Biochemical Pharmacology, Vol. 38, No. 10, pp. 1685–1692, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

MODIFICATION OF VESICULAR DOPAMINE AND NOREPINEPHRINE BY MONOAMINE OXIDASE INHIBITORS

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(*Received* 23 *May* 1988; *accepted* 19 *October* 1988)

Abstract—The possible effects of inhibitors of the two forms of monoamine oxidase (types A and B) on dopamine (DA) and norepinephrine (NE) accumulation and metabolism in the cytoplasmic and microsomal (vesicular) fractions of the rat brain have been examined. It was found that, while L-DOPA treatment raised only cytoplasmic DA without affecting vesicular DA and NE, clorgyline and pargyline treatments caused significant increases in DA and NE concentrations in both cytoplasmic and vesicular fractions. The DA increase in the synaptic vesicles (200–600%) was much more pronounced than that (150%) in the cytoplasm. In contrast, deprenyl treatment increased vesicular DA only slightly without any effect on either vesicular or cytoplasmic NE. L-DOPA administration to rats pretreated with clorgyline and pargyline, but not with deprenyl, further increased cytoplasmic and vesicular DA and NE concentrations. However, excessive increases in vesicular DA lowered vesicular NE. Reserpine drastically reduced vesicular and cytoplasmic DA and NE, and L-DOPA administration to the reserpine-treated rats caused a DA increase only in the cytoplasmic fraction without affecting vesicular DA or NE. The effect of reserpine was abolished by pargyline treatment, which suggests that pargyline may interact with the reserpine-sensitive vesicular uptake. There was a significant correlation between vesicular DA and NE increase.

The interest in inhibitors of monoamine oxidase (MAO: EC 1.4.3.4) is due to the prominence of MAO in the regulation of monoamine levels in the neuronal tissue. It also stems from the fact that, despite their potential clinical importance, the therapeutic use of most of these inhibitors remains severely curtailed because of their side effects. In the metabolism of catecholamines (CA), the mechanism and sites of interaction of inhibitors of the two forms of MAO, MAO-A and MAO-B [1], with the metabolism of dopamine (DA) and norepinephrine (NE) are not yet clear. Previous studies [2, 3] have shown that, although DA and NE are substrates for both forms of the enzyme, clorgyline, an inhibitor of MAO-A, exerts a marked effect on catecholamine levels in the rat brain whereas deprenyl, an inhibitor of MAO-B, has no significant effect. Of particular interest is the influence of the inhibitors, in particular inhibitors of MAO-A, on NE concentrations. MAO inhibitors could affect NE concentrations strongly without having any significant effect on DA [4], although both CA exhibit similar affinity for the enzyme [5]. Furthermore, we have found that prior treatment of rats with an MAO-A inhibitor, but not an MAO-B inhibitor, enhances NE increase following L-DOPA administration [6], whereas administration of L-DOPA alone does not result in any change in NE in rat brain [7], in agreement with other studies [8–11]. The mechanism of this NE enhancement has not been elucidated, but we have proposed [6] that the NE increase is due primarily to increased access of DA to dopamine-β-hydroxylase (DβH), the enzyme responsible for the conversion of DA to NE. Conversely, the absence of an NE increase following L-DOPA administration has been attributed to a lack of entry of DA into the storage vesicle [8, 9] where DβH is exclusively located [12].

The NE increase induced by MAO inhibition may possibly be related to an interaction of the inhibitor with the transvesicular transport of DA. Surprisingly, to our knowledge the effects of MAO inhibitors on the vesicular accumulation of DA and NE have not been studied, although methods for the preparation of viable synaptic vesicle fraction [13, 14] are available. Such a study might reveal whether the different NE responses to deprenyl and clorgyline treatment could be due to the effects of these drugs on DA and NE accumulation at the synaptic vesicles. In addition, it could indicate whether the lack of NE increase following L-DOPA administration is due to lack of entrance of the exogenous DA into the storage vesicle, as suggested by some studies [7–9], or to a displacement of NE from its storage sites by the excess of DA, as proposed by others [10].

MATERIALS AND METHODS

Materials

DA hydrochloride, L-DOPA, pargyline hydrochloride, reserpine and heptane sulfonate were purchased from the Sigma Chemical Co. (St Louis, MO). Clorgyline hydrochloride and L-deprenyl hydrochloride were purchased from Research Chemicals Inc. (Wayland, MA). Acetonitrile (HPLC grade) was from BDH Chemicals (Montreal, Canada), and alumina from Woehm Pharma

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(Eschewe, F.R.G.). All other chemicals of the highest purity were purchased from the Fisher Scientific Co. (Fair Lawn, NJ).

Treatment with MAO inhibitors and L-DOPA

Male Sprague–Dawley rats weighing approximately 175–250 g were used for this study. They were injected intraperitoneally with saline (control group) or with different MAO inhibitors. The doses of the inhibitors (pargyline–HCl, 30 or 80 mg/kg; clorgyline–HCl, 8 mg/kg; *l*-deprenyl–HCl, 1 mg/kg) were chosen to obtain approximately 80–90% inhibition of the enzyme activity [2] in the rat brain. Twenty hours after the MAO inhibitor treatment, the rats were injected with L-DOPA dissolved in 0.01 M HCl or with the vehicle. Sixty minutes after the second treatment, the rats were killed by decapitation, and their brains were quickly excised on ice for the preparation of the synaptic vesicle.

Treatment with reserpine

To determine whether the catecholamines in the vesicular fraction represent the catecholamine content in the synaptic vesicle, a group of rats were injected with reserpine (5 mg/kg, i.p.) dissolved in 0.01 M HCl. Twenty hours later, the rats were either injected with L-DOPA or with the vehicle and were killed by decapitation 60 min after the last injection.

In another series of experiments, to determine whether the MAO inhibitor pargyline interacts with the reserpine-sensitive vesicular uptake of CA, groups of reserpine-treated rats were injected 5 hr later with pargyline at a dose of 80 mg/kg which gave more consistent results than a lower dose (30 mg/kg). Twenty hours after the pargyline treatment, the rats were injected either with L-DOPA or the vehicle and were killed 60 min later. The whole brains were excised quickly and used for preparation of synaptic vesicles.

Preparation of synaptic vesicles

Synaptic vesicle fractions were prepared from whole brains by the method of Ruth *et al.* [14]. Fresh brain tissue was homogenized in 5 vol. of cold buffer (100 mM potassium tartrate, 3 mM potassium bicarbonate, 1 mM potassium chloride, 1 mM magnesium chloride, 4 mM potassium phosphate, 10 μM iproniazid phosphate, 10 μM ascorbic acid and 1 μM EDTA) adjusted to pH 7.5 with potassium hydroxide solution, in a hand-held Dual Teflon/glass homogenizer. The suspension was centrifuged at 4° at 3000 *g* for 20 min. Aliquots of the supernatant fraction were preserved for CA measurement and protein measurements. CA concentrations in this fraction represent cytoplasmic CA concentration. The remaining supernatant fraction was sequentially centrifuged at 20,000 *g* for 30 min and finally at 100,000 *g* for 45 min to yield a crude vesicle pellet (P_3) and a supernatant fraction (S_3). The latter was gently washed twice with 3 ml of cold buffer, and in the third wash was gently resuspended in 1 ml of buffer before centrifugation at 100,000 *g* for 30 min. The pellet was again resuspended in 1 ml of buffer, and aliquots were sampled for protein measurement. The remaining suspension was acidified with 100 μl of 1 M $HClO_4$ containing 1% EDTA, homogenized with a Brinkmann Polytron in 0.1 N $HClO_4$, and analyzed for CA and CA metabolites. Concentrations of CA in this P_3 fraction represent vesicular CA, while those in the S_3 represent cytoplasmic CA. In a previous study [15] we found that more than 90% of radioactive NE or DA added to brain homogenates prior to the synaptic vesicle preparation was recovered intact in the cytoplasmic fraction, indicating that little, if any, CA was metabolized during the preparation.

Methods of analysis

Measurement of DA, NE and DOPAC. The homogenates from the cytoplasmic and vesicular fractions were adjusted to pH 8.4–8.6 with 1 M Tris–HCl, pH 9.6. Free CA and DOPAC were adsorbed onto 20 mg of acid-washed alumina [16]. After repeated washings with water, the alumina was eluted with 200 μl of 0.5 M acetic acid containing 1% EDTA. The eluate was injected directly into an HPLC system equipped with a reverse phase Bondapak C_{18} column (Chromatography Science, Mount Royal, Quebec, Canada) and an amperometric detector (LC-4A, BAS, West Lafayette, IN, U.S.A.) using a glossy carbon electrode. The composition of the mobile phase and the conditions of the assays were exactly as described before [17].

Protein determination. Protein was measured by the method of Lowry *et al.* [18] using bovine serum albumin as standard.

Statistical analysis

The unpaired Student's *t*-test (two-tailed) was used for statistical comparison. A $P < 0.05$ was considered statistically significant.

RESULTS

Figure 1 illustrates the response of DA, NE and DOPAC in the cytoplasmic and vesicular fractions prepared from whole brain of control rats and rats injected with two doses of L-DOPA. There was no change in either cytoplasmic or vesicular NE following L-DOPA, consistent with previous studies [7–10]. Although cytoplasmic DA increased with increased doses of L-DOPA, vesicular DA was not different and remained comparable to that of control rats. Cytoplasmic DOPAC increased in response to L-DOPA administration; the small amount of vesicular DOPAC did not change with either dose of L-DOPA. DA concentrations in the vesicular fraction were one-fifth those in the cytoplasmic fraction, whereas vesicular NE was twice as high as cytoplasmic NE. Table 1 shows the effects of inhibitors of MAO on cytoplasmic and vesicular concentrations of DA, NE and DOPAC following saline or L-DOPA administration. Treatment with clorgyline, at a dose capable of inhibiting 80% of the brain MAO activity [2, 6], only slightly increased cytoplasmic DA and NE but significantly increased vesicular DA and NE concentrations. L-DOPA (30 mg/kg) administration to rats pretreated with clorgyline raised cytoplasmic and vesicular DA and NE levels significantly ($P < 0.05$) above those in rats treated with clorgyline alone. There was also an increase in DOPAC comparable in magnitude to that observed in rats injected

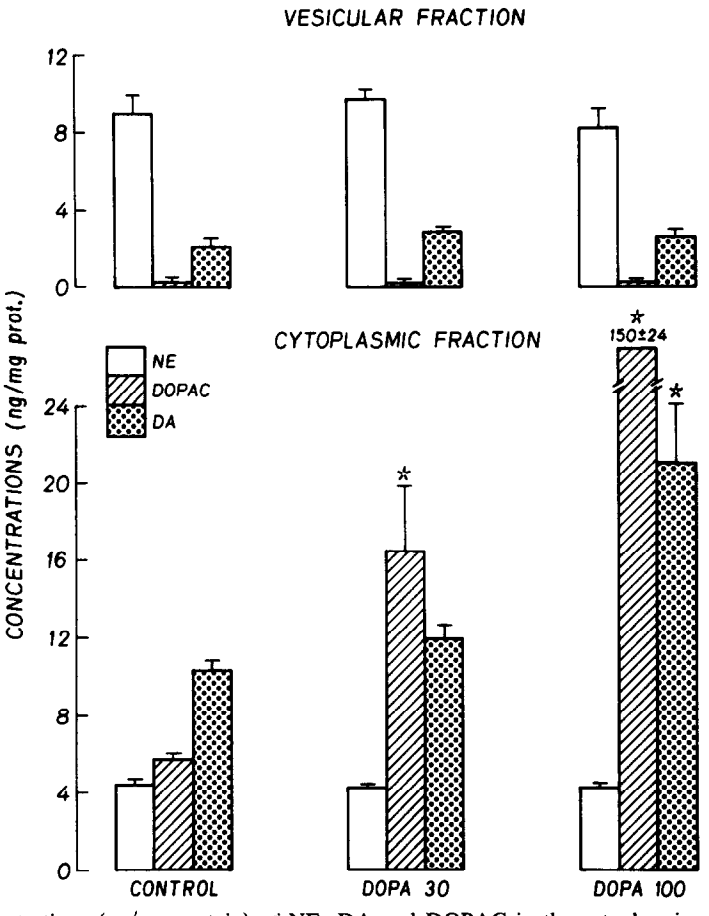

Fig. 1. Concentrations (ng/mg protein) of NE, DA and DOPAC in the cytoplasmic and vesicular fractions prepared from whole brain of rats injected with saline (control) or with different doses (30 or 100 mg/kg) of L-DOPA. The rats (four in each group) were killed by decapitation 60 min following L-DOPA administration. Values are means ± SEM. An asterisk (*) indicates $P < 0.05$ compared to controls. DOPAC was detected in two out of four vesicular fractions. Note the lack of DA increase in the vesicular fraction following L-DOPA administrations.

Table 1. Concentrations of DA, DOPAC and NE in the cytoplasmic and vesicular fractions prepared from whole brain of rats treated with saline, L-DOPA and/or different MAO inhibitors

Treatment	N	Concentrations (ng/mg protein)				
		Cytoplasmic fraction			Vesicular fraction	
		DA	DOPAC	NE	DA	NE
Saline	16	11.4 ± 0.9	5.7 ± 0.3	4.1 ± 0.2	1.9 ± 0.3	8.0 ± 0.4
L-DOPA	9	13.7 ± 0.4*	17.2 ± 2.1*	4.3 ± 0.4	2.9 ± 0.4	9.1 ± 0.6
Clorgyline	11	14.9 ± 1.6	ND†	5.5 ± 0.6*	4.8 ± 0.7*	12.2 ± 1.4*
Clorgyline + DOPA	10	26.6 ± 1.9*	13.5 ± 1.5*	8.3 ± 0.6*	8.4 ± 1.3*	14.8 ± 0.7*
Pargyline	10	15.8 ± 2.2	ND	5.9 ± 0.4*	16.1 ± 1.3*	15.8 ± 1.6*
Pargyline + DOPA	7	181.6 ± 38.8*	ND	11.3 ± 0.6*	37.4 ± 5.4*	17.1 ± 2.1*
Deprenyl	4	12.1 ± 0.3	3.8 ± 0.1	3.7 ± 0.1	2.6 ± 0.2	9.0 ± 0.8
Deprenyl + DOPA	4	14.2 ± 0.5*	22.3 ± 2.3*	4.5 ± 0.3	3.0 ± 0.5	8.1 ± 1.5

The doses of clorgyline, pargyline and deprenyl were: 10 mg/kg, 80 mg/kg and 1 mg/kg respectively. The DOPA dose was 30 mg/kg, injected intraperitoneally as described in Materials and Methods. Values are means ± SE; N denotes the number of rats in each group. DOPAC was not detectable in the vesicular fraction.

* Statistically significant difference compared to the control (saline) group ($P < 0.05$).

† Not detectable by the present method.

Table 2. Effects of reserpine treatment on concentrations of NE, DA and DOPAC in the cytoplasmic and vesicular fractions of rat brain following administration of L-DOPA or pargyline + L-DOPA

Treatment	Concentration (ng/mg protein)				
	Vesicular fraction		Cytoplasmic fraction		
	NE	DA	NE	DOPAC	DA
Saline	8.4 ± 0.7	2.1 ± 0.4	4.1 ± 0.3	4.7 ± 0.7	7.9 ± 0.9
Reserpine	0.7 ± 0.2*	1.1 ± 0.4*	ND†	4.6 ± 0.7	1.0 ± 0.5*
Reserpine + DOPA 30	0.5 ± 0.2*	1.2 ± 0.2*	0.2 ± 0.1*	16.9 ± 4.7	5.0 ± 1.1
Reserpine + DOPA 100	0.6 ± 0.1*	1.1 ± 0.2*	0.2 ± 0.1*	31.7 ± 6.2	26.5 ± 4.7
Reserpine + Pargyline + DOPA 100	6.1 ± 1.1	67.9 ± 8.1*	7.0 ± 0.9	ND	262.1 ± 31.3

Reserpine (5 mg/kg, i.p.) was injected 20 hr prior to L-DOPA administration. Pargyline (80 mg/kg) was administered 5 hr prior to L-DOPA administration. DOPA 30 and DOPA 100 denote the doses of L-DOPA (30 and 100 mg/kg respectively). Values are means ± SEM; there were five to seven rats in each group.

* $P < 0.05$ versus control saline group.

† Not detectable by the present method.

with L-DOPA alone, indicating that clorgyline did not reduce DA deamination despite its inhibition of MAO-A activity. Pargyline treatment gave the same results as clorgyline, except that in rats treated with pargyline vesicular DA was considerably higher than in clorgyline-treated rats. Although both doses (30 or 80 mg/kg) caused the same degree of inhibition of MAO-A and MAO-B and yielded comparable results (data not shown), only results obtained with the higher dose (80 mg/kg) were presented in this table to allow comparison with those obtained in previous studies [2, 6] and to be uniform with results presented in Table 2. Similarly, administration of L-DOPA to the pargyline-pretreated rats led to major increases in cytoplasmic and vesicular DA and NE. However, cytoplasmic DOPAC was significantly lower than in rats injected with L-DOPA alone. Deprenyl, on the other hand, did not change either cytoplasmic or vesicular NE, and injection of L-DOPA to rats previously treated with this inhibitor did not raise vesicular NE concentrations. There was, nevertheless, a significant increase in cytoplasmic DA and DOPAC.

That vesicular DA and NE concentrations do reflect the contents of DA and NE in the synaptic vesicles is suggested by results, presented in Table 2, showing that reserpine treatment drastically reduced vesicular DA and NE. When reserpine-treated rats were injected with L-DOPA, cytoplasmic DA and DOPAC increased significantly, but vesicular DA and NE remained unchanged, in agreement with the action of reserpine in blocking vesicular uptake. Reserpine-treated rats showed a smaller increase in cytoplasmic DA following L-DOPA administration than control rats, suggesting that uptake of L-DOPA and/or its conversion to DA was slower in the reserpine-treated rat. Administration of pargyline to the reserpine-pretreated rat prior to L-DOPA restored the accumulations of vesicular and cytoplasmic DA and NE to levels close to those observed in rats not receiving reserpine, indicating that pargyline treatment may have unblocked vesicular uptake of DA.

Figure 2 compares the percent increases of vesicular and cytoplasmic DA and NE in the brain of rats treated with different MAO inhibitors. The results indicate that, although clorgyline, pargyline and deprenyl induced similar increases in cytoplasmic DA (slightly above the control levels), their effects on vesicular DA concentrations were remarkably different. Thus, pargyline increased vesicular DA almost seven times, whereas deprenyl induced only a 50% increase. Elevations of vesicular DA were accompanied by significant increases in NE in both vesicular and cytoplasmic fractions. Pargyline increased NE more than clorgyline, while deprenyl had no effect on NE in either the vesicular or cytoplasmic fraction, consistent with previous reports [2, 3].

Figure 3 illustrates the relationship between vesicular DA and NE in the control rats and rats pretreated with monoamine oxidase inhibitors and injected with saline or L-DOPA (30 mg/kg). There was a statistically significant correlation between vesicular DA and NE suggesting that their increase may be related, in agreement with our previous report [6] showing that there was a highly significant correlation between tissue DA and NE in the pargyline-pretreated rats injected with different doses of L-DOPA. In contrast, there was no significant correlation between cytoplasmic DA and NE (results not shown) in these rats. However, the increase in vesicular NE was not directly proportional to vesicular DA increases. As shown in Table 3, the NE : DA ratio in the vesicular fraction did not remain constant but varied with different treatments. Thus, administration of different doses of L-DOPA did not alter significantly the NE : DA ratio, whereas clorgyline or pargyline treatment markedly lowered the ratio, suggesting that as vesicular DA increased its conversion to NE decreased.

Results presented in Table 4 show that excessive increases of vesicular DA could, however, trigger decreases in vesicular NE. Thus, in the rats pretreated with clorgyline and pargyline, those given a high (100 mg/kg) dose of DOPA exhibited lower vesicular NE than those receiving a lower (30 mg/kg) dose although their cytoplasmic NE levels were comparable. In contrast, in deprenyl-treated rats

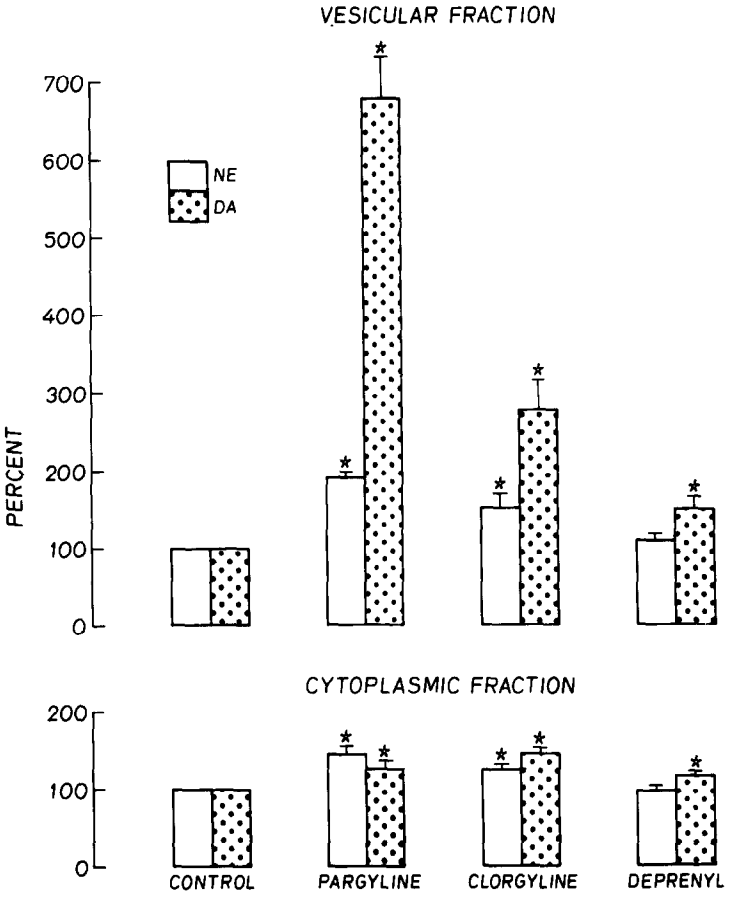

Fig. 2. DA and NE concentrations, expressed as percentage (±SEM) of control values, in the vesicular and cytoplasmic fractions following treatment with MAO inhibitors. See Table 1 for control values. The number of rats in each group and the doses of MAO inhibitors are given in Table 1. (*) $P < 0.05$ versus controls.

which showed no increase in vesicular DA following different doses of L-DOPA, vesicular NE remained unchanged after the L-DOPA treatments. The effect of a large increase of vesicular DA on vesicular NE was also apparent in the very low (0.15 and 0.84 respectively) ratio of vesicular NE/vesicular DA (Table 3) exhibited by the pargyline- and clorgyline-pretreated rats injected with L-DOPA in comparison with those in control rats (4.8) and in rats treated with L-DOPA (3.5) alone.

DISCUSSION

This study showed that treatment of the rat with inhibitors of MAO-A increased synaptic vesicular DA and NE contents several-fold over control levels. In contrast, inhibitors of MAO-B did not have the same effect: treatment of the rat with deprenyl, shown recently to have no significant effect on L-DOPA metabolism in the rat brain [2], raised vesicular DA concentrations only slightly and had no effect on vesicular NE.

To our knowledge there have been no previous reports on DA concentrations in the synaptic vesicles of the brain of rats injected with L-DOPA. It is totally unexpected to find that L-DOPA administration, which significantly raised DA in the brain tissues, in agreement with earlier reports [7–11], scarcely affected DA concentrations in the synaptic vesicles. This is unlikely to be due to a loss of DA during the preparation of the vesicular fraction, since in the same sort of preparation we observed net increases in vesicular DA in the rat treated with inhibitors of MAO-A despite their having similar cytoplasmic DA. Furthermore, in a previous study [15] we showed that neither DA nor NE was metabolized to any significant extent during the purification of the synaptic vesicles. Moreover, the predominance of NE over DA in these synaptic vesicles persisted in all the rats whether they were treated with saline, L-DOPA or MAO inhibitors, suggesting that this was not the result of different metabolism of NE and DA but that DA was located mainly in the cytoplasm and NE in the synaptic vesicle.

The mechanism responsible for the greater DA accumulations in the brain vesicular fraction induced by MAO-A inhibitors is not elucidated by the present data and remains to be studied further. It is, however, unlikely that it was solely a result of possible inhibitory action of these agents on DA degradation

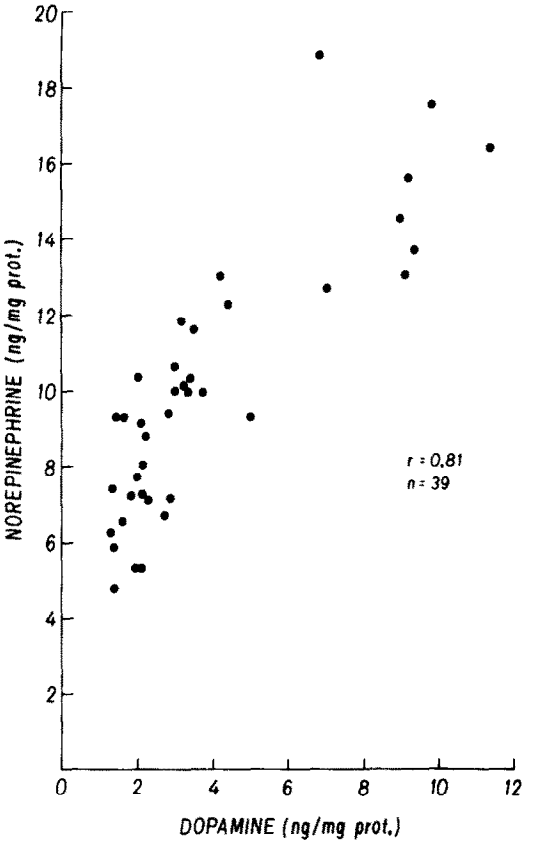

Fig. 3. Relationship between concentrations of DA and NE in the vesicular fractions of brains of control rats and rats pretreated with clorgyline (10 mg/kg), deprenyl (1 mg/kg) or pargyline (80 mg/kg) following saline of L-DOPA (30 mg/kg) administration. Treatments with MAO inhibitors and saline or L-DOPA were as described in Materials and Methods. In contrast to vesicular CA, there was no significant correlation between cytoplasmic DA and NE in these rats.

Table 3. NE:DA ratios in the synaptic vesicle of control (untreated) rats and rats injected with different MAO inhibitors and L-DOPA

Group	N	NE:DA
Control	16	4.84 ± 0.39
Clorgyline	11	2.75 ± 0.32*
Clorgyline + DOPA 30	10	1.58 ± 0.05*
Clorgyline + DOPA 100	6	0.84 ± 0.05*
Pargyline	10	1.05 ± 0.13*
Pargyline + DOPA 100	12	0.15 ± 0.05*
Deprenyl	4	3.42 ± 0.15*
Deprenyl + DOPA 30	4	2.94 ± 0.19*
Deprenyl + DOPA 100	4	2.59 ± 0.12*
DOPA 100	8	3.56 ± 0.46
DOPA 30	6	3.36 ± 0.19

The doses of the inhibitors were as described in Table 1. Values are means ± SE; N denotes the number of rats in each group.

* Statistically significant from control rats ($P < 0.05$).

in the synaptic vesicle, MAO being mainly a mitochondrial enzyme [19] and its activity located principally in the cytoplasm, although some residual activity has also been detected in the microsomal fraction [20]. Nevertheless, it was found that after L-DOPA administration all of the increase in DOPAC was in the cytoplasmic fraction, not in the vesicular fraction, confirming the extravesicular location of MAO activity. It was unexpected that clorgyline treatment, which inhibited MAO-A, the main form of MAO in the rat brain [21–23], did not reduce the DOPAC formation following L-DOPA administration. This is not likely due to a recovery of MAO activity 20 hr following clorgyline treatment. Previous studies [20, 24] have clearly shown that clorgyline, like pargyline, exerts a long-lasting (at least 2 days) inhibitory effect on MAO-A. However, it is entirely possible that inhibition of MAO-A by clorgyline and/or excessive DA increase in the brain could make DA more susceptible to MAO-B degradation [5], DA being a substrate for both MAO-A and MAO-B [3, 25]. Supporting such a view was the finding that in rats pretreated with pargyline, which inhibited both forms of MAO, there was no significant formation of DOPAC following L-DOPA administration.

The increased vesicular accumulation of DA induced by MAO-A inhibition was not simply a result of increased availability of DA in the cytoplasm, for L-DOPA administration to rats not pretreated with a MAO inhibitor caused cytoplasmic DA increases similar in magnitude to those induced by clorgyline or pargyline treatment but did not increase vesicular DA significantly. Moreover, vesicular DA concentrations did not always reflect cytoplasmic DA concentrations. For instance, while cytoplasmic DA levels in the brains of rats treated with clorgyline and pargyline did not differ significantly, vesicular DA concentrations in pargyline-treated rat brain were at least three times higher than those in clorgyline-treated rats. As cytoplasmic DA levels were similar in the brains of control rats and rats treated with these different MAO inhibitors, the higher vesicular DA shown by the MAO-inhibited rats suggested that MAO-A inhibitors induced a shift of cytoplasmic DA towards the synaptic vesicle. Why pargyline uptake of DA exhibited a more pronounced effect at the vesicular level than clorgyline is not known. Both have been found to exert the same degree of MAO inhibition in the rat brain [2] at doses similar to those used in this experiment. The fact that clorgyline pretreatment did not reduce DOPAC increases in the brain of rats following L-DOPA administration as much as did a pargyline pretreatment suggests that the extent of DOPAC formation in brain may be directly linked to vesicular accumulation of DA.

That inhibitors of MAO could affect vesicular uptake of catecholamines is not unexpected; it has been shown [26] that tranylcypromine treatment increased the size of vesicular noradrenaline stores in peripheral nerves of the rat. The present data support possible interaction between MAO inhibitors with vesicular uptake: pargyline treatment reduced or abolished the effect of reserpine on cytoplasmic and vesicular accumulations of DA before

Table 4. Effects of different doses of L-DOPA on concentrations of vesicular and cytoplasmic DA and NE in rats pretreated with MAO inhibitors

Treatment	Concentrations (ng/mg protein)			
	Vesicular fraction		Cytoplasmic fraction	
	NE	DA	NE	DA
Clorgyline + saline	12.2 ± 1.4	4.8 ± 0.7	5.4 ± 0.3	15.1 ± 0.8
Clorgyline + DOPA 30	15.1 ± 0.7*	9.6 ± 0.4*	8.3 ± 0.6*	26.6 ± 1.9*
Clorgyline + DOPA 100	11.4 ± 1.1	13.7 ± 1.4*	8.9 ± 0.4*	82.8 ± 7.0*
Deprenyl + saline	9.0 ± 0.8	2.6 ± 0.3	3.7 ± 0.1	12.1 ± 0.4
Deprenyl + DOPA 30	10.6 ± 1.3	3.6 ± 0.3	4.6 ± 0.3	14.2 ± 0.5*
Deprenyl + DOPA 100	8.0 ± 1.5	3.1 ± 0.5	4.4 ± 0.5	33.4 ± 0.7*

Doses were as in Tables 1 and 2. DOPA was injected 20 hr following the MAO inhibitor treatment. Rats were killed by decapitation 1 hr following L-DOPA. Values are means ± SE; there were four to five rats in each group.

* $P < 0.05$ versus controls.

and following L-DOPA administration. Thus, the greater vesicular accumulation of DA following treatment of rats with inhibitors of MAO-A may be due to an enhanced vesicular uptake of DA.

In addition to provoking a greater accumulation of DA in the synaptic vesicle, clorgyline and pargyline also considerably increased NE concentrations in the cytoplasmic and vesicular fractions of the rat brain. Whether the NE increase resulted from an enhanced vesicular accumulation of DA cannot be deduced from the present data. Nevertheless, there was a significant correlation between vesicular DA and NE in the brain of control rats and rats treated with MAO-A inhibitors. Conversely, in the brains of rats not treated with these inhibitors and in which L-DOPA administrations did not increase vesicular DA significantly, there was no change in either vesicular or cytoplasmic NE and no correlation between DA and NE, as previously reported [7]. The lack of any change in vesicular NE following L-DOPA administration also demonstrated that NE was not displaced from the synaptic vesicle by the L-DOPA-induced excessive increase in DA. That a relationship between vesicular DA and NE may exist is entirely consistent with the notion that the enzyme converting DA to NE, dopamine-β-hydroxylase, is located almost exclusively within the synaptic vesicle. Furthermore, other workers who found that brain NE did not change despite an expanded pool of DOPA-DA in the rat brain following L-DOPA injection had suggested that this may be due to a restricted entry of DA into the CA storage sites [8, 9]. A restricted transvesicular transport of DA has also been suggested to be the origin of the lower NE content in the failing hamster heart [27].

On the other hand, excessive increases in vesicular DA appeared to decrease vesicular NE. Thus, among the clorgyline-pretreated rats those injected with a higher dose of L-DOPA (100 mg/kg) had lower vesicular NE than those receiving a lower dose (30 mg/kg), although their cytoplasmic NE concentrations were similar. Rats pretreated with pargyline exhibited a similar pattern of response to higher doses of L-DOPA as clorgyline-pretreated rats, whereas those pretreated with deprenyl showed no response of vesicular NE to increases in brain DA. The lower vesicular NE is consistent with a possible displacement of NE from the storage sites by an excess of vesicular DA. In effect, we found [6] that L-DOPA administration to pargyline-pretreated rats leads to a marked increase in normetanephrine (NMN) in the brain tissues, indicating that the displaced NE was actively methylated by extraneuronal COMT. Moreover, this NMN increase correlates very closely with increases in 3-methoxytyramine (3-MT) [6], suggesting that the NMN increase is related to an increase in vesicular DA release in the rat brain. It should be noted that without the pretreatment with MAO-A inhibitors there was no change in NMN or 3-MT in the brain of rats following L-DOPA administration. Because NMN and 3-MT are markers of NE and DA release respectively [28], this suggests that there was no visible exocytotic release of NE and DA however elevated the levels of cytoplasmic DA. It also suggests that vesicular DA and NE and not cytoplasmic DA and NE may be responsible for the formation of 3-MT and NMN.

In summary, the results demonstrate that, in rats not treated with an MAO-A inhibitor, the DA increase from exogenous L-DOPA was mainly confined to an extravesicular pool, where it was metabolized almost exclusively by MAO. Inhibition of MAO-A, but not MAO-B, induced a greater vesicular accumulation of DA, and this vesicular DA increase may be responsible for the NE increase in rat brain tissues.

Acknowledgements—The author thanks Ms Monique Angers and Johanne Duhaime for their technical assistance. This work was supported by grants from the Medical Research Council of Canada, the Quebec Heart Foundation, and partly by the Parkinson Foundation of Canada.

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