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Inhibition of phenylethanolamine-*N*-methyl transferase

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THE FINAL step in epinephrine biosynthesis is the transfer of a methyl group from S-adenosylmethionine to the amine nitrogen of norepinephrine.[1] This step is catalyzed by phenylethanolamine-*N*- methyl transferase (PNMT), an enzyme found to be highly localized to the adrenal medulla of several mammalian species.[2] This enzyme *N*-methylates a variety of phenylethanolamines, but not phenethyl or indoleamines.[2] The ability of some phenethyl- and phenylethanolamines to inhibit the *N*-methylation of normetanephrine by PNMT has been demonstrated, but no other classes of compounds have been studied with regard for their capacity to inhibit this enzyme.[3] This report describes the inhibition of PNMT by a variety of amines, with special emphasis on the marked inhibitory ability of tranylcypromine and several related compounds.

PNMT was prepared from beef adrenal medullae according to the method of Axelrod[2] through the ammonium sulfate precipitation step. The enzyme preparation was dialyzed overnight against 0·001 M phosphate buffer, pH 7·0, and the protein content was then adjusted to 2·0 mg/ml. Each assay contained 100 μg protein, 50 μmole phosphate buffer, pH 7·9 1·0 mμmole ^{14}C-methyl S-adenosylmethionine (50·2 mc/m mole, New England Nuclear Corp.). Substrate and inhibitors were added to give a final concentration of 10^{-4} M (unless otherwise stated) in a reaction volume of 300 μl. Incubations and extractions were carried out as previously described.[2] All assays were done in triplicate, and individual samples varied from each other by less than 10 per cent. Percentage figures. for inhibition are based on the mean of the three determinations.

TABLE 1. INHIBITION OF PNMT BY VARIOUS COMPOUNDS

Compound	Inhibition of PNMT (%)
Tranylcypromine	73
2-Cyclohexylcyclopropaneamine (SKF 9722-I)	82
8-Amino-1,2-methanoindane (SKF 9620-A)	66
Tyramine	41
Tryptamine	42
Pargyline	34
Phenethylamine	32
Dopamine	31
5-Hydroxytryptamine (serotonin)	27
Phenelzine	24
Cis-phenylcyclopropaneamine (SKF cis 385-A)	18
Amphetamine	17
Nialamide	15
Iproniazid	11
Trans-*N*,*N*-dimethyl-2-phenylcyclopropylamine (SKF trans-556-A)	0
α-Methyl benzyl hydrazine	0

The substrate was *dl* normetanephrine. Both substrate and inhibitors were 10^{-4} M.

The ability of various compounds to inhibit the methylation of normetanephrine by PNMT is shown in Table 1. None of these compounds was a substrate for the enzyme. Tranylcypromine and two related compounds, 2-cyclohexylcyclopropaneamine maleate (SKF 9722-I) and 8-amino-1,2-methanoindane hydrochloride (SKF 9620-A) were found to have the most potent inhibitory action. The cis isomer of 2-phenylcyclopropylamine HCl (SKF cis 385-A) and trans-*N*,*N*-dimethyl-2-phenylcyclopropylamine hydrochloride (SKF trans-556-A) were much weaker inhibitors. When norepinephrine was used as the substrate in the assay at concentrations equimolar to inhibitor

TABLE 2. INHIBITION OF ENZYMATIC FORMATION OF EPINEPHRINE

Substrate concentration	Inhibition (%)		
	Tranylcypromine	8-Amino-1,2-methanoindane	2-Cyclohexylcyclopropaneamine maleate
10^{-4}M	0	29	42
10^{-5}M	95	89	99

The substrate was *l*-norepinephrine in the concentrations given. Inhibitors were 10^{-4}M.

(Table 2), these compounds were much less effective. At lower concentrations of substrate a striking reduction in epinephrine formation was demonstrated with these compounds. Since tranylcypromine is also a potent monoamine oxidase inhibitor,[4] other MAO the inhibitors were examined (Table 1.) These were found to have slight inhibitory action. In addition, three most potent PMNT inhibtors, tranylcypromine, 2-cyclohexylcyclopropaneamine, and 8-amino-1,2-methanoindane, while sharing the cyclopropylamine structure, differ widely in their ability to inhibit MAO.[5] The inhibition of PNMT by tranylcypromine appears to be of the competitive type, as shown by Lineweaver–Burk plot (Fig. 1).

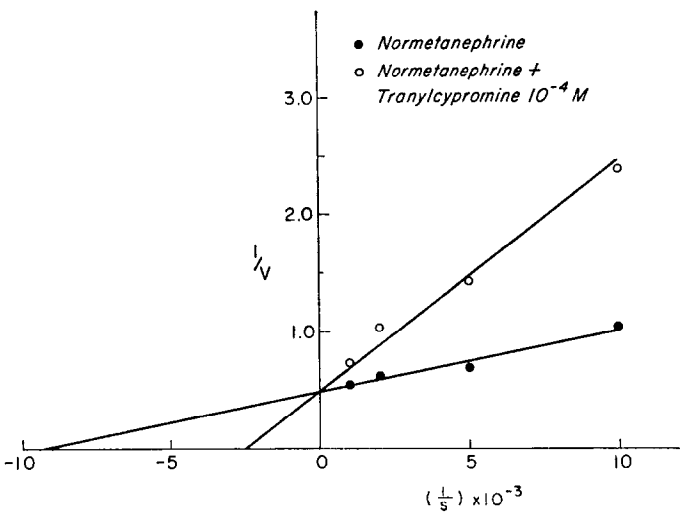

FIG. 1. Lineweaver–Burk plot of the effect of tranylcypromine 10^{-4}M on the *N*-methylation of normetanephrine by PNMT; 1/S is given as 1/M $\times$ 10^{-3}; 1/V is the reciprocal of millimicromoles of product/hour/mg protein of the enzyme preparation.

No significant decrease in adrenal epinephrine content has been observed in rats treated for periods up to one week with tranylcypromine (10 mg/kg/day) s.c. It is possible that more prolonged administration or use of the more potent analogues may permit selective inhibition *in vivo* of epinephrine biosynthesis.

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Electrophoresis of acetylcholine, choline and related compounds*

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IN THE course of biochemical studies of compounds which act at cholinergic synapses we have found paper electrophoresis a considerably more satisfactory method for the separation of such bases than paper or ion-exchange chromatography,[1] fractional crystallization,[2, 3] gas chromatography,[4] techniques based upon solvent extractions,[5] or thin-layer chromatography on silica gel, cellulose, or alumina. The following results extend earlier reports by Chefurka and Smallman,[6] Henschler,[7] Frontali,[8] Ryall *et al.*,[9] and Friesen *et l.*[10, 11]

Chemicals were obtained from commercial sources except as follows. Acetyltriethylcholine was synthesized,[12] and β-methylcholine and thiocholine were prepared by alkaline hydrolysis of their acetyl-esters. ^{14}C-Choline (35 mc/m-mole) was obtained from Nuclear Chicago Corp. and ^{3}H-acetylcholine (45 mc/m-mole) and other isotopes from New England Nuclear Corp. Tritiated acetyltriethylcholine, acetyl-β-methylcholine, and acetylthiocholine (each 4·5 mc/m-mole) were prepared from ^{3}H-acetic anhydride; ^{14}C-benzoycholine was synthesized from benzoic acid.[13] Tritiated scopolamine (4100 mc/m-mole) was prepared by platinum-catalyzed exchange in $^{3}H_2O$.

Electrophoresis was performed at room temperature on 3 × 30·4-cm strips of Whatman 1 paper. Compounds were applied and air-dried on a pencil line drawn across the middle of the strips. Eight strips were mounted as 28-cm bridges in an inverted V-type Durrum cell (Beckman Instrument Co.) and were wetted with buffer to within 1 cm of the applied samples. The movement of fluid through the paper toward the samples then served to concentrate the materials to be separated, at the origin. After the strips had drained for several minutes, electrophoresis was carried out at a constant voltage of 500 (about 18 V/cm) for 1 hr. The strips were then left in a hood until they were barely damp. Separated substances were stained brown in iodine vapor and were outlined in pencil before the iodine evaporated; 0·1–1 μg of the compounds studied could be detected per cm^2 of damp paper. The position and recovery of labeled compounds on dried paper strips after electrophoresis were determined by counting 1·5-mm wide pieces of the paper in ethanol and a toluene-base phosphor by liquid scintillation spectrometry.

The most satisfactory buffer for our purposes was a 1·5 M acetic acid–0·75 M formic acid solution at pH 2; it gave the best distribution of isolated materials, was stable but completely volatile, and could be used to release organic bases from tissue fragments at the time of electrophoresis. The mobilities of 32 compounds in this buffer are given in Table 1, and some are illustrated in Fig. 1. The width of each applied sample was 2–3 mm, and the iodine stained bands are 3–4 mm wide. A mobility difference of 0·5 cm between two known compounds is therefore sufficient for their separate identification. Quaternary amines with more similar mobilities must be separated by longer runs. The mobilities of tertiary amines and of organic bases with carboxyl groups or other negatively charged centers may be altered at different pH values. In 0·1 M phosphate buffer at pH 8, for example,

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