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THE MONOAMINE OXIDASE INHIBITORS CLORGYLINE AND L-DEPRENYL ALSO AFFECT THE UPTAKE OF DOPAMINE, NORADRENALINE AND SEROTONIN BY RAT BRAIN SYNAPTOSOMAL PREPARATIONS

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Abstract—Clorgyline and L-deprenyl are, respectively, specific type A and type B monoamine oxidase (MAO) inhibitors. We investigated the effects of these two drugs as differential inhibitors of synaptosomal amine uptake and determined how far these effects might be predicted from their properties as specific MAO-A and MAO-B inhibitors. The rank order of inhibition of uptake by clorgyline was found to be: serotonin (IC_{50} = 10 μM) > dopamine (IC_{50} = 56 μM) > noradrenaline (IC_{50} = 66 μM). The rank order of inhibition of uptake by L-deprenyl was: noradrenaline (IC_{50} = 26 μM) > serotonin (IC_{50} = 460 μM) > dopamine (IC_{50} = 530 μM). The observation that clorgyline is a more specific inhibitor of the uptake of serotonin (a type A MAO substrate) is consistent with its activity as a type A MAO inhibitor. Paradoxically, L-deprenyl, though a type B MAO inhibitor, is fairly effective at inhibiting the uptake of noradrenaline (a type A MAO substrate).

In brain and other tissues the enzyme primarily responsible for the oxidative deamination of transmitter as well as non-transmitter amines is monoamine oxidase (MAO, E.C. 1.4.3.4) [1, 2]. There is increasing evidence to suggest that MAO may exist in more than one form, although the definitive nature of the multiple forms remains to be fully elucidated [2]. A useful concept has been the classification into two forms, first proposed by Johnston [3]. Type A preferentially deaminates transmitter amines such as serotonin and noradrenaline, and is particularly sensitive to inhibition by clorgyline [3]. Type B oxidizes non-transmitter amines, especially benzylamine and phenylethylamine, and is selectively inhibited by L-deprenyl [2]. Using specific type A and B inhibitors it has been shown that in rat brain *in vivo* serotonin and noradrenaline are metabolized by type A MAO, whereas phenylethylamine is deaminated by type B MAO [4, 5]. However, whether dopamine is preferentially oxidized by type A or type B (or both forms) of brain MAO *in vivo* is a matter of some controversy [4, 5].

Our initial observation (results reported in this paper) that clorgyline inhibited dopamine uptake by synaptosomes prompted us to examine whether any of the inhibitory effects that these inhibitors exerted on the amine transport systems in nerve-ending particles *in vitro* may be predicted on the bases of their known properties as specific inhibitors of the A and B forms of MAO [2]. We now report on the results of such studies.

MATERIALS AND METHODS

Whenever possible analytical grade (AR) chemicals were used and obtained from either BDH Chemicals Ltd., Enfield, U.K., or Sigma (London) Ltd., Poole, U.K. Ficoll-400 was obtained from Pharmacia, Uppsala, Sweden and dialysed against deionized glass-distilled water for at least 4 hr before use. [^{3}H]Dopamine hydrochloride (sp. act. 5 Ci/mmole, final concentration 0.75×10^{-6} M), *l*-[^{3}H]noradrenaline hydrochloride (sp. act. 15 Ci/mmole, final concentration 0.11×10^{-6} M) and [^{3}H]5-hydroxy-tryptamine creatinine sulphate (sp. act. 0.5 Ci/mmole, final concentration 0.2×10^{-6} M) were obtained from the Radiochemical Centre, Amersham, U.K. L-Deprenyl and clorgyline were kind gifts from Dr. J. Knoll, Semmelweis University of Medicine, Hungary and Dr. F. Owen, Division of Psychiatry, Clinical Research Centre, Harrow, U.K., respectively. All solutions were prepared with deionized glass-distilled water.

Sub-cellular fractionation. Forebrain synaptosomes were prepared using four adult male Wistar rats (Porton Strain) (150–180 g, aged 40–55 days). Animals were decapitated. The forebrain was obtained by transsecting each brain at the level of the superior and inferior colliculi and taking the part rostral to this transsection (except the olfactory bulbs). For the preparation of striatal synaptosomes, the brain was dissected according to the method of Glowinski and Iversen [6].

The subcellular fractionation procedure was essentially similar to that of Lai and Clark [7, 8] except that the density gradient used for separating synaptosomes from 'free' mitochondria and myelin consisted of 3 ml of 0.32 M sucrose, 10 mM Tris–HCl, pH 7.4, overlaid on 6 ml of 7.5% (w/w) Ficoll, 0.32 M sucrose, 10 mM Tris–HCl, pH 7.4, overlaid on a suspension of the crude mitochondrial fraction in 10 ml of 12% (w/w) Ficoll, 0.32 M sucrose, 10 mM Tris–HCl, pH 7.4. This gradient was modified from the Booth and Clark procedure [9]. None of the media used contained EDTA.

Amine uptake. The procedure for determining synaptosomal amine was basically that of Nicklas *et al.* [10]. Sets of tubes were set up containing Krebs–Ringer phosphate (135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2.6H_2O$, 10 mM D-glucose, 1 mM sodium phosphate buffer, pH 7.4, 1 mM $CaCl_2$ and 1 mM Tris–HCl, pH 7.4), and the synaptosomal preparation, in the absence or presence of clorgyline or L-deprenyl. Uptake was carried out at 37° for 4 min (with a 3-min preincubation) in a shaking water bath. The content of each tube was filtered through a Millipore filter (pore size 0.65 μm). The filter was washed three times with 1 ml of ice-cold 0.15 M NaCl. The radioactivity on each filter was determined by liquid scintillation counting after solubilization with 2 ml of ethoxyethanol. The difference between the counts in the zero-time sample and the counts retained by subcellular particles after the 4-min incubation was usually used for computing the uptake rates. A tube containing Krebs–Ringer phosphate and the synaptosomal preparation was incubated at 37° for 3 min. At the end of this incubation time [^{3}H]amine substrate was added to the contents of the tube. The tube was cooled on ice and 1 ml of ice-cold 0.15 M NaCl was then added to the contents of the tube. The contents of the tube were filtered through a Millipore filter (pore size 0.65 μm) and the filter was washed three times with 1 ml of ice-cold 0.15 M NaCl. The radioactivity on the filter was determined by scintillation counting and this constituted the zero-time sample. The zero-time sample was usually assayed in duplicate. For all three [^{3}H]amine substrates (serotonin, dopamine and noradrenaline) studied, the zero-time samples contained c.p.m. similar to (within ± 15 per cent) those obtained with the 0° (i.e. non-metabolic) blanks. The 0° blanks were obtained by incubating samples containing Krebs–Ringer phosphate, synaptosomal preparation and the [^{3}H]amine substrate on ice for 4 min, filtering the synaptosomal preparation through a Millipore filter and counting the [^{3}H]amine taken up by the synaptosomes on the filter by liquid scintillation counting.

Protein determination. This was carried out by the method of Lowry *et al.* [11].

Statistics. Analyses of significance of differences were done with the non-paired *t*-test.

RESULTS

Effects of nialamide on the uptake of dopamine, noradrenaline and serotonin by forebrain synaptosomes. Synaptosomal amine uptake is usually determined in the presence of a MAO inhibitor such as nialamide to eliminate the effects of metabolism [12]. Experiments were therefore set up to determine if the presence or absence of a MAO inhibitor made any difference to the rates of amine uptake. As shown in Table 1, nialamide (a MAO inhibitor), at 10^{-5} M and 4×10^{-5} M, appeared to slightly inhibit the uptake of dopamine, but the rates of uptake in the presence or absence of nialamide were not statistically different ($P > 0.05$). At 4×10^{-5} M, nialamide inhibited the uptake of noradrenaline by about 30 per cent ($P < 0.05$, Table 1), whereas it was ineffective at 10^{-5} M (Table 1). At 2×10^{-5} to 4×10^{-5} M, nialamide slightly stimulated the uptake of serotonin. However, the differences between the rates of uptake of serotonin in the presence or absence of nialamide did not reach statistical significance ($P > 0.05$, Table 1). These results provided evidence that metabolism did not play a major role in the control of initial rates of amine uptake by forebrain synaptosomes.

Effects of clorgyline and L-deprenyl on the uptake of dopamine by forebrain synaptosomes. The effects of clorgyline and L-deprenyl on dopamine uptake were as shown in Fig. 1. Both MAO inhibitors inhibited dopamine uptake in a dose-dependent fashion with similar characteristics, except that clorgyline ($IC_{50} = 5.6 \times 10^{-5}$ M) was more effective than L-deprenyl ($IC_{50} = 5.3 \times 10^{-4}$ M). At 10^{-3} M, clorgyline virtually completely inhibited dopamine uptake, whereas dopamine uptake was only partially inhibited by 10^{-3} M L-deprenyl.

Effects of clorgyline and L-deprenyl on the uptake of noradrenaline by forebrain synaptosomes. As shown in Fig. 2, the dose–response curves of the effects of clorgyline and L-deprenyl on the uptake of noradrenaline were almost identical. This suggests

Table 1. Effects of nialamide on the uptake of dopamine, noradrenaline and serotonin by rat forebrain synaptosomes*

Substrate	Nialamide concentration (μM)	Amine uptake (% of Control) (Mean ± S.D.)	P values
Dopamine (0.75 μM)	10 μM	73 ± 15	> 0.05
	40 μM	80 ± 18	> 0.05
Noradrenaline (0.11 μM)	10 μM	82 ± 10	> 0.05
	40 μM	69 ± 5	< 0.05
Serotonin (0.2 μM)	20 μM	128 ± 25	> 0.1
	40 μM	136 ± 34	> 0.05

* All values were mean ± S.D. of three or four experiments. P values were obtained using non-paired *t*-test. The control values were: dopamine uptake, 6.8 ± 1.2 (14) pmoles/min/mg protein; noradrenaline uptake, 1.1 ± 0.2 (10) pmoles/min/mg protein; serotonin uptake, 3.9 ± 0.6 (3) pmoles/min/mg protein (values were mean ± S.D. with number of experiments in parentheses).

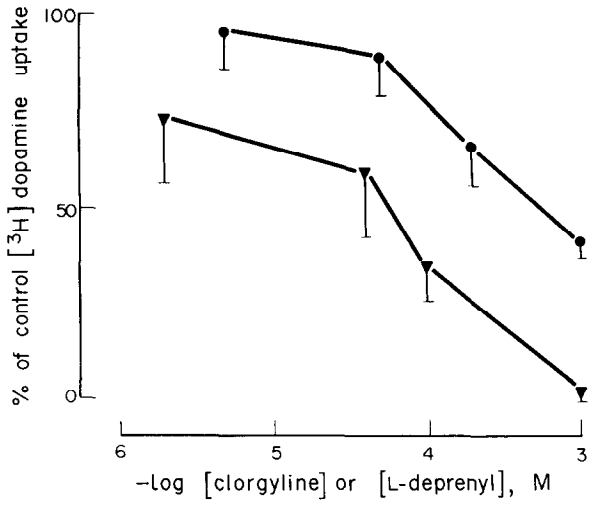

Fig. 1. Forebrain synaptosomes were used for dopamine uptake as described in Materials and Methods. Values were mean ± S.D. of three to five experiments. Control values for dopamine uptake were: 6.5 ± 0.9 (10) pmoles/min/mg protein (mean ± S.D., with the number of experiments in parenthesis). ▲ Clorgyline, ● L-deprenyl.

that both drugs were equally effective in inhibiting noradrenaline uptake. The IC_{50} values for clorgyline and L-deprenyl were 6.6×10^{-5} and 2.6×10^{-5} M, respectively. The latter is compatible with values quoted by Braestrap *et al.* [4]. Knoll and Magyar [13] also found that in cerebral cortical slices of mice, L-deprenyl significantly inhibited the uptake of noradrenaline both *in vivo* and *in vitro*.

Effects of clorgyline and L-deprenyl on the uptake of serotonin by forebrain synaptosomes. From the

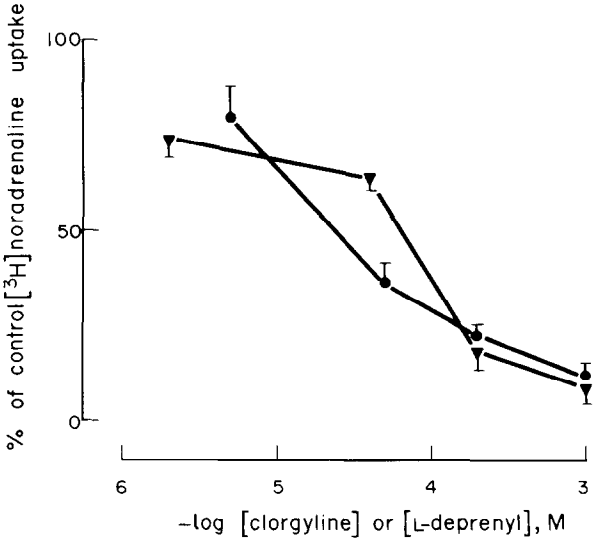

Fig. 2. Forebrain synaptosomes were used for noradrenaline uptake as described in Materials and Methods. Values were means ± S.D. of three to five experiments. Control values for noradrenaline uptake were: 1.2 ± 0.1 (7) pmoles/min/mg protein (mean ± S.D., with the number of experiments in parenthesis). ▲ Clorgyline, ● L-deprenyl.

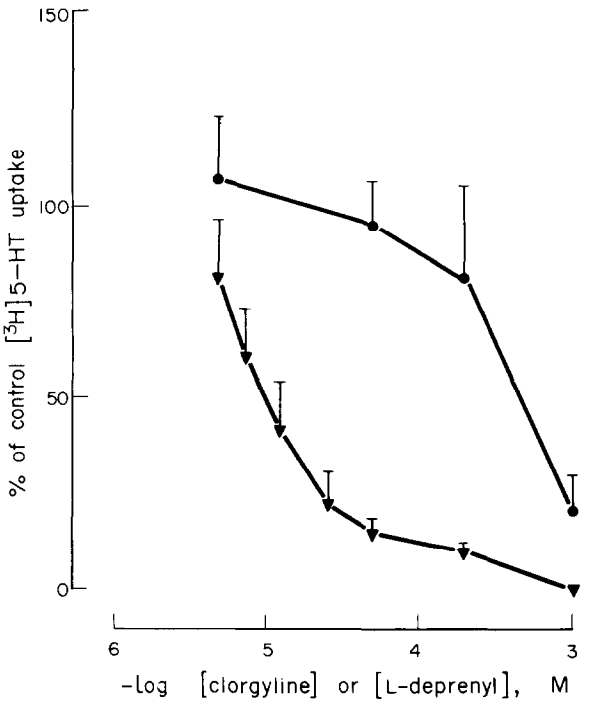

Fig. 3. Forebrain synaptosomes were used for serotonin uptake as described in Materials and Methods. Values were means ± S.D. of three to five experiments. Control values for serotonin uptake were: 4.1 ± 0.5 (5) pmoles/min/mg protein (mean ± S.D., with the number of experiments in parenthesis). ▲ Clorgyline, ● L-deprenyl.

dose–response curves of the effects of clorgyline and L-deprenyl on serotonin uptake shown in Fig. 3 it was evident that clorgyline ($IC_{50} = 10^{-5}$ M) was a more potent inhibitor than L-deprenyl ($IC_{50} = 4.6 \times 10^{-4}$ M). At concentrations around 10^{-4} M, serotonin uptake was nearly completely inhibited by clorgyline, but it was not affected by L-deprenyl at concentrations lower than 10^{-4} M.

Effects of clorgyline and L-deprenyl on the uptake of dopamine, noradrenaline and serotonin by striatal synaptosomes. To determine whether there were regional variations in the differential sensitivity of amine uptake to inhibition by clorgyline and L-deprenyl, studies were carried out on synaptosomes isolated from striatum, a region particularly enriched in dopaminergic nerve-endings. At 7.5×10^{-5} M, clorgyline inhibited dopamine uptake by about 60 per cent ($P < 0.001$) and at a higher concentration of 10^{-3} M it virtually completely inhibited dopamine uptake ($P < 0.001$) (Table 2). The extent of inhibition by clorgyline at these two concentrations was comparable to that observed previously with forebrain synaptosomes (see Fig. 1).

In contrast with clorgyline, L-deprenyl was considerably less effective in inhibiting dopamine uptake by striatal synaptosomes. At 5×10^{-5} M, L-deprenyl was without effect: this was similar to its effect on forebrain synaptosomes (see Fig. 1). However, L-deprenyl, at 10^{-3} M, inhibited dopamine uptake by striatal synaptosomes to about the same extent as forebrain synaptosomes (see Table 2 and Fig. 1).

Table 2. Effects of clorgyline and L-deprenyl on the uptake of dopamine, noradrenaline and serotonin by striatal synaptosomes

Substrate	Inhibitor concentration	Amine uptake (% of control*) (Mean ± S.D. of four to six determinations)	P Values
[^{3}H]Dopamine (0.75 μM)	75 μM Clorgyline	30 ± 6	< 0.001
	1 mM Clorgyline	2 ± 2	< 0.001
	50 μM L-Deprenyl	107 ± 6	> 0.05
	1 mM L-Deprenyl	27 ± 5	< 0.001
[^{3}H]Noradrenaline (0.11 μM)	60 μM Clorgyline	39 ± 6	< 0.001
	1 mM Clorgyline	10 ± 3	< 0.001
	25 μM L-Deprenyl	41 ± 6	< 0.001
	1 mM L-Deprenyl	18 ± 5	< 0.001
[^{3}H]Serotonin (0.22 μM)	10 μM Clorgyline	90 ± 5	> 0.1
	200 μM Clorgyline	6 ± 1	< 0.001
	0.5 mM L-Deprenyl	26 ± 4	< 0.005
	1 mM L-Deprenyl	17 ± 2	< 0.001

* The control values (mean ± S.D. with number of experiments in parentheses) were: dopamine, 34.7 ± 5.6 (4) pmoles/min/mg protein; noradrenaline, 2.1 ± 0.2 (4) pmoles/min/mg protein; serotonin, 6.2 ± 0.7 (3) pmoles/min/mg protein.

L-Deprenyl was just as effective an inhibitor as clorgyline of noradrenaline uptake by striatal synaptosomes (Table 2). From the dose–respone curves (see Fig. 2) it was evident that clorgyline influenced noradrenaline uptake by striatal synaptosomes to the same extent as noradrenaline uptake by forebrain synaptosomes (Table 2). L-Deprenyl also acted in a similar fashion (compare data in Fig. 2 and Table 2).

The effects of clorgyline on serotonin uptake by striatal synaptosomes differed from those on forebrain synaptosomes (compare the dose–response curve in Fig. 3 with results shown in Table 2). At 10^{-5} M, although clorgyline inhibited serotonin uptake by forebrain synaptosomes by 50 per cent (Fig. 3), it did not affect serotonin uptake by striatal synaptosomes (Table 2). At much higher concentrations, it inhibited serotonin uptake by both types of synaptosomes to the same extent (see Fig. 3 and Table 2).

L-Deprenyl was equally effective at inhibiting serotonin uptake by striatal as well as forebrain synaptosomes (see Fig. 3 and Table 2). In contrast with clorgyline, L-deprenyl was a less potent inhibitor of serotonin uptake by striatal synaptosomes (see Table 2).

DISCUSSION

Our finding that clorgyline was an inhibitor of synaptosomal dopamine uptake prompted us to investigate whether clorgyline and L-deprenyl exhibited any selectivity towards inhibiting the synaptosomal amine uptake systems as they obviously do towards the multiple forms of MAO [14]. Can the selectivity of these two drugs in inhibiting amine uptake be predicted in accordance with their known differential effects on the A and B forms of MAO? Since serotonin is a substrate for type A MAO, clorgyline (a type A-specific inhibitor) would be expected to exert a greater inhibitory effect on synaptosomal serotonin uptake than L-deprenyl (a type B-specific inhibitor). The results of the dose–response (see Fig. 3) and the IC_{50} values (see Table 3) appear to substantiate this prediction. However, the paradoxical finding that L-deprenyl (a type B MAO inhibitor) is equally effective as clorgyline in inhibiting noradrenaline (a type A MAO substrate) uptake by forebrain synaptosomes is at variance with this type of prediction (see Fig. 2 and Table 3). Our results (see Fig. 1 and Table 3) also indicate that clorgyline is a more effective inhibitor of dopamine uptake by forebrain synaptosomes than L-deprenyl, as predicted on the assumption that dopamine is a preferred substrate for type A rat brain MAO [2, 4]. The latter assumption has been challenged by some workers who maintained that dopamine is deaminated by both the A and the B forms in the rat brain *in vivo* [5].

Whether the differential inhibition of synaptosomal amine uptake by clorgyline and L-deprenyl, as presented in this paper, can contribute towards the *in vivo* effects of these two drugs on brain amine metabolism cannot be established. However, a comparison of the inhibitory effects of these two drugs on MAO activity in purified preparations of synaptic and non-synaptic mitochondria [15] and on the uptake of amines by synaptosomes (Figs. 1–3 and

Table 3. Summary of IC_{50} values for clorgyline and L-deprenyl inhibition of rat forebrain synaptosomal amine uptake*

Substrate	IC_{50}	
	Clorgyline	L-Deprenyl
[^{3}H]DA (0.75 μM)	5.6×10^{-5} M	5.3×10^{-4} M
[^{3}H]NA (0.11 μM)	6.6×10^{-5} M	2.6×10^{-5} M
[^{3}H]5HT (0.2 μM)	1×10^{-5} M	4.6×10^{-4} M

* Abbreviations: DA, dopamine; NA, noradrenaline; 5HT, serotonin.

Tables 2 and 3) leads to the following conclusions: (i) Clorgyline is much more potent as an inhibitor of rat brain mitochondrial MAO (IC_{50} = 10^{-11}–10^{-7} M; see Fig. 2 of Ref. 15) than of synaptosomal uptake of amines (IC_{50} = 10^{-5}–10^{-4} M; see Table 3 of this paper); (ii) L-Deprenyl is equally effective as an inhibitor of rat brain mitochondrial MAO (IC_{50} = 10^{-5}–10^{-4} M; see Fig. 3 of Ref. 15) as it is of synaptosomal uptake of amines (IC_{50} = 10^{-5}–5.3×10^{-4} M; see Table 3 of this paper).

Under the conditions of our uptake assays it is not possible to rule out the possibility that we had also been measuring some synaptosomal amine binding. If this is indeed the case, then our results suggest that the two MAO inhibitors appear to affect synaptosomal amine binding as they do uptake. However, from the following considerations it seems unlikely that amine binding constitutes a major component in our uptake measurements.

(i) The concentrations of amines we employed for studying uptake were at least two orders of magnitude above the K_d values for specific amine binding [16]. The fact that we observed concentration-dependent increase in amine uptake at concentrations higher than those employed in this study (Refs. 17, 18 and J. C. K. Lai, unpublished observations) indicates that we were measuring amine uptake rather than binding, for the K_m value for amine in the transport processes is two orders of magnitude greater than the K_d value for amine in the binding processes [16]. (ii) The non-metabolic component (cf. Materials and Methods), which may represent the binding component, has already been subtracted from the uptake values quoted by us. Furthermore, it is well established that uptake is inhibited by a variety of metabolic poisons (e.g. dinitrophenol, oligomycin, etc.) [19].

In conclusion, we have presented some evidence to suggest that, *in vitro*, clorgyline and L-deprenyl appear to exert differential inhibitory effects on the uptake of dopamine, noradrenaline and serotonin by rat brain synaptosomes and that these effects are different from their selective effects on rat brain mitochondrial MAO.

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