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EFFECT OF MONOAMINE OXIDASE INHIBITORS ON THE DISPOSITION OF INTRACISTERNALLY ADMINISTERED METARAMINOL-^{3}H*

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Abstract—The effect of several monoamine oxidase inhibitors on the disappearance of intracisternally injected metaraminol-^{3}H, a synthetic monoamine which is resistant to the action of monoamine oxidase, was studied in the rat. Pretreatment with phenelzine, pheniprazine or nialamide markedly accelerated the disappearance of metaraminol-^{3}H from brain, while pargyline pretreatment substantially retarded its escape. In contrast, both pheniprazine and pargyline were found to significantly hasten the disappearance of the labeled amine from heart. Phenelzine or pheniprazine failed to alter the uptake of intracisternally injected metaraminol-^{3}H into brain, and neither pargyline nor pheniprazine affected the disappearance of intracisternally administered urea-^{14}C or α-aminoisobutyric acid-^{14}C. These findings are consistent with the view that pargyline interferes with monoamine transport from brain, while pheniprazine, nialamide, and phenelzine act to displace metaraminol by promoting the accumulation of endogenous amines.

NUMEROUS reports attest to the diverse pharmacological effects of monoamine oxidase (MAO) inhibitors.[1] Under certain circumstances nialamide diminishes the augmentation of norepinephrine release associated with nerve stimulation.[2] Pheniprazine has been shown to retard the disappearance of norepinephrine-^{3}H previously accumulated by peripheral tissues,[3] as well as to increase the cerebral accumulation of intraventricularly administered norepinephrine-^{3}H[4,5] and to slow its subsequent disappearance.[6] More recent studies with intracisternally injected octopamine-^{3}H have demonstrated the ability of pheniprazine and pargyline to retard the efflux of this amine from brain.[7] The foregoing observations are likely the result of inhibition of deamination thus favoring amine storage. The operation of mechanisms in addition to MAO inhibition was suggested recently when it was found that pargyline retarded the disappearance of octopamine-^{3}H from brain to a greater extent than pheniprazine.[7] In an attempt to examine these latter findings, the effect of a series of MAO inhibitors on the disappearance of intracisternally injected metaraminol-^{3}H an α-methylated phenylethanolamine not destroyed by monoamine oxidase, was studied.

METHODS

Male, Sprague–Dawley rats weighing 180–200 g were used in all experiments. Intracisternal injection[8] of 25 μl of Elliott's "B" irrigating solution (Baxter Labs.)

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containing *d,l*-metaraminol-7-^{3}H (5 or 10 μc, 6·0 c/m-mole), urea-^{14}C (660 mμc, 3 mc/m-mole) or α-aminoisobutyric acid-^{14}C (600 mμc 3 mc/m-mole) was carried out while the animals were under light ether anesthesia. In other experiments metaraminol-^{3}H (10 μc) was given intravenously (i.v.). At various times after injection the animals were killed by cervical fracture and their brains or hearts quickly removed. The organs were thoroughly rinsed (after midcoronal sectioning of brains to expose their ventricular system), blotted and then homogenized in 10 ml of ice-cold 0·4 N perchloric acid and centrifuged. Total radioactivity, which was found to have the same chromatographic characteristics (Analtec G, thin-layer plates; butanol: acetic acid: water, 4 : 1 : 1) as authentic metaraminol 1 and 4 hr after its intracisternal injection, was determined in an aliquot of the supernatant by liquid scintillation spectrometry.

Pargyline, 75 mg/kg; nialamide, 50 mg/kg; pheniprazine, 10 or 15 mg/kg; iproniazid, 50 mg/kg; phenelzine, 25 mg/kg; and *d*-amphetamine, 1 or 5 mg/kg were given by intraperitoneal injection. All doses for the MAO inhibitors refer to the salt; *d*-amphetamine doses were given as their base. Control animals received isotonic saline. Radioactive compounds were purchased from New England Nuclear, Boston, Mass.

RESULTS

Effect of pargyline on the disappearance of metaraminol-^{3}H from brain. Pargyline pretreatment substantially retarded the initial disappearance of intracisternally injected metaraminol-^{3}H from brain (Table 1, Fig. 1). When the drug was given 30 min after

TABLE 1. EFFECT OF PARGYLINE ON METARAMINOL-^{3}H DISAPPEARANCE FROM BRAIN*

Treatment	Relation to metaraminol injection	Metaraminol-^{3}H c.p.m./brain
Control		154,000 ±5000
Pargyline	Before	260,000† ±10,000
Pargyline	After	164,000‡ ±12,000

*Pargyline (75 mg/kg) was given either 30 min before or 30 min after the intracisternal injection of metaraminol-^{3}H (10 μc). Animals were sacrificed 6 hr after metaraminol-^{3}H administration. Each value represents the mean ± S.E.M. of six determinations.

† $P < 0.001$ when compared with control values.

‡ $P > 0.1$ when compared with control values.

metaraminol-^{3}H, however, there was no significant difference between control and pargyline treated animals (Table 1). Comparison of the time-course of metaraminol-^{3}H disappearance from the brains of pargyline pretreated and control animals suggests that the drug acts largely on the initial rapid phase of metaraminol-^{3}H clearance, although it may also diminish the rate of metaraminol-^{3}H disappearance at later times (Fig. 1).

Effect of various MAO inhibitors on the disappearance of metaraminol-^{3}H from brain. The finding that pargyline retarded the rate at which intracisternally administered metaraminol-^{3}H disappeared from brain prompted an examination of several other

MAO inhibitors. Pretreatment with nialamide, pheniprazine or phenelzine, in contrast to pargyline, hastened the disappearance of metaraminol-^{3}H from brain (Fig. 2). Iproniazid had no discernible effect.

Effect of pargyline and pheniprazine on the disappearance of metaraminol-^{3}H from heart. The influence of pargyline and pheniprazine, respectively the most effective of the drugs studied in retarding or accelerating the disappearance of metaraminol-^{3}H

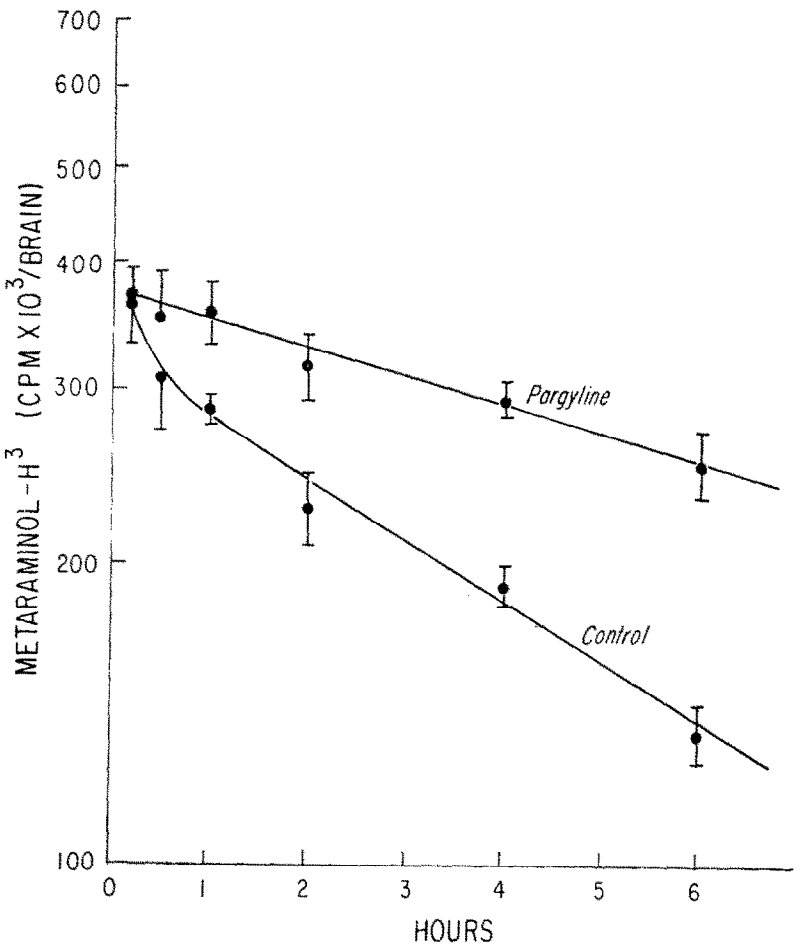

FIG. 1. Effect of pargyline pretreatment on the disappearance of metaraminol-^{3}H from brain. Drug (75 mg/kg) was given 30 min prior to intracisternal injection of metaraminol-^{3}H (10 μc). Each point represents the mean ± S.E.M. of six determinations.

from brain, was tested on heart by giving either of these drugs 30 min prior to the i.v. administration of labeled amine. Both MAO inhibitors reduced the cardiac content of metaraminol-^{3}H to about 65 per cent of control levels (Table 2).

Effects of pheniprazine and phenelzine on the uptake of metaraminol-^{3}H in brain. Pheniprazine and phenelzine given 30 min prior to the intracisternal administration of metaraminol-^{3}H failed to alter the levels of metaraminol-^{3}H found in brain 10 min

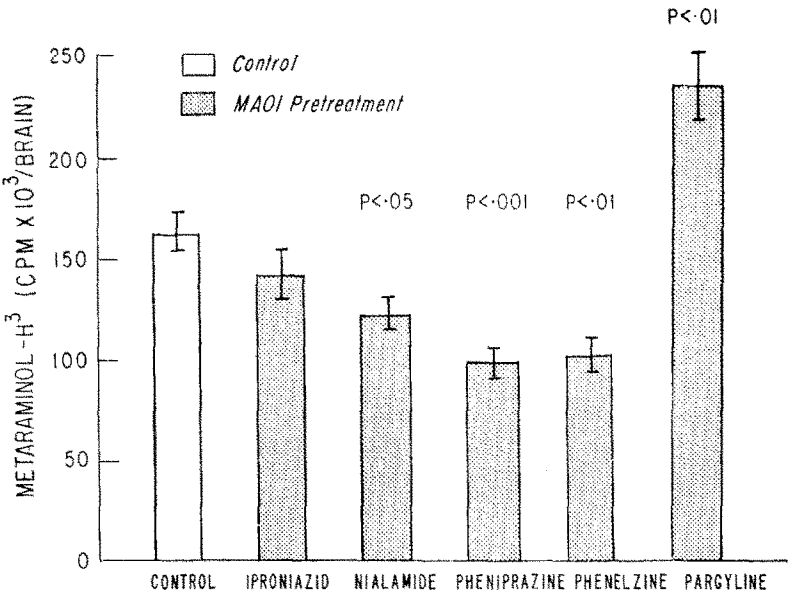

FIG. 2. Effect of MAO inhibitors on metaraminol-^{3}H disappearance from brain. Iproniazid (50 mg/kg), nialamide (50 mg/kg), pheniprazine (10 mg/kg), phenelzine (25 mg/kg) or pargyline (75 mg/kg) was given 30 min before metaraminol-^{3}H (10μc). Animals were killed 5 hr later. Values represent the mean ± S.E.M. of six to nine determinations.

TABLE 2. EFFECT OF PARGYLINE AND PHENIPRAZINE ON THE DISAPPEARANCE OF METARAMINOL-^{3}H FROM HEART*

Treatment	Metaraminol-^{3}H c.p.m./heart
Control	61,000 ± 1700
Pargyline	38,000 ± 1500†
Pheniprazine	41,000 ± 2800†

*Pargyline (75 mg/kg) or pheniprazine (10 mg/kg) was given 30 min before the i.v. injection of metaraminol-^{3}H (10 μc). Animals were killed 4 hr after drug administration. Each value represents the mean ± S.E.M. of six determinations.

† $P < 0.001$ when compared with control values.

after injection (Table 3). Pargyline pretreatment also appeared not to affect the uptake of metaraminol-^{3}H into brain, since cerebral levels of the labeled amine 15 min after its injection were not significantly different than those found in control animals (Fig.1).

Effect of pargyline and pheniprazine on the disappearance of urea-^{14}C and α-aminoisobutyric acid-^{14}C from brain. In an attempt to assess the specificity of the foregoing results, the effect of pargyline or pheniprazine pretreatment on the efflux of intracisternally administered urea-^{14}C or α-aminoisobutyric acid-^{14}C was examined. Neither

TABLE 3. EFFECT OF PHENIPRAZINE AND PHENELZINE ON THE ACCUMULATION OF ^{3}H-METARAMINOL*

Treatment	Metaraminol-^{3}H c.p.m./brain
Control	381,600 ± 14,600
Pheniprazine	335,100 ± 27,300†
Phenelzine	348,600 ± 33,000†

*Pheniprazine (15 mg/kg) or phenelzine (25 mg/kg) was given 30 min before the intracisternal injection of metaraminol-^{3}H (5 μc). Animals were killed 10 min after the ^{3}H-metaraminol. Each value represents the mean ± S.E.M. of eight to twelve determinations.
† $P > 0{\cdot}1$ when compared with control values.

TABLE 4. EFFECT OF PARGYLINE AND PHENIPRAZINE ON THE DISAPPEARANCE OF UREA-^{14}C AND α-AMINO ISOBUTYRIC ACID-^{14}C FROM BRAIN*

Treatment	Urea-^{14}C	AIBA-^{14}C
Control	116,000 ±8000	187,000 ±9000
Pargyline	120,000† ±7000	171,400† ±12,000
Pheniprazine	107,000† ±4000	184,500† ±6000

*Pargyline (75 mg/kg) or pheniprazine (15 mg/kg) was given 30 min before the labeled compound. Animals were sacrificed 2 hr later. AIBA = α-amino isobutyric acid. Values represent the mean ± S.E.M. of six determinations expressed in c.p.m. per brain.
† $P > 0{\cdot}1$ when compared with control values.

drug significantly altered the disappearance of either of the labeled compounds tested (Table 4).

Effect of amphetamine on the disappearance of metaraminol-^{3}H from brain. Amphetamine pretreatment substantially reduced the amount of radioactivity in brain 6 hr after intracisternal injection of metaraminol-^{3}H (Fig. 3). Similar results were obtained when amphetamine was given after metaraminol-^{3}H: labeled amine levels averaged 24 ± 4% ($P < 0{\cdot}05$) below control values in animals receiving amphetamine (1 mg/kg) 30 min after metaraminol-^{3}H administration and killed 4 hr later.

DISCUSSION

Administered metaraminol-^{3}H is rapidly accumulated by tissues rich in sympathetic nerve terminals,[9] where it replaces norepinephrine at intraneuronal binding sites.[9–11] When formed from α-methyl-metatyrosine by decarboxylation and subsequent β-hydroxylation, metaraminol has also been found to displace norepinephrine from its storage sites in brain.[10, 12, 13] Furthermore, brain slices have been observed to concentrate metaraminol-^{3}H when they are incubated in a medium containing the labeled

amine.* It would thus seem reasonable to assume that when injected into the cerebrospinal fluid pathways of the living animal, metaraminol-^{3}H, like its analog norepinephrine-^{3}H,[8, 14] is taken up and bound by cerebral monoaminergic terminals. Since metaraminol is not a substrate for MAO or catechol-*O*-methyl transferase, brain levels after its intracisternal injection reflect the relative rates of uptake into brain and efflux from cerebrospinal fluid or cerebral tissues into the systemic circulation.

In the present experiments pargyline, pheniprazine, phenelzine and nialamide were found to significantly influence the fate of intracisternally injected metaraminol-^{3}H (Fig. 2). It is unlikely that these results reflect nonspecific changes consequent to

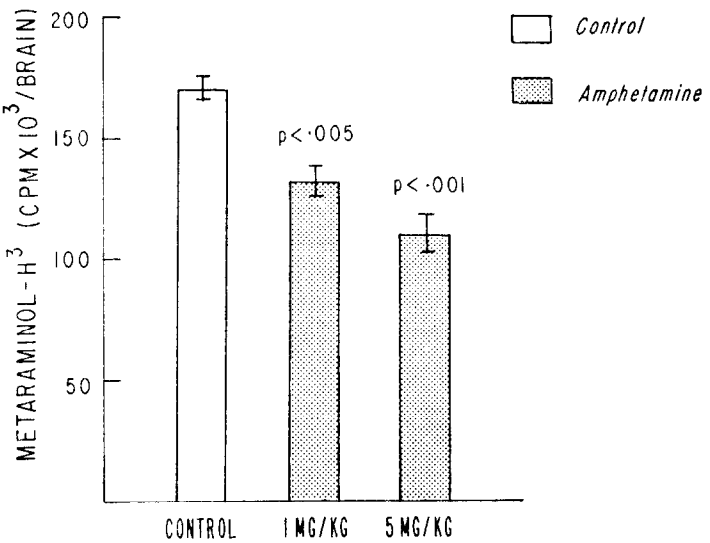

FIG. 3. Effect of amphetamine on metaraminol-^{3}H disappearance from brain. Experimental animals received 1 or 5 mg per kg of drug 30 min before metaraminol-^{3}H injection (10 μc), and were killed 4 hr later. Values represent mean $\pm$ S.E.M. of six determinations.

alterations in blood or cerebrospinal fluid pressure, since neither pargyline nor pheniprazine altered the efflux of intracisternally administered urea-^{14}C or α-amino-isobutyric acid-^{14}C (Table 4).

Metaraminol-^{3}H levels in the brains of animals pretreated with pheniprazine, nialamide or phenelzine were considerably below control values (Fig. 2). A similar effect on metaraminol-^{3}H concentrations in heart was found in rats pretreated with pheniprazine or phenelzine (Table 2). The later observations are in agreement with earlier reports by Carlsson *et al.*[15, 16] on the effect of certain MAO inhibitors on the disappearance of metaraminol-^{3}H from mouse heart. Although an inhibitory influence on metaraminol-^{3}H uptake might account for these results, none was demonstrated in the present experiments in rat brain (Table 3) or in previous studies carried out in mouse heart.[15, 16] It thus appears that certain MAO inhibitors are capable of accelerating the release of metaraminol from central as well as peripheral stores. The ability of decarboxylase inhibitors, which presumably interfere with the synthesis of

*G. R. Breese. T. N. Chase and I. J. Kopin, unpublished observations.

endogenous amines, to block the MAO inhibitor-induced release of α-methylated phenylethanolamines from heart has been cited to support the contention that MAO inhibitors act to displace metaraminol in peripheral neurons by promoting the accumulation of such amines.[16] Conceivably, the effect of pheniprazine, nialamide or phenelzine on metaraminol-^{3}H in brain may reflect this mechanism.

Metaraminol-^{3}H in levels in brain were also substantially reduced by amphetamine (Fig. 3). Since in the dose range used amphetamine does not appear to influence norepinephrine metabolism,[17] the effect of this drug on metaraminol-^{3}H cannot be attributed to an MAO inhibitor-like action. Several studies report that the effect of amphetamine is dependent upon norepinephrine[18,19] and it has recently been found that amphetamine increases the efflux of labeled norepinephrine into fluid perfusing the ventricular system.[20] The activity of amphetamine-like agents on adrenergic systems may reflect an increase in membrane permeability [15, 21] or a displacement of the norepinephrine by a false-transmitter formed by the metabolism of amphetamine.[22] Either of these actions might account for the present results with labeled metaraminol.

Pargyline pretreatment, in striking contrast to amphetamine and all other MAO inhibitors tested, acted to retard the disappearance of metaraminol-^{3}H from brain (Fig. 2). The presumed releasing action of this drug on metaraminol-^{3}H in heart (Table 2) together with its apparent inability to augment the cerebral accumulation of the labeled amine (Fig. 1) suggest that pargyline pretreatment exerts a substantial inhibitory influence on the transfer of metaraminol-^{3}H out of brain. This may be due to a difference in initial distribution of the labeled amine in pargyline pretreated animals since this effect was not evident when pargyline was given after metaraminol-^{3}H. Little is known of the sites or mechanisms subserving amine transfer from the central nervous system. The demonstration by Tochino and Schanker[23] of the ability of the choroid plexus to actively take up monoamines *in vitro* suggests that active amine transport via this organ may occur in the living animal. The relevance, if any, of the effect of MAO inhibitors on the release and transport of amines in brain to the behavioral actions of these drugs remains to be elucidated. Actions of MAO inhibitors on processes other than deamination, however, may account for differences in the clinical efficacy or toxic side effects of these compounds.

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