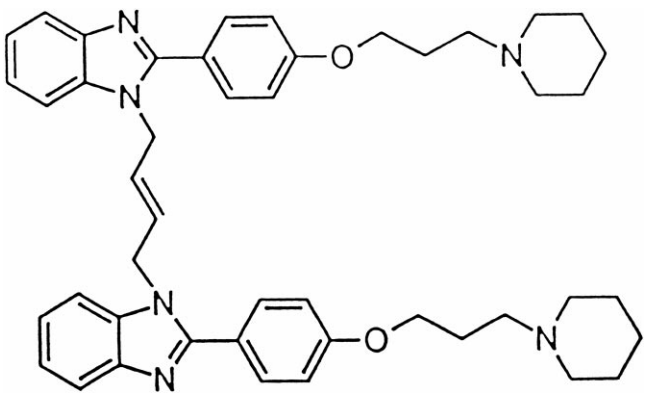

FIG. 1. Structure of PD 152255. *E*-1, 1′-(2-butene-1,4-diyl)bis[2-[4-[3-(1-piperidinyl)propoxy]-phenyl]-1H-benzimidazole].

Cell Culture

Chinese hamster ovary (CHO) cells provided by Dr. James Granneman (Wayne State University, Detroit, MI) were transfected with human D_{2L}, D_3, or $D_{4.2}$ cDNA and maintained under an atmosphere of 95% air and 5% CO_2 at 37°C. The cell lines expressing human D_{2L} receptors were purchased from Dr. O. Civelli (Oregon Health Sciences University), D_3 cDNA was obtained from Dr. K. O'Malley (Washington University), and $D_{4.2}$ cDNA was synthesized as previously described (28,29). CHO cells were grown and subcultured in F-12 medium (Gibco, Grand Island, NY) containing 100 U/ml penicillin–streptomycin (Gibco Laboratories) and dialyzed fetal bovine serum (HyClone Laboratories) as described previously (25). The media was changed every 2–3 days, and at least 18 h before harvesting of the cells. Confluent cultures were harvested and centrifuged at 1000 g for 2 min. The pellets obtained were recentrifuged and used in the assay.

Receptor Binding Assays

Dopamine D_{2L}, D_3, and $D_{4.2}$ receptor binding assays were conducted as described previously (25) with cell membranes from appropriately transfected CHO-K1 cells employing the radioligands [^{3}H]spiperone (0.2 nM for D_{2L}, 0.6 nM for D_3, 0.5 nM for $D_{4.2}$ receptor assays), and haloperidol (1 μM) to define nonspecific binding. Dopamine D_1 receptor binding assays were conducted by rapid filtration assay (4) as described previously (25) with rat striatal membranes employing the radioligand [^{3}H]SCH 23390 (0.3 nM) and SCH 23390 (10 μM) to define nonspecific binding. Inhibition constants (K_i) were determined in the various assays using eight ligand concentrations and triplicate samples for each data point. Data were analyzed by the LIGAND program (23) and the Lundon Receptor Fit Competition program (Lundon Software Inc., Chagrin Falls, OH) to determine the K_i values (18). Muscarinic receptor binding assays were conducted as described previously (27) with membranes from CHO cells transfected with human m_1–m_5 receptors employing the radioligand [^{3}H]N-methyl scopolamine (NMS, 0.1 nM), and atropine (1 μM) to define nonspecific binding. A variety of other receptor binding assays were conducted by PANLABS, Inc. (Bothell, WA). For these assays, PD 152255 was first tested at a concentration of 10 μM and for assays in which greater than 50% inhibition was observed, secondary tests were conducted with four concentrations (0.01–10 μM). The receptors tested (radioligand and receptor source indicated in parentheses) included: adenosine A1 ([^{3}H]DPCPX, rat whole brain), adenosine A_2 ([^{3}H]CGS-21680, rat striatum), adrenergic α_1 ([^{3}H]prazosin, rat whole brain), adrenergic α_2 ([^{3}H]DPCPX, rat whole brain), adrenergic β_1 ([^{3}H]CGP-12177, rabbit lung), adrenergic β_2 ([^{3}H]CGP-12177, rat whole brain), angiotensin II ([^{3}H]angiotensin II, rabbit adrenal gland), bradykinin B_2 ([^{3}H]bradykinin, guinea pig ileum), calcium L channel ([^{3}H]nitrendipine, rat brain cortex), cholecystokinin$_A$ ([^{3}H]L-364718, rat pancreas), cholecystokinin$_B$ ([^{3}H]CCK-8, mouse brain), endothelin ET_A ([^{125}I]endothelin, A10 cells), endothelin ET_B ([^{125}I]endothelin, rat cerebellum), $GABA_A$ ([^{3}H]muscimol, rat whole brain), galanin ([^{125}I]galanin, rat whole brain), kainate ([^{3}H]kainate, rat whole brain), NMDA ([^{3}H]CGS-19755, rat brain cortex), glycine ([^{3}H]strychnine, rat spinal cord), hista-

mine H_1 ([^{3}H]pyrilamine, guinea pig brain), leuokotriene$_{B4}$, ([^{3}H]LTB_4, guinea pig spleen), neurokinin NK_1 ([^{3}H]substance P, guinea pig submaxillary gland), neuropeptide Y_2 ([^{3}H]NPY, rabbit kidney medulla), nicotinic cholinergic ([^{3}H]cystine, rat brain cortex), δ-opiate ([^{3}H]DPDPE, guinea pig brain), κ-opiate ([^{3}H]U-69593, guinea pig brain), μ-opiate ([^{3}H]DAMGO, guinea pig brain), phencyclidine ([^{3}H]TCP, rat brain cortex), serotonin 5-HT_1 ([^{3}H]5-HT, rat brain cortex), serotonin 5-HT_{1A} ([^{3}H]8-OH-DPAT, rat brain cortex), serotonin 5-HT_2 ([^{3}H]ketanserin, rat brain cortex), serotonin 5-HT_3 ([^{3}H]GR-65630, rabbit ileum), sigma$_1$ ([^{3}H]pentazocine, guinea pig whole brain), sodium channel ([^{3}H]batrachotoxin, rat whole brain), thromboxane A_2 ([^{3}H]SQ-29548, rabbit platelet), TRH ([^{3}H] (Me)thyrotropin releasing factor, rat whole brain), and VIP ([^{125}I]vasoactive intestinal peptide, guinea pig lung).

[^{3}H]Thymidine Uptake Assay

The effects of PD 152255 on DA receptor signal transduction were determined with use of a [^{3}H]thymidine uptake assay, as described elsewhere (9,25). The ability of agents to block the stimulation of [^{3}H]thymidine uptake in CHO pro-5 cells transfected with human DA D_{2L}, or D_3 receptor cDNA caused by the DA agonist quinpirole was employed as a measure of DA receptor antagonist activity. Cells were plated on 96-well plates at a density of about 5,000 per well and were grown at 37°C in a minimum essential medium (alpha) with 10% fetal calf serum containing penicillin and streptomycin for 2 days. The cells were then serum deprived by washing and maintained in serum-free media. Twenty-four hours later, 10 μl vehicle, standard or PD 152255 was added to 200 μl serum-free media in each well. After culture for 18 h [^{3}H]thymidine (5 μCi/well) was added for 2 h. Cells were trypsinized (100 μl of 0.25%) and harvested onto filter mats with a 96-well Brandel cell harvester. The filters were counted in a Beta-Plate scintillation counter.

Biogenic Amine Synthesis

To provide an index of the effects of PD 152255 on brain biogenic amine synthesis, the drug was given to rats IP 30 min. before the injection of the L-aromatic amino acid decarboxylase inhibitor, NSD 1015 (100 mg/kg IP). Rats were sacrificed by decapitation 30 min later. The striatum and mesolimbic region (nucleus accumbens plus olfactory tubercle) were rapidly dissected from the brains and stored at −70°C until assayed. Synthesis rates of rat brain catecholamines and serotonin (5-HT) were estimated by measuring the accumulation of the DA precursor, L-3,4-dihydroxyphenylalanine (DOPA) and the 5-HT precursor, 5-hydroxytryptophan (5-HTP) after NSD 1015 administration (7) by HPLC with electrochemical detection (25). Each value represents the mean of four determinations and is expressed as a percent of the control values (ng/g ± SEM).

Locomotor Activity (LMA) in Mice and Rats

Locomotor activity (LMA) was measured in groups of three mice (n = 5–6 groups) or in individual rats (n = 4–10) for 30–60 min in darkened cylindrical photobeam chambers (35 cm diameter × 20 cm deep). Each LMA count represented six consecutive interruptions of the radially arranged photobeams. Standard LMA tests began 30 min after dosing by the IP or SC routes and 60 min after p.o. (gavage) dosing. For LMA tests in habituated rats, rats were placed in the LMA chambers for a 1-h period prior to the SC injection of saline or test compound, after which LMA was measured for 1 h starting immediately after dosing. For studies on the antagonism of amphetamine-stimulated locomotor activity, rats were injected IP with 1.0 mg/kg *d*-amphetamine and placed in LMA chambers for a 20 min acclimation period, after which test compounds or saline were administered SC or IP and LMA was measured for 1 h starting immediately after dosing. Locomotor activity was expressed either as cumulative photobeam breaks or as a percentage of LMA counts determined in vehicle-treated control animals tested concurrently. Drug doses that reduced locomotion by 50% (ED_{50}) were calculated by regression analysis; significant changes in activity levels were determined by analysis of variance and Newman–Keul's multiple-range test (34).

Cholinergic Antagonist Effects In Vivo

An index of in vivo anticholinergic activity was obtained by determining the ability of drugs to antagonize the effects of the muscarinic agonist oxotremorine in rats. Male Sprague–Dawley rats (n = 5) were given either saline (2 ml/kg SC), PD 152255 (1 or 10 mg/kg SC) or the muscarinic antagonist, scopolamine (1 mg/kg SC), together with the muscarinic agonist, oxotremorine (0.4 mg/kg SC). Rats were observed for 1 h and rated for the presence or absence of salivation, licking, and chromodacryorrhea.

RESULTS

Receptor Binding Assays

PD 152255 displaced [^{3}H]spiperone binding from human D_3 and D_{2L} receptors expressed in CHO K1 cells with K_i values of 12.7 and 565 nM, respectively (Fig. 2). This indicated a 45-fold selectivity of PD 152255 for the D_3 vs. D_{2L} receptor. PD 152255 also had 200-fold greater affinity for D_3 vs. $D_{4.2}$ or D_1 receptors; the K_i values for human $D_{4.2}$ and rat striatal D_1 receptors were greater than 3 μM. PD 152255 displayed K_i values of less than 1 μM for human muscarinic m1 (5.1 nM), m2 (5.6 nM), m3 (349 nM), m4 (15.6 nM), and m5 (99.6 nM) receptors.

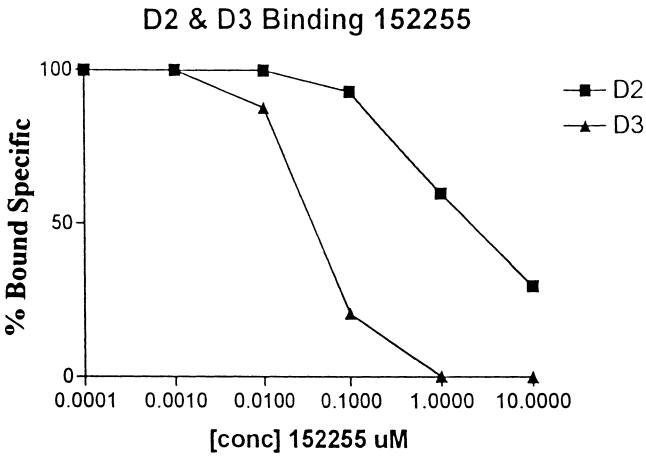

FIG. 2. Concentration-dependent displacement of [^{3}H]spiperone binding with PD 152255 in CHO K1 cells transfected with cloned human DA D_3 and D_{2L} receptors, with corresponding K_i values of 12.7 and 565 nM. The K_i values for human $D_{4.2}$ and rat striatal D_1 receptors were >3 μM. K_i values are mean ± SEM of three experiments.

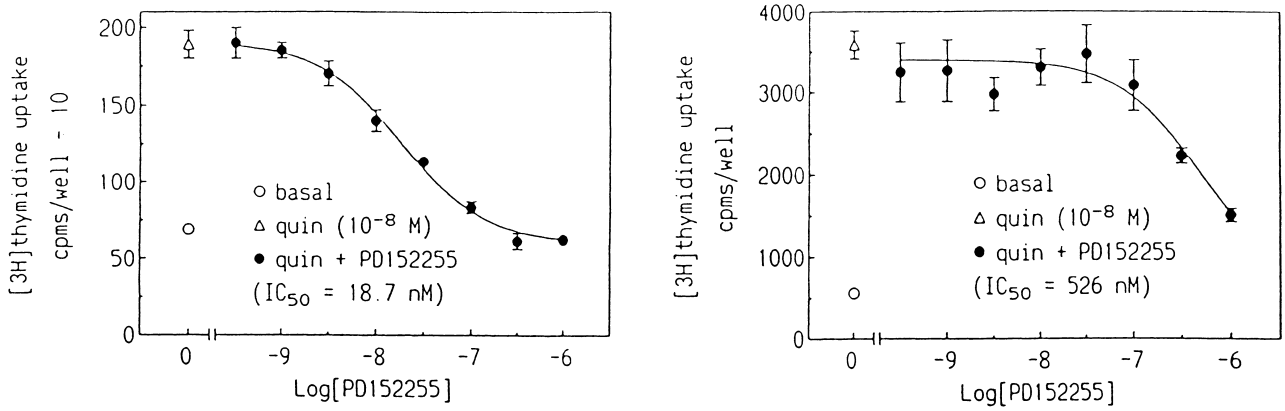

FIG. 3. Dose-dependent effects of PD 152255 on quinpirole-stimulated [^{3}H]thymidine uptake (mitogenesis assay) in CHO pro-5 cells expressing human D_3 (left panel) or D_{2L} (right panel) DA receptors as described in the Method section. Data are results of one experiment in which each point is a mean ± SE of quadruplicate determinations.

Effects on Dopamine Receptor Signal Transduction Activity in CHO Cells

PD 152255 (0.3–1000 nM) alone had no effect on [^{3}H]thymidine uptake in CHO pro-5 cells expressing either the human DA D_{2L} or D_3 receptor. The stimulation of mitogenesis using the DA agonist quinpirole (10 nM) was effectively blocked in a dose-dependent manner by PD 152255 in both the D_3 and D_{2L}-transfected cell lines (IC_{50} values = 18.7 and 525 nM, respectively) (Fig. 3). PD 152255 was 28-fold more potent in blocking this response in D_3-expressing cells than in D_{2L}-expressing cells.

Effects in Cholinergic Muscarinic Functional Assays

Each of five rats treated with saline plus the cholinergic agonist oxotremorine exhibited marked salivation and licking, as well as chromodacryorrhea for at least 30 min after dosing. The muscarinic antagonist scopolamine (1 mg/kg) completely prevented these effects when given in combination with oxotremorine. However, when oxotremorine was given in combination with either 1 or 10 mg/kg PD 152255 SC, the rats appeared identical to rats given saline plus oxotremorine. Thus, PD 152255 does not appear to block muscarinic agonist side effects in this biological assay.

Biogenic Amine Synthesis

PD 152255 (10 mg/kg IP) produced no significant change in biogenic amine synthesis in the striatum or mesolimbic regions of rat brain as reflected by the accumulation of DOPA after administration of NSD 1015 (Fig. 4). PD 152255 also did not appreciably alter serotonin synthesis in these brain regions, as evidenced by unchanged concentrations of the 5-HT precursor 5-HTP (data not shown).

Spontaneous Locomotor Activity (LMA) Tests

PD 152255 dose-dependently reduced spontaneous LMA in mice (1 h test immediately after IP dosing) with an ED_{50} of

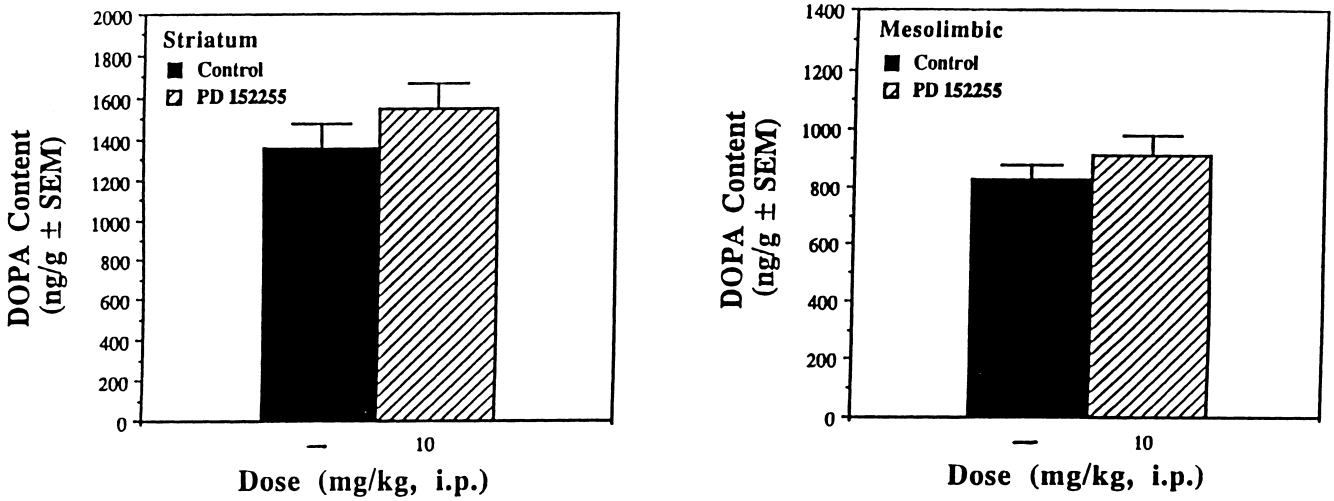

FIG. 4. Effect of PD 152255 (10 mg/kg IP) on DOPA accumulation in rat striatum (left panel) and mesolimbic (right panel) brain regions after NSD 1015 administration. PD 152255 was dosed 30 min prior to NSD 1015 (100 mg/kg IP), and rats were sacrificed by decapitation 30 min after NSD. Each value is a mean ± SE of four animals.

15.4 mg/kg IP (95% CL = 7.7; 30.5) (Fig. 5). In rats dosed SC and tested for 30 min after a 30-min drug absorption period, PD 152255 did not affect spontaneous locomotion ($ED_{50} > 30$ mg/kg SC) (Fig. 5). In fasted rats dosed orally with PD 152255 and tested for 30 min after a 1-h absorption period, PD 152255 only slightly decreased spontaneous activity (LMA ED_{50} of > 30 mg/kg p.o.) (Fig. 5). However, in rats dosed IP and tested for 30 min after a 30 min absorption interval, PD 152255 was much more potent, and dose dependently decreased spontaneous activity levels with an ED_{50} of 1.4 mg/kg (95% CL = 1.0; 1.9) (Fig. 5).

Acclimated Rat Activity Test

PD 152255 significantly increased locomotor activity levels in rats given 3, 10, and 30 mg/kg SC after acclimation to the locomotor activity chambers for 1 h prior to the 1 h test (Fig. 6). This increase in activity was not observed, however, when PD 152255 (1–30 mg/kg) was given p.o. (data not shown). The increased activity level produced by PD 152255 is similar to the effect observed with both (+)-AJ 76 and (+)-UH 232 (Fig. 6) dosed SC in rats acclimated to the locomotor activity chambers.

Amphetamine-Stimulated Activity in Rats

PD 152255 at 1, 3, and 10 mg/kg IP potently and dose dependently reduced the locomotor stimulation produced by the indirect DA agonist *d*-amphetamine in rats (Fig. 7), consistent with the effect observed on spontaneous locomotion when dosed IP. However, when PD 152255 was dosed SC, little reversal of amphetamine-induced hyperactivity was observed (data not shown), consistent with the lack on effect on spontaneous locomotion when dosed SC.

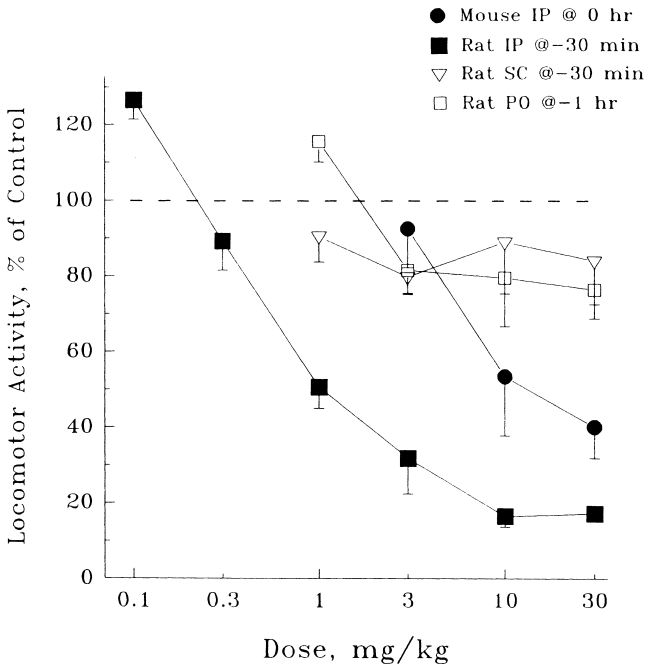

FIG. 5. Effect of PD 152255 on spontaneous locomotor activity in mice (IP) and rats (IP, SC and PO). Locomotion was measured immediately after dosing for 1 h in mice dosed IP. In rats dosed PO the 30 min locomotor activity test began 1 h after dosing, while in rats dosed SC or IP the test began 30 min after dosing. Locomotor activity is expressed relative to vehicle-treated control rats tested concurrently. Each point is the mean ± SE of 4–10 rats.

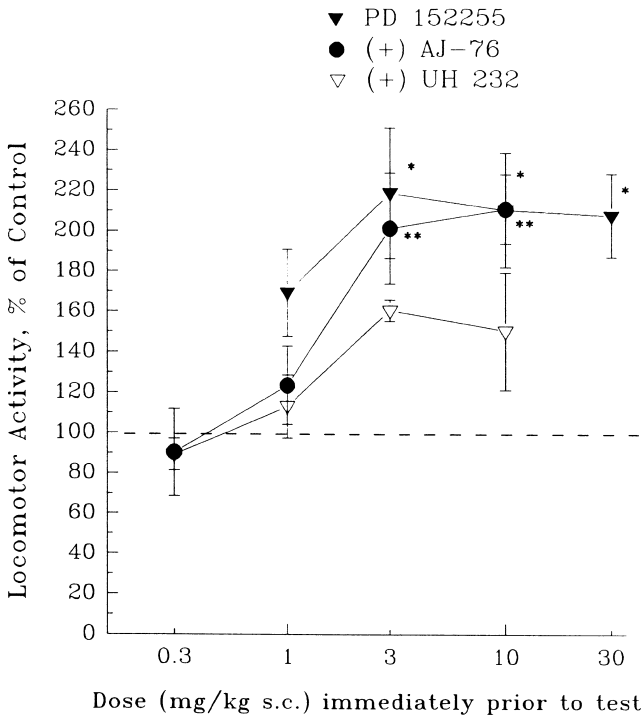

FIG. 6. Dose-dependent increases in activity levels under low baseline conditions with PD 152255, compared to (+)-AJ 76 and (+)-UH 232. Rats were acclimated to chambers for 1 h prior to SC drug injection and subsequent 1-h activity test. Data is expressed as percent of vehicle-treated controls tested concurrently. Each point is a mean ± SE of 5 rats. $*p < 0.05$, $**p < 0.01$.

DISCUSSION

PD 152255 is a structural analog of a DA D_3 antagonist identified from a receptor binding screening program (36). The present results demonstrate that PD 152255 exhibits higher affinity for DA D_3 receptors than D_{2L} receptors expressed in CHO K1 cells, with no appreciable affinity for similarly expressed DA $D_{4.2}$ and rat striatal D_1 receptors. Studies demonstrating the lack of intrinsic agonist activity in cells expressing D_3 receptors and concentration-dependent antagonism of the effects of a DA agonist in such cells indicate that PD 152255 is a DA D_3 receptor antagonist. This would support the validity of comparing PD 152255's relative receptor binding affinities for D_3 vs. D_{2L} receptors with the DA antagonist ligand [^{3}H]spiperone. This comparison suggests that PD 152255 is 45-fold selective for D_3 vs. D_{2L} DA receptors expressed in CHO K1 cells using [^{3}H]spiperone as ligand. A 28-fold selectively for D_3 vs. D_{2L} receptors was found by comparing the relative potency for blockade of DA agonist stimulated [^{3}H]thymidine uptake in CHO pro-5 cells expressing D_3 or D_{2L} receptors (IC_{50} values = 18.7 vs. 525 nM, respectively). In comparison with other DA D_3 antagonists, (+)S-14297 showed a 23-fold preference for D_3 receptors using DA antagonist ([^{3}H]-iodosulpride) binding assays (22). Nafadotride was

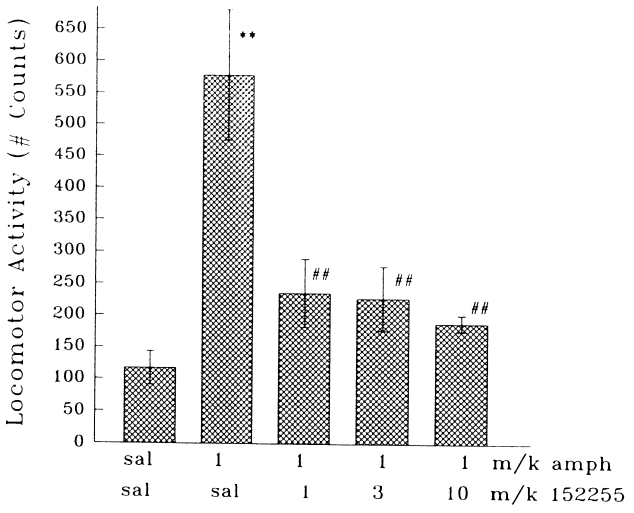

FIG. 7. Dose-dependent inhibition of *d*-amphetamine-stimulated hyperlocomotion by PD 152255. Rats were treated with saline (sal) or *d*-amphetamine (amph) and placed in locomotor activity chambers for 20 min prior to IP PD 152255. Locomotion was then measured for 1 h. Each point is a mean ± SE of four to five rats. $^{**}p < 0.01$ from saline treated control, $^{\#\#}p < 0.01$ from amphetamine-treated control.

reported to be 10-fold selective for D_3 vs. D_2 receptors in [^{125}I]-iodosulpride binding assays and 11-fold D_3-selective in the [^{3}H]thymidine uptake assay (26). U99194A showed a 20-fold preference for D_3 vs. D_2 receptors in [^{3}H]spiperone and [^{3}H]U-86170 binding assays, respectively (35). The DA autoreceptor antagonists (+)-AJ76 and (+)-UH 232 were reported to possess only three-to fivefold preference for D_3 vs. D_2 receptors in [^{3}H]iodosulpride binding assays (31). Thus, by comparison, PD 152255 exhibits a somewhat higher degree of selectivity for D_3 vs. D_2 receptors than other DA D_3 antagonists described to date.

Additional receptor binding studies performed on PD 152255 by PANLABS, Inc. indicated IC_{50} values of less than 1 μM for muscarinic m_1 and m_2 receptor subtypes, histamine H_3 receptors, and sigma binding sites, prompting additional studies. However, in vitro PD 152255 did not enhance or block the agonist (McN-A-343)–induced enhancement of the electrically stimulated rabbit vas deferens preparation (an m_2 test), nor did it induce bradycardia or block methacholine-induced bradycardia in the guinea pig atria (an m_1 test), suggesting that the compound is devoid of functional muscarinic m_1 and m_2 agonist or antagonist effects. These initial PANLABS studies are further supported by the in vivo studies performed at Parke-Davis, in which we found PD 152255 to be without any significant cholinergic agonist behavioral side effects, nor did it antagonize any of the muscarinic agonist side effects of oxotremorine. Taken together, the in vitro and in vivo results indicate that PD 152255 does not exhibit appreciable functional muscarinic agonist or antagonist activity. PD 152255 also exhibited affinity for histamine H_3 receptors in the PANLABS studies, yet it did not exhibit any functional H_3 agonist or antagonist (against R-alpha-methyl-histamine) actions, as assessed by its lack of relaxation and blockade of H_3-agonist–induced relaxation of the guinea pig ileum. Thus, the present results do not reveal a role for histamine or muscarinic activity in the pharmacological effects of PD 152255. Additionally, PD 152255 exhibited significant affinity for sigma sites, as do a number of antipsychotic and other drugs (16, 17). However, the significance of this finding is controversial because no definite function has been associated with these sites.

PANLABS found no significant affinity or significant functional activity for a variety of other nondopaminergic receptors and ion channel binding sites, listed below. For these assays, PD 152255 was first tested at a concentration of 10 μM, and for assays in which greater than 50% inhibition was observed, secondary tests were conducted with four concentrations (0.01–10 μM). Binding affinities (IC_{50}s) of greater than 1 μM were seen at adenosine A_1, adenosine A_2, α_1-adrenergic, α_2-adrenergic, β_1-adrenergic, β_2-adrenergic, angiotensin II, bradykinin B_2, calcium L channel, cholecystokinin$_A$, cholecystokinin$_B$, endothelin ET_A, endothelin ET_B, $GABA_A$, galanin, kainate, NMDA, glycine, histamine H_1, leuokotriene$_{B4}$, neurokinin NK_1, neuropeptide Y_2, nicotinic cholinergic, δ-opiate, κ-opiate, μ-opiate, phencyclidine, 5-HT_1, 5-HT_{1A}, 5-HT_2, 5-HT_3, sodium channel, thromboxane A_2, TRH (thyrotropin releasing hormone), and VIP (vasoactive intestinal peptide) receptors.

It is unclear whether DA D_3 receptors function as autoreceptors (12,20), postsynaptic receptors (33,35), or both. In the present study, PD 152255 did not increase the apparent rate of brain DA synthesis as reflected by DOPA accumulation after decarboxylase inhibition in the striatum or mesolimbic brain regions. The lack of effect of PD 152255 on brain DA synthesis is consistent with the lack of effect of other DA D_3 antagonists on DA release or utilization (13,35) and consistent with the implication of D_2 rather than D_3 affinity in producing this effect associated with DA antagonists (13). Given the strong linkage between presynaptic D_2 autoreceptors and DA synthesis and release, these results suggest that D_3 receptors do not serve in a manner analogous to that of D_2 autoreceptors. However, the hypothesis that PD 152255 reduces LMA by blockade of postsynaptic DA D_3 receptors is in contrast to the proposed existence of inhibitory postsynaptic DA D_3 receptors (35), suggested by the increased locomotion seen with U99194A. When PD 1552255 was given SC in rats that were acclimated to the activity chambers for 1 h prior to test, PD 152255 dose dependently increased activity levels like the DA D_3 antagonists nafadotride (26) and U99194A (35). Thus, PD 152255 produces both elevated locomotion in acclimated animals when dosed SC, and reduced locomotion in normally active animals when dosed IP. Its ability produce these effects as well as block the stimulatory effect of amphetamine is consistent with actions at sites postsynaptic to DA nerve terminals.

Although speculative, certain aspects of the pharmacological profile of PD 152255 suggest potential antipsychotic utility. PD 152255 decreased both spontaneous and amphetamine-stimulated locomotor activity in rats, a profile seen with a variety of antipsychotics (2,14). The lack of effect on brain DA synthesis argues strongly against the involvement of DA D_2 receptors in the antipsychotic-like behavioral effects of PD 152255 and serves to distinguish this agent from other DA antagonists. However, several findings indicate the limitations of PD 152255 as a therapeutic agent. The lack of activity by the oral route in both spontaneous and acclimated locomotor activity tests suggests that PD 152255 has low oral bioavailability. The absence of activity in the amphetamine-stimulated locomotor activity test after SC, but not IP, dosing suggests the possibility that metabolism to an active metabolite is necessary for biological activity. Despite these limitations, this novel dimeric D_3 antagonist may prove useful in elucidating the functional role of brain DA D_3 receptors.

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