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Preclinical Pharmacology of Ropinirole (SK&F 101468-A) a Novel Dopamine D_2 Agonist

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EDEN, R. J., B. COSTALL, A. M. DOMENEY, P. A. GERRARD, C. A. HARVEY, M. E. KELLY, R. J. NAYLOR, D. A. A. OWEN AND A. WRIGHT. *Preclinical pharmacology of ropinirole (SK&F 101468-A) a novel dopamine D_2 agonist.* PHARMACOL BIOCHEM BEHAV **38**(1) 147–154, 1991.—These studies characterise the pharmacology of ropinirole, a selective D-2 agonist. High-affinity human caudate binding revealed a K_i for D_2 receptors of 2.9×10^{-8} M with no affinity for D_1 at 10^{-4} M in the rat. Ropinirole was weakly active at α_2-adrenoceptors and 5-HT_2 receptors but inactive at 5-HT_1, benzodiazepine and gamma-aminobutyric acid receptors or α_1 and β-adrenoceptors. In rodents, ropinirole, like apomorphine, caused biphasic spontaneous locomotor activity and contralateral circling in 6-OHDA-lesioned mice with no tolerance to the latter after 14 days treatment. Amphetamine caused ipsilateral responses in the lesioned mice. Ropinirole did not cause marked stereotypies. In marmosets ropinirole (0.05–1.0 mg/kg SC or 0.1 mg/kg PO) reversed all motor and behavioural deficits induced by MPTP. This response started 10–20 minutes after dosing, and exceeded 2 hours. No tolerance was seen following chronic b.i.d. treatment. Similar results were obtained with l-dopa plus benserazide; however, l-dopa always caused emesis, whereas beneficial effects were shown with ropinirole in the absence of this side effect. These results support the continued clinical assessment of ropinirole for the treatment of Parkinson's disease.

Dopamine D_2 agonist Central nervous system Rodents MPTP Marmosets Parkinson's disease

WHILST the treatment of Parkinson's disease was revolutionised by the advent of l-dopa therapy in the late 1960s, the management of the disease is still far from perfect. Patients on long-term l-dopa treatment experience fluctuations in performance (11,20), the most disabling of these being the "on-off" phenomena. This complicated effect is manifest by the patient experiencing severe dyskinesias and dystonias during the "on" period and the reappearance of Parkinsonism akinesia during the off period. It has been suggested that these therapeutic response fluctuations may parallel l-dopa plasma concentrations (22). Alternatively, it has been proposed that such fluctuations may result from a decreased capacity to store newly synthesised dopamine within the brain as a consequence of the progressive degeneration of dopaminergic nerve terminals (20).

Clearly, there is a need for dopamine agonist therapy for Parkinsonism which is devoid of the problems associated with l-dopa. These problems have been partially overcome by giving either intravenous or subcutaneous infusions of dopamine agonists (13, 23, 26). Whilst the chronic infusion of dopamine agonists appears to be clinically effective, this type of therapy requires the patient to be hospitalised. For practical reasons, a directly acting dopamine agonist which can be administered in a less invasive manner and in doses which reduce the incidence of unpleasant side effects such as nausea, whilst still providing good control of Parkinsonian symptoms, would be highly advantageous. It was considered that ropinirole, SK&F 101468-A (4-[2-(dipropylamino) ethyl]-1,3-dihydro-2H-indol-2-one HCl), a novel, nonergoline, selective dopamine D_2 receptor agonist (10,12) may fulfill these requirements. Studies were, therefore, designed to evaluate the pharmacology of ropinirole in both in vitro and in vivo systems, including the effect of the compound on Parkinsonian-like deficits induced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in a nonhuman primate.

METHOD

Specific binding of ropinirole to receptors from a variety of tissues was measured using standard radioligand binding techniques. Six point displacement curves were used to determine K_i values or EC_{50}s at each of the receptors studied.

Human Platelets Membranes

Platelet concentrates (buffy coats, 24–48 h old, from South

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West Thames Blood Transfusion Service) were centrifuged at 190 × g for 10 min. The supernatant (platelet rich plasma) was centrifuged at 16,000 × g for 12 min, the resulting supernatant discarded and the pellet homogenised (10 strokes at 800 rpm) in washing buffer (50 mM Tris, 20 mM EDTA, 110 mM NaCl pH 6.4) and centrifuged at 16,000 × g for 12 min. The supernatant was discarded and the pellet rehomogenised in washing buffer and centrifuged at 16,000 × g for 12 min. The supernatant was discarded and the pellet homogenised (at 1500 rpm) in hypotonic lysing buffer (5 mM Tris, 5 mM EDTA, pH 7.5), allowed to stand on ice for 20 min, and then centrifuged at 39,000 × g for 15 min. The resulting pellet was resuspended in the appropriate assay buffer and stored in aliquots at −80°C.

On the day of use, aliquots of platelets were allowed to thaw at room temperature, were homogenised in incubation buffer (6 strokes at 1200 rpm) and centrifuged at 39,000 × g for 10 min; the resulting pellet was resuspended in incubation buffer.

Platelet α_2-Adrenoceptors

Platelet membranes were incubated with 1.7 nM ^{3}H-yohimbine (spec.act. 70 Ci/mmol) in 50 mM Tris HCl, pH 7.1, in the presence or absence of test drugs, for 45 min at 25°C (assay volume 250 μl). Specific binding was defined as radioactivity displaceable with 10 μM phentolamine. Samples were filtered through Whatman GF/C filters with a Brandel cell harvester and washed with 16 ml ice-cold incubation buffer.

Platelet BZ Receptors

Platelet membranes were incubated with 1 nM ^{3}H-PK 11195 (spec.act. 90 Ci/mmol) in 50 mM Tris-HCl pH 7.4, in the presence or absence of test drugs for 45 min at 25°C (assay volume 500 μl). Specific binding was defined as radioactivity displaced by 100 μM RO 5-4864. Samples were filtered through Whatman GF/C filters with a Brandel cell harvester and washed with 16 ml ice-cold incubation buffer.

β-Adrenoceptors (Human Cortex)

Temporal cortex (Brodmann area 38) was obtained from 3 male subjects (48–54 years) who had died following acute myocardial infarction. Membranes were prepared from tissue, mean 34 h maximum 72 h postmortem, and binding assays performed (0.1 nM ^{3}H-CGP 12177) as described by De Paermentier (6).

Central BZ Binding (Bovine Cortex)

Bovine cortex was homogenised in 50 mM Tris-citrate pH 7.1 and centrifuged at 39,000 × g for 10 min. The supernatant was discarded and the procedure repeated 2 further times.

Washed membranes were stored at −20°C until used. On the day of assay, membranes were allowed to thaw and were washed 5 times with 50 mM Tris-citrate pH 7.1. Aliquots of membranes were incubated with 0.5 nM ^{3}H-flunitrazepam (83 Ci/mmol) in 50 mM Tris-citrate pH 7.1 in the presence or absence of test drugs for 90 min at 4°C (assay volume 1 ml). Specific binding was defined with 2 μM clonazepam. Samples were filtered through GF/B filters with a Brandel cell harvester and washed with 16 ml ice-cold buffer.

5-HT_2 Receptors (Rat Frontal Cortex)

Membranes were prepared from rats that had been killed by cervical dislocation and ^{3}H-ketanserin binding performed as described by Cross and Horton (5); the ^{3}H-ketanserin concentration used was 0.2 nM.

DA_2 Receptors

Caudate nucleus (head and body) was obtained from 6 subjects (4 males, 2 females aged 39–59 years) who had died from acute myocardial infarcts. Tissue was homogenised in incubation buffer (50 mM Tris-HCl, 10 mM KCl, 2 mM $MgCl_2$ and 4 mM $CaCl_2$, pH 7.6) and centrifuged at 39,000 × g for 10 min. Supernatant was discarded and the pellet rehomogenised and centrifuged as above and resuspended in fresh incubation medium.

Aliquots of membrane were incubated with 0.1 nM ^{3}H-spiperone (spec.act. 67.6 Ci/mmol) in incubation buffer, in the presence and absence of drugs for 60 min at 25°C (assay volume 2 ml). Specific binding was defined with 1 μM (+)butaclamol. Samples were filtered through Whatman GF/C filters using a Brandel cell harvester and washed with 16 ml ice-cold incubation buffer.

GABA

GABA binding was carried out using bovine cerebellum which had been stored frozen. Aliquots of thawed tissue were homogenised using a Polytron in 30 volumes (w/v) ice-cold 50 mM Tris-HCl (pH 7.4, 25°C) for 10 s, then centrifuged (20,000 rpm, 10 min at 4°C). The tissue pellets were resuspended in 30 volumes (w/v) of above solution containing 0.05% Triton X-100 and incubated for 10 min on ice and a further 10 min at room temperature. Pellets were washed 4 times by centrifugation and resuspension in 50 mM Tris-HCl (pH 7.4, 25°C). For use the tissue was resuspended to a concentration of 20 mg/ml (original wet weight) in the Tris-HCl. Each tube received 8 mg tissue (400 μl), 50 μl ^{3}H-GABA (DuPont) and 50 μl drug or vehicle: binding reaction was initiated by addition of the tissue and terminated by rapid vacuum filtration onto presoaked Whatman GF/B filters. The final radioligand concentration was 5 nM and the incubation period 15 min at 0°C. Radioligand was assessed using liquid scintillation spectrophotometry after soaking the filters for 3 h in the scintillation fluid. The reference compound and positive control used was muscimol 5×10^{-11} to 1×10^{-6} M, the K_d for the receptor for ^{3}H-GABA was 370 nM.

5-HT_1- and DA_1-Receptors

The techniques for assessing binding to 5-HT_1, using ^{3}H-5-HT (2×10^{-9}), and dopamine-D_1 receptors, using ^{3}H-SCH23390 (5×10^{-10}), in rat forebrain or striatum were those described by Bennett and Snyder (1) and Billard et al. (2), respectively.

Caudate Adenylate Cyclase Activity

Caudate nuclei were quickly dissected on ice from rat, immediately following decapitation, and homogenised with cold, 50 mM Tris-maleate buffer, pH 7.4, containing 2 mM EGTA. Aliquots (50 μl) of the homogenate were transfered to a solution (250 μl) containing 80 mM Tris-maleate buffer, pH 7.4, containing 0.2 mM EGTA, 2 mM $MgSO_4$, 0.05 mM sodium metabisulphite and 10 mM theophylline and various drugs as required. The incubation tubes were kept at 0°C, while 20 μl ^{14}C-ATP (10 mM) was added making a final concentration of 0.6 mM ATP. Each tube was incubated for 3 min at 30°C and the reaction halted by heating at 100°C for 3 min. Each tube was diluted with 600 μl H_2O and the ^{14}C-cAMP formed isolated by Dowex and alumina chromatography and quantified by liquid scintillation spectrome-

try. In these experiments 50 μM dopamine was used as the internal standard. Antagonist activity was assessed as % inhibition of response to 50 μM dopamine on caudate adenylate cyclase.

In Vivo Studies: Rodent

Irwin primary screen. This dose-ranging test was used to gain an overall assessment of the compound. Three groups of three mice (male, C. River CD derived, 27–31 g) received ropinirole in doses of 1.0, 10.0 and 100 mg/kg PO. A number of behavioural parameters were observed to assess central stimulation, depression, strength, coordination, vision and general demeanour. The animals were observed continuously for thirty minutes and thereafter at one and two hours after dosing. Each parameter was scored on a 0–8 scale, 'normal' being a score of '0' if that particular parameter is absent in the undosed animal, or '4' if it is normally present. Body temperature was measured at each observation period using a rectal thermometer.

Antinociceptive effects. The antinociceptive potential of ropinirole was assessed using a technique based on that of Woolfe and MacDonald (30). Groups of ten mice, obtained by random allocation, were dosed orally (1–100 mg/kg) with the compound or vehicle thirty minutes prior to testing. The mice were placed on a stainless steel surface maintained at 57°C. The time from placement of the animal to observation of the characteristic nociceptive response was measured for each mouse. The end-point response was either lifting or licking of forepaws or 'rattling' of hindpaws on the plate. No animal was allowed to stay on the plate for longer than 30 seconds.

Spontaneous locomotor activity (SLA). The effect of ropinirole, apomorphine, amphetamine or vehicle on spontaneous locomotor activity was measured in mice (male, BKW strain, 25–35 g) using Perspex cages fitted with two photocells located 2.5 cm above the floor. Locomotor activity was recorded for 30 minutes after dosing and expressed as a cumulative count. Spontaneous locomotor responses to ropinirole were also measured in rats (male Sprague-Dawley CD, 250–325 g) using individual cages fitted with one photocell set off centre. Interruptions of the light beam were recorded at 5-minute intervals for 60 minutes to yield an activity count per 60 minutes.

Stereotypic behaviour. The study used adult male Sprague-Dawley rats (Bradford bred, 250–320 g) and male BKW mice (Bradford bred, 30–35 g). The ability to induce stereotyped behaviour in both rats and mice was assessed by observation according to the following scoring system: 0—no stereotypy, behaviour indistinguishable from vehicle-treated animals; 1—periodic sniffing, with head or limb movements; 2—continuous sniffing, with head or limb movements; 3—discontinuous biting, gnawing, licking; 4—continuous biting, gnawing, licking.

Immediately after treatment with ropinirole, apomorphine, amphetamine or the appropriate vehicle, the animals were placed individually in Perspex cages and the degree of stereotypy assessed at 5-minute intervals for 30 minutes. Effects were followed for up to 2.5 hours.

Antagonism of dyskinetic activity. The study used male Sprague-Dawley rats (Bradford bred, 250–320 g). Dyskinesias were induced by the administration of the mixed dopamine agonist 2-di-n-propylamino-5,6-dihydroxytetralin (tetralin, 0.025 mg/kg SC). Rats were pretreated with either tiapride (30 min), ropinirole (10 min) or the appropriate vehicle. After administration of tetralin the rats were placed in individual Perspex cages and the peri-oral dyskinesias assessed at 30-minute intervals for the duration of the effect (2 to 2.5 hours). The biting or peri-oral movements were scored on a 3-point rating scale on each test occasion: 1—infrequent, 2—frequent or 3—continuous.

Intrastriatal administration. Stimulation of the caudate-putamen (striatum) was investigated in rats (male Sprague-Dawley CD, 300–350 g). Under chloral hydrate-induced general anaesthesia, the animals were subjected to standard stereotaxic techniques to implant chronic bilateral cannulae which allowed injection of drug or vehicle into the centre of the caudate-putamen [ant. 7.8, vert. 1.0, lat. ±3.0, atlas of De Groot (7)]. Postoperative care included treatment with antibiotics and a 14–21-day recovery period. Following recovery, ropinirole, apomorphine or amphetamine was injected unilaterally into one striatum and vehicle into the other (random distribution) in a volume of 1 μl over 60 s. Asymmetric behaviour was assessed at 15-minute intervals using the following scoring system: 0—no asymmetry, behaviour indistinguishable from untreated animals; 1—a distinct tendency for animals to move in one direction when handled but still capable of movement in either direction; 2—spontaneous movements in one direction, a twisting of the body in this direction; 3—a marked and intense twisting of the body, active circling movements when disturbed, the animal being unable to move in the opposite direction.

Circling behaviour was measured in revolutions per minute (unidirectionally) in an open area 40 cm in diameter.

6-Hydroxydopamine (6-OHDA)-lesioned mice. Postsynaptic dopamine agonist activity was investigated in mice (male, BKW strain, 30–35 g) 7–25 days after unilateral administration of 6-OHDA into the substantia nigra. The injection unit was positioned, under a general anaesthetic, using standard stereotaxic techniques. The coordinates used were ant. 1.1, lat. −1.6, relative to the zero on a Kopf stereotaxic frame and vert. −5.4 relative to the skull surface. A Hamilton syringe was used to deliver 8 μg/0.5 μl over 60 seconds. Ropinirole was administered acutely IP over the dose range 0.001–100 mg/kg, or for 14 days at 1.0 mg/kg IP, b.i.d.; apomorphine (0.5 mg/kg) and amphetamine (1.25 mg/kg) were used for comparison. The following simplified scoring system was used to assess asymmetry: 0—no asymmetry; 1—weak asymmetry, primarily in one direction, but with ability to move in the other direction; 2—marked asymmetry, animals remained with the body bent in one direction and were unable to turn in the opposite direction; 3—intense body asymmetry pivoting in circles nose to tail.

In further studies circling behaviour (revolutions/minute) was also used as a measure of agonist activity.

In Vivo Studies: Primate

Marmosets (*Callithrix jacchus*) of either sex were subjected to a unilateral, 13-day infusion of MPTP into the zona compacta of the substantia nigra using Alzet osmotic minipumps implanted subcutaneously in the scapula region. The pumps were primed to deliver 20 μg MPTP/24 hours in a volume of 0.48 μl/hour, via a previously implanted cannula. The cannula was implanted 10–14 days prior to the infusion under Saffan® general anaesthesia, using standard stereotaxic techniques. The coordinates for infusion into the zona compacta were: ant. 5.0, lat. ±2.3, vert. 13.7 mm below the dura [with respect to zero of the Kopf stereotaxic frame using the atlas of Stephan et al. (25)]. Motor deficits were evident within 3–4 days and persisted for several weeks postinfusion. Assessment of drug effects commenced 7–10 days after the start of the MPTP infusion when stable baseline values had been attained. Behavioural parameters measured included assessment of spontaneous locomotor activity. This was assessed in the home cage for two 2-minute test periods using an electronic key pad connected to a BBC microcomputer. During the first period, the amount of time the animal spent in locomotor activity was recorded. The second 2-minute period was used to assess the amount

TABLE 1

AFFINITY OF ROPINIROLE IN VARIOUS RECEPTOR BINDING ASSAYS

Receptor Site	Tissue	Ligand	Binding (K_i)
Dopamine-D_2	Human caudate*	^{3}H-Spiperone	2.9×10^{-8} (n = 6)
Dopamine-D_1	Rat striatum	^{3}H-SCH 23390	No affinity at 10^{-4} M
α_2-Adrenoceptors	Human platelets*	^{3}H-Yohimbine	$IC_{50} = 9 \times 10^{-6}$ M
β-Adrenoceptors	Human temporal cortex*	^{3}H-CGP 12177	No affinity at 10^{-4} M
5-HT_1 receptors	Rat forebrain	^{3}H-5-HT	No affinity at 10^{-4} M
5-HT_2 receptors	Rat forebrain*	^{3}H-Ketanserin	$IC_{50} = 5 \times 10^{-5}$ M
Central BZ	Bovine cortex*	^{3}H-Flunitrazepam	No affinity at 10^{-5} M
Peripheral BZ	Human platelets*	^{3}H-PK 11195	No affinity at 10^{-4} M
$GABA_A$ receptors	Bovine frontal cerebellum	^{3}H-GABA	No affinity at 10^{-5} M

*See acknowledgment.

of the animal's head movement. An additional index of locomotor activity was provided by recording the number of jumps the animal made from the back to the front of the cage. This was recorded over the two 2-minute test periods. A behavioural scoring system was employed to assess the intensity of the impairment produced by MPTP such that 0—absent, 2—slight, 4—moderate and 6—marked. Using the system, the following parameters were measured:

a. Reduction in speed of locomotor activity.
b. Reduction in speed of head movement.
c. "Lack of interest in novel stimuli": A normal marmoset will make eye contact with the observer, and grab at novel objects introduced by the observer. This response is markedly reduced by MPTP treatment.
d. Inability to alter facial expression: A novel stimulus (a pencil) is presented, in close proximity to the animal's face. A normal animal will "screw-up" its face in a form of "grimace." This is in contrast to the "mask-like' facial expression seen in MPTP-treated animal.
e. Inability to elevate head: A pencil was placed above the animal's head and the response of the animal was recorded. As marmosets are inquisitive creatures, a normal animal readily lifts its head to observe the novel object. An MPTP-treated animal, however, has difficulty and in some cases failed to do this.

RESULTS

In Vitro Studies

High-affinity radioligand binding studies with ropinirole in human caudate revealed a K_i for D_2 receptors of 2.9×10^{-8} M. Further studies in rat striatum showed ropinirole to have no affinity for D_1 receptors at concentrations up to 10^{-4} M. Additionally, ropinirole had no stimulatory effect on rat caudate dopamine sensitive adenylate cyclase activity up to 10^{-4} M and no antagonism of dopamine-stimulated caudate adenylate cyclase activity at concentrations up to 10^{-5} M. Ropinirole weakly inhibited binding to α_2-adrenoceptors in human platelets (EC_{50} 9×10^{-6} M) and 5-HT_2 receptors in rat frontal cortex (EC_{50} 5×10^{-5} M). The compound was devoid of activity at 5-HT_1, benzodiazepine and GABA receptors or α_2- and β-adrenoceptors; the binding data are summarised in Table 1.

In Vivo Studies: Rodent

Irwin primary screen. No observable effects were seen following the lowest dose, 1.0 mg/kg, of ropinirole. At the highest dose, 100 mg/kg PO, the compound caused an initial depressive appearance 5 minutes after dosing and 2 of the mice developed 'fearfulness' and vocalisation lasting for up to 1 hour. The third mouse showed stereotyped sniffing. Hypothermia was recorded in all animals with the peak effect (mean rectal temperature 32.0°C) occurring 30 minutes after dosing. The group mean temperature returned to 36.0°C by 5 hours after dosing. The mean rectal temperature for the control group was 37.5°C. Of the mice given 10 mg/kg, 2 showed no observable effects and the third showed the depressive appearance with a rectal temperature of 35.2°C. The hypothermia lasted for approximately 2 hours.

Antinociceptive effects. There was no antinociceptive activity following oral administration of ropinirole at doses up to 100 mg/kg.

Spontaneous locomotor activity (SLA). Spontaneous locomotor activity of mice was markedly reduced by lower doses of ropinirole, 1–50 mg/kg IP, but was significantly increased, with reference to control or vehicle groups, by the higher dose of 100 mg/kg (Fig. 1). Similar biphasic responses were obtained with (+)-amphetamine where doses of 0.01 and 0.1 mg/kg IP depressed and 1.0 mg/kg stimulated SLA. In contrast, no stimulation was observed following a relatively high dose (1 mg/kg, SC) of apomorphine. This lack of stimulation may be due to the profound stereotypy seen following this dose of apomorphine. Spontaneous locomotor activity was also affected biphasically in rats, by ropinirole, where 1 mg/kg reduced activity but significant increases were measured following doses of 10 and 100 mg/kg, IP.

Stereotypic behaviour. Amphetamine (2.5–10 mg/kg IP) and apomorphine (0.5–2.0 mg/kg SC) caused statistically significant, dose-related stereotypies in mice. However, the stereotypies observed following administration of ropinirole (1–100 mg/kg IP) only achieved the minimum score and were not dose-dependent (Fig. 2). Similar results, for each compound, were obtained in rats.

Antagonism of dyskinetic activity. Ropinirole dose-dependently inhibited the dyskinesias induced by 2-di-n-propylamino-5,6-dihydroxytetralin at doses of 0.01–10 mg/kg IP. Tiapride also dose-dependently inhibited the dyskinesias but the effective dose range was 1–40 mg/kg SC. These results are summarised in Fig. 3.

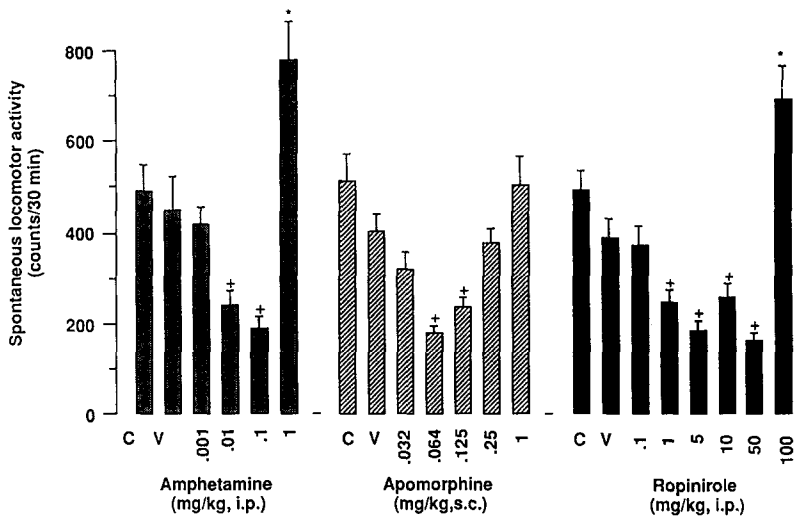

FIG. 1. Comparison of the effects of ropinirole, amphetamine or apomorphine on spontaneous locomotor activity in mice. Values represent mean ± s.e.m. (n = 6–10). Statistical significance was determined using one-way ANOVA followed by Dunnett's *t*-test for multiple comparisons. $+p<0.01$–0.001 significant reduction from vehicle (V) or control (C) activity. $*p<0.001$ significant increase from vehicle (V) or control (C) activity.

Intrastriatal administration. Ropinirole, at doses of 1 and 10 μg, injected unilaterally directly into the striatum of the rat caused marked, contralateral (away from the side of injection) asymmetry and circling. In contrast, both apomorphine and amphetamine (at doses up to 50 or 100 μg respectively) were inactive in this model. Thus ropinirole was able to stimulate striatal dopamine mechanisms to a greater degree than the latter two dopamine agonists.

Unilateral 6-OHDA-lesioned mice. Ropinirole was shown to cause contralateral (with respect to the lesion) asymmetry at doses ranging from 0.01 to 100 mg/kg IP. Apomorphine (0.5 mg/kg) also caused contralateral asymmetry, the maximum intensity of this response being the same as that for ropinirole. In contrast, the dopamine releasing activity of amphetamine (1.25 mg/kg IP) was demonstrated by ipsilateral asymmetry in this model. These results are contained in Table 2. When administered chronically (1.0 mg/kg b.i.d. for 14 days) the contralateral asymmetry response to ropinirole remained unchanged throughout the experimental period (Table 3).

Primate Studies

MPTP infused into the zona compacta of the substantia nigra of the marmoset, at a rate of 20 μg per 24 hours, for 13 days,

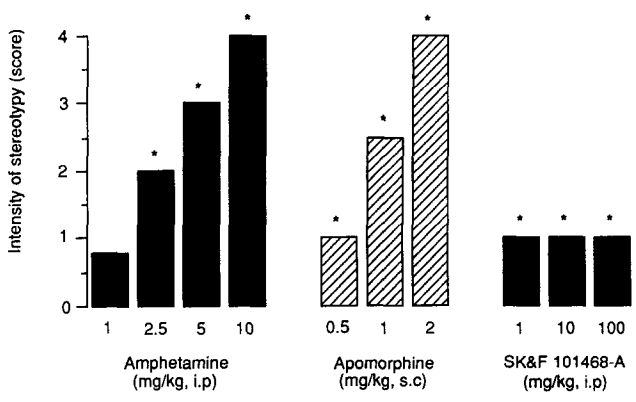

FIG. 2. Comparison of stereotyped behaviour exhibited by mice following treatment with amphetamine, apomorphine or ropinirole. Values represent mean scores (n = 10), s.e.m.s <12%. All values of 1 or greater are statistically significant $p<0.001$ (Mann-Whitney U-test).

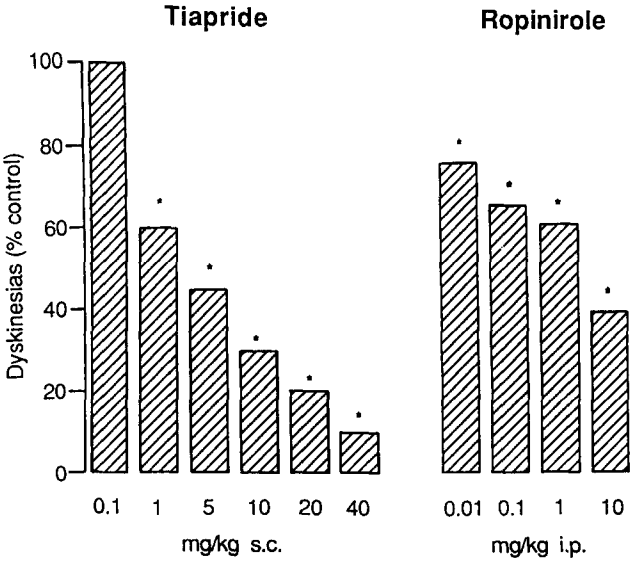

FIG. 3. Comparison of the dose-related inhibition of 2-di-n-propylamino-5,6-dihydroxytetralin-induced dyskinesias by tiapride or ropinirole in the rat. Bars represent mean values, s.e.m. 9.8–12.7% (n = 6). Statistical significance was determined using ANOVA plus Dunnett's *t*-test. $*p<0.05$–0.001, significantly different from vehicle-treated animals.

TABLE 2

ASYMMETRY INDUCED BY ACUTE ADMINISTRATION OF APOMORPHINE, AMPHETAMINE OR ROPINIROLE IN MICE WITH 6-OHDA-INDUCED UNILATERAL LESIONS OF THE SUBSTANTIA NIGRA

Treatment	Dose (mg/kg) Route	Direction of Asymmetry*	Asymmetry Score
Apomorphine	0.5 SC	Contralateral	2.0 ± 0.0
Amphetamine	1.25 IP	Ipsilateral	1.6 ± 0.3
Ropinirole	0.001 IP	None	none
Ropinirole	0.01 IP	Contralateral	1.3 ± 0.4
Ropinirole	0.1 IP	Contralateral	2.0 ± 0.0
Ropinirole	1.0 IP	Contralateral	1.8 ± 0.2

*Designated with respect to side of the lesion.
Values represent the mean ± s.e.m. of n = 6 animals.

caused severe motor impairment which was evident within 3–4 days following the implantation of osmotic minipumps. Locomotor activity was reduced to 28% of control, the time spent in spontaneous locomotor activity was reduced by 93% and the time spent in exhibiting head movements was reduced by 72%. Additionally, the animals lost interest in novel stimuli and were unable to alter their facial expression or elevate their head.

l-Dopa (50 mg/kg PO) given 30 minutes after benserazide (12.5 mg/kg PO) reversed both the MPTP-induced behavioural and locomotor deficits. Emesis occurred following treatment with l-dopa, and this was accompanied by 'nose rubbing' on cage surfaces and ptosis; this observation has been defined as a 'nausea response' (3). Bromocriptine, 1 mg/kg PO, failed to significantly alter any of the locomotor or behavioural parameters. Ropinirole (0.01 to 1.0 mg/kg SC) caused dose-related reversal of all the MPTP-induced deficits. The threshold dose was 0.05 mg/kg and maximal response occurred at 0.1 mg/kg when the behavioural response of marmosets could not be distinguished from that of untreated (pre-MPTP) animals. Onset of activity following ropinirole was within 10–20 minutes of administration, with duration exceeding 2 hours. Emesis occurred following doses of 0.1 mg/kg and above, and this was sometimes accompanied by the 'nausea response'; no emesis occurred following treatment with 0.01 or 0.05 mg/kg SC ropinirole. These results are shown in Figs. 4 and 5.

Ropinirole was also administered directly into the stomach by gastric gavage in the dose range 0.1 to 1.0 mg/kg. At the highest oral dose given, 1.0 mg/kg, ropinirole, assessed 60 minutes after

TABLE 3

CONTRALATERAL ASYMMETRY AND CIRCLING INDUCED BY ACUTE ADMINISTRATION OF APOMORPHINE OR CHRONIC (b.i.d.) TREATMENT WITH ROPINIROLE IN MICE WITH 6-OHDA-INDUCED UNILATERAL LESIONS OF THE SUBSTANTIA NIGRA

Treatment (day)	Dose (mg/kg) Route	Asymmetry Score	Circling (rev/min)
Apomorphine (−1)	0.25 SC	2.0 ± 0.2	6.1 ± 0.6
Ropinirole (1)	1.0 IP	1.8 ± 0.2	6.0 ± 0.7
Ropinirole (5)	1.0 IP	2.0 ± 0.0	8.4 ± 1.0
Ropinirole (10)	1.0 IP	2.0 ± 0.1	7.4 ± 0.9
Ropinirole (14)	1.0 IP	1.9 ± 0.2	7.5 ± 0.7

Values represent the mean ± s.e.m. of n = 13 animals.

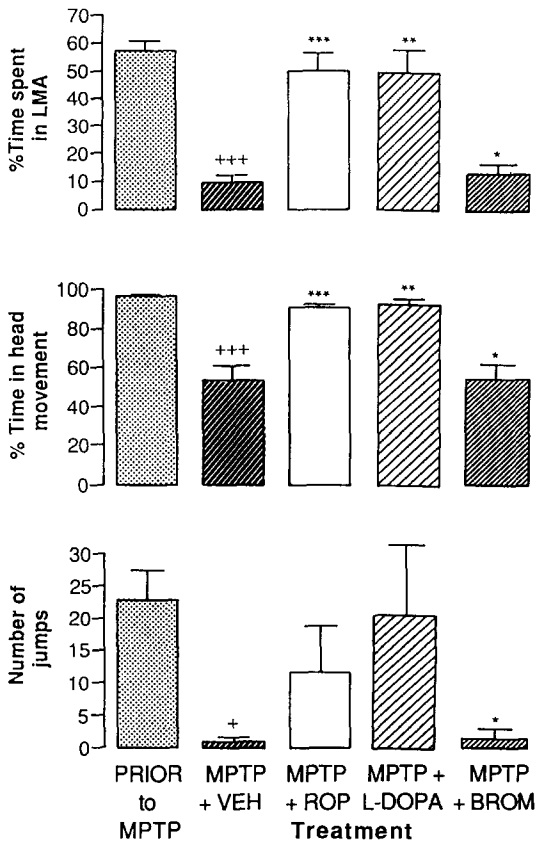

FIG. 4. Comparison of ropinirole, l-dopa, and bromocriptine, administered by mouth, on reversal of MPTP-induced locomotor (LMA) and behavioural deficits in the common marmoset. All studies were carried out in the home cage. M + VEH, M + Rop, M + l-dopa and M + Brom = responses following treatment with vehicle, ropinirole 0.1 mg/kg, l-dopa 50 mg/kg, 30 min after benserazide 12.5 mg/kg SC, or bromocriptine 1 mg/kg respectively, in animals treated with MPTP. Observations were made 60 min after dosing (n = 6). Statistical significance was determined using paired *t*-test. $+p<0.05$ compared to animals prior to MPTP. $*p<0.05$ reversal of MPTP-induced deficit.

dosing, caused stimulation of locomotor activity with activity counts up to 5 times those obtained prior to MPTP administration. Emesis occurred at this dose after 10 minutes. MPTP-induced deficits were completely reversed followed 0.5 mg/kg PO ropinirole which also caused mild emetic episodes and the 'nausea response' in some animals. The behavioural motor deficits were also reversed, to a lesser extent, by the lower dose of 0.1 mg/kg; no emesis or nausea was observed following treatment with this dose.

There was no diminution in the responses to ropinirole in the MPTP-lesioned marmosets during chronic treatment with 0.5 mg/kg PO b.i.d. for 4 days (Fig. 6).

DISCUSSION

The binding studies showed that ropinirole binds selectively to dopamine D_2 receptors with little or no affinity for any of the other receptor sites investigated. No gross signs of CNS stimulation or depression, other than those expected from a dopamine agonist, were seen in the Irwin screen or in the test for antinociception. Emesis was seen in both dog and cynomolgus monkey (in experiments not reported here) as well as the marmoset. Ropinirole elicited biphasic changes in spontaneous locomotor activ-

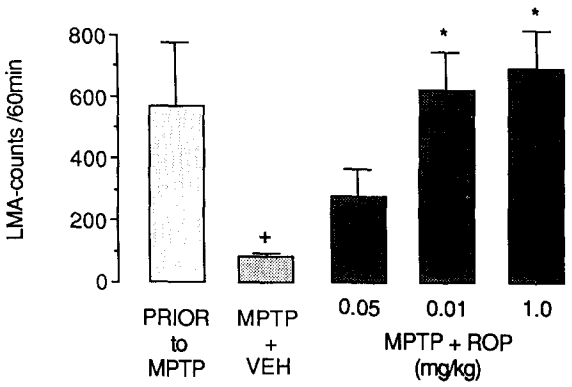

FIG. 5. Dose-response relationship of ropinirole reversal of MPTP-induced locomotor (LMA) deficits in the common marmoset. Ropinirole (Rop), 0.05, 0.1 or 1.0 mg/kg, or vehicle (VEH) SC was administered 40 min prior to assessment, activity was measured for 60 minutes. Values represent mean ± s.e.m. (n = 3 or 4). Statistical significance was determined using ANOVA plus Dunnett's *t*-test. $+p<0.05$ MPTP-induced reduction of activity. $*p<0.05$ reversal of MPTP-induced deficit.

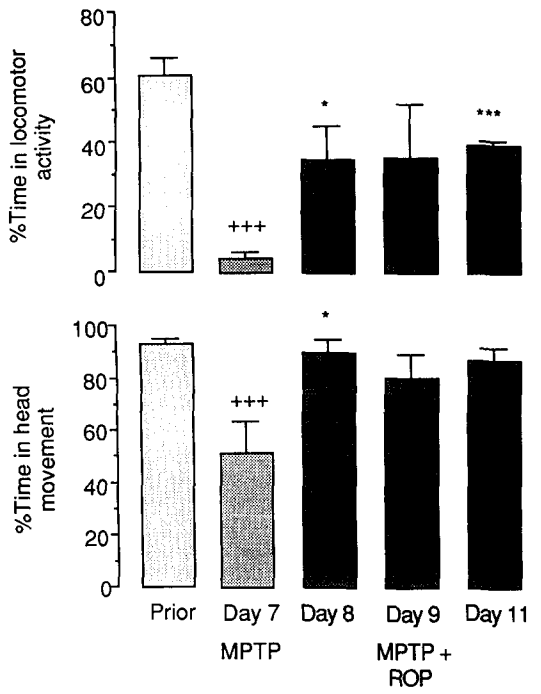

FIG. 6. The effect of repeated administration of ropinirole (0.5 mg/kg PO b.i.d.) for four days on MPTP-induced deficits in the marmoset. Dosing with ropinirole was started on day 8 of the MPTP infusion. Values represent mean ± s.e.m. of percentage time the animals spent in locomotor activity and head movement during two separate 2-min test periods (n = 3 or 4). Statistical significance was determined using paired *t*-test. + + + $p<0.001$ MPTP-induced reduction of activity. $*p<0.05$ reversal of MPTP-induced deficit. $***p=0.001$ reversal of MPTP-induced deficit.

ity in rats and mice, which would indicate presynaptic agonist activity at the lower and postsynaptic activity at the higher doses (21). Increases in locomotor activity in response to apomorphine have been shown by other workers (19) and the lack of effect in these studies was probably due to variations of methodology, since with many compounds it is only possible to see this biphasic response when the animals have been habituated to their environment. Alternatively, the profound stereotypy seen in mice after the higher doses of apomorphine will mask any stimulation of locomotor activity.

In contrast to amphetamine and apomorphine, ropinirole failed to cause marked or dose-related stereotypies, thus differentiating the locomotor stimulant properties of the compound from possible indications of prodyskinetic activity. This result probably reflects the selective D_2 agonist properties of ropinirole, whereas apomorphine and amphetamine cause D_1/D_2 effects (29). As with tiapride, ropinirole dose-dependently inhibited the 2-di-n-propylamino-5,6-dihydroxytetralin-induced dyskinesias in the rat. This effect was rather unexpected since addition of another dopamine agonist might be expected to enhance the dyskinesias rather than result in a neuroleptic type action (4).

Marked contralateral (with respect to the drug injection) asymmetries and circling behaviour following unilateral administration into the rat striatum provided further evidence of the dopamine agonist potency of ropinirole. Dopamine agonists such as apomorphine and amphetamine alone are not sufficiently active to initiate such a response. That this action was due to direct postsynaptic agonist activity was supported by the contralateral turning, relative to the lesion, observed in the unilateral 6-OHDA-lesioned mice, in response to systemic administration of the compound, an effect well characterised by others (24,28). In contrast, amphetamine (1.25 mg/kg IP) caused ipsilateral turning responses in this model reflecting the indirect, presynaptic dopamine releasing action of this agent.

When ropinirole was given chronically, b.i.d. for 14 days, there was no diminution in this response, which suggests that tolerance to the central effects does not develop as it does to the responses in the periphery (8,9).

In the marmoset, treatment with MPTP resulted in bradykinesia and hypokinesia, with loss of facial expression and reduced blink reflex, symptoms that have been well documented in man and other nonhuman primates (14, 16, 17). All the observed deficits were antagonised by ropinirole, given SC or orally with an onset of activity within 10–20 minutes of administration and a duration greater than 2 hours. Following chronic oral b.i.d. treatment with ropinirole for 4 days there was no diminution of the reversal of the MPTP-induced deficits. In contrast, in the same model, bromocriptine failed to restore the motor activity of the marmosets following a single, oral administration of a dose extrapolated from that used in man, reflecting the lack of acute efficacy of this compound. Similar reversals of MPTP deficits to those affected by ropinirole were seen following l-dopa in combination with benserazide. However, l-dopa treatment was always accompanied by emesis, whereas antagonism of the MPTP-induced deficits could be shown with ropinirole in the absence of this side effect.

These results are consistent with ropinirole being a specific, directly acting dopamine-D_2 agonist in rodents with activity in the extrapyramidal system suggesting a possible utility for the treatment of Parkinson's disease since compounds with a similar profile have been shown to be effective in this disease (26). Human and nonhuman primates are exquisitely sensitive to MPTP in whom the neurotoxin induces symptoms indistinguishable from Parkinson's disease. All the MPTP-induced deficits, in monkey or man, are reversed by current anti-Parkinsonian treatments (15). Thus the results obtained in the MPTP-marmoset reported here confirm direct dopamine-D_2 agonist action for ropinirole, seen in in vitro and in vivo studies in both rodent and primate, and support clinical assessment of this compound for the treatment of Parkinson's disease.

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