Sumanirole, a Highly Dopamine D₂-Selective Receptor Agonist: In Vitro and in Vivo Pharmacological Characterization and Efficacy in Animal Models of Parkinson’s Disease

Robert B. McCall, Keith J. Lookingland, Paul J. Bédard, and Rita M. Huff

ABSTRACT

The purpose of this study is to demonstrate that sumanirole is a novel dopamine receptor agonist with high in vitro and in vivo selectivity for the D₂ receptor subtype. Sumanirole, (R)-5,6-dihydro-5-((methylamino)-4H-imidazo[4,5,1-j]quinolin-2(1H)-one; (Z)-2-butenedioate (1:1), is unique; it has greater than 200-fold selectivity for the D₂ receptor subtype versus the other dopamine receptor subtypes in radioligand binding assays. In cell-based assays, sumanirole is a fully efficacious agonist, with EC₅₀ values between 17 and 75 nM. In animals, sumanirole elicits many physiological responses attributed to D₂-like receptor function. In rats, sumanirole is a full agonist for elevation of striatal acetylcholine levels (ED₅₀ = 12.1 μmol/kg i.p.). Sumanirole s.c. dose dependently decreased plasma prolactin levels and depressed dopamine neuron firing rates in the substantia nigra pars compacta with an ED₅₀ of 2.3 μmol/kg i.v. This high selectivity for D₂ receptors translates into excellent locomotor stimulant activity in animal models of Parkinson’s disease. In reserpinized, α-methyl-para-tyrosine-treated rats, sumanirole caused a significant and sustained increase in horizontal activity at doses ≥12.5 μmol/kg s.c. In unilateral 6-hydroxydopamine-lesioned rats, sumanirole caused profound, sustained rotational behavior and was substantially more efficacious than any other agonist tested. Sumanirole-stimulated rotational behavior was blocked by the dopamine receptor antagonist haloperidol. Sumanirole dose dependently improved disability scores and locomotor activities of two of three 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned monkeys. In summary, sumanirole is the first published selective D₂ receptor agonist. The compound has activity in animal models of dopamine hypofunction and has a high level of efficacy in animal models of Parkinson’s disease.

Parkinson’s disease results from degeneration of the dopaminergic cells in the substantia nigra and is characterized by bradykinesia, tremors, and muscular rigidity. Several dopamine receptor agonists have been used as antiparkinsonian therapy, including bromocriptine, cabergoline, pergolide, pramipexole, and ropinirole (Hagan et al., 1997). Pramipexole, cabergoline, and ropinirole are effective as monotherapy in early stage Parkinson’s disease and their use significantly delays the initiation of L-dopa therapy (Inzelberg et al., 2003; Bracco et al., 2004). Dopamine agonists are also used as adjuuncts to L-dopa. The combined treatment enables lower doses of L-dopa, which decreases the incidence of response fluctu-
The data in this study demonstrate sumanirole, (5R)-5,6-dihydro-5-(methy lamino)-4H-imidazo[4,5,1-ij]quinolin-2(1H)-one (22Z)-2-butenedioate (1:1), is a novel dopamine agonist with high affinity and efficacy at D2 dopamine receptors and has a substantial degree of selectivity for the D2 receptor over other dopamine receptor subtypes. The in vitro and in vivo properties of sumanirole described here suggest that the compound may have utility in treating Parkinson’s disease.

Materials and Methods

The chemical structure of sumanirole is shown in Fig. 1, and the synthesis was described previously (Heier et al., 1997). The drug has been tested as either of two salt forms: the monohydrochloride salt with a molecular weight of 239.71 g/mol and the maleate salt with a molecular weight of 319.92 g/mol.

Cell Growth. Chinese hamster ovary (CHO) cells expressing human D2, D3, or D4 receptors or transfected with the 3C vector alone were grown in minimal essential medium (α modification (α-MEM)) supplemented with 10% fetal calf serum, 2 mM glutamine, 10 U/ml penicillin, and 100 μg/ml streptomycin. Media contained 1 mg/ml G418 for growth of CHO-3C (mock-transfected), D2-L6, D2-S8, and D3-3 cells. HEK293 cells expressing human D2 receptors were grown in Dulbecco’s modified Eagle’s medium high glucose supplemented with 10% fetal bovine serum, 4 mM glutamine, 100 U/ml penicillin G, 100 μg/ml streptomycin, and 100 U/ml hygromycin B. D2-L6 cells express 3.5 pmol/mg human D2α receptors measured in Dulbecco’s modified Eagle’s medium high glucose supplemented with 10% fetal bovine serum, 4 mM glutamine, 100 U/ml penicillin G, 100 μg/ml streptomycin, and 100 U/ml hygromycin B. D2-L6 cells express 3.5 pmol/mg human D2α receptors measured with [3H]spiroperidol, and 1.0 pmol/mg protein measured with [3H]HU-86170, an agonist ligand. D2-S8 cells express 3.0 pmol/mg human D2α receptors measured with [3H]spiroperidol. D3-3 cells express D2 receptors at 2.5 pmol/mg protein measured with [3H]spiroperidol (Chio et al., 1994b).

Receptor Binding Methods. Radioligands used were [3H][3H]spiroperidol (D2, dopamine, 70 Ci/mmol, 1 nM), [3H][3H]HU-86170 (Lahli et al., 1991) (D2, dopamine, 62 Ci/mmol, 2 nM), and [3H]spiperone (D2, and D4, dopamine, 96 Ci/mmol, 0.2 nM). Rat striatal membranes were the source of D1 receptors. CHO cells and HEK293 cells expressing D2, D3, and D4 receptors were rinsed with ice-cold Ca2+/Mg2+-free phosphate-buffered saline and harvested in the same buffer. Cells were pelleted (500g, 5 min), resuspended in 25 mM Tris, 5 mM EDTA, and 5 mM EGTA, pH 7.5, and frozen in liquid nitrogen. After thawing, the cells were homogenized and centrifuged at 1000g to remove nuclei and unbroken cells. The supernatant was centrifuged at 47,000g; the membrane pellet was washed once with Tris, EGTA, EDTA, resuspended in 20 mM HEPES, pH 7.4, 150 mM NaCl, 10 mM MgCl2, and 1 mM EDTA, and frozen in liquid nitrogen. Membrane aliquots were stored at −70°C. For the receptor binding assays, the membranes were thawed and diluted into 20 mM HEPES, pH 7.4, 150 mM NaCl, 10 mM MgCl2, or 1 mM EDTA, 10 mM MgSO4. Nonspecific binding was determined using 3 μM SCH23390 (D1 receptor antagonist) or 3 μM haloperidol (D2, D3, and D4 receptor antagonist). Binding reactions were carried out in 0.9-ml total volume for 1 h at room temperature. Reactions were stopped by vacuum filtration. Competition binding experiments employed 11 concentrations of test compound run in duplicate. IC50 values were determined by fitting the data to a one-site model by nonlinear least-squares minimization. Ki values were calculated with the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

CAMP Measurements. CAMP accumulation was measured in intact CHO cells plated at a density of 15,000 cells/well in a 24-well plate 48 h before the experiment. The cells were incubated in serum-free medium 1 h before the experiment. Fresh medium (0.5 ml) containing 100 μM forskolin, 100 μM isobutyl methylxanthine, and varying concentrations of drugs were added to each well and cAMP was allowed to accumulate for 15 min at room temperature. The reactions were terminated by the removal of the medium and the addition of 100 μl of cold 7.5% trichloroacetic acid. The samples were diluted by the addition of 1.0 ml of 50 mM sodium acetate, pH 6.2, and aliquots were assayed by radioimmunoassay using the Biomedical Technologies Incorporated (Stoughton, MA) cAMP radioimmunoassay kit.

Mitogenesis Assays. CHO cells were seeded into 96-well plates at a density of 5000 cells/well and were grown at 37°C in a α-MEM, with 10% fetal calf serum for 48 h. The wells were rinsed three times with serum-free α-MEM. Ninety microliters of fresh α-MEM was added along with 10 μl of drug (diluted in sterile water and filtered through 0.2-μm filters) or sterile water alone. Eight wells of every plate received 100 μl of α-MEM with 10% fetal calf serum. After culture for 16 to 17 h, [3H]thymidine (1 μCi/well) was added for 2 h. The cells were trypsinized and harvested onto filter mats with a Skatron cell harvester (Molecular Devices, Sunnyvale, CA). The filters were counted in a Betaplate counter (PerkinElmer Life and Analytical Sciences, Boston, MA).

Measurement of [3H]Arachidonic Acid Release. Cells were plated in 24-well plates at a density of 15,000 cells/well 48 h before use. Cells were labeled by incubation with [3H]arachidonic acid (210 Ci/mmol, 0.4 μCi/ml; Amersham Biosciences Inc., Piscataway, NJ) in α-MEM (1 ml) supplemented with 10 mM HEPES, pH 7.5, and 0.5% fatty acid-free bovine serum albumin for 2 h at 37°C. The cells were then washed twice with 1 ml of the same buffer. Drugs were added in 1 ml of the same buffer, and the cells were incubated at 37°C for 30 min. Samples (0.5 ml) from each well were counted by liquid scintillation spectroscopy.

In Vivo: General Methods, Rat. Unless otherwise stated, the following generalizations apply to all in vivo rat testing. Animals were male Sprague-Dawley rats from Harlan (Indianapolis, IN) and were maintained in a temperature-controlled environment at 22 ± 1°C, with a 12:12-h light/dark cycle (lights on from 6:00 AM to 6:00 PM). They were group-housed and received food (Rodent Laboratory Chow #5001; Purina Mills Inc., Richmond, IN) and water ad libitum. All rodent testing was in compliance with the Animal Welfare Act Regulations, 9CFR Parts 1, 2, and 3, and also with the Guide for the Care and Use of Laboratory Animals (National Academy Press, National Academy of Sciences, 1996).

Electrophysiology. Rats weighing between 280 and 330 g were anesthetized with chloral hydrate (400 mg/kg i.p.). The femoral artery and vein were catheterized for monitoring blood pressure and

![Fig. 1. Chemical structure of sumanirole.](image-url)
administration of drugs, respectively. Glass microelectrodes filled with pontamine sky blue in 2 M NaCl used for extracellular recordings were lowered through a small hole burred through the calvarium by means of a hydraulic microdrive. Stereotaxic coordinates for placement of recording electrodes were AP, –4.8 to –5.0; L, +2.0; and V, –6.8 to –7.5 mm, relative to bregma (Paxinos and Watson, 1988). Substantia nigra pars compacta (SNPC) neurons were identified by waveform and firing patterns (Bunney et al., 1973). Histological localization of iontophoresed pontamine sky blue dye spots verified electrode locations. Drug solutions were made in distilled water with equimolar citric acid added as needed. Drug effects were measured as changes in firing rates monitored by an integrated rate meter.

**Plasma Prolactin.** Male Long Evans rats weighing 200 to 225 g (Harlan) were used in this assay. Sumanriole was dissolved in saline and administered s.c. in a volume of 1 ml/kg at various times. Thirty minutes after drug or vehicle administration, animals were killed by decapitation, and trunk blood was collected and plasma was stored. Plasma prolactin was measured by a double-antibody radioimmunoassay using the reagents and procedures of the National Institute of Diabetes and Digestive and Kidney Diseases assay kit (kindly supplied by Drs. A. F. Parlow and S. Raiti, National Institute of Diabetes and Digestive and Kidney Diseases National Hormone and Pituitary Program, Torrance, CA). National Institute of Diabetes and Digestive and Kidney Diseases rat prolactin (RP-3) was used as the standard. Using a 100-μl aliquot of plasma, the lower limit of sensitivity for prolactin was 0.1 ng/ml. The intra-assay coefficient of variation is usually about 8.6%.

**Striatal Acetylcholine Concentration.** Rats (130–150 g; Charles River Laboratories, Inc., Wilmington, MA) were used in this study. Solutions of sumanriole and ropinirole were prepared in 0.25% carboxymethylcellulose. Drug treatments were given i.p., and control rats received an equal volume of vehicle (2 ml/kg). Thirty minutes after treatment, animals were killed by decapitation; the brain was quickly removed from the skull and placed in ice-cold 0.32 M sucrose. Bilateral striata were dissected and homogenized in 0.05 N perchloric acid containing ethylhomocholine as an internal standard. Acetylcholine concentration was determined by high-pressure liquid chromatography. Each point represents five to six animals, and statistical analysis was done by a one-way ANOVA, followed by Student’s t test.

**Locomotor Activity Measurements in Reserpinized Rats.** Rats pretreated with reserpine and the dopamine synthesis inhibitor α-methyl-para-tyrosine (AMPT) are akinetic and cataleptic. These effects can be reversed by L-dopa (Carlsson et al., 1957). Reserpine/AMPT-treated rats have been used as a model of dopamine depletion to mimic parkinsonian conditions. Rats weighing 200 to 250 g were treated with MPTP i.v. at different dosages until variable, but stable, parkinsonian features occurred. The monkeys were used to previously screen a number of dopamine agonists. Sumanriole was injected s.c. During the period of peak drug effect, behavioral responses were scored every 30 min using an MPTP monkey disability scale (Gomez-Mancilla et al., 1993), and locomotor activity was recorded every 15 min by photocells mounted in the cages. Results obtained with L-dopa/benserazide 50:12.5 mg/kg (Prolopa; Roche Diagnostics, Mississauga, ON, Canada) and 0.1 mg/kg apomorphine were used as reference standards.

**Results**

**Studies with Cloned Receptors.** Sumanriole is an agonist selective for the D2 subtype of dopamine receptors (Table 1). To measure high-affinity, guanine nucleotide-sensitive agonist interactions at D2 receptors, an agonist ligand, [3H]HU-86170, was used (Lahti et al., 1991). The affinity of sumanriole for D2 receptors is 9.0 ± 1.0 nM. This is similar to the affinity of D2 receptors for ropinirole and slightly higher than bromocriptine. However, sumanriole is less than 1% as potent as pergolide at the D2 receptor.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Sumanriole</th>
<th>Ropinirole</th>
<th>Bromocriptine</th>
<th>Pergolide</th>
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<tr>
<td>D2</td>
<td>9.0 ± 1</td>
<td>7.2 ± 0.8</td>
<td>27 ± 9</td>
<td>1 ± 0.2</td>
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<tr>
<td>D3</td>
<td>1940 ± 142</td>
<td>22 ± 4</td>
<td>18 ± 2</td>
<td>0.4 ± 0.03</td>
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<tr>
<td>D4</td>
<td>&gt;2190</td>
<td>1450 ± 390</td>
<td>373 ± 15</td>
<td>9.3 ± 1</td>
</tr>
<tr>
<td>D1</td>
<td>&gt;7140</td>
<td>&gt;7140</td>
<td>3420 ± 130</td>
<td>1300 ± 132</td>
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Rats (225–280 g) were pretreated with desmethylamphetamine 25 mg/kg i.p. 1 h before surgery. They were anesthetized with Chloropent (Sigma Chemical Co., St. Louis, MO) given at 3 ml/kg i.p. and placed in a stereotaxic apparatus with the incisor bar raised to 4 mm (smaller rats) or 5 mm (larger rats). A small hole was drilled through the skull and a 30-gauge stainless tubing was lowered to the right substantia nigra using the following coordinates: for smaller rats, AP, –1.5 mm; L, +1.8 mm; and V, –8.0 mm; for larger rats, AP, –2.8 mm; L, +2.0 mm; and V, –8.0 mm (Pellegrino et al., 1979). 6-OHDA, hydrogen bromide solution was injected into the substantia nigra at 12 μg/μl free base) in 0.9% saline/0.1% acetic acid at 1 μl/min, using a Hamilton syringe pump (Hamilton Co., Reno, NV). After surgery, the scalp incision was closed with stainless steel wound clips, and Mycitracin Plus (Johnson & Johnson, Skillman, NJ) ointment containing lidocaine and antibiotics (bacitracin, neomycin, and polymyxin B) was applied to the incision area. Rats were replaced in group housing, and clips were removed after 1 week.

Two weeks after surgery, the effects of the lesions were tested by monitoring the turning rate of rats given 0.5 mg/kg s.c. apomorphine HCl in 0.9% saline/0.1% acetic acid. Total turns were recorded at 10-min intervals in automated monitors (Rotoscan; Omnitech Inc.). Each rat was connected by a lightweight harness and tether to a rotometer at the top of a clear plastic cylindrical cage (11 in. in diameter). The cage floor paper was changed for each animal. Rats were used for sumanriole experiments if they had at least 30 turns/10 min (range 30–160 turns/10 min) in this screen. Whenever possible, animals were balanced across treatment groups to accord each treatment a similar mix of animals from within this range. Groups were compared statistically by an F-test for variance of means, followed by a one-way analysis of variance and Student’s t test for individual data points and area under the curve. Analysis was done using RS/1 software (Brooks Automation Inc., Chelmsford, MA), customized in-house.

**MPTP-Lesioned Monkeys.** Three female cynomolgus monkeys (Macaca fascicularis), weighing approximately 3 kg each, were treated with MPTP i.v. at different dosages until variable, but stable, parkinsonian features occurred. The monkeys were used to previously screen a number of dopamine agonists. Sumanriole was injected s.c. During the period of peak drug effect, behavioral responses were scored every 30 min using an MPTP monkey disability scale (Gomez-Mancilla et al., 1993), and locomotor activity was recorded every 15 min by photocells mounted in the cages. Results obtained with L-dopa/benserazide 50:12.5 mg/kg (Prolopa; Roche Diagnostics, Mississauga, ON, Canada) and 0.1 mg/kg apomorphine were used as reference standards.
and lower than the receptor affinities for bromocriptine and pergolide, respectively, all agents used clinically for the treatment of Parkinson's disease (Eden et al., 1991; De Keyser et al., 1995; Rascol et al., 1996). The affinity of D3, D4, and D1 receptors for sumanirole was calculated to be at least 200-fold lower than at D2 receptors. In contrast, ropinirole, bromocriptine, and pergolide all showed high affinity for D3 receptors; pergolide also showed high affinity for the D4 receptor subtype.

We next examined whether sumanirole is an agonist or antagonist at the D2 receptor using three measures: cAMP, arachidonic acid release, and mitosis. Forskolin-stimulated cAMP accumulation is inhibited by sumanirole and ropinirole in CHO cells (Fig. 2). Both compounds caused near maximal inhibition of forskolin-stimulated cAMP at the highest concentrations. Sumanirole had no effect on forskolin-stimulated cAMP accumulation at concentrations up to 1 μM, suggesting that the response in L6 cells occurs via the transfected D2 receptor. Table 2 compares the potencies of sumanirole, ropinirole, and dopamine in two assays for activation of D2 receptors in CHO-L6 cells: cAMP inhibition and potentiation of ATP-stimulated arachidonic acid release (Lajiness et al., 1994). Sumanirole is approximately 3 times less potent than ropinirole at each measurement (Table 2). Both compounds were efficacious as dopamine at maximal concentrations, indicative of high levels of intrinsic activity. Sumanirole activated D2 receptor-stimulated mitogenesis with an EC50 of 4.6 nM, but it had no activity at D3 and D1 receptors in measurements of receptor-activated mitogenesis at concentrations up to 1 μM; this was consistent with the very low affinity of these receptors for this compound. In comparison, ropinirole potentiates activated D2 and D3 receptors in the mitogenesis assay [EC50 values of 12 ± 1.0 nM (n = 5) and 7.5 ± 2.9 nM (n = 5), respectively].

Studies in Animals. Dopamine agonists that activate the D2-like subfamily of dopamine receptors inhibit the release of prolactin from anterior pituitary cells (Ho and Thorner, 1988). The effects of four doses of sumanirole on plasma prolactin levels in male Sprague-Dawley were tested. Sumanirole decreased plasma prolactin at all doses studied with significant decreases at all doses (Table 3). The effects at 31 μmol/kg lasted at least 120 min postinjection.

Dopamine agonists depress dopamine neuron firing rates in the SNPC by activation of presynaptic dopamine receptors of the D2-like subfamily. As demonstrated by population dose-response curves, sumanirole resulted in inhibition of SNPC dopamine neuronal firing with an ED50 ± S.E.M. of 2.3 ± 0.9 μmol/kg i.v. (n = 6) (Fig. 3). Complete inhibition of basal firing rate was seen at 16.8 μmol/kg i.v. The inhibition of cell firing was reversed by haloperidol.

Activation of striatal postsynaptic D2-like receptors decreases acetylcholine release and raises striatal acetylcholine concentrations (Sethy, 1979). Administration of both ropinirole and sumanirole to rats caused a dose-dependent increase in striatal acetylcholine levels (Fig. 4). The maximal effects of both compounds were similar, with a near doubling of striatal acetylcholine concentration. The ED50 values for the effects (calculated as the fitted half-maximal response ± S.E.M. of the fit using the dose-response fit equation of Sigma Plot (SPSS Inc., Chicago, IL) were 6.0 ± 8.2 μmol/kg i.p. for ropinirole and 12.1 ± 4.1 μmol/kg i.p. for sumanirole.

Effects in Animal Models of Parkinson's Disease. Sumanirole increased locomotor activity in reserpine/AMPT-pretreated rats with pronounced effects at 12.5 μmol/kg (Fig. 5). The onset of this effect was approximately 30 min postinjection, and the duration of the 12.5 μmol/kg dose was at least 2.5 h. Even greater locomotor activation was observed in reserpinized/AMPT-treated rats at 42 μmol/kg s.c., and the activity was completely blocked by 0.3 mg/kg haloperidol.

Table 2. In vitro D2 potency of sumanirole and other agonists

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<tr>
<th>Assay</th>
<th>Potency Measurements at D2 Receptors</th>
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<tr>
<td></td>
<td>Sumanirole</td>
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<tr>
<td>cAMP Inhibition</td>
<td>17 ± 4.8 (4)</td>
</tr>
<tr>
<td>(3H)Arachidonate release</td>
<td>75 ± 16 (6)</td>
</tr>
<tr>
<td>Mitogenesis</td>
<td>32 ± 6.4 (3)</td>
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*With permission from Lajiness et al. (1994).
operidol. These data suggest that sumanirole induces strong behavioral activation through a D₂ dopamine receptor.

Sumanirole was also highly efficacious at causing contralateral turning in unilateral 6-OHDA-lesioned rats after either oral or s.c. administration. A dose-response and time-course study of sumanirole given orally at 4.2, 12.5, or 42 μmol/kg showed turning activity in substantia nigra-lesioned rats, with significant effects seen at the two higher doses (Fig. 6). Maximal turning behavior was reached at 2 to 3 h after oral administration. Additionally, in this assay, sumanirole had a long duration of action as 12.5 and 42 μmol/kg still showed near-maximal efficacy at 4.5 h postinjection.

Ropinirole also caused dose-dependent increases in contralateral turning in the 6-OHDA-lesioned rats (Fig. 7). Significant turning behavior was measured at 19 μmol/kg s.c. A maximally effective dose of ropinirole was not as efficacious as sumanirole, as measured by the degree of turning. In the same set of rats, sumanirole (12.5 μmol/kg s.c.) induced approximately 3 times as many turns per 10-min interval than 19 μmol/kg s.c. ropinirole. Note that the effects of 12.5 μmol/kg sumanirole s.c. (Fig. 6) are equivalent to the effects produced by 42 mol/kg sumanirole administered orally (Fig. 7). Thus, the oral potency is approximately one-third the potency of the parenteral route. The peak response of sumanirole was also greater than the maximally effective doses of the following drugs given s.c.: 0.37 μmol/kg apomorphine, 4.6 μmol/kg bromocriptine, 9.5 μmol/kg pergolide, 13 μmol/kg quinpirole, and 11.7 μmol/kg SKF 38393. The turning activity induced by sumanirole is completely blocked by the coadministration of 0.27 or 2.7 μmol/kg haloperidol (Fig. 8), indicating that turning is mediated by a D₂-like dopamine receptor.

Drug-experienced cynomolgus monkeys rendered parkin-
sonian with MPTP-induced lesions of the substantia nigra were treated with different dosages of sumanirole and tested for improvement in scores using a MPTP monkey disability scale (Fig. 9). Two monkeys (A and B) had dose-dependent reductions in the disability scores after sumanirole, especially at the 12.5 μmol/kg dose. Monkey A had a dramatic recovery at 2.5 μmol/kg s.c. lasting for 5 to 6 h after a delay of 0.5 to 1 h. This monkey also had a dyskinesia accompanying the recovery. Monkey B required 12.5 μmol/kg sumanirole to show a dramatic response, and the response also lasted for 5 to 6 h after a 0.5 to 1 h delay. No dyskinesias were observed in monkey B to either sumanirole or other dopamine agonists. Monkey C did not show dramatic improvement even with the 12.5 μmol/kg dose; however, this monkey was unresponsive to apomorphine as well. In locomotion measurements, sumanirole at 12.5 μmol/kg s.c. increased the activity of monkeys A and B more than Prolopa. Monkey C did not increase locomotion with sumanirole or with apomorphine, although this monkey did respond to Prolopa (Fig. 10).

Discussion

The striking difference between sumanirole and the other examined dopamine agonists (ropinirole, bromocriptine, and pergolide) lies in sumanirole's lack of affinity for D₃ and D₄ receptors, whereas the other dopamine agonists all showed high affinity for D₃ and/or D₄ receptors. Sumanirole is an imidazoquinolinone and the first compound described as a highly selective agonist for D₂ receptors over the other D₂-like subtypes, D₃ and D₄. The selectivity of this compound was demonstrated in radioligand binding assays, where it has at least 200-fold higher affinity for D₂ receptors than for the other dopamine receptor subtypes. The other dopamine agonists examined did not demonstrate D₂ receptor-selectivity. Sumanirole has been shown to be inactive in more than 80 enzyme and receptor assays. The compound does have moderate affinity at the 5-HT₁A receptor (Kᵢ = 95 nM; unpublished observations).

D₂ receptors are abundant in motor areas such as the basal ganglia; therefore, their activation may be associated with therapeutic efficacy of dopamine agonists in Parkinson's disease. In contrast, D₃ and D₄ receptors are located in limbic and cortical areas and may contribute to the psychiatric disturbances that accompany dopamine agonist and L-dopa therapeutics. In support of this hypothesis, clozapine, a dopamine receptor blocker with higher affinity for the D₂ subtype, has been used to ameliorate the psychoses accompany-
ing antiparkinsonian treatment (Friedman, 1995). D₃ receptor agonists have also been shown to decrease locomotor activity (Lagos et al., 1998). This suggests that sumanirrole’s unique selectivity may confer beneficial effects in the treatment of Parkinson’s disease. Eliminating D₃- and D₄-activating properties of dopamine receptor agonists may potentially reduce nonmotor side effects and improve tolerability. Whether the unique selectivity of sumanirrole translates into a clinical advantage over other dopamine agonists remains to be determined.

The unique selectivity of sumanirrole allows the compound to be used to characterize D₂-specific activity in the central nervous system that had previously been only inferred by using nonselective D₂-like receptor agonists. Sumanirrole inhibits prolactin release. D₂ receptor mRNA but not D₃ receptor mRNA is found in the pituitary gland (Bunzow et al., 1988; Sokoloff et al., 1990), and D₂ receptors inhibit prolactin release from lactotrophs (Missale et al., 1991). Thus, it is not surprising that the D₂-selective agonist sumanirrole can mediate the prolactin inhibition response, but these results provide evidence that sumanirrole has anticipated in vivo D₂ activity. D₂-like receptors inhibit the firing rates of SNPC dopamine neurons (Pinnock, 1984; Piercey et al., 1996a,b).

Both mRNA for D₂ and D₃ receptors have been localized to these dopaminergic neurons (Meador-Woodruff et al., 1989; Bouthenet et al., 1991). Sumanirrole inhibited the firing rate of these cells in a similar dose range as the doses shown to inhibit prolactin release. These findings support a role for the molecularly defined D₂ receptors as autoreceptors that regulate the firing rates of SNPC neurons. Furthermore, activation of D₂-like receptors decreases acetylcholine release (Consolo et al., 1987). Changes in acetylcholine content in the striatum were used as an in vivo measure of D₂-like receptor activation. By this measurement, sumanirrole is equally efficacious in vivo as other compounds used as antiparkinsonian agents. In three animal models of Parkinson’s disease, sumanirrole was shown to have good locomotor stimulant properties, including reserpine/AMPT-treated rats, 6-OHDA-lesioned rats, and MPTP monkeys. Reserpine treatment of rats to deplete catecholamine stores results in a hypokinetic animal that can be activated by l-dopa and provides an animal model for Parkinson’s disease (Carlsson et al., 1957). Reserpine-induced akinesia was dose dependently reversed by sumanirrole and the activation effect was blocked by haloperidol. The mechanism for this locomotor activation is likely to be direct stimulation of postsynaptic D₂ dopamine receptors.
Sumanirole was highly efficacious at causing contralateral turning in unilateral 6-OHDA-lesioned rats after either oral or s.c. administration, demonstrating superior efficacy over other dopamine agonists tested in this model. Ropinirole was slightly more potent but equally efficacious as sumanirole in assays of D₂ receptor activation in cell-based assays, and at D₂-like receptor activation in other in vivo responses. However, even maximally effective doses of ropinirole did not produce the same degree of response as sumanirole on rotational behavior. Furthermore, sumanirole was more efficacious than other agonists (bromocriptine, pergolide, and apomorphine) tested at maximally effective doses in this model. The enhanced efficacy of sumanirole in the 6-OHDA turning model could be a result of other undiscovered activities of this compound; however, turning was completely blocked by the dopamine receptor antagonist haloperidol. This suggests the enhanced efficacy in this model resulted from sumanirole’s unique D₂-receptor selectivity.

MPTP-lesioned monkeys represent another animal model for Parkinson’s disease. The toxin MPTP causes selective destruction of dopaminergic neurons resulting in behavioral deficits similar to those observed in diseased humans (Burns et al., 1983). L-Dopa and dopamine agonists reverse the behavioral deficits in MPTP-treated monkeys. Chronic L-dopa can also cause dyskinesias in monkeys as it does in patients with Parkinson’s disease (Bedard et al., 1986). Sumanirole has only been tested to a limited extent in the MPTP-lesioned monkey model and only in monkeys tested extensively with other dopaminergic agents. Sumanirole caused improvement in Parkinson’s disability scores in two of the three monkeys. Sumanirole also increased the locomotion of the two responding monkeys to a greater extent than Prolopa. This is consistent with the high efficacy effects of sumanirole in the rat 6-OHDA turning model. The studies in the MPTP-lesioned monkey suggest that sumanirole is efficacious in this model but are too limited to draw solid conclusions about the relative efficacy or the dyskinetic potential of this compound. The current issue of *Journal of Pharmacology and Experimental Therapeutics* reports results of a subsequent study in MPTP-treated monkeys in which sumanirole shows antiparkinsonian effects comparable with existing dopaminergic therapies without inducing dyskinesias using both behavioral and pathological assessments (Stephenson et al., 2005).
Conclusions

Sumanire represents the first dopamine agonist with high selectivity for the D2 receptor subtype over the other closely related D3 and D4 receptor subtypes. In vitro studies demonstrate that sumanire is a selective D2 receptor agonist and can therefore be used to evaluate the importance of the D2 receptor in the physiological effects of dopamine agonists. The D2-selective nature of sumanire is assumed to allow for the assessment of D2 agonist effects on locomotor activity in the absence of D3 and D4 receptor coactivation. Studies presented here suggest that, in animals, many of the physiological responses thought to be mediated via the D2 receptor subtype, namely, prolactin release, regulation of striatal acetylcholine content, and autoreceptor-mediated inhibition of nigrostriatal firing rates, are indeed mediated by the molecularly defined D2 receptor subtype, since sumanire was fully efficacious at in vivo concentrations expected to selectively activate D2 receptors. Sumanire thus represents a potential tool for studying D2 receptor subtype specificity both in vitro and in vivo.

Acknowledgments

We appreciate the following people for the contribution to the research which is the focus of this manuscript: Nanette F. Nichols, Peggy J. K. D. Schreur, Kjell A. Svensson, Philip F. VonVoigtlander, Martin W. Smith, and Vimala H. Sethy.

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