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Motor Response to a Dopamine D3 Receptor Preferring Agonist Compared to Apomorphine in Levodopa-Primed 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine Monkeys[1]

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ABSTRACT

The profile of dopamine receptor subtype activation contributing to the therapeutic efficacy and motor response complications of levodopa (nonselective pro-agonist) in Parkinson's disease remains unclear. Potent, selective, short-acting dopamine D2 receptor subfamily agonists show good antiparkinsonian efficacy but produce dyskinesias comparable to levodopa. Nonetheless, agonists displaying higher affinity for dopamine receptors other than the D2 subtype may have a better therapeutic index. To clarify this issue, we compared the nonselective dopamine D1/D2 receptor subfamilies agonist apomorphine to the dopamine D3 receptor preferring agonist [R-(+)-trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol] (PD 128,907) in 6 levodopa-primed , 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned parkinsonian monkeys with reproducible dyskinesias. Single s.c. dosing with the lowest fully effective dose of apomorphine (averaging 27.9 $\pm$ 4.5 μg/kg) and PD 128,907 (averaging 41.7 $\pm$ 4.4 μg/kg) yielded equivalent antiparkinsonian efficacy on the behavioral scale and portable activity monitoring used. A comparable significant dose-dependent increase in the response magnitude and duration was seen with two higher doses. The severity of dyskinesia was also similar between the two drugs. When the lower dose for each drug was administered six times at a fixed 90-min interval, both drugs remained efficacious with no significant tolerance observed. The D3 receptor preferring antagonist U-99194A significantly reduced the motor effects of both apomorphine and PD 128,907. Thus, increased D3 receptor tone does not acutely ameliorate dyskinesias in levodopa-primed parkinsonian monkeys. Given the reported lack of affinity of PD 128,907 for central D1 receptors, our data support the concept that the pharmacological activation of D1 receptors is not mandatory for relief of parkinsonism and production of dyskinesia.

Chronic levodopa replacement treatment in Parkinson's disease patients is associated with the development of motor response complications including end-of-dose deterioration ("wearing-off" effect), sudden and seemingly erratic fluctuations ("on-off" effect) and dyskinesia in a majority of cases, commonly within the first 3 to 4 yr of therapy (Peppe *et al.*, 1993; Blanchet *et al.*, 1996). Both simple and complex fluctuations are now thought to reflect changes at or downstream from striatal postsynaptic dopaminergic receptors (Mouradian *et al.*, 1988; Grandas *et al.*, 1992a; Bravi *et al.*, 1994; Verhagen Metman *et al.*, 1997a). Differential activation of certain dopamine receptor subtypes expressed in the striatopallidal complex may account for the antiparkinsonian efficacy and motor complications of levodopa. Currently, at least five distinct genes encoding different dopamine receptor subtypes have been identified and grouped in D1 (D1a, D1b or D5) and D2 (D2, D3, D4) subfamilies based on pharmacological and biochemical characteristics (reviewed by Neve and Neve, 1997). The precise distribution and degree of coexpression of the different dopamine receptor subtypes on the medium spiny output neurons of origin of the striatonigral ("direct") pathway and the striatopallidal ("indirect") pathway are still debated (Le Moine *et al.*, 1991; Surmeier *et al.*, 1993, 1996; Gerfen and Keefe, 1994). While the medium spiny neurons of the "indirect" striatopallidal pathway mainly bear D2 receptors, those at the origin of the "direct" striatonigral pathway expressing substance P mainly but not exclusively bear D1 receptors (Surmeier *et al.*, 1996).

The D1 and D2 receptor subtypes are abundantly expressed in the basal ganglia (Schwartz *et al.*, 1992) and have traditionally been implicated in mediating the motor response of the dopaminergic drugs used in the treatment of Parkinson's disease. Although the other dopamine receptors

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ABBREVIATIONS: ANOVA, analysis of variance; APO, apomorphine; Levodopa, L-DOPA or L-3,4-dihydroxyphenylalanine; LFED, lowest fully effective dose; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PAM, portable activity monitor; PD, PD 128,907 or [R-(+)-trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol].

and receptor messages have a more restricted pattern of distribution than D1/D2 receptors, they may still contribute to motor function. One possible candidate is the D3 receptor, which is thought to subserve cognitive, emotional, neuroendocrine and autonomic functions (Sokoloff *et al.*, 1990). Arguments in favor of its involvement in motor control come from *in situ* hybridization histochemistry studies of the D3 receptor mRNA message in the rat brain that extended its expression to include not only "limbic" brain structures connected with the ventral tegmental area (strong expression in the olfactory tubercle-island of Calleja complex and nucleus accumbens), but also the globus pallidus, parts of the neostriatum and the subthalamic nucleus (Bouthenet *et al.*, 1991). In addition, autoradiographic studies with different ligands in the human brain also revealed significant binding in the internal segment of the globus pallidus (Murray *et al.*, 1994), albeit weaker in another study using a D3 agonist (Herroelen *et al.*, 1994), and significant binding in the neostriatum and throughout the cerebral cortex (Herroelen *et al.*, 1994). Recently, the D3 receptor mRNA was estimated to be present in half of the striatal neurons of origin of the "direct" pathway (Surmeier *et al.*, 1996). Of importance also is the lack of significant difference in D3 receptor binding and mRNA expression between the normal and parkinsonian striatum chronically exposed to exogenous levodopa treatment (Hurley *et al.*, 1996).

The experimental evidence on the motor effects of dopamine agonists obtained in MPTP monkeys and clinical data indicate that short-acting drugs with D2-preferring or mixed D1/D2 properties eventually create problems in Parkinson's disease. Indeed, the pharmacological activation of dopamine D1/D2 receptors or of D2 receptors alone has been repeatedly shown to produce dyskinesias both in drug-naive (Clarke *et al.*, 1989; Luquin *et al.*, 1992; Bédard *et al.*, 1993; Graham *et al.*, 1993) and drug-primed (Blanchet *et al.*, 1993) MPTP monkeys and levodopa-treated parkinsonian patients (Grandas *et al.*, 1987; Vidailhet *et al.*, 1990; Mouradian *et al.*, 1991; Hughes *et al.*, 1993), with the notable exception of the D2 agonist bromocriptine (Lees and Stern, 1981; Bédard *et al.*, 1986). Furthermore, repeated dosing with the mixed D1/D2 agonist apomorphine reportedly loses efficacy during the day compared to the first dose (Grandas and Obeso, 1989), and this apparent partial hyposensitivity is thought to be dependent on a short dosing interval of 30 min (Luquin *et al.*, 1993) or 2 hr (Grandas *et al.*, 1992b) and linked to pulsatile dopamine D1 receptor stimulation in the MPTP primate model (Luquin *et al.*, 1996). Thus, alternatives to levodopa showing the same antiparkinsonian efficacy and rapidity of onset, along with a better consistency from dose to dose and a lesser dyskinesigenic potential, could be advantageously used by patients. We herein report on the behavioral effects of PD 128,907 [R-(+)-trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol], a short-acting dopaminomimetic agonist with no affinity for D1 receptors and several-fold higher affinity for the human dopamine D3 *vs.* D2 receptor in different assays (table 1), and compare its behavioral motor profile with the nonselective drug apomorphine. These two agonists were also tested in combination with the 20-fold D3 *vs*. D2 dopamine receptor preferring antagonist U-99194A (Waters *et al.*, 1993, 1994) exhibiting only weak affinity for the D2 receptor subtype (K_i = 1,572 nM, table 1) in an attempt to distinguish the relative contribution of D2 and D3 receptors to the motor response observed.

TABLE 1

Dissociation constants (K_i values in nM) of tested drugs for D1, D2 and D3 dopamine receptors

Drug	Receptor Subtype			
	D1	D2	D3	Ratio D2/D3
Dopamine[a]	2,000	2,000	30	67
Apomorphine[a]	700	70	70	1
Apomorphine[b] (K_H)	48	18	389	0.05
Apomorphine[c]		2.9	8.9	0.3
PD 128,907[c]	>10,000[d]	42	2.3	18
PD 128,907[e]		389	1.8	216
PD 128,907[f]		7,470	5.9	1,266
U-99194A[g]	>1,000	1,572	78	20

[a] Inhibition of antagonist binding (Schwartz *et al.*, 1992).
[b] Values for the high affinity state of the receptors in the human striatum (De Keyser *et al.*, 1995).
[c] Values for the high affinity agonist binding receptor sites (Pugsley *et al.*, 1995).
[d] Based on IC_{50} value >10,000.
[e] Inhibition of antagonist binding (Sautel *et al.*, 1995).
[f] Inhibition of antagonist binding (Lachowicz and Sibley, 1997).
[g] Inhibition of agonist binding (D2 sites) and antagonist binding (D3 sites) (Waters *et al.*, 1993).

Methods

Animals

Six cynomolgus (*Macaca fascicularis*) monkeys of both sexes weighing 2 to 6.7 kg were tested under an approved protocol that met the ethical standards of the National Institutes of Health Animal Care and Use Committee. They were housed individually, fed with a standard biscuit diet twice a day supplemented with fruits, and had free access to water. They were kept under stable room conditions and exposed to a 12-hr light/dark cycle. All monkeys were exposed to MPTP hydrochloride (Research Biochemicals Intl., Natick, MA) administered s.c. while awake at a weekly dose of 0.5 to 1 mg/kg until definite parkinsonian features with a sustained action tremor appeared. The average cumulative MPTP dose was 4.4 mg/kg (range 2.1–9.75 mg/kg). All animals were left drug-free for 6 to 8 wk and scored on a regular basis using the Laval University Disability Scale for MPTP Monkeys (Gomez-Mancilla *et al.*, 1993), where the normal state extends from 0 to 2 points and maximal disability is 10 points (see below). Once a mild to moderate parkinsonian syndrome had stabilized (baseline disability scores between four to six points), monkeys began chronic treatment with carbidopa/levodopa (Sinemet, 25 mg/100 mg tablets; Roane-Barker Inc., Greenville, SC), 0.5 to 2 tablets once daily, hidden in food pellets or fruit bites and ingested spontaneously. All developed dyskinesias of a predominantly choreic nature within 1 mo, which were subsequently produced consistently and predictably with each oral dose of carbidopa/levodopa. At that point, they were put on a maintenance dose of carbidopa/levodopa administered three times a week. On testing days, their morning meal and dose of carbidopa/levodopa were withheld.

Drug Experiments

Single dosing. R-(–)-apomorphine and PD 128,907 (Research Biochemicals Intl., Natick, MA) solutions were prepared fresh before every experiment and dissolved easily in sterile normal saline. Ascorbic acid (0.2 mg/ml) was added to the apomorphine solution for stability. First, a dose-finding study was conducted to determine the LFED that consistently reversed parkinsonian signs in each animal for 30 to 45 min. The starting dose for each drug was 5 μg/kg and the dose was gradually increased by 25 to 50% until a satisfactory response magnitude and duration was attained. This dose was then increased 2-fold (2LFED) and 4-fold (4LFED) to document the be-

havioral effects at suprathreshold doses. For this phase of the study, a single daily injection was given and the dosing interval was at least 24 hr. Both drugs were administered s.c. in the abdomen or flank and sites of injection carefully rotated to avoid local inflammation.

In three monkeys, the LFED dose of each drug was also administered 30 min after the dopamine D3 receptor preferring antagonist U-99194A 1 mg/kg s.c. (Research Biochemicals Intl.) (see table 1 for affinity constants). The drug was first administered in monotherapy (dose range 0.05–5 mg/kg) and the highest well-tolerated dose selected. Doses of 2.5 and 5 mg/kg alone produced myoclonic jerks, sedation and retching. U-99194A was dissolved in saline only.

Repeated dosing. After a washout period of at least 72 hr, the LFED for each drug was administered six times the same day to every animal at a fixed interval of 90 min consistent with the dosing intervals ranging from 30 min to 3 hr (Grandas *et al.*, 1992b; Luquin *et al.*, 1993) used previously to study behavioral tolerance. The drugs were prepared and administered as described above. The drug solutions were kept at room temperature and protected from light to prevent spontaneous oxidation.

Assessment of the Response

Both qualitative and quantitative assessments of the drug responses were obtained. The monkeys were moved to an observation room and watched for 20 min for habituation and determination of a reliable baseline rating using the Laval University Disability Scale for MPTP Monkeys (Gomez-Mancilla *et al.*, 1993). Once injected, they were maintained under direct observation until complete return to baseline and rated every 15 min in a nonblinded fashion by two investigators familiar with the scale. The following motor and behavioral parameters were scored: posture: normal = 0, flexed = 1, crouched = 2; mobility: normal = 0, passive = 1; climbing: present = 0, absent = 1; gait: normal = 0, abnormal = 1; tremor: absent = 0, present = 1; holding food: present = 0, absent = 1; vocalizing: present = 0, absent = 1; grooming: present = 0, absent = 1; social interaction: present = 0, absent = 1. The parkinsonian disability score is equal to the sum of subscores and a definite antiparkinsonian response considered present as long as the baseline rating was improved by at least two points. Dyskinesias were scored as follows: occasional, mild = 1; intermittent, moderate = 2; continuous, severe = 3; intermediate scores (0.5, 1.5, 2.5) were allowed to reflect slight bodily asymmetries, and each segment (face, neck, trunk, each limb) scored separately. A dyskinesia index was then calculated using the formula: (sum of all dyskinesia scores/duration of antiparkinsonian effect) $\times$ 100. Nonmotor adverse effects like emesis, somnolence and hallucinatory-like behavior were also recorded. In cynomolgus monkeys, hallucinatory-like behavior is characterized by neck extension and side-to-side oculocephalic movements as if following a moving object, which can be interrupted by stimulation.

A continuous objective assessment of the total motor activity resulting from each injection was also obtained from a PAM tied underneath a primate jacket and providing a motor count every 2 min. The same PAM was used for each monkey and retrieved under sedation only once the whole protocol was completed. Motor counts were averaged for 16 consecutive min at peak effect, as a measure of the peak motor response, and accrued for 60 min after a drug injection as a measure of the total drug effect (reflecting both the duration and intensity of the drug response). Motor stimulation was determined to be present as long as the baseline motor counts were improved by at least 20%.

For both the single and repeated dosing studies, the duration (min) of the response as determined by the behavioral scale scores and by the motor improvement from the PAM counts, the peak motor response, the total motor activity for 60 min, and the calculated dyskinesia index values were pooled for all animals and differences determined using a ANOVA for repeated measures, followed by pairwise group comparisons performed using Student's t test. A P value $< .05$ was considered statistically significant.

Results

Antiparkinsonian efficacy. The selected LFED for apomorphine averaged 27.9 $\pm$ 4.5 μg/kg (range 12.5–40). That dose yielded a full antiparkinsonian response in all animals after a mean latency of 4.5 $\pm$ 0.3 min, the peak motor response and total motor activity over 60 min increasing 5.5-fold and 4.6-fold, respectively, compared to saline administration. Clinical efficacy persisted for a mean duration of 37.2 $\pm$ 2.5 min, a value similar to that derived from the PAM (table 2). Compared to the LFED, the 2LFED had a more robust effect and produced statistically significant increases on the mean clinical response duration (54 $\pm$ 2.8 min) (P = .018), duration of PAM improvement (P = .004) (table 2) and total motor activity (P = .035) (fig. 1), whereas the mean peak motor response did not rise significantly (fig. 2). After the 4LFED, the motor response further lengthened to 64.5 $\pm$ 3.4 min (P = .008 *vs.* 2LFED) but no statistical difference was found between the 2LFED and 4LFED for any other parameter (figs. 1 and 2), indicating a plateau effect.

The selected LFED for PD 128,907 averaged 41.7 $\pm$ 4.4 μg/kg (range 20–50). That dose produced essentially identical results to the LFED for apomorphine for all parameters of response considered (duration, peak motor response, total motor activity) after a mean latency of 3.0 $\pm$ 0.5 min. The quality of the antiparkinsonian response was similar. The 2LFED markedly enhanced the effect size of the LFED for the mean clinical response duration (P = .027), duration of PAM improvement (P = .027), total motor activity (P = .006) (fig. 1) and peak motor response (P = .005). The 4LFED further increased the mean duration of efficacy that reached 91.2 $\pm$ 8.5 min (P = .0005 *vs.* 2LFED) but other parameters were less affected, suggesting a relative plateau effect.

The LFED and 2LFED for each drug provided similar durations of efficacy for the corresponding injection (table 2),

TABLE 2

Mean duration of efficacy (in min) from activity monitoring counts obtained following single or repeated injections (INJ.) of apomorphine (APO) and PD 128,907 (PD) in six MPTP-lesioned parkinsonian monkeys

Drug		Duration of Efficacy								
		Single Dosing			Repeated Dosing					
		LFED[a]	2 LFED	4 LFED	INJ. 1	INJ. 2	INJ. 3	INJ. 4	INJ. 5	INJ. 6
APO	Mean	34.2	48.3	64.0	30.7	28.3	30.3	31.7	27.0	25.7
	S.E.M.	3.8	2.3	3.9	2.1	2.9	3.5	3.2	3.8	5.2
PD	Mean	36.0	53.3	91.3	30.7	27.3	30.0	31.7	27.0	37.7
	S.E.M.	1.4	5.9	6.1	4.3	7.3	3.4	5.5	2.4	6.3

[a] LFED, lowest fully effective dose.

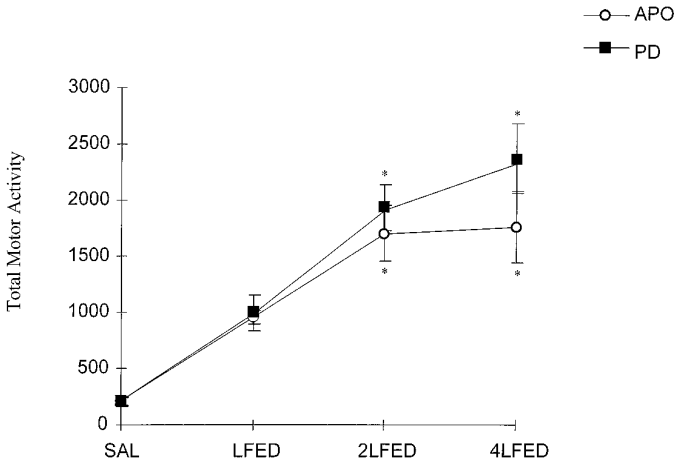

Fig. 1. Total motor activity reflecting the combined motor activity recorded by monitors for all six animals over 60 min after single dosing with apomorphine (APO), PD 128,907 (PD) or saline (SAL) administration. The drug doses consisted of the lowest fully effective dose (LFED) (27.9 ± 4.5 and 41.7 ± 4.4 μg/kg for APO and PD, respectively) and multiples of LFED. Mean motor counts for 60 min ± S.E.M. *P < .05 *vs.* LFED of respective drug.

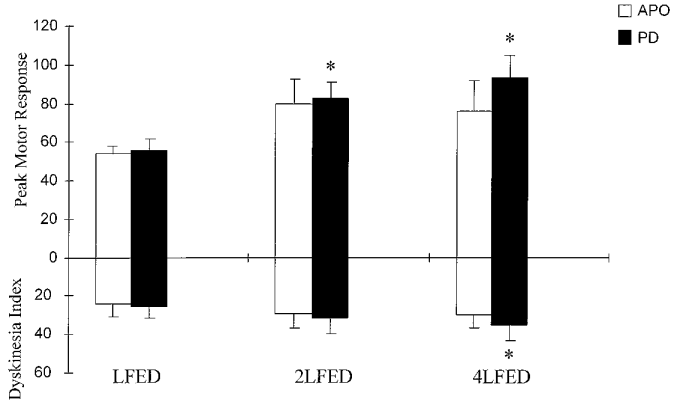

Fig. 2. Peak motor response representing the mean motor count recorded by monitors for 16 consecutive min at peak effect (top histogram) and dyskinesia index values (bottom histogram) after single dosing with apomorphine (APO) or PD 128,907 (PD) administration. The drug doses consisted of the lowest fully effective dose (LFED) (27.9 ± 4.5 and 41.7 ± 4.4 μg/kg for APO and PD, respectively) and multiples of LFED. Mean values ± S.E.M. *P < .05 *vs.* LFED of respective drug.

but the response to the 4LFED was 41% longer for PD 128,907 compared to the 4LFED for apomorphine (P = .031). The mean total motor activity (fig. 1) and peak motor response (fig. 2) obtained with all three doses (LFED, 2LFED, 4LFED) of PD 128,907 were statistically similar to those obtained with each corresponding injection of apomorphine.

The dopamine D3 receptor preferring antagonist U-99194A (1 mg/kg s.c.) totally blocked the behavioral response to the LFED for apomorphine in one of three animals tested with this combination, and totally (two animals) or partially (one animal) blocked the behavioral response to the LFED for PD 128,907.

Dyskinesia. The LFED for apomorphine produced choreic dyskinesias in five animals with a mean dyskinesia index value of 24 (range 0–52). All six animals exhibited dyskinesias at higher doses and dyskinesia severity was slightly dose-dependent with mean dyskinesia index values rising to 29 (range 2–55) and 30 (range 14–62) following the 2LFED and 4LFED, respectively (fig. 2). This difference was statistically insignificant. After the LFED for PD 128,907, all animals exhibited choreic dyskinesias with a mean dyskinesia index value of 26 (range 6–51). A similar dose-dependent increase in severity was observed with the 2LFED (mean index of 32, range 7–70) that reached statistical significance for the 4LFED (mean index of 35, range 9–74; P = .048 Vs. LFED) (fig 2). U-99194A (1 mg/kg s.c.) totally blocked dyskinesias in response to the LFED for apomorphine in two of three animals, and totally (two animals) or partially (one animal) blocked dyskinesias to the LFED for PD 128,907.

Repeated dosing. In the multiple injection protocol, the first dose of apomorphine and PD 128,907 provided similar antiparkinsonian effect and mean duration of efficacy (table 2). No total response failure was seen at any dose for both drugs, although one animal displayed only a partial behavioral response to the last three apomorphine injections and to the last PD 128,907 injection that failed to reach our threshold for definite clinical improvement on the rating scale on these occasions. The mean dyskinesia index values obtained for each drug did not significantly decline with successive injections (data not shown). The difference between the two drugs for each successive injection as determined by ANOVA did not reach statistical significance for any response parameter.

Adverse effects. Both drugs were well tolerated. Only two animals showed mild behavioral stereotypies including licking (one), repetitive, quick, lateral tongue movements (one) and searching and picking (one), present when the LFED was administered and not clearly worsening in a dose-dependent fashion. The 4LFED dose was responsible for apparent hallucinatory-like behavior for both apomorphine (two subjects) and PD 128,907 (one).

Discussion

The results of the single dosing experiment document for the first time the behavioral effects of the dopamine D2 receptor subfamily agonist the most selective (up to 1000-fold, see table 1) for the D3 receptor subtype in MPTP-lesioned parkinsonian monkeys. These data are in accordance with the increased mobility reported following high doses of PD 128,907 in normal and reserpinized rats (Pugsley *et al.*, 1995). The therapeutic profile (antiparkinsonian efficacy, latency of onset, peak motor response, total motor activity) of PD 128,907 was essentially identical to apomorphine in our animals. Thus, the antiparkinsonian potency of PD 128,907 was not altered by the absence of affinity for D1 receptors or the 14-fold lower affinity for human D2L receptors in agonist binding compared to apomorphine (table 1), nor was its greater D3 receptor tone found detrimental to motor behavior. Thus, the pharmacological coactivation of dopamine D1/D2 receptors is not an absolute requirement for optimal motor improvement in conditions of chronic dopamine deficiency, although one cannot entirely rule out the possibility that the endogenous residual dopamine may have provided enough D1 receptor activation to synergistically enhance the effects of PD 128,907.

The antiparkinsonian benefit derived from the two dopamine agonists studied might conceivably originate from their D2 receptor agonist activity alone, and one might argue that the expectedly lower D2 receptor effect resulting from the administration of PD 128,907 was still sufficient to account

for the motor improvement. Indeed, the density of D2 receptors is far more abundant and its distribution more widespread than the D3 receptor. Furthermore, studies in parkinsonian monkeys and patients showed that potent selective D2 agonists such as lisuride (Parkes *et al.*, 1981), the non-ergot drugs (+)-4-propyl-9-hydroxynaphthoxazine (Grandas *et al.*, 1987; Clarke *et al.*, 1988) and ropinirole (Vidailhet *et al.*, 1990), and the aminotetralin N-0923 (Belluzzi *et al.*, 1994; Roberts *et al.*, 1994) display full antiparkinsonian efficacy comparable to levodopa. Thus, the D3 receptor occupancy in the dorsal neostriatum provided by PD 128,907 may be behaviorally silent and provide no advantage compared to selective D2 agonists or nonselective D1/D2 receptor subfamilies agonists such as apomorphine. An alternative explanation for the symptomatic motor effects of PD 128,907 is that D3 receptor activation in the nonlimbic parts of the basal ganglia (see above) mediates a significant part of the antiparkinsonian effect observed, perhaps in synergy with D2 receptors. The negative impact of the dopamine D3 receptor preferring antagonist U-99194A on the motor response to both PD 128,907 and apomorphine may also be supportive of this hypothesis. It is of interest that several antiparkinsonian drugs including lisuride, pergolide (De Keyser *et al.*, 1995) and pramipexole (Svensson *et al.*, 1994; Mierau, 1995) also bind with significant affinity to the D3 receptor. The question of whether the dopamine D3 receptor physiological action is mainly presynaptic or postsynaptic remains unanswered, but one study in rats suggested a predominant postsynaptic role and proposed that the D2 receptor subtype was more likely to represent the autoreceptor (Svensson *et al.*, 1994). Our data with PD 128,907 provide no evidence of decreased motor activity at any dose as expected from the activation of a dopamine autoreceptor, but this may hold true only in the parkinsonian brain and not in normal conditions. Although the D4 mRNA has recently been found at low to moderate levels in a subset of neostriatal medium spiny neurons (Surmeier *et al.*, 1996), the 105-fold selectivity of PD 128,907 for dopamine D3 *vs.* D4 receptors (Pugsley *et al.*, 1995) makes unlikely a significant contribution of D4 receptor activation to the motor effects of the drug.

The foregoing results also indicate that the administration of a short-acting dopamine D3 preferring agonist does not reduce dyskinesia severity in this model. Unless longer acting D3 agonists display a better therapeutic index in the future, it is unlikely that these drugs will provide an alternative to dyskinesigenic drugs targeting dopamine D2 receptors (Bédard *et al.*, 1993; Blanchet *et al.*, 1993). The low affinity of bromocriptine for human striatal D3 receptors has been proposed as a key feature for its much lower dyskinesia induction properties compared to levodopa (De Keyser *et al.*, 1995). A role for the D3 receptor subtype in the dyskinetic process is also suggested by the antidyskinetic properties of the dopamine D3 receptor preferring antagonist U-99194A. The absence of affinity of PD 128,907 for the D1 receptor subtype, and lack of a strong coactivation of D1/D2 receptors thereof, did not reduce the severity of dyskinesia in our animals. Thus, these pharmacological conditions appear unnecessary to reproduce levodopa-induced dyskinesia in drug-primed parkinsonian animals. Other effects at adrenergic, serotonergic, muscarinic and γ-aminobutyric acid type A and B receptors are probably not functionally important because PD 128,907 was found to have insignificant affinity for these receptors (reported in Pugsley *et al.*, 1995). Finally, the fact that the dyskinesia index values did not rise in every animal and not as much as other parameters of response during the single dosing experiment could be used as a supportive argument for the idea that the severity of levodopa-induced dyskinesia is not tightly dose-dependent (Nutt and Holford, 1996; Verhagen Metman *et al.*, 1997b).

The lack of behavioral tolerance during the repeated dosing experiment is compatible with the results previously obtained in parkinsonian patients with subcutaneous apomorphine (mean dose of 52 μg/kg) (Hughes *et al.*, 1991) or intravenous levodopa (Davis *et al.*, 1991), validating the non-human primate model. Previously, the repeated administration of a minimally effective dose (2.4 μg/kg) of apomorphine led to a significant reduction in the duration of the response (Luquin *et al.*, 1993). In that experiment, the results from each successive injection were not substantiated by a more objective quantitative monitoring of the motor response. This apparent behavioral hyposensitivity is probably best explained by the use of a near threshold dose that may have exaggerated the effect of any spontaneous variations in absorption, as suggested previously (Hughes *et al.*, 1991), or the effect of a transient change in endogenous dopamine turnover that could not totally recover between doses. Acute enzymatic autoinduction may also modify the peripheral pharmacokinetics of a drug, compounding possible random variations in absorption. In any case, apparent hyposensitivity to repeated dosing was not observed when a higher dose of apomorphine closer to ours was repeatedly injected at 30-min interval (Luquin *et al.*, 1993). Because no total response failure was observed with the two direct-acting dopamine agonists studied, no acute swings in postsynaptic dopamine receptor responsiveness are likely to explain the "on-off" effect that occurs in levodopa-treated parkinsonian patients. In fact, these troublesome and sudden akinetic episodes are acutely reversed by supra-threshold s.c. doses of apomorphine and greatly reduced by a continuous intravenous infusion of levodopa (Hardie *et al.*, 1984).

Our results clearly show that a dopamine D3 receptor preferring agonist can mirror the behavioral effects of a nonselective agonist for dopamine D1/D2 receptor subfamilies in an experimental primate model of parkinsonism. The therapeutic index is similar and not associated with less severe dyskinesias. It is remarkable that hallucinatory-like behavior was not more frequently observed with a D3-preferring drug presumably acting more strongly in "limbic" brain structures. The contribution of the dopamine D3 receptor subtype to motor function deserves further attention.

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