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The novel type B MAO inhibitor PF9601N enhances the duration of L-DOPA-induced contralateral turning in 6-hydroxydopamine lesioned rats

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Summary. The present study examined the effect of the highly potent and selective MAO B inhibitor PF9601N on L-DOPA-induced rotational behavior in unilateral nigrostriatal 6-hydroxydopamine lesioned rats. Three doses of PF9601N (20, 40 and 60 mg/kg) were administered 30 min before an injection of L-DOPA (25 mg/kg), and both contralateral and ipsilateral rotational behavior was measured. In addition, we also studied the effect produced by another MAO B inhibitor, deprenyl (20 mg/kg), the MAO A inhibitor, clorgyline (20 mg/kg), and the dopamine reuptake inhibitor, GBR2909 (7.5 mg/kg) on L-DOPA-induced rotational behavior. The results showed that PF9601N plus L-DOPA significantly enhanced the duration of contralateral rotational behavior with respect to L-DOPA plus vehicle in a dose-related manner. At the dose of 40 and 60 mg/kg, PF9601N produced significantly more overall contralateral turning than L-DOPA plus vehicle, and at the dose of 60 mg/kg, PF9601N produced significantly more turning behavior than L-DOPA plus deprenyl. These results suggest that PF9601N may be used as a novel tool in the treatment of Parkinson's disease.

Keywords: MAO B inhibitors, L-DOPA, turning behavior, Parkinson's disease.

Introduction

The monoamine oxidase (MAO) type B inhibitors such as deprenyl, produce significant increases in striatal dopamine (DA) concentrations by diminishing dopamine metabolism in the nigrostriatal system, promoting release, and blocking the reuptake of dopamine (Knoll, 1978). In animal studies, it has been shown that deprenyl (Heikkila et al., 1981), as well as another MAO B inhibitor, MDL 72145 (Fozard et al., 1986) enhance L-DOPA (L-3,4-

dihydroxyphenylalanine)-induced contralateral rotational behavior in unilateral nigrostriatal 6-hydroxydopamine denervated animals. More recently, Heeringa et al. (1997) have shown that Ro 19-6327 (MAO B inhibitor) in conjunction with the MAO A inhibitor, Ro 41-1049, prolonged the duration of L-DOPA-induced rotation, although the total number of turns were not increased. Moreover, type B MAO inhibitors have been shown to protect dopamine neurons from MPTP neurotoxicity in mice and monkeys, as well as from 6-hydroxydopamine neurotoxicity (for reviews see Heinonen and Lammintausta, 1991; Lange et al., 1994). These agents are currently used in the clinic for the treatment of Parkinson's disease, either alone or in combination with L-DOPA (Marsden, 1990; Galler et al., 1996).

PF9601N is a novel, potent, and selective MAO B inhibitor. In biochemical studies we have shown that under similar experimental conditions, this compound resembles deprenyl in that it blocks the reuptake of amines, and increases the release of dopamine in human and rat slices (Pérez et al., 1999a,b). However, this compound showed greater selectivity and greater potency than deprenyl in inhibiting MAO B, and unlike deprenyl, PF9601N is not transformed to amphetamine or methamphetamine (Pérez et al., 1999a). In addition, like other MAO B inhibitors, PF9601N can also protect nigrostriatal dopamine neurons against MPTP neurotoxicity in C57BL/6 mice (unpublished observations).

In the present study, we investigated the effects of PF9601N on L-DOPA-induced contralateral rotational behavior in unilateral 6-hydroxydopamine nigrostriatal lesioned rats. The actions of PF9601N were compared with those of deprenyl, the MAO A inhibitor clorgyline, and the selective dopamine reuptake blocker, GBR12909.

Methods

Animals

Male Sprague-Dawley rats were used in all groups. Initially, rats (150 ± 10 g) were housed eight to a cage (59 × 38 × 20 cm), with free access to rat chow and water. They were maintained in a temperature controlled environment (21 ± 1°C) on a 12 h. light/dark cycle (lights on at 8 a.m.) when they were not in experimental sessions. This experiment was carried out in compliance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) for care and use of laboratory animals.

Surgical procedure

Rats were anaesthetized with a sodium pentobarbital solution (40 mg/kg, i.p.) and placed in a David Kopf stereotaxic frame with the incisor bar set at 2.4 mm (König and Klippel, 1963). Unilateral microinjections of 8 μg of 6-hydroxydopamine HCl (calculated as free base, Sigma, USA) in 4 μl of physiological saline with 0.2% ascorbic acid were performed using a Hamilton syringe with a conically shaped needle of maximum diameter equal to 0.4 mm. The rate of infusion was 1 μl/min. The infusions were aimed at the medial forebrain bundle (A −4.4, L −1.2, V −7.8 mm, calculated from bregma and dura). This lesion has been shown to extensively denervate the dopaminergic nigrostriatal system (Ungerstedt, 1971). After surgery, rats were housed four to a cage for the remainder of the experiment.

Rotational behavior

Rotational behavior was recorded using a computerized system (Panlab S. A., Spain). Animals were placed individually in plastic hemispherical bowls (40 cm in diameter), attached to a harness and connected to photoelectric detectors. The number of contralateral and ipsilateral half turns was monitored.

Animal selection

In order to select the successfully denervated animals, thirty days post-surgery, all rats were challenged with a low dose of apomorphine (0.05 mg/kg, s.c.). The apomorphine test was repeated four times with an interval of one week between treatments. Only rats showing more than 500 contralateral half (180°) turns in one hour during the last two tests with apomorphine were used in this study. Contralateral turning reflects the stimulation of supersensitive dopamine receptors in the lesioned hemisphere (Schwarting and Huston, 1996). Several authors have demonstrated that at least 90% dopamine depletion is needed for apomorphine to induce contralateral rotational behavior (Hefti et al., 1980; Hudson et al., 1993).

Drugs

Apomorphine and L-DOPA (Sigma, USA) were dissolved in 0.9% physiological saline. Clorgyline (Sigma, USA), deprenyl, GBR-12909 (RBI), as well as PF9601N (Cruces et al., 1991), were dissolved in a 10% solution of β-cyclodextrin (Cerestar, USA) in distilled water. All drugs were injected intraperitoneally in a volume of 5 ml/kg, except apomorphine that was injected subcutaneously in a volume of 1 ml/kg.

Biochemical assays

At the end of the behavioral experiments, rats were sacrificed, their brains extracted, and both lesioned and non-lesioned striatal tissue was dissected and stored at −80°C (approximately 5 to 6 h after L-DOPA administration). The concentration of DA and 3,4-dihidroxyphenylacetic acid (DOPAC) in striatal tissue was determined by high performance liquid chromatography (HPLC) coupled with an electrochemical detector. The mobile phase was comprised of citric acid (0.1 M), EDTA (0.05 mM) and octane sulfonic acid (1 mM) in 1% acetonitrile at pH 2.5. Briefly, tissues were homogenized in perchloric acid (0.25 M), EDTA (0.1 mM) and sodium metabisulphite (0.25 mM; 1 mg/25 μl), centrifuged (16,000 × g, 15 min). A 20 μl aliquot of supernatant was injected onto a 5 μ Kromasil C-18 column (Teknokroma, Spain) attached to an HPLC system equipped with a Coulochem II coulometric detector (ESA, UK). The monoamines were quantified by peak height comparisons with standards prepared in the homogenate medium.

General procedure

Sixty-nine well denervated animals were randomly allocated in 7 different groups. Rats were placed in the rotometers, and following a 15 min habituation period, group 1 received an injection of the vehicle solution (5 ml/kg, n = 10), group 2 received an injection of PF9601N (20 mg/kg, n = 10), group 3 received an injection of PF9601N (40 mg/kg, n = 10), group 4 received an injection of PF9601N (60 mg/kg, n = 10), group 5 received an injection of deprenyl (20 mg/kg, n = 10), group 6 received an injection of clorgyline (20 mg/kg, n = 10), and group 7 received and injection of GBR-12909 (7.5 mg/kg, n = 9). Thirty minutes after the first injection, all rats received an injection of L-DOPA (25 mg/kg). Ipsilateral and contralateral rotational behavior was recorded for 30 min after the first injection, and for 240 min after the second injection.

Data analysis

Differences in total number of turns between groups were analyzed using a one-way ANOVA, followed by the Duncan post-hoc test. Rotational behavior was also compared between groups in 7 different time periods (blocks of 30 min) after L-DOPA administration using a repeated measures MANOVA, post-hoc analyses were carried out with the Contrast option in MANOVA.

Results

DA and DOPAC concentrations

Dopamine concentration measured in striatal tissue from the lesioned side (87.0 ± 48.0 pg/mg) was found to be significantly smaller than the concentration measured in the intact side (10,596 ± 1,031 pg/mg) ($p < 0.001$). Similarly, DOPAC concentration measured in striatal tissue from the lesioned side (29.0 ± 15 pg/mg) was significantly smaller than the concentration observed in the intact side (651.0 ± 111.0 pg/mg) ($p < 0.001$). These values represent more than 99% dopamine depletion in the lesioned striatum.

Rotational behavior

Systemic administration of PF9601N, deprenyl, clorgyline or GBR12909, 30 minutes before L-DOPA (25 mg/kg) administration, produced very little ipsilateral (PF9601N 20 mg/kg: 13.10 ± 2.11; 40 mg/kg: 12.0 ± 4.22; 60 mg/kg: 8.30 ± 1.74; clorgyline 20 mg/kg: 16.80 ± 3.39; GBR 7.5 mg/kg: 16.89 ± 3.85; deprenyl 20 mg/kg: 10.40 ± 2.88; vehicle: 11.30 ± 2.92) and contralateral (PF9601N 20 mg/kg: 36.40 ± 12.24; 40 mg/kg: 46.70 ± 17.66; 60 mg/kg: 32.70 ± 7.72; clorgyline 20 mg/kg: 87.0 ± 24.71; GBR 7.5 mg/kg: 42.22 ± 15.43; deprenyl 20 mg/kg: 19.80 ± 7.04; vehicle: 40.0 ± 20.16) rotational behavior, which was not significantly different between groups.

As shown in Fig. 1, contralateral rotational behavior following treatment with L-DOPA plus vehicle produced increasing levels of contralateral rotational behavior during the first 30 min after administration. Turning behavior was maintained at high levels during 60 min, and then started to decrease at 90 min. L-DOPA in combination with the different doses of PF9601N, deprenyl, clorgyline or GBR12909 produced lower levels of contralateral turning with respect to L-DOPA plus vehicle during the first 90 min (Deprenyl > PF9601N 60 mg/kg > PF9601N 40 mg/kg > PF9601N 60 mg/kg > clorgyline > GBR12909), but no significantly differences were found between groups at this time point. At 150 min, the groups treated with L-DOPA plus PF9601N (20, 40 and 60 mg/kg), deprenyl or clorgyline still showed rotational behavior, while the groups treated with L-DOPA plus vehicle or plus GBR12909 reached values near zero. At 180 min, the groups injected with L-DOPA plus clorgyline, or plus PF9601N at the doses of 40 and 60 mg/kg showed significantly more rotational behavior than L-DOPA plus vehicle, plus deprenyl or plus PF9601N at the dose of 20 mg/kg. At 210 min, rotational behavior in the groups treated with L-DOPA plus deprenyl, or plus PF9601N at the dose of 20 mg/kg, decreased to near zero values, while the groups

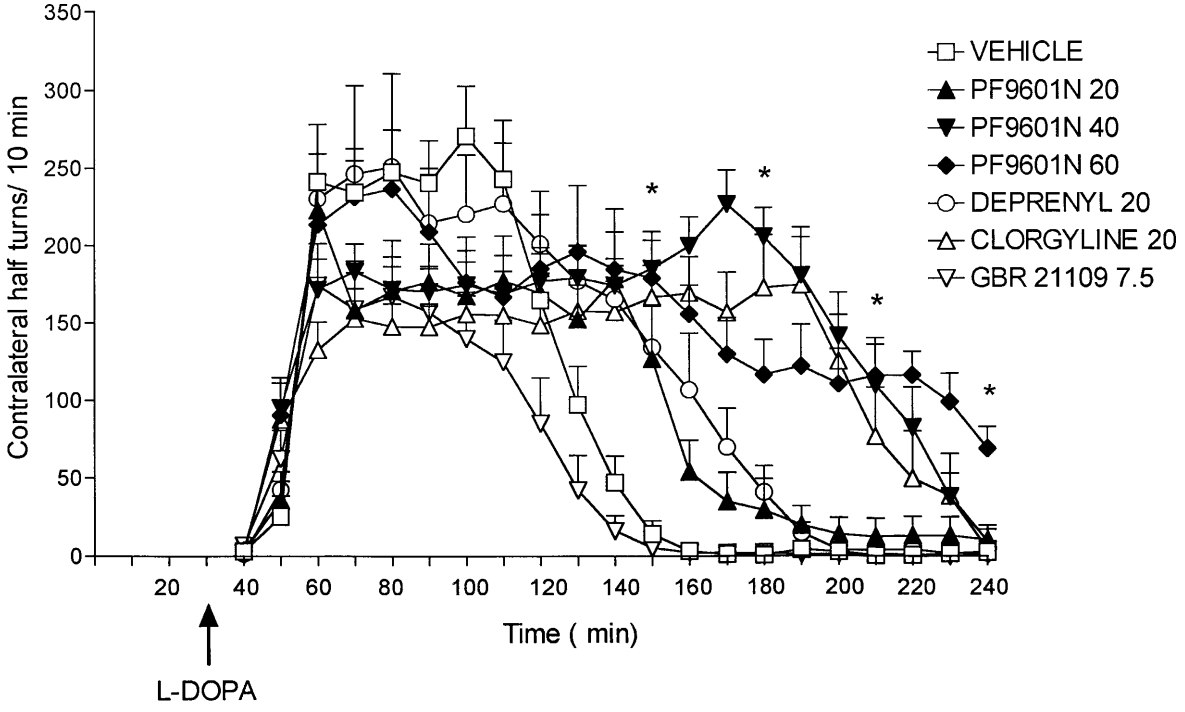

Fig. 1. Time-related effects of PF9601N (20, 40 and 60 mg/kg), deprenyl (20 mg/kg), clorgyline (20 mg/kg), GBR 21109 (7.5 mg/kg), or vehicle (1 ml/kg) on contralateral rotational behavior induced by L-DOPA (25 mg/kg) in unilateral 6-hydroxydopamine denervated rats. L-DOPA was administered 30 min after pretreatment with the various substances. The data are expressed as half turns/10 minutes + S.E.M. Significant differences between groups were found at 150 min: [$F(6,60) = 8.17$, $p < 0.001$], (* $t < 4.25$, $p < 0.002$, L-DOPA plus PF9601N 20, 40 and 60 mg/kg or plus clorgyline > L-DOPA plus vehicle or plus GBR12909). At 180 min: [$F(6,60) = 19.33$, $p < 0.001$], (* $t < 5.18$, $p < 0.03$, L-DOPA plus PF9601N 40 and 60 mg/kg or plus clorgyline > L-DOPA plus vehicle, plus GBR12909, or PF9601N 20 mg/kg, or plus deprenyl). At 210 min: [$F(6,60) = 13.37$, $p < 0.001$], (* $t < 5.28$, $p < 0.001$, L-DOPA plus PF9601N 40 and 60 mg/kg or plus clorgyline > L-DOPA plus vehicle, plus GBR12909, plus PF9601N 20 mg/kg, or plus deprenyl). At 240 min: [$F(6,60) = 7.77$, $p < 0.001$], (* $t < 4.98$, $p < 0.03$, L-DOPA plus PF9601N 60 mg/kg > L-DOPA plus vehicle, plus GBR12909, plus PF9601N 20 mg/kg, plus deprenyl, or plus clorgyline)

injected with L-DOPA plus PF9601N at the doses of 40 and 60 mg/kg, or plus clorgyline still showed turning behavior. At 240 min, the groups treated with L-DOPA plus PF9601N at the doses of 40 and 60 mg/kg showed more contralateral turning than the groups treated with L-DOPA plus vehicle, plus PF9601N at the dose of 20 mg/kg, plus deprenyl and plus GBR12909. At this time point, no significant differences were observed between the group treated with L-DOPA plus PF9601N at the dose of 40 mg/kg and the group treated with L-DOPA plus clorgyline. In addition, the group treated with L-DOPA plus PF9601N at the dose of 60 mg/kg, showed significantly more turning than the group treated with L-DOPA plus PF9601N at the dose of 40 mg/kg.

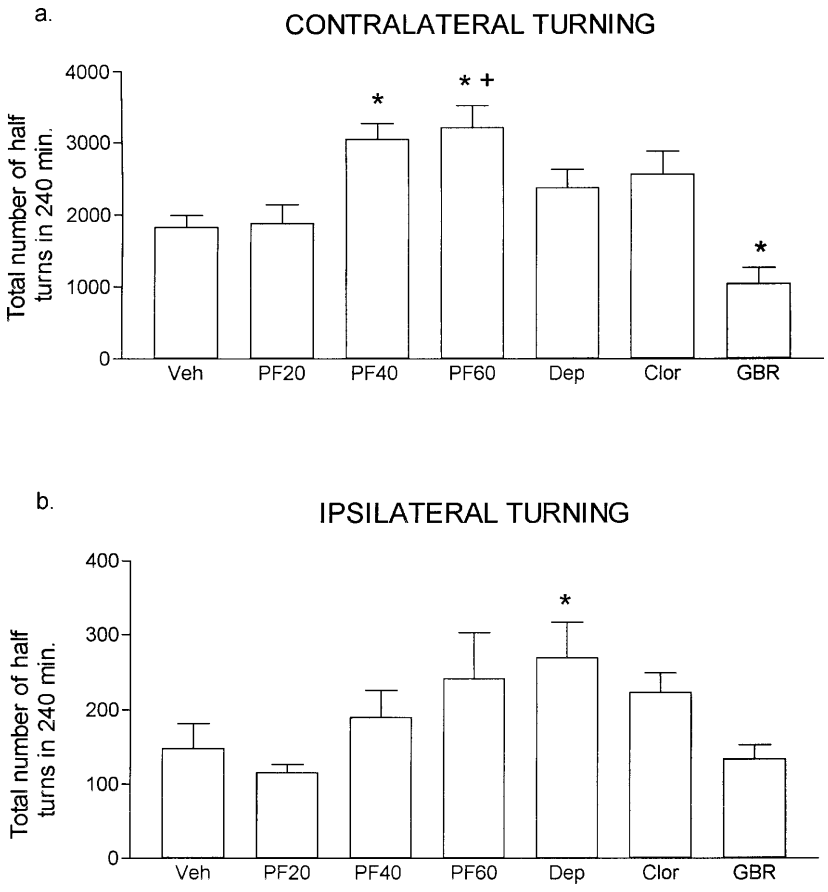

Fig. 2. Effects of PF9601N (PF: 20, 40 and 60 mg/kg), deprenyl (Dep: 20 mg/kg), clorgyline (Clor: 20 mg/kg), GBR21109 (GBR: 7.5 mg/kg), or vehicle (Veh: 1 ml/kg) on the overall contralateral (**a**) and ipsilateral (**b**) rotational behavior induced by L-DOPA (25 mg/kg). Data are expressed as total number of half turns + S.E.M in 240 min. The asterisks represent significant differences with respect to vehicle plus L-DOPA (Duncan, $p < 0.05$). The plus sign (+) represents significant differences with respect to deprenyl plus L-DOPA (Duncan, $p < 0.05$)

Figure 2 shows the total number of ipsilateral and contralateral turns in 240 min induced by the various drugs studied plus an injection of L-DOPA. For contralateral turning, statistical comparisons revealed significant differences between groups [$F(6,62) = 8.76$, $p < 0.0001$]. The Duncan post-hoc test showed that the groups injected with PF9601N at the doses of 40 and 60 mg/kg plus L-DOPA showed significantly more overall contralateral turning as compared to the group injected with vehicle plus L-DOPA ($p < 0.05$). In addition, PF9601N at the dose of 60 mg/kg plus L-DOPA, produced significantly more contralateral rotational behavior than deprenyl (20 mg/kg) plus L-DOPA ($p < 0.05$). In contrast, GBR12909 (7.5 mg/kg) plus L-DOPA significantly reduced contralateral turning with respect to saline plus L-DOPA ($p < 0.05$). Low levels of ipsilateral turning were observed following L-DOPA administration plus the different compounds. Statistical compari-

sons showed that PF9601N plus L-DOPA did not significantly enhance ipsilateral turning with respect to vehicle plus L-DOPA at any of the doses tested. In contrast, deprenyl (20 mg/kg) plus L-DOPA significantly enhanced ipsilateral turning with respect to vehicle plus L-DOPA ($p < 0.05$).

Discussion

In this study we show that, while PF9601N had no effect on rotational behavior by itself, it increased the duration of L-DOPA-induced contralateral rotational behavior in 6-hydroxydopamine denervated rats in a dose-related manner. Similarly, both deprenyl and clorgyline significantly enhanced the duration of L-DOPA-induced contralateral turning. However, clorgyline as well as high doses of PF9601N prolonged the duration of L-DOPA's effect to a greater extent than did deprenyl. In contrast, the selective dopamine uptake inhibitor GBR12909 (Anderson, 1989) reduced the contralateral turning induced by L-DOPA, resembling the actions of amphetamine in 6-hydroxydopamine denervated rats (Pycok, 1980). However, it did not increase the ipsilateral turning produced by L-DOPA plus vehicle, indicating that the action of L-DOPA in the denervated striatum primes over the action of GBR12909 in the non-denervated striatum.

Previous studies have shown that non-selective doses of deprenyl (20 mg/kg), and clorgyline (5–20 mg/kg), can potentiate the total number of turns induced by L-DOPA, as well as, its duration of action (Heikkila et al., 1981; Fozard et al., 1986). In our study deprenyl did not potentiate the total number of contralateral turns induced by L-DOPA probably because it also significantly increased the amount of ipsilateral rotational behavior. This increase in ipsilateral turning may be explained by the fact that deprenyl is rapidly metabolized to amphetamine and methamphetamine (Reynolds et al., 1978), thus promoting dopaminergic activity in the non-denervated striatum.

In 6-OHDA-lesioned rats, the dopamine produced by the decarboxylation of L-DOPA can be broken down by MAO present in glia (MAO A), and in the nerve terminals that have not been destroyed by the lesion (MAO A and MAO B). Thus, the ability of non-selective doses of deprenyl and clorgyline to potentiate L-DOPA-induced contralateral turning in this animal model is likely due to an increase of dopamine in the lesioned striatum produced by the inhibition of both type A and type B MAO. A recent report however, suggests that MAO B inhibition may not account for the potentiation of L-DOPA-induced rotational behavior in denervated rats since the potent type B MAO inhibitor, RO19-6327, dose not potentiate L-DOPA-induced rotational behavior (Heeringa et al., 1997). In addition, *in vivo* microdialysis experiments have shown that MAO B inhibition increases the levels of dopamine derived from L-DOPA in intact rats, but not in rats lesioned with 6-hydroxydopamine (Wachtel and Abercrombie, 1994; Finberg et al., 1995). Alternatively, MAO A inhibition has been shown to play a major role in dopamine metabolism both in non-denervated and denervated striatum (Finberg et al., 1995), and there is data showing that type B MAO inhibitors at doses that produce more

than 90% inhibition of type B MAO, still caused 25% inhibition of type A MAO (Finberg et al., 1995).

Our results showing that PF9601N potentiates L-DOPA-induced contralateral turning in an animal model of hemiparkinson can be due to its ability to increase the levels of dopamine in the denervated striatum through inhibition of both type A MAO present in intact nerve terminals, and type B MAO present in the glia. An alternative mechanism of action for PF9601N may involve direct stimulation of hypersensitive dopamine receptors in the denervated striatum by the compound itself or by an active metabolite. In this respect, we have found that PF9601N is metabolized to its corresponding amine form within 30 min (unpublished observations). However, more studies are needed in order to confirm this hypothesis.

PF9601N may also increase dopamine levels by its ability to increase release and inhibit the reuptake of dopamine (Pérez et al., 1999a,b). In unilateral 6-hydroxydopamine lesioned rats, this mechanism of action would be present predominantly in the non-lesioned striatum to produce ipsilateral turning. However, in this study PF9601N plus L-DOPA did not significantly increase ipsilateral turning with respect to vehicle plus L-DOPA.

In conclusion, our results showing that the new type B MAO inhibitor, PF9601N, increases the duration of action produced by L-DOPA in an animal model of hemiparkinson, suggest that this compound may be a useful therapeutic adjuvant in the treatment of Parkinson's disease.

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References

Anderson PH (1989) The dopamine uptake inhibitor GBR12909: selectivity and molecular mechanism of action. Eur J Pharmacol 166: 493–504

Cruces MA, Elorriaga C, Fernández-Alvarez E (1991) Acetylenic and allenic derivatives of 2-(5-metoxyindolyl) and 2-(5-hidroxyindolyl)methylamines: synthesis and in vitro evaluation as monoamine oxidase inhibitors. Eur J Med Chem 26: 33–41

Finberg JPM, Wang J, Goldstein DS, Kopin IJ, Bankiewicz KS (1995) Influence of selective inhibition of monoamine oxidase A or B on striatal metabolism of L-DOPA in hemiparkinsonian rats. J Neurochem 65: 1213–1220

Fozard JR, Palfreyman MG, Robin M, Zreika M (1986) Selective inhibition of monoamine oxidase type B by MDL 72145 increases the central effects of L-DOPA without modifying its cardiovascular effects. Br J Pharmacol 87: 257–264

Galler RM, Hallas BH, Fazzini E (1996) Current trends in the pharmacologic and surgical treatment of Parkinson's disease. J Am Osteopath Assoc 96: 228–232

Heeringa MJ, d'Agostini F, DeBoer P, DaPrada M, Damsma G (1997) Effect of monoamine oxidase A and B and of catechol-O-methyltransferase inhibition on L-DOPA-induced circling behavior. J Neural Transm 104: 593–603

Hefti F, Melamed E, Wurtman RJ (1980) Partial lesions of the dopaminergic nigrostriatal system in rat brain: biochemical characterization. Brain Res 195: 123–137

Heikkila RE, Cabbat FS, Manzino L, Duvoisin RC (1981) Potentiation by deprenyl of 1-dopa induced circling in nigral-lesioned rats. Pharmacol Biochem Behav 15: 75–79

Heinonen EH, Lammintausta R (1991) A review of the pharmacology of selegiline. Acta Neurol Scand 84 [Suppl] 136: 44–59
Hudson JL, van Horne CG, Strömberg Y, Brock S, Clyton J, Masserano J, Hofferm BJ, Gerhardt GA (1993) Correlation of apomorphine- and amphetamine-induced turning with nigrostriatal dopamine content in unilateral 6-hydroxydopamine lesioned rats. Brain Res 626: 167–174
Knoll J (1978) The possible mechanism of action of (−)deprenyl in Parkinson's disease. J Neural Transm 43: 177–198
König JFR, Klippel RA (1963) The rat brain: a stereotaxic atlas of the forebrain and lower parts of the brain stem. Williams & Wilkins, Baltimore
Lange KW, Riederer P, Youdim MBH (1994) Biochemical actions of l-deprenyl. Clin Pharmacol Ther 56: 734–741
Marsden CD (1990) Parkinson's disease. Lancet i: 948–952
Pérez V, Marco JL, Fernández-Alvarez E, Unzeta M (1999a) Relevance of benzyloxy group in 2-indolyl methylamines in the selective MAO-B inhibition. Br J Pharmacol 127: 869–876
Pérez V, Marco JL, Fernández-Alvarez E, Unzeta M (1999b) Involvement of Ca2+ in dopamine release in striatal slices by PF9601N and 1-deprenyl. Neurobiology (in press)
Pycock CJ (1980) Turning behaviour in animals. Neuroscience 8: 461–514
Reynolds GP, Elsworth JD, Blau K, Sandler M, Lees AJ, Stern GM (1978) Deprenyl is metabolized to methamphetamine and amphetamine in man. Br J Clin Pharmacol 6: 542–544
Schwarting RKW, Huston JP (1996) The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments. Prog Neurobiol 50: 275–331
Ungerstedt U (1971) Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. Acta Physiol Scand 367: 69–93
Watchel SR, Abercrombie DE (1994) L-3,4-Dihydroxyphenylalanine-induced dopamine release in the striatum of intact and 6-hydroxydopamine-treated rats: differential effects of monoamine oxidase A and B inhibitors. J Neurochem 63: 108–117

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