Biochemical Pharmacology, Vol. 36, No. 10, pp. 1731–1735, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

EFFECTS OF DIFFERENT MONOAMINE OXIDASE INHIBITORS ON THE METABOLISM OF L-DOPA IN THE RAT BRAIN

NGUYEN T. BUU* and MONIQUE ANGERS

Laboratory of the Autonomic Nervous System, Clinical Research Institute of Montreal, and Université de Montréal, Montreal, Quebec, Canada H2W 1R7

(*Received* 1 *July* 1986; *accepted* 25 *November* 1986)

Abstract—The influence of monoamine oxidases A and B on the metabolism of dopamine or the expanded dopamine pool following L-dopa administration remains unclear. This study found that treatment of Sprague–Dawley rats with monoamine oxidase inhibitors strongly affected L-dopa metabolism in the brain, but the influence varied with each individual inhibitor. In animals pretreated with pargyline or clorgyline, L-dopa administration led to huge accumulations of dopamine and significantly raised central norepinephrine concentrations. In contrast, similar L-dopa injections in deprenyl-pretreated rats caused only a moderate rise in dopamine and no change in norepinephrine. There seems to be little relationship between the degree of monoamine oxidase inhibition and the accumulation of catecholamines and their metabolites in the rat brain. The effects of monoamine oxidase inhibitors on dopamine accumulation appeared to occur outside the catecholaminergic neurons since in the animals pretreated with 6-hydroxydopamine, which decreased significantly the content of brain catecholamines, dopamine accumulation following L-dopa administration still remained considerable. On the other hand, the influence of monoamine oxidase inhibitors on brain norepinephrine concentrations seemed to originate in the noradrenergic neurons because norepinephrine increase was greatly reduced in rats treated with 6-hydroxydopamine but was restored when the treatment with 6-hydroxydopamine was accompanied by desimipramine which specifically protects noradrenergic stores.

Despite more than two decades of levodopa use in the treatment of patients with Parkinson's disease, certain aspects of the metabolism of L-dopa in the brain remain unresolved. For instance the effects of L-dopa on norepinephrine (NE) concentrations [1–3], as well as the role of catechol-*O*-methyltransferase [4] in such a metabolism, are not yet clear. Of particular interest are the effects of inhibitors of monoamine oxidase (MAO) in the central metabolism of L-dopa. This interest arises because, on the one hand, monoamine oxidase inhibitors, because of their blocking of the inactivation of catecholamines, can potentiate the efficacy of levodopa treatment. On the other hand, monoamine oxidase inhibitors caused such adverse effects [5] in patients that their use with L-dopa had to be discontinued.

However, several forms of MAO (i.e. type A and type B) have been described in a variety of tissues, including brain [6, 7]. Recently, a selective inhibitor of MAO-B, deprenyl, has been found to produce no adverse effects when administered together with levodopa [8]. The reasons for the difference between the influence of these MAO inhibitors on L-dopa metabolism were not clear. There has not been, to our knowledge, any study which compared the metabolism of L-dopa in the normal brain with that in the brain of animals treated with MAO inhibitors. Such a study may reveal metabolic factors that may be associated with the side effects of the combined treatment of L-dopa with such inhibitors in Parkinsonian patients.

In the present study, we measured catecholamines (CA) and CA metabolites in the brains of normal rats and rats pretreated with specific and nonspecific MAO inhibitors after L-dopa injections. The results indicated that not only important differences in dopamine (DA) accumulation were found in the brain of animals treated with different inhibitors but differences in the accumulations of NE and CA metabolites exist between these differently treated rats.

MATERIALS AND METHODS

Materials

DA hydrochloride, L-dopa, pargyline hydrochloride, 6-hydroxydopamine hydrochloride, desimipramine hydrochloride and heptanesulfonate were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.) Clorgyline hydrochloride and L-deprenyl hydrochloride were from Research Biochemicals Inc. (Wayland, MA, U.S.A.). Acetonitrile (HPLC grade) was from BDH Chemicals (Montreal, Canada), and Sephadex G-10 from Pharmacia Chemicals (Uppsala, Sweden). All other chemicals of the highest purity (analytical grade) were purchased from the Fisher Scientific Co. (Fairlawn, NJ, U.S.A.). [^{3}H]Hydroxytryptamine binoxalate (29 Ci/mmol) and [^{14}C]phenylethylamine

* Correspondence: Nguyen T. Buu, Ph.D., Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec H2W 1R7, Canada.

Table 1. Concentrations of DA, NE and their metabolites in brain tissues of rats injected with different MAO inhibitors

	Dose (mg/kg)	DA	NE	NMN	3-MT
			(ng/g tissue)		
Hypothalamus					
Control		299 ± 41	928 ± 135	ND*	ND
Pargyline	80	331 ± 20	1602 ± 155†	201 ± 25†	119 ± 17†
Clorgyline	4	352 ± 33	1745 ± 22†	75 ± 8†	56 ± 5†
Clorgyline	8	324 ± 38	1498 ± 146†	81 ± 17†	49 ± 11†
Deprenyl	1	244 ± 29	985 ± 46	ND	ND
Deprenyl	10	266 ± 18	994 ± 64	ND	ND
Striatum					
Control		3488 ± 520	208 ± 27	ND	58 ± 9
Pargyline	80	4107 ± 684	309 ± 37†	273 ± 37	596 ± 79
Clorgyline	4	6377 ± 852†	388 ± 83†	72 ± 14	517 ± 57
Clorgyline	8	5590 ± 583†	376 ± 43†	123 ± 27	517 ± 63
Deprenyl	1	3656 ± 590	214 ± 17	ND	72 ± 8
Deprenyl	10	4289 ± 251	207 ± 32	ND	122 ± 21

Values are means ± SEM. There were seven rats in each group. Rats were killed by decapitation 20 hr following injection of the drug.

* Not detectable by the present method of HPLC.

† Statistically significant ($P < 0.05$) when compared to control group.

hydrochloride (50 mCi/mmol) were from New England Nuclear (Boston, MA, U.S.A.).

Treatment with MAO inhibitors

Male Sprague–Dawley rats weighing approximately 200–250 g were used for this study. They were injected intraperitoneally with saline (control group) or with different doses of MAO inhibitors dissolved in 0.9% saline solution. Twenty hours later the rats were injected intraperitoneally with L-dopa (100 mg/kg). Rats were killed by decapitation 60 min after the second treatment, and the brains were quickly excised and dissected on ice along the demarcations described by Glowinski and Iversen [9]. The brain tissues were frozen on Dry Ice and stored at −80° until analysis. The time between decapitation and freezing of tissue was approximately 1 min.

Treatment with 6-hydroxydopamine and desimipramine

To determine whether the changes in DA metabolism occurred inside or outside noradrenergic neurons, rats were injected intraventricularly (i.v.t.) with 6-hydroxydopamine (200 μg) freshly dissolved in 20 μl of 0.9% saline containing 0.1% ascorbic acid. Other rats were injected with desimipramine (30 mg/kg, i.p.), an inhibitor of NE uptake [10], 1 hr before the intraventricular injection of 6-hydroxydopamine. Seven days after the treatment with the neurotoxins, the rats were injected with pargyline or with pargyline and L-dopa as described above. Control rats received saline. All rats were killed 2 hr after pargyline or 1 hr after L-dopa.

Methods of analysis

Tissue treatment. Before analysis, each tissue was homogenized in ten times its weight of 0.1 M perchloric acid. The homogenate was centrifuged at 15,000 *g* for 20 min, and the supernatant fraction was used for determination of DA, NE, dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and normetanephrine (NMN).

Measurement of DA, NE, NMN, 3-MT, DOPAC. The tissue extracts were eluted through Sephadex G-10 columns (5 × 70 mm) prepared in Pasteur pipettes as described by Westerink [11], and the CA and their metabolites were measured by reverse phase HPLC with electrochemical detection.

Monoamine oxidase activities. Whole brain was homogenized in 10% (w/v) 0.08 M phosphate buffer (pH 7.2) and centrifuged at 900 *g* for 10 min at 4°. The supernatant fraction was measured for MAO-A and MAO-B activities by the method of Campbell *et al.* [12] using [^{3}H]5-hydroxytryptamine as substrate for MAO-A and [^{14}C]phenylethylamine for MAO-B. Protein was measured by the method of Lowry *et al.* [13].

Statistical analysis. Statistical comparisons between two groups of rats in each series of experiments used the unpaired Student's *t*-test (two-tailed). When comparisons were made between several groups the Neuman–Keuls test was used. In all cases, a $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the effects of different MAO inhibitors on the concentrations of DA, NE, NMN and 3-MT in the hypothalamus and striatum. With the exception of deprenyl, all other MAO inhibitors induced significant increases in NE in the hypothalamus and to a lesser extent in the striatum. Surprisingly, in the hypothalamus the increases in NE were not accompanied by any change in DA levels. In the striatum, only clorgyline raised significantly the levels of DA, whereas deprenyl and pargyline had no effect. These results are, in general, in agreement with those of Yang and Neff [14] who

Table 2. Inhibition of MAO-A and MAO-B activities by different MAO inhibitors

Inhibitors	Dose (mg/kg)	MAO-A (% inhibition)	MAO-B
Pargyline	80	84	85
Clorgyline	4	88	0
Clorgyline	8	92	7
Deprenyl	0.5	7	75
Deprenyl	1.0	13	87
Deprenyl	10	35	91

Activities were measured in whole brain 20 hr after the injection of the drugs. There were six rats in each group.

found that clorgyline but not deprenyl raised significantly central NE in the rats. However, these authors reported an increase in DA concentrations in the brains of rats treated with either clorgyline or deprenyl. The discrepancy between their finding pertaining to DA and ours may be due to the different mode of drug administration and to the fact that these authors measured whole brain concentrations where DA levels exceeded NE by a ratio of 3 to 1. Both clorgyline and pargyline caused significant increases in NMN and 3-MT which became measurable in the hypothalamus and striatum. By contrast, deprenyl produced neither an increase in NE nor a change in NMN and 3-MT. There was no appreciable difference in CA and metabolite levels between rats treated with clorgyline at doses of 4 and 8 mg/kg, or with deprenyl at doses of 1 and 10 mg/kg. Similar changes in CA and their metabolites were also observed when the rats were killed only 2 hr following their treatment with similar doses of MAO inhibitors (results not shown).

Table 2 shows that clorgyline had no appreciable effect on MAO-B activity, whereas deprenyl, predominantly an inhibitor of MAO-B activity, exhibited increasing mixed effects at higher doses. Thus, at 0.5 mg/kg, deprenyl strongly (75%) inhibited MAO-B but had only a weak effect (7%) on MAO-A, whereas at 10 mg/kg it inhibited significantly both MAO-A (35%) and MAO-B (91%). Pargyline, a mixed inhibitor, strongly inhibited both MAO-A and MAO-B activities. In a separate experiment, when MAO-A and MAO-B activities were measured in the brain of rats killed 2 hr following the injections with these same inhibitors, the degree of inhibition of the respective enzyme (results not shown) was, in general, lower and less consistent than results presented in this table (except for pargyline), due perhaps to distribution of the drug or interference of the drug with the enzyme assay.

Table 3 shows the effects of these MAO inhibitors on the metabolism of L-dopa. All MAO inhibitors provoked at approximately the same degree of enzyme inhibition (80%) significant increases in DA accumulation following L-dopa administration, but the degree of this increase varied greatly from inhibitor to inhibitor. Pargyline caused the highest DA accumulation, whereas deprenyl produced the lowest accumulation which remained nonetheless 2–3 times higher than control levels. The DA increase in the deprenyl-treated rats was not accompanied by any increase in NE, NMN or 3-MT. On the other hand, the DA increase in the clorgyline- and pargyline-treated rats was accompanied by significant increases in NE, NMN and 3-MT. Surprisingly, after L-dopa the concentrations of DOPAC in the deprenyl- and clorgyline-treated rats were not different from those of control rats, indicating that DA metabolism by MAO was not reduced significantly in rats in which MAO-A or MAO-B were strongly inhibited (Table 2). This is consistent with an earlier report [15] indicating that neither clorgyline nor deprenyl alone reduced synaptosomal DA deamination.

To determine the site of action of the MAO inhibitors on L-dopa metabolism, the above experiment was repeated in rats that had been treated with the neurotoxin 6-hydroxydopamine (6-OHDA) to destroy catecholaminergic neurons or with the combination of 6-OHDA and desimipramine, which is designed to protect specifically the noradrenergic stores [10] against the action of 6-OHDA. Results presented in Table 4 indicate that destruction of the catecholaminergic neurons did not lead to any decrease in DA accumulation after L-dopa. The addition of desimipramine to the treatment did not affect the DA accumulation. 6-Hydroxydopamine treatment, on the other hand, sharply reduced both the NE concentrations in brain and the NE increase following pargyline and L-dopa treatment. Protection of the NE store with desimipramine restored the NE increase to levels observed in the control rats. It may be of interest to notice that unlike the intact rats (Table 3) the rats treated with 6-OHDA had measurable levels of NMN and 3-MT.

DISCUSSION

The present results demonstrate the profound effect of MAO inhibitors on the metabolism of L-dopa in the brain. The most noticeable effect was the large accumulation of DA in the rats in which MAO was inhibited by pargyline and clorgyline. The DA increase after L-dopa was much higher than that observed in rats treated only with L-dopa, or with L-dopa and a peripheral dopa decarboxylase inhibitor [3], underlining the importance of central MAO in the metabolism of the expanded L-dopa/DA pool. The increase was much more marked in the hypothalamus (10–30 times as much as basal levels) than in the striatum (almost 2-fold) which suggests that the hypothalamus was more exposed to exogenous DA than the striatum. This DA accumulation originated outside the catecholaminergic neurons since treatment with the neurotoxin 6-OHDA did not prevent its occurrence.

There were nonetheless marked differences in the effects of individual MAO inhibitors on DA accumulation following L-dopa. Deprenyl induced a smaller DA increase than either clorgyline or pargyline. It is clear that the different effects of MAO inhibitors on DA accumulation were not directly related to their potency in inhibiting MAO-A and MAO-B activities in the brain. Thus, at approximately the same degree of MAO-A inhibition, DA accumulation was twice higher in pargyline-treated than in clorgyline-treated rats (Table 3). The reasons for the

Table 3. Concentrations of DA, NE and their metabolites following L-dopa administration in the rats pretreated with different MAO inhibitors

	DA	NE	NMN (ng/g tissue)	3-MT	DOPAC
Hypothalamus					
L-Dopa alone	1,113 ± 127	1,022 ± 44	ND	ND	6,578 ± 716
Pargyline + L-dopa	9,136 ± 396*	1,562 ± 100*	618 ± 33*	4,730 ± 201*	3,597 ± 420*
Clorgyline + L-dopa	5,252 ± 261*	2,001 ± 175*	486 ± 87*	2,476 ± 198*	8,179 ± 1,660
Deprenyl + L-dopa	1,277 ± 105	1,035 ± 93	ND	ND	7,511 ± 533
Striatum					
L-Dopa alone	8,740 ± 172	189 ± 19	ND	52 ± 21	11,035 ± 970
Pargyline + L-dopa	20,340 ± 1,029*	576 ± 38*	285 ± 21*	5,656 ± 148*	3,088 ± 181*
Clorgyline + L-dopa	25,110 ± 3,670*	468 ± 55*	219 ± 21*	2,860 ± 434*	5,390 ± 788*
Deprenyl + L-dopa	7,848 ± 938	206 ± 8	ND	35 ± 25	9,857 ± 876

Values are means ± SEM. The number of rats in each group was eight. The doses of pargyline, clorgyline and deprenyl used in this experiment were 80, 4 and 1 mg/kg respectively. Results with other doses (25 mg/kg of pargyline, 4 mg/kg clorgyline and 10 mg/kg deprenyl) were comparable to those reported in this table. All rats were killed 20 hr after the injection of the MAO inhibitor and 1 hr after L-dopa. ND = not detectable.

* Statistically significant ($P < 0.05$) from L-dopa treated group.

Table 4. Effects of pretreatment with 6-hydroxydopamine (6-OHDA) or the combined pretreatment with 6-OHDA and desimipramine on the DA, NE and NMN concentrations of rats injected with saline or with pargyline and L-dopa

	DA	NE	NMN (ng/g tissue)	3-MT
Hypothalamus				
Control (saline)	499 ± 31	975 ± 103	ND	ND
6-OHDA	184 ± 26*	345 ± 34*	85 ± 6*	164 ± 13*
6-OHDA pretreated + pargyline	323 ± 36*†	382 ± 49*	130 ± 9*	204 ± 27*
6-OHDA pretreated + pargyline + L-dopa	24,900 ± 1,100*†	782 ± 56†	213 ± 22*†	7,620 ± 265*†
6-OHDA + desimipramine pretreated	257 ± 17*	1,100 ± 25*†	101 ± 21*	128 ± 128*
6-OHDA + desimipramine pretreated + pargyline + L-dopa	24,600 ± 1,293*†	1,420 ± 373†	472 ± 26*†	8,980 ± 360*†
Saline pretreated + pargyline + L-dopa	12,600 ± 1,030*†	1,840 ± 53*†	759 ± 90*†	9,580 ± 500*†

6-Hydroxydopamine (200 μg, i.v.t.) and desimipramine (30 mg/kg, i.p.) were injected into the rats 7 days before their treatment with pargyline and L-dopa. DOPAC values were not detectable in the hypothalamus of control rats and in the rats treated with 6-OHDA. Values are means ± SEM. There were six to eight rats in each group.

* Statistically significant ($P < 0.05$) when compared to control group.

† Statistically significant ($P < 0.05$) when compared to the 6-OHDA group.

difference are unknown, but these results suggest that the influence of the inhibitor on DA accumulation may be through some other mechanism than that of reducing DA metabolism.

Deprenyl, the only MAO inhibitor still being used in conjunction with L-dopa in the treatment of Parkinson's disease [8], had no effect on NE levels, in agreement with a previous study [14]. Thus, unlike the other MAO inhibitors, its injection alone or in conjunction with L-dopa did not change NE levels in the rat brain. The lack of NE response was not due to lack of precursor DA since after L-dopa injection the DA concentrations in the hypothalamus were 3 times higher than control levels. In previous studies it has been shown that, in the brain of rats not treated with a MAO inhibitor, there is little relationship between DA and NE levels [2, 3, 16]. The reasons why deprenyl promotes DA accumulation without affecting NE levels remain to be determined but may explain why it is the only MAO inhibitor still being in use in conjunction with L-dopa for treating Parkinsonian patients [8]. In contrast to deprenyl, pargyline and clorgyline strongly affected NE levels in the brain. There was a significant increase in NE concentrations in the rats treated with the inhibitors alone and, when these rats were further treated with L-dopa, the NE increase became even greater, which indicates that in these rats DA levels may influence NE levels. The effect of the MAO inhibitors on NE concentrations most probably occurs within the noradrenergic neurons since prior treatment with 6-OHDA greatly reduced this effect whereas concurrent pretreatment with desimipramine restored the full effect.

The finding of high concentrations of DOPAC in the brain of rats in which MAO activity was strongly inhibited was unexpected. In view of an earlier report that clorgyline and deprenyl did not reduce synaptosomal DA deamination [15], the presence of these huge levels of DOPAC may indicate that brain DOPAC is formed mostly at the synaptic level.

Because both NMN and 3-MT can be further metabolized by MAO, their increase in the brain of rats treated with MAO inhibitors was not surprising. However, inhibition of metabolism alone may not account for the increase in these metabolites, for deprenyl did not provoke any increase in NMN and 3-MT in contrast to clorgyline. Moreover, as the results with DOPAC concentrations seemed to suggest, deamination at the synaptic level was not reduced significantly and, therefore, the marked increase in 3-MT and NMN in the brains of rats treated with pargyline or clorgyline may not be totally due to reduced deamination. On the other hand, since 3-MT and NMN are markers of DA and NE release [17], respectively, their increase may be indicative of possible effects of these MAO inhibitors (e.g. pargyline, clorgyline) on CA release. It is interesting that inhibitors that promote NE increases (e.g. pargyline, clorgyline) also induce increases in NMN and 3-MT, whereas an inhibitor (e.g. deprenyl) which did not affect NE also had no effect on these metabolites. Since NE synthesis (through dopamine-β-hydroxylase) and CA release (through exocytosis) occur at the vesicular level, it is tempting to speculate that pargyline and clorgyline influence the vesicular accumulation of CA, whereas deprenyl does not.

In summary, our findings show that different MAO inhibitors affect DA and NE accumulation, and that of their metabolites, differently. Among these inhibitors, deprenyl had the least effect on DA accumulation and no effect on NE levels. The difference in their effects on L-dopa metabolism does not appear to be related solely to their inhibitory action on MAO-A and MAO-B activities. Finally, the results show that DA is generated from L-dopa outside catecholaminergic neurons.

Acknowledgements—The authors thank Dr. Theodore Sourkes for the gift of clorgyline and L-deprenyl which helped to start this study and Mrs. Sylvie Ouellet for typing the manuscript. This work was supported by funds from the Medical Research Council of Canada and the Canadian Heart Foundation (Québec).

REFERENCES

1. J. P. Chalmers, R. J. Baldessarini and R. J. Wurtman, *Proc. natn. Acad. Sci. U.S.A.* **68**, 662 (1971).
2. P. Doshi and D. J. Edwards, *J. Chromat.* **210**, 505 (1981).
3. N. T. Buu, J. Duhaime and O. Kuchel, *J. Neurochem.* **44**, 787 (1985).
4. N. T. Buu, *J. Neurochem.* **45**, 1612 (1985).
5. A. Fletscher, *Pharmac. Rev.* **18**, 121 (1966).
6. H. C. Kim and A. D'Iorio, *Can. J. Biochem.* **46**, 295 (1968).
7. M. B. H. Youdim, G. G. S. Collins and M. Sandler, *Nature, Lond.* **223**, 626 (1969).
8. J. Knoll, *J. neural Transm.* **43**, 177 (1978).
9. J. Glowinski and L. L. Iversen, *J. Neurochem.* **13**, 655 (1966).
10. M. F. Callahan, M. Beales, G. Oltmans, S. A. Berenbaum, P. E. Meyers, G. Pullen and T. Hansen, *Soc. Neurosci. Abstr.* **8**, 428 (1982).
11. B. H. C. Westerink, *J. Neurochem.* **42**, 934 (1984).
12. I. C. Campbell, D. S. Robinson, W. Lowenberg and D. L. Murphy, *J. Neurochem.* **32**, 49 (1979).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. H. Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **189**, 733 (1974).
15. A. J. Azzaro, J. King, J. Kotzuk, D. D. Schoepp, J. Frost and S. S. Schochet, *J. Neurochem.* **45**, 949 (1985).
16. C. R. Freed and R. C. Murphy, *J. Pharmac. exp. Ther.* **205**, 702 (1978).
17. W. Kehr, *J. neural Transm.* **50**, 165 (1981).