The Selective Dopamine D1 Receptor Agonist A-86929 Maintains Efficacy with Repeated Treatment in Rodent and Primate Models of Parkinson’s Disease

K. E. ASIN, E. F. DOMINO, A. NIKKEL and K. SHIOSAKI
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ABSTRACT
The ability of the selective dopamine D1 receptor agonist (5aR,11bS)-4,5,5a,6,7,11b-hexahydro-2-propyl-3-thia-5-azacyclopent-1-ena[c]-phenanthrene-9,10-diol (A-86929) to induce contralateral rotation after repeated administration was determined in rodent and primate models of Parkinson’s disease. Testing was conducted in rats previously given unilateral 6-hydroxydopamine injections and in macaques previously given unilateral, intracarotid infusions of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Both treatments have been shown to reduce forebrain dopamine levels on the side of the infusion. Such animals rotate contralaterally after injections of direct-acting dopamine receptor agonists. Rats were administered A-86929 (0.11 or 0.22 μmol/kg s.c.) three times daily for 10 days, with injections spaced 3 h apart, and rotation was measured across a 9-h period on various treatment days. Initially, monkeys were given various doses of A-86929 (0.03, 0.10 or 0.30 μmol/kg i.m.), and rotation was monitored for 3 h after each dose. Significant, dose-dependent levels of contralateral rotation were achieved. Monkeys were next treated three times daily at 3-h intervals with A-86929 (0.3 μmol/kg). Analysis of total, daily rotation scores indicated that the magnitude of the behavioral response did not change significantly across the 10-day treatment period in monkeys, although it increased in rats (0.22 μmol/kg). The first daily injection tended to elicit greater and longer-lived responses than the subsequent daily injections in both species. In monkeys, this was particularly true on the first test day and was not seen by the last test. This study suggests that a selective D1 receptor agonist, such as A-86929, with full intrinsic activity relative to dopamine, may be useful for the treatment of Parkinson’s disease.

A-86929 is a selective ligand for the DA D1 receptor with full agonist activity relative to DA (Shiosaki et al., 1996); the diacetyl derivative, ABT-431, is currently under development for the treatment of PD. One useful model for testing compounds for potential antiparkinsonian properties involves the use of rats or nonhuman primates with unilateral DA depletions. In such animals, direct-acting DA receptor agonists induce rotation away from the lesioned side, presumably because of the increased sensitivity of postsynaptic DA receptors on the denervated side. In rats, unilateral injections of the neurotoxin 6-OHDA reduce forebrain DA levels, whereas in primates, unilateral brain depletions of DA can be achieved through the intracarotid infusion of the neurotoxin MPTP. Results generated in the MPTP primate model have, in particular, proved useful for extrapolation to humans. Although the partial agonist SKF 38393 proved efficacious in rat models of PD, it was unable to ameliorate parkinsonian signs in MPTP-treated primates, perhaps because of its partial agonist activity (Bedard and Boucher, 1989; Braun et al., 1986; Falardeau et al., 1988). Subsequently, D1 receptor agonists with greater intrinsic activity have been demonstrated to relieve parkinsonian signs and symptoms in MPTP-treated primates and in PD patients (Blanchet et al., 1993; Emre et al., 1992; Gnanalingham et al., 1995; Luquin et al., 1994; Taylor et al., 1991; Temlett et al., 1989) and to induce dose-dependent contralateral rotation in monkeys rendered hemiparkinsonian via unilateral, intracarotid infusions of MPTP (Domino and Sheng, 1993a,b; Johnson et al., 1995; Vermeulen et al., 1993, 1995). The behavioral effects of these compounds after repeated daily treatment have not been reported.

The ability of A-86929 to produce rotation in rats bearing unilateral 6-OHDA lesions and in nonhuman primates rendered hemiparkinsonian through intracarotid infusions of MPTP was investigated in the present study. Rotation was examined across a thrice-daily, repeated-treatment regimen across a 10-day period. The results indicate that a D1 receptor agonist with full intrinsic activity may be useful as a chronic treatment for PD.

ABBREVIATIONS: A86929, (5aR,11bS)-4,5,5a,6,7,11b-hexahydro-2-propyl-3-thia-5-azacyclopent-1-ena[c]-phenanthrene-9,10-diol; DA, dopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; PD, Parkinson’s disease; ANOVA, analysis of variance.
Materials and Methods

Subjects

Twelve adult male Sprague-Dawley rats (Charles River, Portage, MI), weighing approximately 300 g, were used as subjects. Animals were single-housed in hanging wire-mesh cages, with food and water available ad libitum. The light:dark schedule was 12:12 h.

Five adult female Macaca nemestrina (pig-tailed macaque) monkeys weighing 5.4 to 6.9 kg at the time of study were originally obtained from Charles River Co., Port Washington, NY. The animal care and use program conforms to the standards in the Guide for the Care and Use of Laboratory Animals (1985), and we followed the B Virus Working Group Guidelines for Prevention of Herpes Virus Simian Infection in Monkey Handlers (1988). Animals were single housed and maintained on a 12:12 light:dark schedule, with water and monkey chow available ad libitum each morning, and fruit supplements, as warranted.

Surgery

Rats. Twenty minutes after an injection of desmethylimipramine HCl (25 mg/kg i.p.), rats were anesthetized with sodium pentobarbital (50 mg/kg) and given unilateral injections of 6-OHDA into the medial forebrain bundle, as described previously (Asin et al., 1995). Rats were prescreened for rotation in response to apomorphine about 2 to 3 weeks after lesioning, as also described elsewhere (Asin et al., 1995). Animals were used in the present studies approximately 2 months after surgery. There were six animals in each of the two treatment groups.

Monkeys. The method of Bankiewicz et al. (1986) was used to infuse MPTP unilaterally into one common carotid artery (also see Domino and Sheng, 1993a,b). Animals were anesthetized with ketamine hydrochloride (5–10 mg/kg, base dose) followed by 30 mg/kg i.v. sodium pentobarbital to maintain deep anesthesia. Animals were secured on the operating table, and one common carotid artery was exposed at its bifurcation. MPTP HCl (approximately 3 mg in 60 ml) was infused slowly over a 20-min period, and the wound was then closed. Each animal was given 300,000 U sterile penicillin G benzathine and penicillin G procaine in an aqueous suspension. At the time of the current studies, animals had been displaying hemiparkinsonian symptoms for 3 to 5 years and had been given various selective D1 and D2 DA receptor agonists and other compounds (e.g., amphetamine, cocaine, ketamine, phencyclidine and MK-801) during this time. However, animals had been free of medication for approximately 1 month before the present study.

Apparatus and Procedure

Rats. Animals were tested for rotation in automated rotometers (San Diego Instruments, San Diego, CA), as described previously (Asin et al., 1995). Animals were allowed to habituate to the rotometer bowls for approximately 20 min before the first daily injection of A-86929 (0.11 or 0.22 μmol/kg s.c.); the second and third injections were administered at 3-h intervals. These doses are approximately 50% and 100%, respectively, of the ED₅₀ value for this compound to induce rotation (Shiosaki et al., 1996). During the 10 days of treatment, rotation was measured on days 1, 3, 5, 8 and 10 for 3 h after each injection (total test time of 9 h); on the remaining days, rats were given the three daily injections in their home cages.

Monkeys. Methods were similar to those described elsewhere (see Domino and Sheng, 1993a,b). Briefly, monkeys were placed individually into a standard primate cage modified with a clear plexiglas front. Gross animal behaviors were observed and recorded via three color video cameras (Panasonic VHS model PV-420; Magnavox VR 9344-AV01 and Sears LX-1 model 1934) connected individually to videocassette recorders (Mitsubishi model U-32 VCR). The behavior of the animals was recorded for 30 min after vehicle injection and for 3 h after each drug injection without humans present. Videotapes were scored for the number and direction of complete, 360° turns demonstrated by each animal during each consecutive 5-min period.

Initially, we administered A-86929 in doses of 0.03, 0.10 and 0.30 μmol/kg i.m. and gave it in increasing doses in order to determine the effect and the duration of action of each dose. A 2- to 3-day washout period intervened between tests. On the basis of the results of the dose-response determination, the 0.30-μmol/kg dose was chosen for use in the repeated-treatment study. This dose was administered thrice daily for 10 days at approximately 9:00 a.m., noon and 3:00 p.m. At approximately 8:30 a.m. each day, animals were given a control injection of 5% dextrose in water (i.m.) followed 30 min later by the active drug.

Compounds

A-86929 was synthesized at Abbott Laboratories (Michaeldes et al., 1995). It was administered to rats in sterile water and to monkeys in sterile 5% glucose in water, purchased from Abbott Laboratories (Abbott Park, IL). 6-OHDA and desmethyl-imipramine HCl were purchased from Sigma Chemical (St. Louis, MO), and MPTP HCl was purchased from Aldrich Chemical Co (Milwaukee, WI).

Results

Rats

Separate one-way ANOVAs with repeated measures for each of the two doses of A-86929 were conducted on the total net number of contralateral rotations (contralateral minus ipsilateral) shown by 6-OHDA-lesioned rats. There was a significant effect of Days for both the low [F(4,20) = 4.16; P < .02] and the high [F(4,20) = 5.73; P < .003] doses. Post hoc analysis (Tukey HSD) indicated that for the lower dose, the mean total number of responses on days 3 and 8 differed from each other, whereas for the higher dose, responses on days 8 and 10 both differed significantly from the response on day 1 (P < .05) (see fig. 1).

Separate two-way ANOVAs (Injection number × Days) on rotation scores after the three daily injections across the five testing days were also conducted for each dose. For the low dose, there were significant effects of Days [F(4,20) = 4.16; P < .02] and Injection number [F(2,10) = 23.12; P < .001] and a significant Days × Injection number interaction [F(8,40) = 4.75; P < .001]. For the high dose, there were also significant effects of Days [F(4,20) = 5.73; P < .003] and Injection number [F(2,10) = 13.62; P < .001] and a significant Days × Injection number interaction [F(8,40) = 3.72; P < .002]. As can be seen in figure 2, and as borne out in post-hoc analysis (one-way ANOVAs with repeated measures conducted across days for each dose and each injection number), the behavioral response to the first daily injection increased significantly across days for both the low [F(4,20) = 4.28; P < .02] and the high [F(4,20) = 3.85; P < .02] doses. The levels of rotation seen over days in response to the second daily injection remained unchanged at the low dose [F(4,20) < 1.0] but increased significantly at the high dose [F(4,20) = 5.82; P < .004]. The response to the third daily injection tended to decline in rats administered the low dose [F(4,20) = 2.60; P < .07] and remained unchanged at the high dose [F(4,20) < 1.0].

In order to compare the initial and final rotation responses over time after the three daily injections, three-way ANOVAs (Injection number × Time × Days) were also conducted for each dose on rotation scores after each injection on treatment days 1 and 10 (fig. 3). For the low dose, there were significant

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effects of Injection number \[F(2,10) = 10.89; P < .004\] and Time \[F(5,25) = 12.37; P < .001\] but not Days \[F(1,5) < 1.0\]. Significant interactions were obtained for Days \times\) Injection number \[F(2,10) = 5.35; p < .03\], Injection number \times\) Time \[F(10,50) = 9.03; P < .001\] but not Days \times\) Time \[F(5,25) < 1.0\]. The Days \times\) Injection number \times\) Time interaction was also significant \[F(10,50) = 3.13; P < .005\]. The results of the ANOVA conducted on the lower dose can be summarized as follows: On the first day of treatment, the response over time to the three injections was similar. By Day 10, post-hoc analysis (Tukey HSD) indicated that the third injection elicited a smaller, shorter-lived response, both within and between days \(P < .01\), although the overall total number of rotations did not differ significantly between the first and last treatment days.

For the high dose, there were significant effects of Days \[F(1,5) = 7.85; P < .04\] and Time \[F(5,25) = 76.14; P < .001\] but not Injection number \[F(2,10) = 2.70; P > .10\]. Significant interactions terms were obtained for Days \times\) Injection number \[F(2,10) = 21.60; P < .001\] and Days \times\) Time \[F(5,25) = 12.43; P < .001\] but not Injection number \times\) Time \[F(10,50) < 1.0\]. The Days \times\) Injection number \times\) Time interaction was not statistically significant \[F(10,50) = 1.08; P > .35\]. The results of the ANOVA conducted on data from rats at the higher dose can be summarized as follows: The total number of rotations was greater on day 10 than on day 1, and this was true across each of the three injections (although there was no difference within a day in the behavioral response occurring after each injection). As may be seen in figure 3, response sensitization occurred over the treatment period and was approximately equal in magnitude after each of the three injections.

Monkeys

Dose-response determination. A one-way ANOVA with repeated measures was conducted on the total net number of contralateral rotations (contralateral minus ipsilateral) demonstrated by 6-OHDA-lesioned rats after three daily injections of A-86929. *From Michaelides et al., 1995.
onstrated by monkeys at each dose. A-86929 produced significant, dose-dependent increases in the net number of contralateral rotations demonstrated by each monkey (F(2,8) = 9.71; P < .008) (table 1). The duration of action was also dose-dependent (fig. 4). These results indicate that acute administration of A-86929 is able to produce robust contralateral rotation in a primate model of Parkinson’s disease.

Repeated treatment. On the basis of the initial dose-response study, a dose of 0.30 μmol/kg was chosen for the repeated-administration study. The data generated in the former study indicated that although the duration of the rotation at this dose was approximately 3 h, the duration was reduced to approximately 2 h in the repeated-treatment study after the first day of treatment. Therefore, only data recorded across the 2-h period after each drug injection were analyzed. Initially, the total, net number of contralateral rotations for the first, second and third daily injections were summed and analyzed over days. This one-way (Days) ANOVA with repeated measures failed to indicate a significant effect of Days [F(9,36) = 1.60; P > .15], which indicated that there was no significant change in the effects of A-86929 across the 10-day injection period (fig. 5A). A second ANOVA (Injection number × Days, with repeated measures on both factors) was conducted on the net number of contralateral rotations shown by each monkey during the 2-h period after the first, second and third daily injection (fig. 5B). There was a marginally significant effect of Injection number [F(2,8) = 4.03; P = .06]. As expected, there was no significant effect of Days [F(9,36) = 1.60; P > .15] and no Injection number × Days interaction [F(18,72) = 1.40; P > .15]. A graph of the data (fig. 5B) indicates that although there was no change in drug efficacy over days, the first daily injection tended to elicit greater contralateral rotation than the second and third daily injections. Because this difference in response magnitude appeared to diminish over time, additional data analyses were performed on the data generated on the first and tenth treatment days. For each day, a two-way (Injection number × Time) ANOVA was conducted on 30-min rotation scores after each injection (fig. 6). On day 1 of treatment, there was a significant effect of Time [F(3,12) = 14.16; P < .001] and a marginally significant effect of Injection number [F(2,8) = 3.96; P < .065]. The interaction between Injection number and Time was significant (F(2.52; p < .05). These results indicate a significant difference between the response pattern over time after the three injections. In contrast, only a significant effect of Time (F(3,12) = 7.65; P < .005) was obtained from the analysis of data from day 10 [Injection number (F(2,8) = 2.78; P > .10; Injection number × Time (F(6,24) = 1.31; P > .25)]. Thus, by the last treatment day, the responses to the three daily injections were statistically similar. In contrast to the robust contralateral rotation seen after treatment with A-86929, treatment with vehicle elicited ipsilateral rotation during the 30-min period after injection (mean ± S.E.M. = 12.40 ± 5.70).

**Table 1**

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<thead>
<tr>
<th>Dose</th>
<th>Mean ± S.E.M.</th>
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<tr>
<td>0.03 μmol/kg</td>
<td>28.6 ± 78.3</td>
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<tr>
<td>0.10 μmol/kg</td>
<td>329.6 ± 160.7</td>
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<tr>
<td>0.30 μmol/kg</td>
<td>973.2 ± 369.1</td>
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**Fig. 4.** Time course of the behavioral response to various doses of A-86929 in MPTP-lesioned primates. Graphed are the mean (± S.E.) net number of contralateral rotations (contralateral minus ipsilateral).

**Fig. 5.** A) Mean (± S.E.) daily total numbers of net contralateral rotations across the 10 days of repeated treatment in primates. Total testing time was 6 h. B) Mean (± S.E.) net numbers of contralateral rotations 2 h after the first, second and third daily injections.

**Discussion**

In the present study, thrice-daily injections of the full DA D1 receptor agonist A-86929 promoted rotation across a 10-day treatment period in both rats and primates bearing unilateral forebrain DA depletions. The daily total number of rotations did not decline over the 10-day period, and in rats, evidence for response sensitization was obtained with the higher dose (0.22 μmol/kg). These observations are unlike those we reported previously after daily treatment of le-
dosed, primate hemiparkinsonian animals were found to display dose-dependent, contralateral rotation in monkeys rendered hemiparkinsonian via unilateral, intracarotid infusions of MPTP (Domino and Sheng, 1993a; Johnson et al., 1995; Vermue, et al., 1993). The results of these more recent studies using D1 receptor agonists with full intrinsic activity certainly suggest that such compounds may be efficacious in treating PD.

In MPTP-lesioned monkeys given repeated L-dopa treatment, D2 receptor agonists, including quinpirole, bromocriptine and (+)-4-propyl-9-hydroxynaphthoxazine were found to alleviate parkinsonian symptoms but also to induce dyskinesias, as a consequence of L-dopa priming. In contrast, the D1 receptor agonists SKF 82958 and A-77636 also alleviated parkinsonian symptoms but had less propensity for inducing dyskinesias (Blanchet et al., 1993). Although somewhat controversial (Luquin et al., 1994), selective D1 receptor agonists may therefore produce fewer dyskinetic movements than D2 selective agonists in L-dopa-primed animals, and they appear even to potentiate the antiparkinsonian effects of selective D2 agonists (Blanchet et al., 1993; Gagnon et al., 1995; Rouillard et al., 1990) in MPTP-treated primates.

Luquin et al. (1996) recently reported that the behavioral response of MPTP-treated monkeys to the partial D1 agonist CY 204-234 decayed when animals were given four drug injections spaced 3 h apart. The decay was primarily due to a more rapid decline in the motor response to the drug relative to the first injection. A diminution in behavioral efficacy was not seen after (+)-PHNO administered under similar conditions, which suggests that the behavioral tolerance seen in MPTP-treated monkeys after repeated apomorphine injections may involve D1 receptor mechanisms (Luquin et al., 1993). In the present study, the first daily injection of A-86929 tended to elicit a greater and longer-lived response than either the second or the third injection across the 10-day treatment period. In monkeys, this difference was particularly apparent on the first day of treatment, where the magnitude of the rotation during the first 30 min after the first injection was greater than that after the second and third injections. By the tenth treatment day, however, there was no temporal difference in the behavioral response to the first, second or third daily injections, although the response did decay more rapidly after each injection on the tenth day than on the first day. Thus the reduction in the behavioral response seen after short-term, repeated D1 agonist treatment in both this study and the study by Luquin et al. (1996) may subsequently plateau during longer-term treatment. If so, it may be possible to overcome this reduction through dose adjustment.

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References


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Send reprint requests to: Dr. K. E. Asin, Dept. of Toxicology, Abbott Laboratories, Dept. 4.68, Blvd. AP13A, 100 Abbott Park Rd., Abbott Park, IL 60064-3500.