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N-propargylbenzylamine, a major metabolite of pargyline, is a potent inhibitor of monoamine oxidase type B in rats *in vivo*: a comparison with deprenyl

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1 In an effort to explore the contribution of the metabolites of pargyline towards the *in vivo* inhibition of monoamine oxidase (MAO), the effects of pargyline and its major metabolites on the production and metabolism of a number of biogenic amines were studied in rats. The administration of pargyline gave rise to three major ethyl acetate extractable metabolites: benzylamine, N-methylbenzylamine and N-propargylbenzylamine (NPB). Only NPB demonstrated *in vivo* monoamine oxidase inhibitory properties at an acute dose of 30 mg kg^{-1}.

2 The acute effects of pargyline, NPB, and deprenyl on urine and brain concentrations of a number of biogenic amines (phenylethylamine (PEA), *m*- and *p*-tyramine, noradrenaline (NA), dopamine, and 5-hydroxytryptamine (5-HT) and their metabolites were evaluated. Increased urine and brain concentrations of PEA were considered to represent *in vivo* inhibition of type B MAO while decreased concentrations of NA and 5-HT metabolites were regarded as indicators of an *in vivo* inhibition of MAO type A. NPB, like deprenyl and pargyline, significantly increased urine and brain PEA while only pargyline reduced 5-HT metabolism, suggesting that the metabolism of pargyline to NPB may contribute towards the MAO type B inhibitory effects of pargyline *in vivo*.

3 Since the therapeutic benefits of MAO inhibitors in clinical practice usually require some period of chronic treatment, the chronic effects of repeated 14 daily doses of the above MAO inhibitors on central and peripheral biogenic amines were evaluated at the following times: during treatment, one day and five days after termination of treatment. The biochemical changes observed during the course of chronic NPB, pargyline and deprenyl treatments generally follow the expected *in vitro* characteristics of these drugs, but the detailed changes observed suggest clear differences. For example, the *in vivo* effect of pargyline on urine 5-hydroxyindoleacetic acid excretion was considerably weaker than its effect on the excretion of NA and dopamine metabolites. These changes are opposite to the *in vitro* effects of pargyline on 5-HT, dopamine and NA oxidative deamination.

4 Inhibitions of the metabolism of all the amines studied were clearly observed during chronic MAOI treatments, but these effects were less evident five days after the end of treatment, suggesting an almost normal metabolism of biogenic amines.

5 It is concluded that while MAO inhibitors may be the primary compound responsible for MAO inhibition, the effects of their metabolites in some cases may also play equally important roles in the regulation of monoamines both in the periphery and the brain. Thus, as demonstrated here, NPB was found to be as potent as pargyline and deprenyl with regard to its *in vivo* MAO type B inhibitory properties.

Introduction

The use of monoamine oxidase (MAO) inhibitors (MAOI) to reduce biogenic amine metabolism and hence to increase their availability both peripherally and centrally is a strategy exploited in the treatment of a number of psychiatric disorders that are believed associated with deficient monoamine availability, especially the catecholamines. Recent trends in the clinical application of MAOIs is towards the use of the so called selective MAOIs; drugs that at low doses preferentially block one of the two major types of

MAO (A- and B-types) (Knoll, 1981; Fowler, 1982). 5-Hydroxytryptamine (5-HT) and phenylethylamine are the preferred substrates for the A and B types of MAO respectively. Unfortunately, the therapeutic benefits of these MAOIs are frequently attributed to MAO inhibition by the parent compounds with little or no consideration of other possible pharmacological effects. Among these effects may be blockade of biogenic amines release and uptake, direct drug effects on biogenic amine receptors and above all the effects of the drugs' metabolites. Indeed, we have recently demonstrated that chronic deprenyl (an MAO type B inhibitor) administration to man produced changes in urinary excretion of biogenic amines metabolites that cannot wholely be attributed to MAO inhibition (Karoum *et al.*, 1982) and have suggested that the metabolites of deprenyl (Reynolds *et al.*, 1978a, b), amphetamine and methamphetamine, may play equally prominent roles as their parent compounds. There is therefore a need for a better understanding of the *in vivo* pharmacological and biochemical properties of MAOIs commonly employed in research and medicine. Such an evaluation should ideally consider a wide range of relevant biogenic amines and should also consider the effects of the drugs' metabolites.

In this paper are described the acute and chronic effects of pargyline, its metabolites and deprenyl on urine and brain concentrations of a number of biogenic amines and their metabolites in rats. The metabolites of pargyline employed are benzylamine, N-methyl benzylamine and N-propargylbenzylamine (NPB).

Methods

Male Sprague-Dawley rats (150–200 g) obtained from Zivic Miller (Allison Park, Pa., U.S.A.) were used. Urine was collected (usually for 4 h) from rats placed in 'Econo Metabolic Units' (American Scientific Products, Springfield, Va. U.S.A.). A fused silica capillary column, 0.32 mm i.d. bond coated with a mixture of SP-2250 (methyl-phenylpropyl silicone gum), SPB 5 (methyl phenyl-vinyl-silicone gum) and SP 2401 (fluoropropyl silicone) (with ratios of 1:2:1 respectively) was used for the mass fragmentography of phenylethylamine and tyramine (custom made by Supelco Inc., Pa. U.S.A.). For the mass fragmentography of catecholamines and their metabolites a 10 ft, $\frac{1}{8}$ inch o.d. stainless steel column packed with 3% SE 54 was used. 5-Hydroxyindoleacetic acid (5-HIAA) was chromatographed on a 5 ft, 3% SE 54 packed column. The fragments focused upon are summarized in Table 1. A model 4000 Finnigan gas chromatograph-quadrupole mass spectrometer (Finnigam Corp., Sunnyvale, Ca.) was used. All chemicals and reagents we used were of the highest grades commercially available. Rats were decapitated and their brains were dissected according to Glowinski & Iversen (1966).

Brain tissues were homogenized in 0.5 ml 1N HCl containing 50 ng of each of the following deuterated isomers: phenylethylamine (PEA) ([$^2H_9$]-PEA), noradrenaline (NA) ([$^2H_3$]-NA), dopamine ([$^2H_4$]-dopamine), 3,4-dihydroxyphenylacetic acid (DOPAC) ([$^2H_5$]-DOPAC), homovanillic acid (HVA) ([$^2H_5$]-HVA) and 5-HIAA ([$^2H_2$]-5-HIAA). Five or ten μl of

Table 1 Mass to charge ratio (m/z) of amines and metabolites employed for mass fragementography

Compound	*Derivative*	*Molecular ion (M ±)*	*m/z of Fragment*
Phenylethylamine (PEA)	PFP	267	104
[$^2H_9$]-PEA	PFP	276	112
p-Tyramine (*p*-Ty)	PFP	429	266
[$^2H_4$]-*p*-Ty	PFP	433	269
Noradrenaline (NA)	PFP	753	590
[$^2H_3$]-NA	PFP	756	592
Dopamine (DA)	PFP	591	428
[$^2H_4$]-DA	PFP	595	431
3,4-Dihydroxyphenylacetic acid (DOPAC)	EE/PFP	488	488
[$^2H_5$]-DOPAC	EE/PFP	493	493
Homovanillic acid (HVA)	EE/PFP	356	356
[$^2H_5$]-HVA	EE/PFP	361	361
3-Methoxy-4-hydroxy-phenylglycol (MHPG)	PFP	622	622
[$^2H_3$]-MHPG	PFP	625	625
5-Hydroxyindoleactic acid (5-HIAA)	EE/PFP	614	438
[$^2H_2$]-5-HIAA	EE/PFP	616	440

PFP = pentafluoropropionate derivative; EE/PFP = ethylester/pentafluoropropionate derivative.

the homogenates were pipetted out for protein determination (Lowry *et al.*, 1951). The rest of the homogenates were centrifuged at 15,000 *g* (Eppendorf 5412 Centrifuge, American Scientific Products) and the clear supernatant stored at − 10°C until analysed.

Urine creatinine was measured by a modification of the original method of Here (1950). In brief, 100 µl of urine was mixed with 500 µl of 1N NaOH and 500 µl of 24.5 µmol ml^{-1} solution of picric acid. After 10 min, 800 µl of the mixture was discarded, followed by the addition of 2 ml water and the optical density of the yellow colour read at 510° nm on a Perkin-Elmer 35 spectrometer. In each batch of analysis, triplicates of reagent blank (a tube containing 100 µl of H_2O instead of urine) and tubes containing standard creatinine solutions (concentrations ranging from 0.1 to 1.0 mg ml^{-1}) were included. Urine creatinine concentrations were calculated from a constructed standard curve.

Catecholamines and their metabolites were measured in urine and brain tissues as previously described (Karoum *et al.*, 1980; Karoum, 1983., 1985). Brain and urine free PEA, *m*- and *p*-tyramine were extracted and prepared for mass fragmentography as previously described (Karoum, 1983) except that a fused silica capillary column was used instead of a packed column.

Total urine PEA, *m*-tyramine and *p*-tyramine were measured as follows: 100 µl of urine was mixed with [$^2H_9$]-PEA (50 ng), [$^2H_9$]-*p*-tyramine (500 ng) and 100 µl 10 N HCl and heated at 100°C for 1 h. Heating urine in 5N HCl and at 100°C for 1 h renders complete hydrolysis of conjugated PEA and *p*-tyramine. After hydrolysis, the mixture was added to 100 µl 10 N NaOH in a 3 ml polypropylene 'Eppendorf' micro centrifuge tube (Brinkmann Instrument Inc., Westburn, N.Y. U.S.A.) followed by the addition of phosphate buffer (prepared by mixing one part 0.5 M Na_2HPO_4 and 3 parts 0.5 M Na_3PO_4, pH13), 0.5 ml. The mixture was extracted twice with ethyl acetate and the amines assayed as described for free PEA and free tyramine (Karoum, 1983; 1985).

Drug schedules

All drugs were dissolved in 0.9% saline and administered intraperitoneally, (i.p.). The doses and time schedules are described in the appropriate Tables and Figures. NPB was first dissolved in 10 or 20 µl of formic acid then made up to the appropriate volume with saline.

Results

Preliminary studies revealed three major ethyl acetate-extractable, dealkylated metabolites of pargyline; benzylamine ($C_6H_5CH_2NH_2$), N-methyl-benzylamine ($C_6H_5CH_2NHCH_3$) and N-propargylbenzylamine (NPB) ($C_6H_5NHCH_2CCH$). Because of their close structures to PEA, these metabolites cannot easily be separated from PEA on a packed column, and hence a capillary column was used.

Relatively high doses of the MAOI were used in the acute studies in order to assure complete inhibition of MAO. For example, acute doses of pargyline ranging from 1 to 5 mg kg^{-1} failed to produce a clear change in the urinary excretion of PEA, *p*-Ty, catecholamine metabolites or 5-HIAA, 5 or 24 h after treatment.

For chronic treatments, the doses selected were those that acutely produced minimal but significant changes in catecholamine metabolism and/or PEA excretion.

The main objective of the present investigation was to explore the contribution of MAOI metabolites in the overall effects of the parent drugs on the *in vivo* production and metabolism of biogenic amines. For this, the metabolites of pargyline were investigated. A comparison of the peripheral (as reflected upon urinary rates of excretion) and the central effects of MAO inhibiton in rats was also made in order to gain a better insight (by extrapolation) into how these drugs may affect human brain amines.

Acute and chronic effects of high doses of pargyline and its three major metabolites as well as deprenyl on brain and urinary biogenic amines

A comparison of the acute *in vivo* effects of 50 mg kg^{-1} of pargyline and those of its three major metabolites, 30 mg kg^{-1}, on urine excretion of free PEA, *m*-, *p*-tyramine, catecholamine and 5-HT metabolites revealed NPB to be the only metabolite that resembles pargyline in its *in vivo* effects on monoamines metabolism (Table 2). Both pargyline and NPB increased free PEA excretion. These two compounds, however, were not identical. Acute NPB treatment in contrast to that of pargyline, failed to reduce significantly urine HVA, MHPG or 5-HIAA. In fact, NPB together with benzylamine and N-methylbenzylamine significantly increased 5-HIAA excretion.

To explore further the MAO B-type inhibitory properties of NPB, its effects were evaluated on hypothalamic and caudate nucleus PEA at doses ranging from 1 to 40 mg kg^{-1}. In good agreement with its effects on urine PEA (Table 1), NPB increased brain PEA in a dose-related manner (Figure 1).

In order to differentiate between the contribution of NPB alone towards PEA excretion from that of unmetabolized pargyline plus its metabolites, we chronically administered pargyline, 50 mg kg^{-1}, to rats for 10 days and collected urine for 4 h at 0, 24 and 48 h after the last treatment. The urines were analysed

Table 2 The acute effects of pargyline, deprenyl, N-methylbenzylamine, benzylamine and N-propargylbenzylamine (NPB) on urinary excretion of biogenic amines and metabolites

Drug	*Free m-tyramine* (ng)	*Free p-tyramine* (ng)	*Free PEA* (ng)	*HVA* (μg)	*MHPG* (μg)	*5-HIAA* (μg)
Control	774 ± 82	1697 ± 264	304 ± 26	4.6 ± 0.2	5.5 ± 0.5	9.3 ± 1.1
Benzylamine	771 ± 36	1614 ± 174	345 ± 26	4.9 ± 0.2	4.55 ± 0.53	16.6 ± 2.3**
N-methyl-benzylamine	704 ± 46	1005 ± 88	372 ± 38	4.9 ± 0.2	5.4 ± 0.3	14.8 ± 1.7**
NPB	783 ± 124	1436 ± 176	2154 ± 690***	3.8 ± 0.1	5.4 ± 0.3	14.6 ± 1.1***
Pargyline	750 ± 200	1830 ± 500	3766 ± 417***	3.3 ± 0.1*	3.3 ± 0.2**	5.6 ± 0.4**
Deprenyl	—	900 ± 100*	1926 ± 529***	1.8 ± 0.2**	3.1 ± 0.2*	—

Benzylamine (30 mg kg^{-1}), N-methylbenzylamine (30 mg kg^{-1}), NPB (30 mg kg^{-1}), deprenyl (10 mg kg^{-1}) and pargyline (50 mg kg^{-1}) were administered i.p. and urine collected for 4 h. Five rats were included in each experiment. Results are expressed as mean ± s.e.mean (unit mg^{-1} creatinine). PEA = phenylethylamine, HVA = homovanillic acid, MHPG = 3-methoxy-4-hydroxyphenylglycol, 5-HIAA = 5-hydroxyindoleacetic acid.
*$P < 0.05$; **$P < 0.005$; ***$P < 0.001$ by unpaired t test.

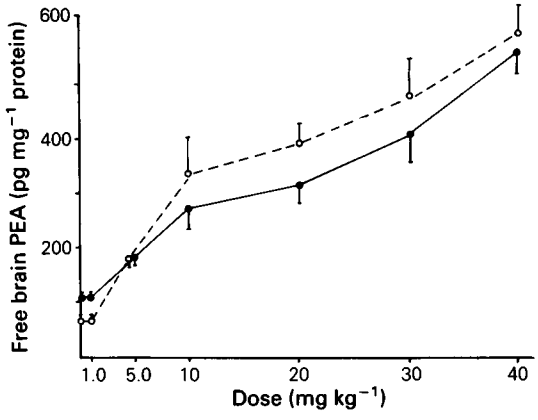

Figure 1 The effects of different doses of N-propargylbenzylamine on brain phenylethylamine (PEA) 3 h after treatment: (O———O) caudate nucleus; (●——●) hypothalamus. Five rats were included in each point. All points corresponding to 5 mg kg^{-1} and above were statistically significantly different ($P < 0.05$) from the baseline values.

for most of the compounds listed in Table 2 and for the concentrations of pargyline and its three major metabolites (Table 3). There appears to be a close association between the amount of NPB excreted and the output of free PEA, *m*- and *p*-tyramine. No such association was observed for HVA and MHPG excretions. We were not able to detect pargyline in any of the urine samples analyzed.

The acute effects of the above MAOIs on brain PEA, NA, dopamine and 5-HT metabolism are summarized in Table 4. NPB like deprenyl and pargyline markedly elevated hypothalamic and caudate nucleus PEA while only pargyline significantly reduced caudate nucleus 5-HIAA. Both NPB and pargyline significantly reduced hypothalamic MHPG. All three drugs reduced caudate nucleus DOPAC and HVA. Apparently MAO type A inhibition, as reflected in 5-HT metabolism, is more associated with the effects of pargyline itself than with its metabolites. Inhibition of MAO type B on the other hand can be attributed to both pargyline and NPB.

Chronic effects of relatively low doses of pargyline, NPB and deprenyl on urine and brain concentrations of some non-catecholic biogenic amines and metabolites

Since repeated doses are required to produce the therapeutic benefits of most MAOIs employed in the treatment of mental illnesses, the chronic effects of the above MAOIs on urine and brain biogenic amines

Table 3 The excretion of pargyline metabolites[a], biogenic amines and their metabolites on different days following chronic pargyline treatment

Description (n)	*Free PEA*	*Free m-tyramine*	*Free p-tyramine*	*BZ*[B]	*NM-BZ*[b]	*NPB*	*HVA*	*MHPG*
Controls (5)	210 ± 43	370 ± 60	1460 ± 330	—	—	—	3.8 ± 0.5	3.5 ± 0.5
0 h (5)	8988 ± 1000***	—	—	c	c	d	1.0 ± 0.2***	0.8 ± 0.2***
24 h (5)	2171 ± 512***	1700 ± 120***	7160 ± 1970***	13.4 ± 3.6	13.3 ± 2.6	80.5 ± 9.5	1.2 ± 0.1***	0.6 ± 0.1***
48 h (5)	528 ± 93**	700 ± 180*	1890 ± 600	ND	ND	ND	1.4 ± 0.2***	2.4 ± 0.2**

Pargyline (50 mg kg^{-1} was administered i.p. daily for 10 days. Urine collected for 4 h immediately after the last dose, 24 and 48 h later. Results are expressed (mean ± s.e.mean) in ng mg^{-1} creatinine for PEA and tyramine. BZ, NM-BZ, NPB, HVA and MHPG are expressed in μg mg^{-1}

[a]BZ,NM-BZ and NPB were assayed by employing [$^2H_9$]-PEA as the deuterated reference standard. Their concentrations were calculated from a standard curve constructed from the responses related to the addition of three different levels of each of the three compounds to the same urine.

[b]BZ = benzylamine, NM-BZ = N-methylbenzylamine, NPB = N-propargylbenzylamine; other abbreviations, see Table 1.

[c]Over 500 μg mg^{-1} creatinine

[d]Over 2000 μg mg^{-1} creatinine

ND = Not detected

*$P < 0.02$; **$P < 0.005$; ***$P < 0.001$ by unpaired t test

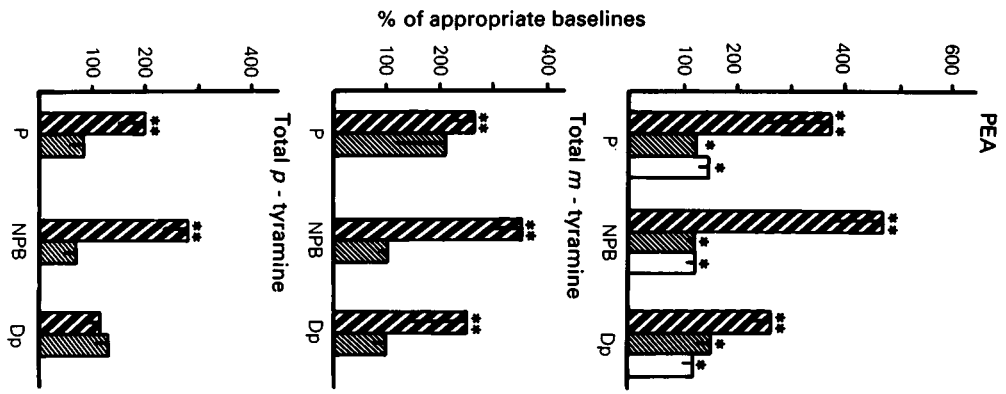

Figure 2 The chronic effects of a number of monoamine oxidase inhibitors (MAOIs) and their metabolites on urinary non-catecholic biogenic amines excretion during and after chronic treatments. Pargyline (35 μmol kg^{-1}; 5 mg kg^{-1}), NPB (35 μmol kg^{-1}; 6.8 mg kg^{-1}) and deprenyl (20 μmol kg^{-1}; 4.5 mg kg^{-1}), were administered i.p. daily for 14 days and urine collected on the 13th day (▨), 24 h (▨) and 5 days (□) after the last treatment. The results are expressed (mean ± s.e.mean) as the % of the mean of the appropriate baseline values of untreated rats. Five rats were included in each experiment. The mean ± s.e.mean concentrations of total PEA, *m*- and *p*-tyramine in urines of control rats were respectively, 0.486 ± 0.033, 0.486 ± 0.063 and 13.16 ± 1.84 μg ml^{-1} creatinine. *$P < 0.05$; **$P < 0.005$. P = pargyline, NPB = propargylbenzylamine, Dp = deprenyl.

Table 4 The acute effects of pargyline, N-propargylbenzylamine (NPB) and deprenyl on brain biogenic amines and metabolites

Tissue/Drug	*PEA* (pg)	*MHPG* (ng)	*DA* (ng)	*DOPAC* (ng)	*HVA* (ng)	*5-HIAA* (ng)
Hypothalamus						
Control	126.8 ± 18.4	1.30 ± 0.01	—	—	—	—
Pargyline	404 ± 51**	0.41 ± 0.04**	—	—	—	—
NPB	538 ± 121**	0.90 ± 0.04**	—	—	—	—
	Deprenyl	250 ± 25**	1.19 ± 0.25	—	—	—
Caudate nucleus						
Control	70.4 ± 5.2	—	98.3 ± 9.6	8.7 ± 0.4	6.5 ± 0.6	12.1 ± 0.6
Pargyline	457.0 ± 68.9**	—	135.1 ± 5.0**	1.0	1.4 ± 0.1**	4.2 ± 0.7**
NPB	652.0 ± 172**	—	119.2 ± 5.5**	2.6 ± 0.4**	3.6 ± 0.2	10.7 ± 0.7
Deprenyl	109.2 ± 10*	—	121.9 ± 6.7**	3.0 ± 0.2**	3.8 ± 0.1	11.5 ± 0.7

Pargyline, 50 mg kg^{-1}, NPB, 30 mg kg^{-1} and deprenyl, 10 mg kg^{-1} were administered i.p. and rats killed 4 h after treatment. Five rats were included in each experiment. Results are expressed (mean ± s.e.mean) in the units indicated per mg protein.
*$P < 0.02$; **$P < 0.002$ by unpaired t test.

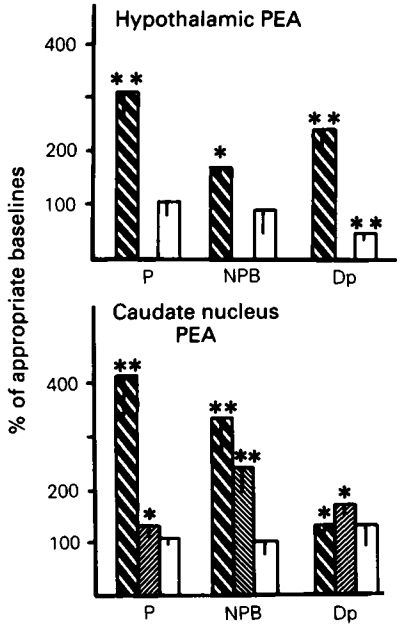

Figure 3 The chronic effects of a number of monoamine oxidase inhibitors (MAOIs) and their metabolites on brain phenylethylamine (PEA) during and after chronic treatments. See Figure 2 for drug schedule and abbreviations. Different groups of rats were killed 3 h after 13 daily doses (■), 24 h (▨) and five days (□) after the end of 14 daily doses. See Table 4 for the normal values of the amines and metabolites. *$P < 0.05$; **$P < 0.005$.

were assessed. The doses selected corresponded to the minimum amounts of drug required to produce a significant increase in urine PEA excretion and/or a significant reduction in catecholamine metabolism. As can be deduced from Figure 1, deprenyl 5 mg kg^{-1} appears close to such a dose. Deprenyl 5 mg kg^{-1} (corresponding to 35 μmol), was therefore selected as the reference dose against which the doses of NPB and pargyline were adjusted. The objective of this study was: (a) to follow the profile of changes in biogenic amines disposition during treatment (after 13 daily doses), 24 h and five days after the end of treatments (14 daily treatments); (b) to correlate the changes observed in the brain with those found in the urine.

During the course of this investigation we discovered that free PEA constitutes about 50% of total urine PEA. In contrast, free *p*-tyramine represented only about 10% of the total tyramine normally excreted. Since changes in free and total PEA, *m*- and *p*-tyramaine in all these studies were parallel, only the results on total excretion of these amines are shown.

Figures 2 and 3 summarize the changes observed in total PEA, *m*- and *p*-tyramine during and after chronic drug treatments. All drugs tested increased PEA excretion during treatment (Figure 2). Comparing the changes in PEA in the urine and brain during treatment and the subsequent two periods (24 h and 5 days), a rapid return towards normal PEA concentrations was observed. Interestingly, hypothalamic PEA concentration did not only cease to be high but was significantly reduced five days after the end of treatment with deprenyl (Figure 3).

The profile of changes in *m*-tyramine excretion

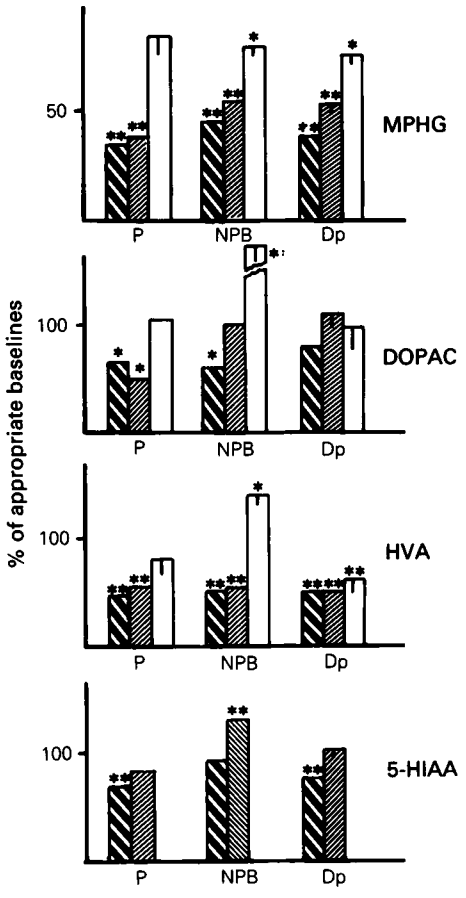

Figure 4 The chronic effects of a number of monoamine oxidase inhibitors (MAOIs) and their metabolites on urinary catecholamine and 5-hydroxytryptamine metabolites after chronic treatments. See Figure 2 for drug schedule and abbreviations and Figure 3 for times when rats were killed. See Table 2 for the normal values of HVA, MHPG and 5-HIAA. The mean ± s.e.mean of DOPAC excretion was 0.657 ± 110 and 22.0 ± 5.3 g mg^{-1} creatinine. $*P<0.05$; $**P<0.005$. HVA = homovanillic acid, MHPG = 3-methoxy-4-hydroxy-phenylglycol, 5-HIAA = 5-hydroxyindole-acetic acid.

observed during treatment with all the above drugs was very similar to that observed for PEA (Figure 3). *p*-Tyramine excretion showed similar correlations only after pargyline and NPB treatments. Deprenyl, chronic treatment did not increase *p*-tyramine excretion. Furthermore, the direction of change in PEA excretion and brain content of PEA were similar.

Chronic effects of pargyline, NPB and deprenyl on urinary catecholamine and 5-HT metabolites

The chronic effects of the above drugs on urinary biogenic amine metabolites are summarized in Figure 4. All the drug treatments significantly reduced MHPG and HVA excretion during treatment and 24 h after treatment. The reductions in MHPG and HVA observed during drug treatment were almost completely abolished or reversed five days after pargyline and NPB. While DOPAC excretion generally followed the same direction as HVA, the changes observed were not as marked. 5-HIAA excretion rate was reduced by about 40% during treatment with pargyline and deprenyl but not by NPB. This reduction was abolished 24 h later.

Chronic effects of NPB, pargyline and deprenyl on brain catecholamine metabolism

The chronic effects of the above three drugs on hypothalamic MHPG and caudate nucleus concentrations of DOPAC and HVA were generally parallel to the effects observed in the excretion of these metabolites. There were, however, some differences in the details of these changes. The percentage of reductions in MHPG induced in the hypothalamus was less than that observed in the urine, (Figure 5). The opposite effects were found for DOPAC in the caudate nucleus. Thus, while in the urine, DOPAC rate of excretion was less responsive to drug treatment than was that of HVA, the opposite was true in the caudate nucleus, (compare results in Figure 4 with Figure 5).

Discussion

Since most urinary amines and their metabolites probably originate from peripheral pools, changes in their excretion can be attributed primarily to changes in their peripheral disposition. This latter assumption has been tested and found to be valid for NA metabolism in rats (Karoum & Costa, 1974). It has also been assumed, with some support from available literature (Neff & Goridis, 1972; Neff *et al.*, 1973; Campbell *et al.*, 1979), that the *in vitro* substrate affinities of MAO A and B to the amines investigated here are similar to their *in vivo* affinities. Thus a marked elevation in PEA excretion or brain content are generally taken to indicate efficient MAO type B inhibition while a marked reduction in MHPG and/or 5-HIAA is considered the result of MAO type A inhibition. An extension to this assumption can be made regarding the possible existence of multiple forms of MAO type A and B *in vivo*. For example, if two amines are equally preferred by the same type of MAO *in vitro* but the *in vivo* inhibition of their

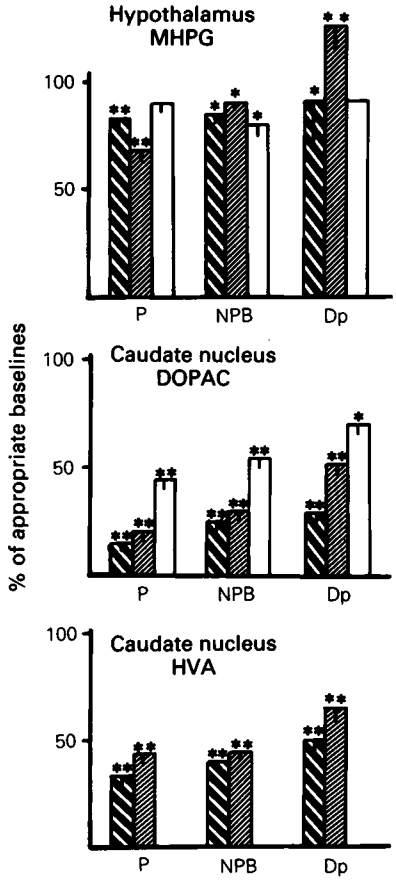

Figure 5 The chronic effects of a number of MAOIs and their metabolites on brain catecholamine metabolites during and after chronic treatment. See Figure 2 for drug schedule and abbreviations and Figure 3 for times when rats were killed. See Table 4 for the normal values of the amines and metabolites. $*P<0.05$; $**P<0.005$. HVA = homovanillic acid, MHPG = 3-methoxy-4-hydroxy-phenylglycol, DOPAC = 3, 4-dihydroxyphenyl acetic acid.

metabolism are different, then the *in vivo* involvement of more than one enzyme or the same enzyme belonging to different compartments should be suspected. Although their structural authenticity has been questioned (Houslay & Tipton, 1973), evidence suggesting the existence *in vivo* of multiple forms of MAO in different organs as well as in the same tissue from different species are quite convincing (Neff & Goridis, 1972; Sandler & Youdim, 1972; Sandler *et al.*, 1974; Neff & Fuentes, 1976).

Three major ethyl acetate extractable metabolites of pargyline were detected; benzylamine, N-methylbenzylamine and NPB. The effects of these metabolites *in vivo* on central and peripheral biogenic amines are described here for the first time (Tables 2 and 5). Weli *et al.* (1982) reported four major metabolites of pargyline, they included NPB, N-methylbenzylamine, benzylamine and N-oxide pargyline (an unstable compound). In that study, incubation with rat liver microsomes revealed that pargyline was very rapidly metabolized (92% of the substrate was metabolized within 1 min) and that the four metabolites account for over 80% of those formed. Assuming pargyline is similarly metabolized *in vivo*, most of its acute effects may well be produced by its metabolites, especially NPB. Consistent with this view is our failure to detect unchanged pargyline in any of the urines analysed. It is therefore possible that during acute treatment with pargyline, exposure of tissue MAO to pargyline is considerably shorter than their exposure to its metabolites. As shown in Table 3, NPB was detected in the urine in substantial quantities up to 24 h following pargyline administration.

Among the three metabolites of pargyline detected, only NPB was found capable of inhibiting MAO *in vivo* (Table 2). These two compounds, however, differ with regard to their abilities to inhibit 5-HT metabolism. As shown in Tables 2 and 4, NPB did not reduce the excretion or brain concentration of 5-HIAA. Additionally, NPB was found to share many similarities with deprenyl, a purported potent MAO B inhibitor (Table 2 and 4) (Knoll, 1980; 1981). At the doses used, NPB and deprenyl appeared to be equipotent. Although MAO activities in the brain were not measured after either deprenyl or NPB, the acute dose of $10\,mg\,kg^{-1}$ deprenyl employed was expected to render an almost complete inhibition of MAO type B in both the periphery and the brain (Neff & Fuentes, 1976; Elsworth *et al.*, 1978). By inference therefore, a similar MAO B inhibition is expected from NPB.

Judging from their acute effects on PEA and 5-HT metabolism, it is easy to surmise that at the doses used, both NPB and deprenyl showed specific MAO type B inhibitions. However, when NA and dopamine metabolisms were considered, these two MAOIs showed clear inhibition of their metabolism in the periphery (Tables 2) and/or the brain (Table 4). It therefore appears that MAO type A was also inhibited by these two drugs. This paradox is difficult to explain without evoking the influence of at least two variants of MAO type A; one sensitive to deprenyl and NPB and the other relatively insensitive to these inhibitors. The former may be primarily located in noradrenergic and dopaminergic neurones while the latter may be associated with 5-HTergic neurones. Although NPB and deprenyl generally have similar properties they are

not identical in their detailed effects on biogenic amine disposition especially dopamine metabolism (see Figures 4 and 5).

The pattern of PEA, *m*- and *p*-tyramine excretions during chronic pargyline treatment suggest a positive correlation between the excretion of these amines and NPB excretion (Table 3). NPB may therefore contribute towards the *in vivo* MAO type B inhibitory properties of pargyline. In this context, it is possible that MAIOs that do not have active metabolites or those whose metabolites behave similarly to their parent compounds as is the case with deprenyl (Karoum *et al.*, 1982), may be labelled specific inhibitors although their metabolites may exhibit additional pharmacological effects. These characteristics may underlie some of the therapeutic differences reported among various forms of the so-called selective MAOIs (Knoll, 1980; 1981). The therapeutic benefits of deprenyl as an adjunct to the regular therapy of Parkinsonism (Birkmayer *et al.*, 1977); Yahr, 1978) is apparently influenced to some extent by its metabolites, amphetamine and methamphetamine (Eisler *et al.*, 1981; Karoum *et al.*, 1982).

The chronic effects of pargyline, NBP and deprenyl have been evaluated on central and peripheral biogenic amines at three different times: during treatment, 24 h and five days after termination of treatment. The results obtained five days after treatment are expected to represent primarily MAO inhibition because by then, most of the drugs and their metabolites are expected to be almost completely eliminated from the body. Considering that pargyline and deprenyl are irreversible inhibitors with half lives of enzyme inhibition extending over 10 days (Neff & Goridis, 1972), MAO inhibition by pargyline, deprenyl and possible NPB is expected to remain relatively unchanged five days after the end of treatment. The results of our study, however, indicated a substantial return to normal metabolism five days after the end of treatment. This effect may be attributed to overcompensation by newly formed MAO. Further, comparing the peripheral changes observed with those produced in the brain, all three MAOIs tested showed parallel peripheral and central effects. Apparently, the use of urine excretion of biogenic amine metabolites may provide a useful index to the central effects of MAIOs.

At first glance the relatively weak and short lived paradoxical (Tables 2 and 4), because pargyline is a highly selective MAO type A inhibitor *in vitro*, and because it inhibits 5-HT deamination *in vitro* better than that of NA in brain tissues (Glover *et al.*, 1977; White & Glassman, 1977). At the 50 mg kg^{-1} dose of pargyline employed, type A MAO is expected to be almost completely inhibited (Neff & Fuentes, 1976; Fowler *et al.*, 1982). On close scrutiny, however, our *in vivo* observations appeared in good agreement with a number of studies reported in man and monkeys. Both chronic clorgyline and pargyline were found to reduce NA and dopamine metabolism *in vivo* better than that of 5-HT (Murphy, 1986). In the cerebrospinal fluid (CSF) (Major *et al.*, 1979; Murphy *et al.*, 1981) and urine (Linnoila *et al.*, 1982) both clorgyline (a potent MAO A inhibitor; Knoll, 1980) and pargyline markedly reduced CSF concentrations of MHPG and HVA with less than 50% reductions in 5-HIAA (Major *et al.*, 1979). Similar alterations were observed in MHPG and 5-HIAA excretions in man (Linnoila *et al.*, 1982). The relatively poor inhibitory effects of pargyline on the *in vivo* metabolism of 5-HT may partly be explained in terms of its rapid metabolism to products that lack effects on 5-HT metabolism. This latter may explain the effects of pargyline but not clorgyline. Whether or not the presence of MAO type B in 5-HTergic neurones in the brain (Levitt *et al.*, 1982) and perhaps in the periphery is responsible for the lack of *in vivo* MAO A inhibition of 5-HT metabolism is an issue that awaits further investigation. Furthermore, the paradox surrounding the *in vivo* characteristics of MAO oxidation of 5-HT as compared to other biogenic amines may partly arise from the fact that 5-HTergic neurones interact with physiological and pharmacological changes in ways that may not be identical to catecholaminergic neurones (Murphy, 1986; Kuhn *et al.*, 1986).

In conclusion, among the three major metabolites of pargyline, NPB was found to exhibit potent *in vivo* MAO inhibitory properties. Thus compared to pargyline and deprenyl, NPB appears to show characteristics that may label it more as an *in vivo* type B than type A MAO inhibitor. Its administration markedly elevated urine and brain concentrations of PEA but had no effect on 5-HIAA concentration in both the urine and the brain. It is therefore possible that the metabolism of pargyline to NPB plays an important role in the *in vivo* MAO type B inhibition by pargyline.

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