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# *In vivo*, noradrenaline is a substrate for rat brain monoamine oxidase A and B

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**1** *In vivo* clorgyline (5 mg/kg) and (−)-deprenyl (5 mg/kg) selectively inhibit monoamine oxidase (MAO) type A and B activities in rat brain hypothalamus and caudate nucleus using 5-hydroxytryptamine (5-HT), noradrenaline (NA), and β-phenylethylamine (PEA) as substrates.
**2** Clorgyline induces a significant increase in NA concentrations of hypothalamus and caudate nucleus; however (−)-deprenyl is without effect.
**3** The combination of clorgyline and (−)-deprenyl at the above doses completely inhibits both forms of MAO, resulting in an even greater increase in NA levels in both brain areas than observed with clorgyline. The non-selective inhibitor tranylcypromine (5 mg/kg) produced a similar effect.
**4** Rats pretreated with the selective or the non-selective inhibitors but given L-DOPA (50 mg/kg) have a similar pattern of brain NA, but its concentrations are higher in both brain regions.
**5** The results indicate that although *in vitro* NA may be an exclusive substrate for MAO type A, *in vivo*, when this enzyme form is selectively inhibited, NA at high concentrations can be a substrate for MAO type B.

## Introduction

Previously, Green & Youdim (1975), observed that, while *in vitro* 5-hydroxytryptamine (5-HT) was deaminated exclusively by brain monoamine oxidase (MAO) type A (Johnston, 1968), nevertheless when the latter enzyme form is selectively inhibited *in vivo* by clorgyline, MAO type B can continue to deaminate the amine. These results have been confirmed by the *in vitro* studies of Ekstedt (1976) and Fowler & Tipton (1982) using liver and brain mitochondrial preparations respectively. Their results indicated that the *K*m of MAO B for 5-HT is much higher than that of MAO type A.

There are many reports that noradrenaline, like 5-HT, is an exclusive substrate for brain MAO type A *in vitro* and *in vivo* (Yang & Neff, 1974; Tipton, Houslay & Mantle, 1976; Neff & Fuentes, 1976). In this paper evidence will be presented to show that *in vivo*, when brain noradrenaline (NA) concentrations are high enough, it can be deaminated by the B form of MAO, a finding similar to that which has been reported for 5-HT (Green & Youdim, 1975) and dopamine (Green, Mitchell, Tordoff & Youdim, 1977), and thus differentiation of MAO substrates into A and B types is not absolute, since β-phenylethylamine (PEA), the MAO B substrate, at high concentration can be a substrate for MAO A (Suzuki, Katsumata & Oya, 1979; Kinemuchi, Wakui, Toyoshima Hayashi & Kamijo, 1979).

## Methods

Male Wistar rats (150–200 g) were used in all experiments.

### *Monoamine oxidase inhibitor experiments*

Tranylcypromine, clorgyline or (−)-deprenyl were dissolved in 0.9% w/v NaCl solution (saline) and injected intraperitoneally to two groups of rats. Thirty minutes later L-DOPA (50 mg/kg) suspended in saline containing 1% carboxymethylcellulose was given to one group and saline to the other group. Ninety minutes later the rats were killed, the brains removed and hypothalamus and caudate were dissected out. Half the hypothalamus and caudate nucleus and the rest of the brain were homogenized in 0.32 M sucrose and MAO activity was estimated by the procedure of Tipton & Youdim (1976) using [1-$^{14}$C]-5-hydroxytryptamine, [1-$^{14}$C]-noradrenaline and [1-$^{14}$C]-phenylethylamine. The final concentrations of 5-HT, NA and phenylethylamine (PEA) were

0.5 mM, 0.5 mM and 20 μM respectively. The other halves of hypothalamus and caudate nucleus were homogenized in cold (4°C) acidified butanol for determination of noradrenaline (Chang, 1964).

*Protein determination*

Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard. Enzyme activity was calculated as nmol of deaminated product formed $mg^{-1}$ protein $min^{-1}$ and the results are expressed as the mean ± s.e.mean of the percentage inhibition compared to control-saline injected group.

*Drugs and chemicals*

Tranylcypromine was a gift from Smith, Kline and French Ltd., U.S.A. as were clorgyline (May and Baker, Ltd.) and (−)-deprenyl (Prof. J. Knoll, Budapest, Hungary). Radioactive noradrenaline and 5-HT were purchased from Radiochemical Centre, Amersham and β-phenylethylamine from NEN Chemicals GmbH. All other agents were of highest purity and were obtained from Sigma Co. (U.S.A.).

## Results

When L-DOPA was given without an MAO inhibitor there was a slight increase in NA concentrations of hypothalamus and caudate nucleus which were not significantly different from those found in the saline-treated group (Table 1). In contrast to (−)-deprenyl (5 mg/kg), clorgyline (5 mg/kg) caused a significant increase in NA but the rise in both brain regions was even larger after L-DOPA treatment. When both clorgyline and (−)-deprenyl were given together (5 mg/kg of each) the rise in hypothalamus and caudate nucleus NA was greater than that observed after clorgyline alone, but was similar to that which was obtained with tranylcypromine (5 mg/kg). In all cases L-DOPA treatment of rats previously given the MAO inhibitors caused an even larger rise in NA (Table 1). Similar results were obtained for the brains in which hypothalamus and caudate nucleus were dissected out (unpublished data).

*Effect of clorgyline, (−)-deprenyl and tranylcypromine on monoamine oxidase activity*

It can observed (Table 2) that at the *in vivo* doses used, clorgyline and (−)-deprenyl selectively inhibit MAO type A (5-HT and NA) and MAO type B (PEA) activity respectively, as measured *in vitro*. In combination, the two inhibitors totally inhibit both forms of the enzyme, a result shared by the action of tranylcypromine.

## Discussion

In agreement with *in vivo* results of Yang & Neff (1974) and Green *et al.* (1977) who used the whole

**Tabe 1** Effect of selective and non-selective monoamine oxidose (MAO) inhibitors with and without L-DOPA on rat brain noradrenaline cocentrations

| *Injection* | | NA (μg/g wet wt.) | |
|---|---|---|---|
| *0 min* | *30 min* | *Hypothalamus* | *Caudate nucleus* |
| Saline | Saline | 1.01 ± 0.18 | 0.57 ± 0.07 |
| Saline | L-DOPA | 1.15 ± 0.10 | 0.71 ± 0.06 |
| Clorgyline | Saline | 1.73 ± 0.14 | 0.92 ± 0.06 |
| Clorgyline | L-DOPA | 2.00 ± 0.15 | 1.20 ± 0.08 |
| Deprenyl | Saline | 1.14 ± 0.12 | 0.70 ± 0.08 |
| Deprenyl | L-DOPA | 1.38 ± 0.10 | 0.68 ± 0.09 |
| Deprenyl + clorgyline | Saline | 2.50 ± 0.17 | 1.58 ± 0.10 |
| Deprenyl + clorgyline | L-DOPA | 2.93 ± 0.24 | 2.08 ± 0.16 |
| Tranylcypromine | Saline | 2.57 ± 0.17 | 1.39 ± 0.16 |
| Tranylcypromine | L-DOPA | 3.16 ± 0.25 | 1.97 ± 0.17 |

Brain NA concentrations are shown 90 min after the intraperitoneal injection of the inhibitors and when L-DOPA had been given 30 min later. The results are expressed as mean ± s.e.mean and are from 6–8 individual experiments (3 rats in each experiment). Each determination was performed in duplicate. The dosage of the drugs was as the following: L-DOPA = 50 mg/kg; clorgyline = 5 mg/kg; deprenyl = 5 mg/kg; clorgyline + deprenyl = 5 mg/kg of each; and tranylcypromine = 5 mg/kg.

**Table 2** Monoamine oxidase (MAO) activity in the hypothalamus and caudate nucleus of rats pretreated with MAO inhibitors

| *Injection* | *% inhibition of MAO activity* | | | | | |
|---|---|---|---|---|---|---|
| | *Hypolalamus* | | | *Caudate Nucleus* | | |
| | 5-HT | NA | PEA | 5-HT | NA | PEA |
| Saline | – | – | – | – | – | – |
| Clorgyline | 92±8 | 97±4 | 24±8 | 95±6 | 98±2 | 31±10 |
| Deprenyl | 36±5 | 27±5 | 97±8 | 41±4 | 35±8 | 95±10 |
| Deprenyl + clorgyline | 100 | 100 | 100 | 100 | 100 | 100 |
| Tranylcypromine | 100 | 100 | 100 | 100 | 100 | 100 |

Brain MAO activity of rats treated with MAO inhibitors and L-DOPA. Results were obtained from the brains of animals in Table 1. See Table 1 for experimental procedures.

brain, in the present study it was found that clorgyline rather than deprenyl caused a rise in NA concentrations of hypothalamus, caudate nucleus and the rest of the brain without the latter regions. This rise is even larger when L-DOPA is given to rats in combination with clorgyline, but there is very little change either after (−)-deprenyl alone or (−)-deprenyl plus L-DOPA treatment. Thus, these results taken together with those of selective *in vivo* inhibition of MAO A in the brain regions imply that NA is exclusively a selective substrate for MAO type A. However, the NA concentrations after clorgyline are not as high as those when clorgyline and (−)-deprenyl are given together and where both enzyme forms are totally inhibited. After administration of tranylcypromine, the non-selective MAO inhibitor, the brain NA level also rises to the concentrations seen after the combination of clorgyline and (−)-deprenyl, with a further increase after L-DOPA.

These results are very similar to those obtained for the *in vivo* deamination of 5-HT and dopamine in the rat brain (Green & Youdim, 1975; Green *et al.*, 1977). Like 5-HT, NA may in normal circumstances be predominantly deaminated by MAO type A *in vivo*. However, when the latter enzyme is selectively inhibited (e.g. by clorgyline), NA concentrations rise more slowly as compared to those found after administration of the non-selective inhibitor (e.g. tranylcypromine) or the combination of clorgyline and (−)-deprenyl. In conditions when MAO A is selectively inhibited by clorgyline, the larger increase in NA concentrations satisfy the higher *K*m of MAO type B for NA and the latter enzyme continues to act on the amine but at a lower rate. This indeed may be the case, because Fowler & Tipton (1982) who confirmed our previous data pertaining to 5-HT deamination by rat brain MAO type A and B, reported a *K*m of 1170 μM for MAO B as compared to 178 μM for MAO A using 5-HT as substrate in the rat brain homogenate.

The present study thus indicates that while *in vitro* noradrenaline may be deaminated exclusively by MAO A (Tipton *et al.*, 1976; Suzuki *et al.*, 1979; Kinemuchi *et al.*, 1979), when this enzyme form is fully inhibited *in vivo*, MAO B can continue to act on noradrenaline, albeit at a slower rate, indicating that the affinity of MAO B as compared to MAO A for NA is much lower. These results are not in agreement with the studies of Neff, Yang & Goridis (1973) and others (Goridis & Neff, 1971; Tipton *et al.*, 1976; Neff & Fuentes, 1976) who reported that noradrenaline was a specific substrate for MAO A alone in the rat brain. However, these investigators based their conclusions on the determination of brain NA and its metabolites after treatment of rats with either clorgyline or (−)-deprenyl and not with a combination of the two selective inhibitors or a non-selective inhibitor. Whether MAO B is located intraneuronally or extraneuronally is now being investigated.

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