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Pharmacology of U-91356A, an agonist for the dopamine D_2 receptor subtype

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Abstract

U-91356A [(*R*)-5,6-dihydro-5-(propylamino)4*H*-imidazo[4,5,1-*ij*]quinolin-2-(1*H*)-one, monohydrochloride], bound with highest affinity to the dopamine D_2 receptor subtype, although it also bound with somewhat lower affinities to the dopamine D_3 and D_4, as well as the 5-HT_{1A} receptor subtypes. In addition to depressing dopamine synthesis and turnover, injection of U-91356A increased striatal acetylcholine concentrations. U-91356A also depressed firing rates of dopamine neurons. In mice, this compound stimulated cage climbing and locomotor activity in reserpinized animals; it also antagonized D-amphetamine-stimulated locomotor activity. It produced contralateral turning in rats with unilateral lesions of the substantia nigra. These data are consistent with roles for the dopamine D_2 receptor subtype as a dopamine autoreceptor and as a stimulatory, postsynaptic dopamine receptor.

Keywords: Dopamine D_2 receptor; Dopamine agonist; Dopamine autoreceptor; Postsynaptic dopamine receptor

1. Introduction

Although the techniques of molecular biology have permitted the identification of at least three dopamine receptor subtypes in the D2 subfamily [1] (D_2, D_3 and D_4, Kebabian, 1992; Sibley and Monsma, 1992), it has been difficult to find dopamine agonists that are selective for the dopamine D_2 receptor subtype so that the unique functional properties of this receptor can be characterized. In this paper, we report a dopamine D_2 receptor agonist, U-91356A ((*R*)-5,6-dihydro-5-(propylamino)4*H*-imidazo[4,5,1-*ij*]quinolin-2-(1*H*)-one, monohydrochloride; Fig. 1), that binds with highest affinity to the dopamine D_2 receptor subtype.

It was shown previously that the (*R*) enantiomer of U-86170F, a dipropyl analog of U-91356A, binds at the dopamine D2 receptor in a manner similar to that of dopamine, as demonstrated by inhibition of [^{3}H]raclopride binding (Lahti et al., 1991a,b). U-86170F does not bind at the dopamine D_1 receptor, but it does have moderate affinity for the serotonin 5-HT_{1A} receptor (Lahti et al., 1991a,b). U-86170F reduces brain dopamine synthesis but not serotonin synthesis when given s.c. at 0.1 and 1 mg/kg (Moon et al., 1992). In other tests of pharmacologic activity, U-86170F performs as a potent dopamine autoreceptor agonist. A very low dose of the (*R*) enantiomer of U-86170F (1.4 ± 0.2 μg/kg) suppresses neuronal firing by 50% in dopamine neurons. U-86170F suppresses the γ-butyrolactone-induced increase in mouse striatal dopamine, as well as the α-methyl-*p*-tyrosine-induced increase in prolactin (Lahti et al., 1991b). Due to its lack of interaction with dopamine D_1 receptors, this compound was not as effective as apomorphine in the reserpinized mouse model of postsynaptic dopaminergic activity, producing only 65% of the increase in locomotor activity induced by apomorphine (Lahti et al., 1991b).

The present study was designed to characterize, both at dopamine autoreceptor and postsynaptic sites, the pharmacology of U-91356A (Moon et al., 1993), a dopamine

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[1] Because of the confusion of the similarities in the names of the D_2 receptor subfamily and the actual D_2 receptor subtype, the distinction will be made throughout this document by the use of subscripts for the subtypes (i.e., D_2 subtypes), in conformity with current nomenclature practices (Watson and Girdlestone, 1995), and the lack of subscripts for the subfamilies (i.e., D1-subfamily and D2-subfamily).

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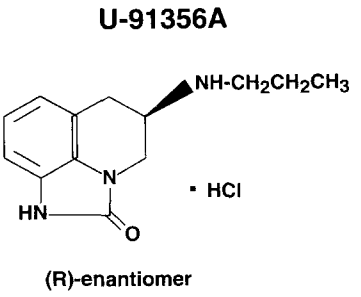

Fig. 1. Chemical structure of U-91356A [(*R*)-5,6-dihydro-5-(propylamino)4*H*-imidazo[4,5,1-*ij*]quinolin-2-(1*H*)-one, monohydrochloride].

receptor agonist with its highest affinity found at the dopamine D_2 receptor subtype.

2. Materials and methods

2.1. Binding assays

The binding profiles of U-91356A and quinpirole were evaluated in competition binding experiments using 11 half-log dilutions of drugs run in duplicate tubes (Piercey et al., 1994). The starting concentrations ranged from 1 to 10 μM, depending on the results of pilot studies using drug at the 1 μM concentration. The radioligands, all tritiated, were prazosin (α_1-adrenoceptor sites, 76 Ci/mmol, 1.2 nM, New England Nuclear, Boston, MA, USA), clonidine (α_2-adrenoceptor sites, 60 Ci/mmol, 3.8 nM, New England Nuclear), dihydroalprenolol (β adrenoceptor sites, 52 Ci/mmol, 1.9 nM, New England Nuclear), oxotremorine-M (muscarinic sites, 87 Ci/mmol, 0.4 nM, New England Nuclear), SCH 23390 (D_1 sites, 71 Ci/mmol, 0.3 nM), U-86170 (dopamine D_2 sites, 62 Ci/mmol, 1–2 nM, Pharmacia and Upjohn, Kalamazoo, MI, USA; Lahti, 1991; Lahti et al., 1991a,b), R(+)7-OH-DPAT (dopamine D_3 sites, 139 Ci/mmol, 0.9 nM, Amersham, Arlington Heights, IL, USA), spiperone (dopamine D_4 sites, 97 Ci/mmol, 0.7 nM, Amersham), 8-OH-DPAT (5-HT_{1A} sites, 85 Ci/mmol, 1.2 nM, New England Nuclear) and ketanserin (5-HT_2 sites, 62 Ci/mmol, 0.8 nM, New England Nuclear). Non-specific binding (5–20% of total) was defined with 3 μM of the following cold compounds (listed in the same order as the radioligands above): phentolamine, clonidine, alprenolol, atropine, SCH23390, haloperidol (dopamine D_2, D_3 and D_4 sites), lisuride and spiperone (all of these compounds were obtained from RBI, Natick, MA, except for aprenolol, atropine and haloperidol, which were synthesized at Pharmacia and Upjohn. The sources of binding sites were as follows: homogenized rat cortex (α_1 adrenoceptors, α_2 adrenoceptors, β-adrenoceptors, acetylcholine muscarinic and 5-HT_2 sites), chinese hamster ovary (CHO) cell membranes prepared from cells expressing the rat dopamine D_1 receptor (Sunahara et al., 1990), CHO cell membranes prepared from cells expressing the rat dopamine D_{2i} receptor (Chio et al., 1990), CHO cell membranes from cells expressing the rat dopamine D_3 receptor (Chio et al., 1994a), HEK293 cell membranes from cells expressing the human dopamine $D_{4.2}$ receptor (Chio et al., 1994b) and CHO cell membranes prepared from cells expressing the human 5-HT_{1A} receptor (Fargin et al., 1988). Buffers used were 50 mM Tris, 5 mM $MgCl_2$, pH 7.4 (α_1- and α_2-adrenoceptor, acetylcholine muscarinic receptor, 5-HT_{1A} receptor and 5-HT_2 receptor assays), 50 mM Tris, 120 mM NaCl, 5 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, pH 7.4 (dopamine D_1 receptor assay), 20 mM HEPES, 10 mM $MgSO_4$, pH 7.4 (dopamine D_2 receptor assay) and 20 mM HEPES, 10 mM $MgSO_4$, 150 mM NaCl, 1 mM EDTA, pH 7.4 (dopamine D_3 and D_4 receptor assays). Incubation of the 0.9 ml binding reactions was for one hour at room temperature. Reactions were stopped by vacuum filtration using ice cold 50 mM Tris, 5 mM $MgCl_2$, pH 7.4. Filter paper was Skatron no. 11734 previously soaked for five minutes in 0.05% polyethylenimine. IC_{50} values were estimated by fitting the data to a one-site model by non-linear least squares minimization using GraphPad Prism. K_i values were calculated according to Cheng and Prusoff (1973). These are expressed in Table 1 with 95% confidence intervals for the weighted average of the mean (n = 1 to 5) constructed using individual standard deviations (Finney, 1978). In some cases, the drug failed to produce 50% inhibition at the 1 μM concentration in the pilot study. Here, dose-response studies were not performed and the IC_{50} is expressed as greater than 1,000 nM.

Table 1
Binding affinities (K_i with 95% confidence interval) of U-91356A and quinpirole [a]

Receptor	U-91356A (nM)	(−)-quinpirole (nM)
α_1-adrenoceptor	> 2427	> 1000 [b]
α_2-adrenoceptor	> 1042	> 1000 [b]
β-adrenoceptor	> 1000 [b]	> 1000 [b]
Acetylcholine receptor	> 2336	> 1000 [b]
Dopamine D_1 receptor	> 4348	> 1000 [b]
Dopamine D_2 receptor	1.6 (1.4–1.9)	3.3 (3.0–3.6)
Dopamine D_3 receptor	36 (33–40)	5.0 (4.6–5.4)
Dopamine D_4 receptor	195 (151–252)	18 (16–20)
5-HT_{1A} receptor	58 (53–64)	> 1000 [b]
5-HT_2 receptor	> 1131	> 1000 [b]

[a] Numbers of experiments were as follows: For U-91356A, 1 for α_1-adrenoceptor, α_2-adrenoceptor, acetylcholine receptor and 5-HT_2 receptor, 2 for dopamine D_1 and 5-HT_{1A} receptor, 3 for β-adrenoceptor, dopamine D_3 receptor and dopamine D_4 receptor and 5 for dopamine D_2 receptor and for (−)-quinpirole, 2 for α_1-adrenoceptor, α_2-adrenoceptor, acetylcholine receptor, dopamine D_1 receptor, dopamine D_4 receptor, 5-HT_{1A} receptor and 5-HT_2 receptor, 3 for β-adrenoceptor and dopamine D_3 receptor and 4 for dopamine D_2 receptor.
[b] IC_{50} (nM) estimated from competition with 1000 nM drug.

2.2. Neurochemical effects

The effects of U-91356A on brain levels of catechols and indoles in the rat were determined by procedures similar to those previously described (VonVoigtlander et al., 1989). Briefly, Charles River bred Sprague-Dawley rats, weighing about 150 g, were injected s.c. with U-91356A (1 mg/kg) or vehicle at time zero. Fifteen min later, rats received an aromatic decarboxylase inhibitor (NSD 1015, 100 mg/kg, i.p.). The rats were sacrificed 30 min later and tissues in the ventral limbic brain area (septal regions, nucleus accumbens, olfactory tubercle) were removed and frozen for later analysis. Tissues were weighed and extracted in perchloric acid containing an internal standard. The extract was then injected onto a Bioanalytical Systems 25-cc ODS column. Catechols and indoles were detected using a BAS electrochemical detector and peaks were quantified by peak integration using Waters Maxima® software. Biochemical differences were compared between the vehicle ($n = 6$) and the U-91356A ($n = 6$) groups using Student's t-test for unpaired samples. The following precursors and metabolites of dopamine and serotonin (5-HT) were assayed: 3,4-dihydrophenylalanine (DOPA), norepinephrine, 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine, 3-methoxy-4-hydroxy-phenylacetic acid (HVA), 3-methoxytyramine (3-MT), 5 hydroxytryptophan (5-HTP) and 5-hydroxyindole-acetic acid (5-HIAA).

The effect of U-91356A on acetylcholine concentration in rat brain striatum was determined using male Charles River Sprague-Dawley rats weighing 130 to 140 g. U-91356A (0.1 to 30 mg/kg), quinpirole hydrochloride (RBI, 0.01 to 3 mg/kg), pergolide mesylate (Eli Lilly, 0.3 to 10 mg/kg) and lisuride maleate (Schering, 0.1 to 3 mg/kg) were dissolved or suspended in either distilled water or 0.25% methylcellulose solution. All drugs were injected i.p. Control rats received an equal volume of the vehicle (2 ml/kg). Thirty min after treatment with drugs, animals were sacrificed by decapitation. The brain was quickly removed from the skull and was placed in ice-cold (0°C) 0.32 M sucrose. The bilateral striata were dissected out and immediately homogenized in 0.05 M perchloric acid containing ethylhomocholine iodide as an internal standard. Acetylcholine was estimated by the high pressure liquid chromatographic methods of Eva et al. (1984) and Potter et al. (1983). The results were expressed as percent increase over control levels, run in parallel with the test drug (controls ranged from 30.50 to 35.76 nmol/g of tissue). The ED_{50} values of dopamine agonists for elevating striatal acetylcholine by 50% of the maximum, along with their 95% confidence limits, were calculated by linear regression analysis. If the overall F statistic was significant at the 0.05 error level, the individual dose levels were compared with controls using a t-test. Following the definition of Ariëns (1964), efficacy was defined as the maximum increase in acetylcholine levels expressed as a percentage of the maximal effect observed with the most effective drug tested. Efficacy results were expressed as means and 95% confidence limits based on variances.

2.3. Electrophysiological effects

The effects of U-91356A on neuronal firing rates of dopamine neurons in the rat substantia nigra pars compacta (SNPC) were evaluated in male Charles River-bred Sprague-Dawley rats weighing 240 to 250 g. So that U-91356A's effects could be evaluated both acutely and chronically, effects on firing rate were measured in animals that had received 14 days of chronic U-91356A ($n = 7$) or vehicle control ($n = 5$). The study drugs were administered in the back via an Alzet model 2ML2 osmotic minipump which was implanted s.c under fluothane and nitrous oxide anesthesia. Test drugs were infused at a rate of 5 μl/h for up to 14 days. Pumps were filled with either U-91356A (11.6 mg/ml) or vehicle (H_2O). The resulting average daily dose was 5 mg/kg, assuming each rat had a mean weight of 280 g throughout the 10 day experiment. Twenty-four hours prior to acute challenge with U-91356A during electrophysiology experiments on day 10, pumps were surgically removed under fluothane and nitrous oxide anesthesia.

During the acute challenge, rats were anesthetized with chloral hydrate (400 mg/kg i.p.). The femoral artery and vein were implanted with cannulae for monitoring blood pressure and administration of drugs, respectively. Glass microelectrodes filled with pontamine sky blue in 2 M sodium chloride (suitable for extracellular recording) were lowered through a small hole burred through the calvarium by means of a hydraulic microdrive. The atlas of Paxinos and Watson (1986) was used for electrode placement. Stereotaxic coordinates for electrode placement relative to Bregma were: A 4.8–5.0 mm, L 2.0 mm and V 6.8–7.8 mm for dopamine neurons and A 0.5–1.7 mm, L 0 mm, V 3.5–4.2 mm (at a posterior angle of 34° to the vertical axis) for serotonin neurons. Substantia nigra pars compacta dopamine neurons were identified by the criteria of Bunney et al. (1973), with firing rates of 2.5–6 spikes/s. Serotonin neurons were identified as biphasic, large positive-negative action potentials with slow and regular firing rates (approximately 0.8–2.5 spikes/s), located in dorsal raphe according to the criteria of Aghajanian et al. (1970).

Once a cell was located, spontaneous firing rates were monitored for several minutes to assure a stable baseline. Then, following an initial dose of 1 μg/kg U-91356A (DA neurons) or 30 μg/kg (serotonin neurons), doses were increased in half-log intervals until the neuron completely or nearly ceased firing. Injections were timed to allow a maximal response to occur, but to accumulate drug sufficiently quickly so that cumulative doses approximated what could be expected with single bolus injections. Thus, the timing of the intervals between injections was determined by how long it took for a maximal response to a drug injection to occur (typically 1–2 min). Histologic

localization of iontophoresed pontamine sky blue dye spots was used to verify electrode placement. Drug effects were measured as changes in firing rates monitored by an integrated rate meter.

2.4. Behavioral effects

Effects on locomotor activity of U-91356A were assessed in reserpinized Harlan male NSA (CF-1) mice weighing, 13 to 19 g. Mice were injected s.c. with 5 mg/kg reserpine (in a vehicle of 1% acetic acid, 5% glucose) 18 to 24 h before testing, as well as with 300 mg/kg DL-α-methyl-*p*-tyrosine methyl ester in water, given i.p. 1 to 5 h before testing. U-91356A, quinpirole HCl, apomorphine HCl, or vehicle was given i.p. in doses ranging from 0.01 to 10 mg/kg 15 min before the mice were placed into 8″ × 8″ cages (Omnitech Digiscan Monitors), with cage papers replaced before each animal. All groups contained 6 mice. Locomotor activity (horizontal activity counts) was recorded for 10 min. Immediately after this test of stimulant locomotor activity for dopamine agonist activity, all mice were injected s.c. with 1 mg/kg apomorphine HCl (kept on ice to prevent oxidation) and were returned to the cages for 10 min to evaluate the ability of the compound to antagonize apomorphine stimulation in these reserpinized mice. Data were presented as percent of control counts for apomorphine alone (which stimulated activity in reserpinized mice). Groups were compared by the least significant difference test.

The effect of U-91356A, quinpirole HCl and apomorphine HCl on amphetamine-stimulated locomotor activity was measured in drug-naive, Harlan male B6C3F1 mice, weighing 23 to 27 g. Mice were placed singly into 8″ × 8″ cages (Omnitech Digiscan Monitors) for 20 min of partial habituation prior to drug treatment. Then, all mice were given 3 mg/kg D-amphetamine sulfate s.c. plus test drug, or vehicle i.p. Locomotor activity (horizontal activity counts) was recorded for two 10 min intervals and reported as percent of control (amphetamine plus vehicle) for the two intervals. Cage papers in the test chambers were replaced after each animal. Data were analyzed by Student's *t*-test; each point represented 6 mice.

The effects of U-91356A and quinpirole HCl on turning in rats with unilateral lesions of the substantia nigra were measured in male Sprague-Dawley rats (Harlan, 280 to 320 g). Rats were anesthetized with Chloropent and lesioned unilaterally in the right substantia nigra (AP 2.8 mm, L + 2.0 mm, V 8.0 mm) by stereotactically injecting 6-OHDA HBr (12 μg/2 μl, 8 μg free base in 2 μl 0.9% saline/0.1% ascorbic acid). Two weeks after lesioning, rats were screened for contralateral turning in response to subcutaneous apomorphine HCl (0.5 to 1.0 mg/kg); rats had to turn at least 3 turns/min to be included in the experiment. Test-drug groups were balanced according to screening test results. Turning was measured with a customized automated rotometer (Omnitech Roto-Scan®). Rats were tethered to rotation sensors via a stainless steel wire harness covered by Technicon tubing. The system continuously monitored the turning of animals and reported data in 5 min intervals. Test groups ($n = 4$ rats) were compared using analysis of variance with least significant difference test.

The effects of U-91356A, quinpirole and apomorphine, with or without pretreatment with SKF 38393 (a dopamine D_1 receptor-selective agonist, RBI) on cage climbing were studied in male CF-1 mice weighing 18 to 20 g. Test drugs were injected s.c. and animals were immediately placed individually in cylindrical test cages (14 cm high, 12 cm diameter) with vertical metal bars 2 mm in diameter and a replaceable waffle bedding paper on the floor. Animals were observed for climbing at 10, 20 and 30 min after cage introduction. Climbing was scored as follows: 0 = all four feet on the floor; 1 = one or two feet on the wall; and 2 = all four feet on the wall. The sum of the three observations was used for statistical evaluation (Dunnett's *t*-test following ANOVAR, $n = 6$ for each group). To evaluate interactions with a D_1 receptor agonist, SKF 38393 (10 mg/kg i.p.) was injected 30 min prior to testing and test drugs were injected s.c. as before. In this study, the test groups were compared to a group that received only apomorphine (1 mg/kg s.c.) using analysis of variance.

3. Results

3.1. Binding assays

The results of the binding assays are shown in Table 1. In contrast to a previous report (Sokoloff et al., 1990), quinpirole had relatively high affinities for all of the dopamine receptors within the D2 subfamily (D_2, D_3 and D_4). U-91356A showed roughly equivalent or very slightly higher affinity for the dopamine D_2 receptor subtype as quinpirole did, but had lower affinities for the dopamine D_3 and D_4 receptor subtypes. U-91356A, but not quinpirole, bound with moderate affinity to 5-HT_{1A} receptors. Both compounds had very low affinities for adrenoceptors, acetylcholine receptors, dopamine D_1 and 5-HT_2 receptors.

3.2. Neurochemical effects

The effects of U-91356A on catechol and indole levels in the rat brain ventral limbic region are shown in Fig. 2. As compared to vehicle, U-91356A significantly reduced tissue levels of DOPA, DOPAC and HVA ($P < 0.05$). However, it did not affect the concentrations of serotonin, its precursor, or its metabolite.

All of the dopamine agonists effectively increased acetylcholine concentration in rat striatum (Table 2, Fig. 3). In this model, the ED_{50} of U-91356A was 0.47 mg/kg. U-91356A and quinpirole were full agonists in this test. In contrast, lisuride and pergolide were only partial agonists,

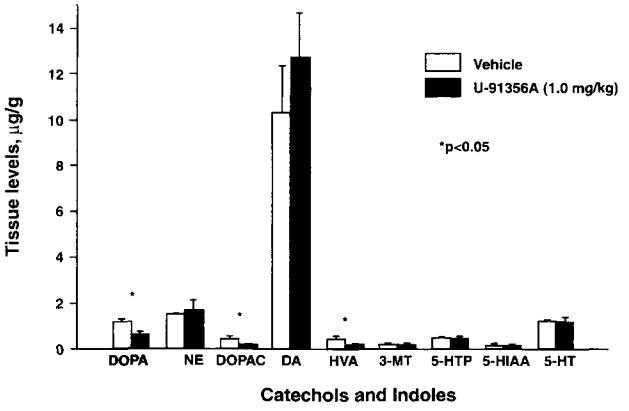

Fig. 2. Effects of U-91356 ($n = 6$) and vehicle control ($n = 6$) on catechols and indoles in the rat limbic region. Asterisks indicate those values where U-93156A-treated animals differed significantly from control animals. DOPA = dihydroxyphenylalanine, NE = norepinephrine, DOPAC = dihydroxyphenylacetic acid, DA = dopamine, HVA = homovanillic acid, 3-MT = 3-methyltyramine, 5-HTP = 5-hydroxytryptophan, 5-HIAA = 5-hydroxyindolacetic acid and 5-HT = 5-hydroxytryptamine or serotonin.

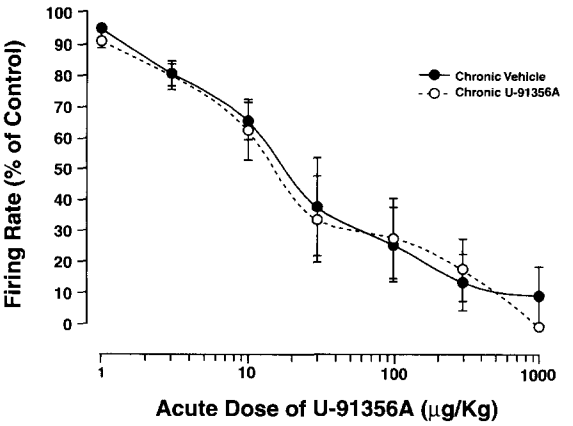

Fig. 4. Effects of treatment with chronic U-91356A ($n = 7$) or vehicle control ($n = 5$) on acute effects of U-91356A on SNPC neuron firing rates in chloralhydrate-anesthetized rats. Ordinate represents the firing rates of SNPC dopamine neurons, expressed as percent of control (mean ± S.E.M.). Abscissa represents cumulative i.v. dose of U-91356A injected acutely 24 h after removal of Alza minipump delivering chronic treatment (see Section 2 for further details).

Table 2
$ED_{50\ value}$s and efficacies of dopamine agonists for increasing rat striatal acetylcholine concentration

Drug	ED_{50} (95% C.L.) [a] (mg/kg, i.p.)	Efficacy (95% C.L.) [b] (%)
U-91356A	0.47 (0.034–3.88)	100 (94–105)
Quinpirole	0.11 (0.036–0.36)	95 (81–103)
Pergolide	1.17 (0.004–47.41)	67 (54–77)
Lisuride	0.20 [c]	29 (28–30)

[a] Doses to produce 50% of maximal effect, using data graphically illustrated in Fig. 3.
[b] Maximal effects as a percent of the mean maximal effect for U-91356A, using same data graphically illustrated in Fig. 3.
[c] Confidence limits could not be calculated.

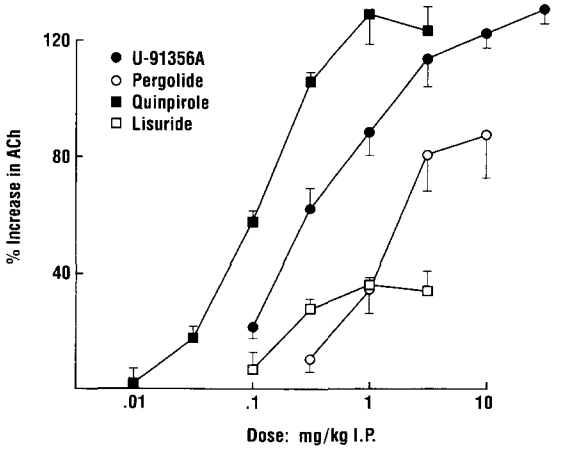

Fig. 3. Effect of dopamine agonists on rat striatal ACh concentration. Each point represents the mean ± S.E.M. of the acetylcholine level, expressed as percent of the parallel control group, found for each group of 6–8 animals treated with the indicated dose of each drug.

their maximal effects being clearly outside the 95% confidence limits of those for both quinpirole and U-91356A (Table 2).

3.3. Electrophysiological effects

For effects on dopamine neurons, firing rates were measured in 12 substantia nigra pars compacta cells (7 from animals given chronic U-91356A and 5 from animals given chronic vehicle control). After acute exposure to U-91356A, all cells tested were inhibited. The cumulative dose-response curves obtained for chronic U-91356A and chronic vehicle control cells are shown in Fig. 4. Chronic administration of U-91356A did not desensitize receptors to acute effects of U-91356A administration on neuronal firing rates. The mean individual $ED_{50} \pm$ S.E.M. for cells from chronic U-91356A ($n = 7$) was 63 ± 35 µg/kg; the mean individual ED_{50} for control animals ($n = 5$) was 71 ± 45 µg/kg.

Consistent with its weaker 5-HT_{1A} binding, U-91356A was weaker in depressing dorsal raphe 5-HT neurons ($ED_{50} = 204 \pm 54$ µg/kg, $n = 6$) than in depressing dopamine neurons (Fig. 5).

3.4. Behavioral effects

Mice given reserpine plus DL-α-methyl-*p*-tyrosine were nearly motionless during the test period. Both U-91356A and quinpirole significantly stimulated locomotor activity (as compared to vehicle control) at doses of 1 and 10 mg/kg, as did apomorphine HCl at doses of 0.1, 1 and 10 mg/kg. Expressed in terms of the maximal amount of stimulant activity observed, apomorphine was the most effective and U-91356A was the least effective of the agonists. Moreover, U-91356A, but not quinpirole, signifi-

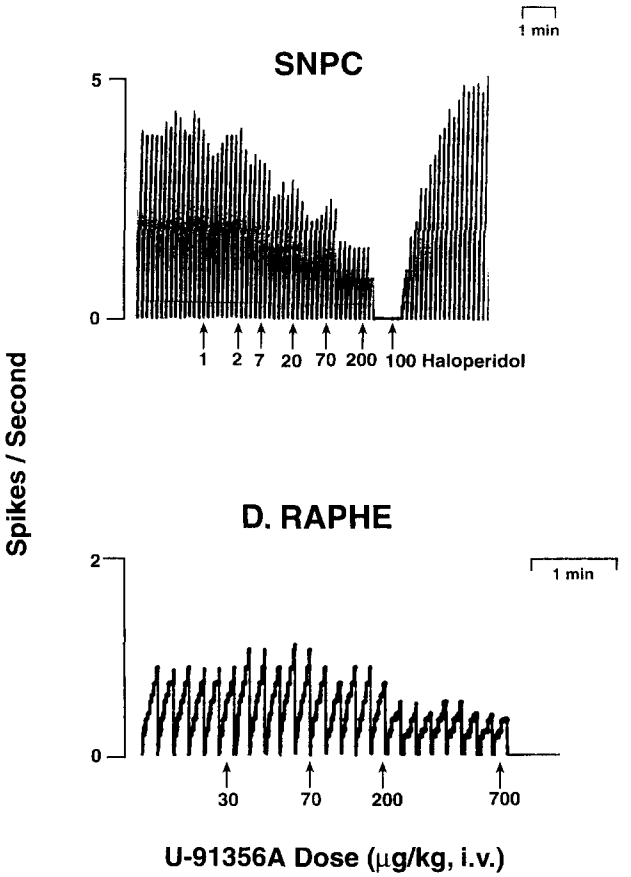

Fig. 5. Effects of U-91356A on a single dopamine neuron in SNPC (top) and a single 5-HT neuron in dorsal raphe (bottom). Ordinates indicate firing rats (spikes/s) and abscissae indicate times. Injections were given at the arrows with the individual (non-cumulative) doses in μg/kg.

cantly antagonized apomorphine-induced locomotor stimulation (Table 3).

U-91356A and quinpirole significantly antagonized the effects of amphetamine-stimulated locomotor activity in drug naive (i.e., non-reserpinized) mice (Table 4).

U-91356A and quinpirole caused contralateral turning in rats with unilateral substantia nigra lesions at both 0.3 and 3 mg/kg (Fig. 6A and B).

Table 3
Effect on locomotion of U-91356A, quinpirole and apomorphine in reserpinized mice (mean ± S.E.M.)

Compound	Dose (mg/kg, i.p.)	Stimulation (mean counts)	Antagonism of apomorphine (% of apomorphine counts)
Vehicle		14 ± 10	100 ± 13
U-91356A	0.1	86 ± 83	82 ± 14
	1.0	565 ± 164 [a]	59 ± 11 [a]
	10.0	208 ± 55 [a]	18 ± 7 [a]
Quinpirole HCl	0.1	110 ± 60	133 ± 35
	1.0	713 ± 197 [a]	124 ± 24
	10.0	767 ± 243 [a]	118 ± 31
Apomorphine HCl	0.01	22 ± 10	119 ± 26
	0.1	156 ± 51 [a]	135 ± 36
	1.0	1777 ± 427 [a]	139 ± 37
	10.0	1184 ± 178 [a]	113 ± 30

[a] $P < 0.05$, least significant differences.

Table 4
Effect of U-91356A, quinpirole HCl and apomorphine on amphetamine-stimulated locomotor activity in mice (means ± S.E.M.)

Compound	Dose (mg/kg, i.p.)	% of Amphetamine Counts	
		0–10 min	10–20 min
Vehicle		100 ± 28	100 ± 17
U-91356A	0.1	36 ± 10 [a]	68 ± 14
	1.0	38 ± 15 [a]	38 ± 13 [a]
	10.0	84 ± 21	5 ± 10 [a]
Quinpirole HCl	0.1	53 ± 17	67 ± 15
	1.0	30 ± 9 [a]	29 ± 8 [a]
	10.0	28 ± 8 [a]	26 ± 6 [a]
Apomorphine HCl	0.01	78 ± 12	86 ± 16
	0.1	73 ± 17	97 ± 18
	1	52 ± 17 [a]	42 ± 10 [a]
	10	68 ± 23	66 ± 18

[a] $P < 0.05$, t-test.

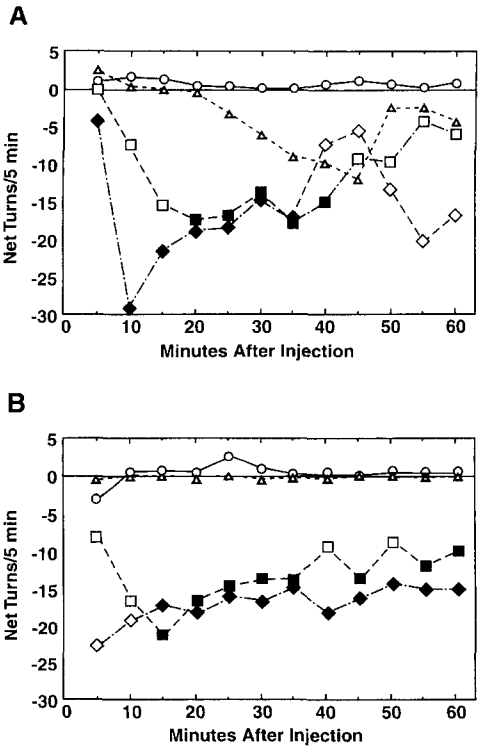

Fig. 6. Contralateral turning in rats with unilateral lesions of the substantia nigra, measured automatically at 5 min intervals. Filled symbols indicate data points which are statistically different from vehicle controls (circles represent vehicle; triangles represent 0.03 mg/kg, squares represent 0.3 mg/kg; diamonds represent 3 mg/kg). (A) Quinpirole HCl s.c., (B) U-91356A s.c.

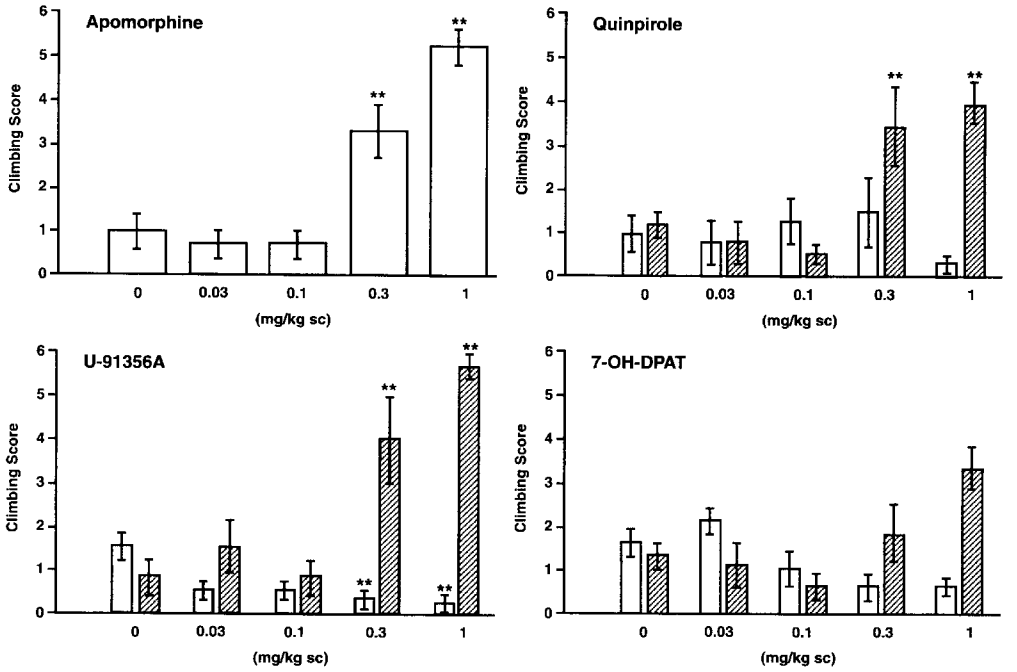

Fig. 7. Cage climbing induced in male CF-1 mice by dopamine D_2 receptor agonists with (hatched bars) or without (open bars) pretreatment with the D_1 agonist SKF 38393. Group sizes were 6 each.

Subcutaneous injection of apomorphine at doses of 0.3 and 1 mg/kg produced cage climbing, while little climbing was noted in the saline control group. U-91356A and quinpirole produced no significant cage climbing at either 0.3 or 1 mg/kg s.c. U-91356A, 0.3 and 1 mg/kg, was the only dopamine agonist to significantly depress cage climbing below control levels (Fig. 7). When pretreated with the D_1-selective agonist SKF 38393, both U-91356A and quinpirole produced cage climbing at 0.3 and 1 mg/kg, respectively (Fig. 7). The D_3-preferring agonist, 7-OH-DPAT (Sokoloff et al., 1990; Chio et al., 1994a) did not produce significant cage climbing, even when combined with SKF 38393.

4. Discussion

When measuring affinities of compounds for many G-protein receptors, it is important to recognize that such receptors can exist in two states or conformations. Antagonists induce a state which has low affinity for agonists and lacks functional activity. Agonists induce a state which has high affinity for agonists and which is responsible for functional activity associated with agonist–receptor interaction. Agonist affinities, when measured by competition with radiolabeled antagonist ligands, are artifactually low and not relevant for their agonist potencies. Thus, agonist affinities should be assessed by their ability to compete with radiolabeled agonist ligands. Antagonists, on the other hand, do not show large differences in affinities whether competing with agonists or antagonists. This difference in agonist affinities for the two different receptor states is particularly high for dopamine D_2 receptors. However, agonist affinities for the two states do not differ greatly for dopamine D_3 receptors, at least for those expressed in cell lines (Sokoloff et al., 1990; Castro and Strange, 1993; Chio et al., 1994a) where the G-protein coupling is very weak. Thus, when D2 subfamily agonist affinities are evaluated with radiolabeled antagonist ligands, the results will artifactually demonstrate high selectivity for dopamine D_3 receptors compared to dopamine D_2 receptors, unless the compound has extraordinarily high selectivity for the D_2 subtype (Gonzalez and Sibley, 1995). In the present study, we used agonist ligand competition studies for both dopamine D_2 and D_3 receptor binding studies.

The data presented here indicate that U-91356A is a dopamine agonist with preferential affinity for the dopamine D_2 receptor subtype. Like quinpirole, U-91356A had a high affinity for the dopamine D_2 receptor subtype but, unlike quinpirole, it bound relatively poorly to the dopamine D_3 and D_4 receptor subtypes. U-91356A had moderate affinity for 5-HT_{1A} receptors and very low affinity for adrenoceptors, acetylcholine receptors, dopamine D_1 receptors and 5-HT_2 receptors. This binding pattern suggests that U-91356A is the first dopamine receptor agonist reported which binds to the dopamine D_2 receptor subtype with a higher affinity than to any other receptor.

The aminotetralin, (+)-7-OH-DPAT, has been frequently used as a tool for evaluating potential functional roles for dopamine D_3 receptors (Levesque et al., 1992; Damsma et al., 1993; McElroy et al., 1993; Svensson et al., 1993; Millan et al., 1994). Initially, when (+)-7-OH-

DPAT's affinities for dopamine receptors were estimated by competition with antagonist ligands, it appeared that this aminotetralin was a very selective dopamine D_3 receptor agonist (Sokoloff et al., 1990). However, more recently, it has been appreciated that when affinities are expressed in terms of the high affinity binding to the agonist conformations of the receptors, that (+)-7-OH-DPAT has preferential, rather than selective, affinity for the dopamine D_3 receptor (Chio et al., 1994a). The data presented for U-91356A binding at dopamine D_2 and D_3 receptors in this report are for the agonist high-affinity state of the receptors. Interestingly, based on relative affinities for different subtypes, U-91356A, as reported here, is more selective for dopamine D_2 receptors than (+)-7-OH-DPAT is for dopamine D_3 receptors (Chio et al., 1994a).

It should be noted that antagonist radioligands were used in the α_1-adrenoceptor, β-adrenoceptor, dopamine D_1 and D_4 receptor and 5-HT_2 receptor binding assays. Because these radioligands presumably occupy both coupled and uncoupled receptors at the concentrations used, the affinities reported in Table 1 for these assays may be underestimated. However, the in vivo pharmacological evaluations yielded results consistent with the in vitro receptor binding data (i.e., strong dopaminergic and weak serotonergic effects).

The fact that U-91356A had agonist activity at both presynaptic and postsynaptic dopamine receptors indicates that there is a probable role for the dopamine D_2 receptor subtype at both receptor sites.

It has long been appreciated that dopamine autoreceptors belong to the dopamine D2 receptor subfamily (Lehmann et al., 1983; White and Wang, 1984; Brown et al., 1985; Bowyer and Weiner, 1987), but there is little evidence demonstrating which of the three subtypes comprising this subfamily contribute to dopamine autoreceptor function. Northern blots and in situ mRNA distribution provide data for roles of D_2, D_3 and D_4 receptor subtypes as dopamine autoreceptors (Bouthenet et al., 1991; Van Tol et al., 1991). The current data with a preferential dopamine D_2 receptor agonist are consistent with a dopamine autoreceptor role for the dopamine D_2 receptor subtype. The activity of U-91356A at the dopamine autoreceptor is supported by several lines of evidence. First, U-91356A significantly reduced levels of DOPA (the precursor of dopamine), as well as DOPAC and HVA (metabolites of dopamine), indicating that U-91356A inhibited the rate of dopamine synthesis and/or release, both of which are known to be controlled by dopamine autoreceptors (Roth, 1979; VonVoigtlander et al., 1989; Lahti et al., 1991b). Second, U-91356A significantly inhibited firing of dopamine substantia nigra pars compacta neurons, which results from stimulation of somatodendritic dopamine autoreceptors; similar results have been reported for other dopamine autoreceptor agonists and partial agonists (Aghajanian and Bunney, 1977; Piercey et al., 1987).

In situ hybridization and northern blot studies (Bouthenet et al., 1991; Van Tol et al., 1991) indicate that, compared to dopamine D_3 and D_4 receptor subtypes, the dopamine D_2 receptor subtype is both more prevalent and has a wider distribution in dopamine postsynaptic regions. Consistent with these findings, U-91356A has stimulatory activity at the postsynaptic dopamine D_2 receptor subtype. For example, it increases rat striatal acetylcholine with an efficacy similar to quinpirole. The behavioral effects of U-91356A, including stimulation of locomotor activity and increased cage climbing after pretreatment with the D_1 receptor selective agonist SKF 38393, are those expected of a postsynaptic dopamine D_2 receptor agonist (Walters et al., 1987). The ability to promote contralateral turning in animals with unilateral 6-OHDA lesions suggests that dopamine D_2 receptor agonists may have some utility in treating Parkinson's disease because of their stimulation of postsynaptic dopamine receptors.

U-91356A appears to be a full dopamine agonist. It completely silences dopamine neuron firing and increases striatal acetylcholine levels to the same extent as quinpirole, also a full dopamine agonist. Consistent with its full agonist profile, U-91356A has much higher affinity for the agonist conformation than it does for the low affinity (antagonist) conformation of dopamine D_2 receptors (Moon et al., 1993). The locomotor effects of U-91356A in mice, like those of U-86170E (Lahti et al., 1991a,b), are less than those of quinpirole and apomorphine. And, even though U-91356A antagonized some of the locomotor effects of apomorphine in this assay, it is unlikely this effect results from partial agonist activity for U-91356A at the dopamine D_2 receptor. If U-91356A's limited locomotor effects in mice were due to limited D_2 receptor intrinsic activity, the effects would reach a plateau. Instead, the locomotor effects of U-91356A were biphasic (Table 3), suggesting that an additional process masks the D_2 receptor effects associated with higher doses of U-91356A. One possible mechanism limiting U-91356A's locomotor effects in this assay is its 5-HT_{1A} receptor agonist properties, since we find that 5-HT_{1A} receptor agonists such as 8-OH-DPAT antagonize the locomotor effects of apomorphine and amphetamine in this assay (unpublished results). Chronic U-91356A did not result in a loss of potency for depressing dopamine neuron firing. This indicates that the effects of U-91356A on somatodendritic autoreceptors do not change with continuous administration. However, in monkeys made Parkinsonian by administration of the neurotoxin MPTP, continuous administration of U-91356A does result in decreased anti-parkinsonian effects; opposite results are found when U-91356A is repeatedly administered in a pulsatile fashion (Blanchet et al., 1995). The reasons for the differences between the effects of continuous U-91356A treatment on dopamine neuron firing and anti-parkinsonian effects could be due to differences between postsynaptic dopamine D_2 receptors and dopamine D_2 autoreceptors. Alternatively, it is possible that it is

simply easier to reverse receptor up-regulation induced by dopamine neuron degeneration, as occurs in the MPTP model, than it is to down-regulate normal receptor levels in unlesioned animals.

Other than the effects observed with high doses in the mouse locomotor assay, it is unlikely that U-91356A's weaker effects at 5-HT_{1A} receptors contributed dramatically to the results we observed. U-91356A did not affect serotonin levels or metabolism. Additionally, the doses required for inhibition of serotonin neuron firing rates were higher than those required to depress dopamine neuron firing rates. By inference, since the affinities of U-91356A for the dopamine D_3 and D_4 receptor subtypes are similar to or less than that for 5-HT_{1A} receptors, it is reasonable to suggest that the majority of the functional effects we observed with U-91356A are mediated primarily via the D_2 subtype, for which its affinity is much higher.

The data presented here indicate that U-91356A acts as an agonist at both dopamine autoreceptors and postsynaptic receptors. Although somewhat less biological data is available for U-86170F, the dipropyl analog, it also appears to be active at both autoreceptors and postsynaptic receptors (Lahti et al., 1991b). In addition, we find that, using the same binding conditions as we describe in this manuscript, U-86170F also binds preferentially to the D_2 receptor subtype, with a selectivity only slightly less than that for U-91356A (unpublished data). These data, along with in situ hybridization studies demonstrating D_2 receptor mRNA in presynaptic and postsynaptic sites (Bouthenet et al., 1991), are consistent with a dualistic dopamine autoreceptor/postsynaptic receptor role for the dopamine D_2 receptor subtype. Additional studies (currently underway) which compare U-91356A pharmacology with that for preferential D_3 receptor agonists, will enhance appreciation of the relative importance of these subtypes in the physiology and pharmacology of dopamine (Hoffmann et al., 1993; Hyslop et al., 1993).

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