Biochemical Pharmacology, Vol. 30, No. 11, pp. 1295–1298, 1981.
Printed in Great Britain.

0006-2952/81/111295-04 $02.00/0

INFLUENCE OF HARMALINE ON THE ABILITY OF PARGYLINE TO ALTER CATECHOLAMINE METABOLISM IN RATS

RAY W. FULLER,* SUSAN K. HEMRICK-LUECKE and KENNETH W. PERRY
The Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285, U.S.A.

(*Received* 22 *September* 1980; *accepted* 1 *December* 1980)

Abstract—When pargyline hydrochloride (20 mg/kg, i.p.) was injected into rats 48 hr before the measurement of monoamine oxidase (MAO) activity, the oxidation of [^{14}C]phenylethylamine (type B MAO) and of [^{14}C]-serotonin (type A MAO) was inhibited. Neither type A nor type B MAO was inhibited 48 hr after the injection of harmaline hydrochloride (30 mg/kg, i.p.) a short-acting, reversible, highly selective inhibitor of type A MAO. When harmaline was given just before pargyline, it prevented the inhibition of type A MAO by pargyline but not the inhibition of type B MAO. Pargyline alone elevated epinephrine, norepinephrine, and dopamine concentrations in brain regions and norepinephrine concentration in heart. The concentration of dopamine metabolites (3,4-dihydroxyphenylacetic acid and homovanillic acid) was decreased. Pretreatment with harmaline prevented all of these effects of pargyline. The findings suggest that inhibition of type A MAO is involved in the inhibition of catecholamine metabolism by pargyline, since harmaline pretreatment did not prevent inhibition of type B MAO and would not be expected to alter any other possible actions of pargyline. These findings support the idea that type A MAO is primarily responsible for the oxidation of epinephrine, norepinephrine, and dopamine in rat brain and of norepinephrine in rat heart.

Pargyline is a potent inhibitor of monoamine oxidase [MAO; monoamine: oxygen oxidoreductase (deaminating), EC 1.4.3.4] inhibiting preferentially type B rather than type A MAO [1,2]. Pargyline is a "k_{cat} inhibitor" or a "suicide substrate" for MAO; the enzyme acts on pargyline to form a reaction intermediate that reacts with the enzyme to inactivate it [3]. We had shown earlier that harmaline, a highly selective, reversible inhibitor of type A MAO, prevented the inactivation of type A MAO in rat tissues by pargyline [4]. Harmaline, a relatively short-acting inhibitor of MAO *in vivo*, apparently prevented type A MAO catalysis of pargyline conversion to a covalent-binding intermediate. Within 24–48 hr after the drugs were injected into rats, the reversible inhibition of type A MAO by harmaline had ended, and only the irreversible inactivation of type B MAO by pargyline remained.

Comparing pharmacologic effects of pargyline alone with those of pargyline given together with harmaline offers the opportunity to deduce which effects were due to inhibition of type A MAO, since these are the only actions of pargyline that harmaline would be expected to prevent. In the present study, we have determined the ability of pargyline alone or of pargyline + harmaline to increase catecholamine concentrations in rat brain and heart and to lower brain concentrations of catecholamine metabolites.

METHODS

Male Wistar rats (150–200 g) from Harlan Industries, Cumberland, IN, were given food and water *ad lib*. Pargyline hydrochloride and harmaline hydrochloride were purchased from the Sigma Chemical Co., St. Louis, MO. These compounds were injected i.p. in aqueous solution. Rats were decapitated, and brains or hearts were removed quickly and frozen on dry ice. In one experiment brain regions were dissected before the tissue was frozen. Tissues were stored at −15° prior to analysis. Catecholamines and their metabolites were measured by high performance liquid chromatography with electrochemical detection [5, 6]. MAO activity was assayed radiometrically with [^{14}C]-serotonin (100 μM) as substrate for type A MAO or [^{14}C]-phenylethylamine (12.5 μM) as substrate for type B MAO [7].

In all experiments, pargyline hydrochloride (20 mg/kg) and harmaline hydrochloride (30 mg/kg) were injected i.p. 48 hr before the rats were killed. All data are shown as mean values ± standard errors for five rats per group. Statistical comparisons were made by Student's *t*-test.

RESULTS

Norepinephrine and epinephrine concentrations were elevated in the hypothalamus and the brain stem in pargyline-treated rats (Table 1). The percentage increase in epinephrine concentration was greater both in hypothalamus and brain stem (+194 and +155 per cent, respectively) than was the increase in norepinephrine (+47 in hypothalamus and +40 per cent in brain stem). There were no differences from control in catecholamine concentrations in rats treated with harmaline alone, and harmaline completely prevented the increase in epinephrine and norepinephrine concentrations caused by pargyline in both brain regions.

In the cerebral hemispheres (Table 2), the pre-

* Address all correspondence to: Dr. Ray W. Fuller, Lilly Research Laboratories, 307 East McCarty St., Indianapolis, IN 46285, U.S.A.

Table 1. Norepinephrine and epinephrine concentrations in rat brain regions*

Treatment group	Catecholamine (nmoles/g) Norepinephrine	Epinephrine
	Hypothalamus	
Control	8.01 ± 0.51	0.145 ± 0.006
Pargyline	11.77 ± 0.77†	0.427 ± 0.028†
Harmaline	7.27 ± 0.32	0.126 ± 0.008
Harmaline + pargyline	7.41 ± 0.38†	0.138 ± 0.007†
	Brain stem	
Control	4.44 ± 0.16	0.053 ± 0.008
Pargyline	6.17 ± 0.15†	0.135 ± 0.009†
Harmaline	4.51 ± 0.11	0.054 ± 0.004
Harmaline + pargyline	4.79 ± 0.23‡	0.057 ± 0.003‡

* Pargyline hydrochloride (20 mg/kg, i.p.) was injected 48 hr before rats were killed and 10 min after the injection of harmaline hydrochloride (30 mg/kg, i.p.). Mean values ± S.E. for five rats are shown.
† Significant elevation compared to control group ($P < 0.05$).
‡ Significantly different from group treated with pargyline alone ($P < 0.05$).

Table 2. Dopamine and norepinephrine concentrations in whole cerebral hemispheres of rats*

Treatment group	Catecholamine (nmoles/g) Dopamine	Norepinephrine
Control	4.59 ± 0.13	1.87 ± 0.05
Pargyline	5.92 ± 0.10†	2.77 ± 0.06†
Harmaline	4.51 ± 0.07	1.86 ± 0.06
Harmaline + pargyline	4.85 ± 0.10‡	1.85 ± 0.09‡

* Conditions were as in Table 1.
† Significant elevation compared to control group ($P < 0.05$).
‡ Significantly different from group treated with pargyline alone ($P < 0.05$).

dominant catecholamine was dopamine. Dopamine concentration was increased significantly by pargyline in this brain region, as was norepinephrine concentration. Epinephrine concentration was below the limits of detection in this brain region. No significant changes in these catecholamines were present in rats treated with harmaline either alone or in combination with pargyline. Thus, harmaline pretreatment completely prevented the elevation of dopamine and norepinephrine by pargyline.

Table 3 shows that in the conditions of this experiment harmaline prevented the inactivation of type A but not of type B MAO by pargyline. Pargyline alone inhibited the oxidation both of serotonin and of phenylethylamine. At this time after harmaline (48 hr), no effects on either form of MAO remained, although at early times harmaline selectively inhibited serotonin oxidation (type A MAO) (Fig. 1). The inactivation of type A MAO by pargyline was completely prevented in the rats co-treated with harmaline, whereas the inactivation of type B MAO was changed very little (Table 3). Thus, in the rats treated with the combination of pargyline and harmaline, phenylethylamine oxidation but not serotonin oxidation was inhibited. The results in Table 3 are similar to those we published earlier [4], the only differences in the earlier experimental conditions being that the rats were killed at 24 hr instead of at 48 hr as in the present study and that a higher concentration of phenylethylamine had been used to assay type B MAO.

Table 4 shows heart norepinephrine concentra-

Table 3. Inhibition of MAO in rat brain*

Treatment group	MAO activity ($nmoles \cdot min^{-1} \cdot g^{-1}$) [^{14}C]-Serotonin	[^{14}C]-Phenylethylamine
Control	82 ± 0.6	26 ± 1
Pargyline	26 ± 1† (−68%)	9 ± 0.07† (−65%)
Harmaline	83 ± 1	24 ± 1
Harmaline + pargyline	80 ± 1‡	12 ± 0.3†‡ (−56%)

* Conditions were as in Table 1.
† Significant inhibition of MAO activity ($P < 0.01$).
‡ Significantly different from group treated with pargyline alone ($P < 0.05$).

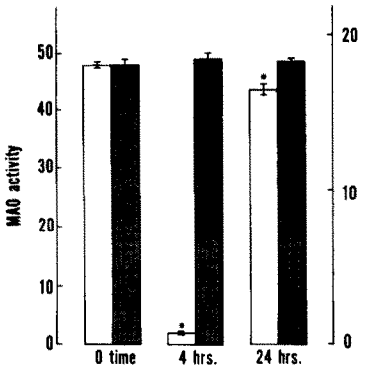

Fig. 1. Transient inhibition of type A MAO selectively by harmaline. MAO activity in brain homogenates was assayed with [^{14}C]-serotonin (100 μM) (left ordinate; open bars) or [^{14}C]-phenylethylamine (12.5 μM) (right ordinate; shaded bars) as substrate at 0 time and at 4 and 24 hr after the injection of harmaline hydrochloride (30 mg/kg, i.p.). Units of enzyme activity are in nmoles·g brain^{-1}min^{-1}. Mean values ± S. E. for five rats per group are shown. An asterisk (*) indicates significant inhibition of enzyme activity ($P < 0.01$).

Table 4. Norepinephrine concentration in rat heart*

Treatment group	Norepinephrine (nmoles/g)
Control	3.54 ± 0.60
Pargyline	5.36 ± 0.46† (+52%)
Harmaline	3.66 ± 0.32
Harmaline + pargyline	4.12 ± 0.42‡

* Conditions were as in Table 1.
† Significant elevation compared to control group ($P < 0.05$).
‡ Significantly different from group treated with pargyline alone ($P < 0.05$).

tions. Pargyline alone caused a significant (52 per cent) increase in norepinephrine concentration. Harmaline alone had no effect, and harmaline prevented any significant elevation of norepinephrine concentration by pargyline. Compared to rats treated with harmaline alone, the rats co-treated with pargyline had an apparent slight (13 per cent) but not statistically significant increase in norepinephrine concentration.

Table 5 shows the concentration of two dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in rat brain. Pargyline caused a statistically significant decrease in the concentration of both metabolites. DOPAC was reduced by 51 per cent and HVA by 34 per cent. Harmaline, which alone had no significant effect on the metabolites, completely prevented their lowering by pargyline.

DISCUSSION

The present results confirm, as we had reported earlier [4], that the inactivation of type A, but not of type B, MAO by pargyline can be prevented by co-treatment with harmaline, a short-acting, highly selective, reversible inhibitor of type A MAO. In rats treated with harmaline alone, no significant effects on type A or type B MAO, on the concentration of catecholamines measured in brain or in heart, or on DOPAC or HVA concentrations in brain remained at 48 hr. Pargyline alone inhibited type B MAO and type A MAO, elevated epinephrine, dopamine and norepinephrine concentrations in brain regions and norepinephrine concentration in heart, and reduced DOPAC and HVA concentrations in brain. In rats treated with pargyline and harmaline together, the inhibition of type B MAO persisted essentially unchanged whereas the inhibition of type A MAO was prevented. The increases in catecholamines in brain and heart and the decreases in dopamine metabolites in brain were also prevented. These findings suggest that type A MAO is chiefly responsible for the oxidation of the catecholamines in these tissues.

A comparison of Tables 3 and 5 reveals that the percentage inhibition of type A MAO by pargyline (68 per cent) was greater than the percentage reduction of DOPAC and HVA (51 and 34 per cent respectively). This finding is consistent with the earlier data of Waldmeier *et al.* [8], who observed that the reductions in DOPAC and HVA paralleled the dose dependence for inhibition of type A MAO by clorgyline and deprenyl, although the percentage change in the metabolites was always less than the percentage inhibition of the enzyme. This relationship was not unexpected; the enzyme presumably is present in excess, so that dopamine oxidation is impaired only when the enzyme is inhibited to a substantial degree.

Earlier evidence has been presented that type A MAO is mainly responsible for the oxidation of norepinephrine and dopamine in rat brain *in vivo*

Table 5. 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentration in rat brain*

Treatment group	Dopamine metabolites in brain (nmoles/g) DOPAC	HVA
Control	0.61 ± 0.02	0.47 ± 0.02
Pargyline	0.30 ± 0.02†	0.31 ± 0.01†
Harmaline	0.67 ± 0.01	0.49 ± 0.02
Harmaline + pargyline	0.63 ± 0.03‡	0.46 ± 0.04‡

* Conditions were as in Table 1.
† Significant reduction compared to control group ($P < 0.01$).
‡ Significantly different from group treated with pargyline alone ($P < 0.01$).

[8–13], although dopamine had sometimes been regarded as a mixed substrate for types A and B MAO [14–16]. Our findings represent a new type of evidence for that conclusion and show additionally that epinephrine in rat brain apparently is deaminated mainly by type A MAO *in vivo*.

REFERENCES

1. R. W. Fuller, *Archs. int. Pharmacodyn. Thér.* **174**, 32 (1968).
2. C. J. Fowler, B. A. Callingham, T. J. Mantle and K. F. Tipton, *Biochem. Pharmac.* **27**, 97 (1978).
3. R. R. Rando, *Science* **185**, 320 (1974).
4. R. W. Fuller and S. K. Hemrick, *Life Sci.* **22**, 1083 (1978).
5. R. W. Fuller and K. W. Perry, *Biochem. Pharmac.* **26**, 2087 (1977).
6. K. W. Perry and R. W. Fuller, *Soc. Neurosci. Abstr.* **5**, 348 (1979).
7. R. W. Fuller and B. W. Roush, *Archs. int. Pharmacodyn. Thér.* **198**, 270 (1972).
8. P. C. Waldmeier, A. Delini-Stula and L. Maitre, *Naunyn-Schmiedeberg's Archs. Pharmac.* **292**, 9 (1976).
9. C. Braestrup, H. Andersen and A. Randrup, *Eur. J. Pharmac.* **34**, 181 (1975).
10. I. C. Campbell, D. S. Robinson, W. Lovenberg and D. L. Murphy, *J. Neurochem.* **32**, 49 (1979).
11. K. T. Demarest and A. J. Azzaro, in *Monoamine Oxidase: Structure, Function, and Altered Functions* (Eds. T. P. Singer, R. W. Von Korff and D. L. Murphy), p. 423. Academic Press, New York (1979).
12. P. E. Keane, F. Chanoine and M. Strolin Benedetti, in *Monoamine Oxidase: Structure, Function, and Altered Functions* (Eds. T. P. Singer, R. W. Von Korff and D. L. Murphy), p. 341. Academic Press, New York (1979).
13. J. Timar, B. Knoll, Z. Gyarmati and J. Knoll, *Pol. J. Pharmac. Pharm.* **31**, 251 (1979).
14. H-Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **189**, 733 (1974).
15. N. H. Neff and H-Y. T. Yang, *Life Sci.* **14**, 2061 (1974).
16. K. F. Tipton, M. D. Houslay and T. J. Mantle, in *Monoamine Oxidase and Its Inhibition* (Ciba Foundation Symposium 39), p. 5. Elsevier, Amsterdam (1976).