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INHIBITION OF MONOAMINE OXIDASE ACTION ON KYNURAMINE BY SUBSTRATE AMINES AND STEREOISOMERIC α-METHYL AMINES

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Abstract—Kynuramine oxidation by rat liver monoamine oxidase was inhibited by dopamine, norepinephrine, phenethylamine, phenylbutylamine, serotonin, or tyramine. Although these were all substrates for the enzyme preparation, there was no consistent relationship between degree of inhibition and efficacy as substrate. The α-methyl analogues of dopamine, norepinephrine, phenethylamine, tyramine, or 4-chlorophenethylamine also inhibited kynuramine oxidation. The degree of inhibition by the α-methyl compounds was, in most cases, less than that by the parent compounds. Inhibition of MAO by α-methyl amines (amphetamine, 2, 4-dichloroamphetamine, 4-chloro-N-methyl-amphetamine, or 3, 4-dichloroamphetamine) was greater with the dextrorotatory isomers than with the levorotatory isomers.

MONOAMINE oxidase (E.C. 1.4.3.4. monoamine: O_2 oxidoreductase (deaminating)) represents an enzyme or a group of closely related enzymes catalyzing the oxidative deamination of a variety of biogenic amines. Monoamine oxidase (MAO) occurs in many species and tissues and appears to be localized intracellularly in the mitochondrial fraction.[1] Inhibition of MAO by α-methyl amines[2, 3] and competition between substrates[1] have been reported. Comparison of the degree of MAO inhibition by α-methyl ethylamines and the corresponding ethylamines (when the latter are substrates for MAO) is not possible with manometric procedures. Spectrophotometric determination of kynuramine oxidation affords the opportunity to measure oxidation of a single substrate in the presence of other substrates. The present work was undertaken to investigate the inhibition of MAO oxidation of kynuramine by other substrates or their α-methyl derivatives.

EXPERIMENTAL

The MAO preparation was purified from rat liver by the method of Hogeboom[4] for the isolation of mitochondria from animal tissues. The final suspension (50% w/v in 0·25 M sucrose) was lyophilized and stored at −15°.

Phenethylamine, phenylbutylamine, and the *d* and *l* isomers of the chlorinated amphetamines (all as hydrochlorides) were synthesized at Eli Lilly and Co. Other chemicals were obtained as follows: dopamine hydrochloride and tyramine hydrochloride (which was then recrystallized) from Nutritional Biochemicals; serotonin creatinine sulfate from Aldrich Chemical Co.; L-norepinephrine bitartrate (L-arterenol bitartrate) from Winthrop Laboratories; kynuramine dihydrobromide from Regis Chemical Co.; *d*-amphetamine sulfate (Dexedrine) and *p*-hydroxyamphetamine

(Paredrine) from Smith, Kline & French Laboratories; *l*-α-methyl norepinephrine HCl from Sterling-Winthrop Research Institute; and *l*-amphetamine sulfate from Ott Chemical Co.

Kynuramine oxidation was determined spectrophotometrically[5] by measuring absorbance decrease at 360 mμ in the Gilford model 2000 multiple sample absorbance recorder. Routine assay mixtures contained 2 mg of the lyophilized MAO preparation, 0·3 μmoles kynuramine, and 0·3 ml of 0·5 M sodium phosphate buffer (pH 7·4) in a total volume of 3·0 ml. Temperature in the cuvet compartment was recorded continuously and was maintained at 37° ± 1° by circulating water from a constant-temperature bath through thermospacers. The initial rate of absorbance change was used to calculate enzyme activity. A cuvet containing no substrate was used as the zero reference to which the base line on the recorder was automatically adjusted by means of the Gilford automatic blank compensator. Substrates and inhibitors were added simultaneously, and absorbance recording was begun immediately, except in the experiment reported in Fig. 2, in which inhibitor and enzyme were preincubated 30 min at 37° prior to kynuramine addition. All determinations were made in duplicate, and average values for per cent inhibition are reported.

The relative activities of the various substrates were determined manometrically. Assay mixtures contained 100 mg of the lyophilized MAO preparation, 40 μmoles semicarbazide, 4 μmoles potassium cyanide, 15 μmoles substrate, and 0·4 ml of 0·5 M sodium phosphate buffer (pH 7·4) in a total volume of 2·2 ml. Incubation was carried out in oxygen atmosphere at 37°. The rate of oxygen consumption was taken as a measure of substrate activity.

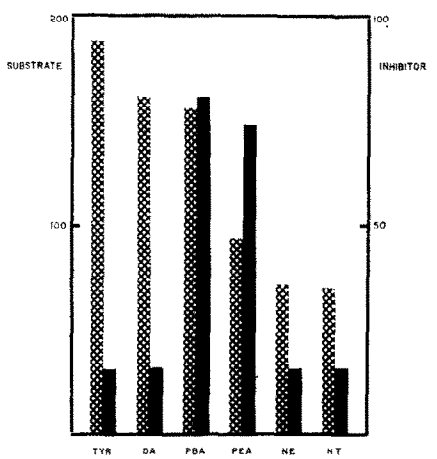


Fig. 1. Inhibition of kynuramine oxidation by other MAO substrates. The activity of the various substrates as determined manometrically is expressed relative to kynuramine as 100 (cross-hatched bars). Inhibition of kynuramine oxidation was determined spectrophotometrically and is expressed as per cent inhibition (solid bars). There was 0·3 μmole of each substrate present in the assay mixtures. TYR = tyramine, DA = dopamine, PBA = phenylbutylamine, PEA = phenethylamine, NE = norepinephrine, and HT = serotonin.

RESULTS AND DISCUSSION

Inhibition by substrate amines

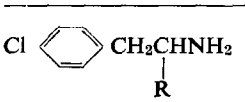
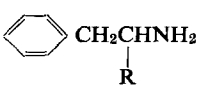
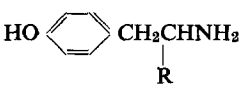
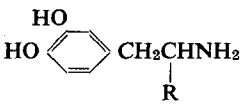
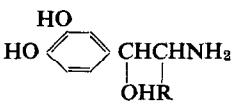
Tyramine, dopamine, phenylbutylamine, phenethylamine, norepinephrine, or serotonin inhibited the oxidation of kynuramine and were also substrates for the rat

liver MAO preparation (Fig. 1). There was no consistent relationship between degree of inhibition of kynuramine oxidation and efficacy of an amine as substrate. This might be considered in terms of Zeller's concept of *eutopic* and *dystopic* complexes formed between enzyme and substrate molecules.[6] The formation of dystopic complexes would influence the degree of inhibition, whereas the formation of eutopic complexes would determine the ability as substrate. These would not necessarily be related. Apparent competition between two substrates for MAO was reported also by Oswald and Strittmatter,[1] who found that the rate of oxygen consumption in the presence of two substrates was intermediate between the rates when either substrate was present alone. Our data would be consistent with a concept of a single MAO acting on all these amines or a family of MAO enzymes with broad or overlapping substrate specificities.

Inhibition by α-methyl amines

α-Methyl phenethylamine (amphetamine), α-methyl tyramine (*p*-hydroxyamphetamine), α-methyl dopamine, α-methyl norepinephrine, and α-methyl 4-chlorophenethylamine were weaker inhibitors of kynuramine oxidation than were the corresponding unbranched amines (Table 1). This was true only at the high concentration of the

TABLE 1. INHIBITION OF KYNURAMINE OXIDATION BY AMINES AND α-METHYL AMINES

Structure	Molar concentration	Per cent inhibition R=H	Per cent inhibition R=CH_3*
	10^{-3}	100	76
	10^{-4}	87	49
	10^{-5}	41	21
	10^{-3}	95	66
	10^{-4}	74	31
	10^{-5}	27	14
	10^{-3}	70	41
	10^{-4}	16	21
	10^{-5}	6	8
	10^{-3}	68	37
	10^{-4}	16	17
	10^{-5}	7	5
	10^{-3}	49	24
	10^{-4}	16	9
	10^{-5}	7	3

* *dl* Mixtures of the α-methyl amines were used. Norepinephrine and α-methyl norepinephrine had the L configuration of the carbinol carbon, but the latter compound was not resolved with respect to the α carbon.

hydroxylated compounds which inhibited only slightly at 10^{-4} or 10^{-5} M. The inhibition of MAO by amphetamine or 4-chloroamphetamine was reversible, according to the criterion of Ackerman and Potter[7] that the degree of inhibition by a reversible inhibitor does not vary with the amount of enzyme present (Fig. 2).

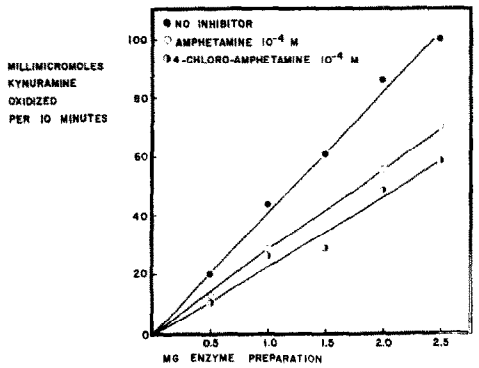


Fig. 2. Reversible inhibition of MAO by amphetamine and 4-chloroamphetamine.

α-Methyl amines contain an asymmetric carbon and exist, therefore, in stereoisomeric forms. The configuration around this asymmetric carbon influenced the inhibition of MAO by amphetamine, 2, 4-dichloroamphetamine, 4-chloro-N-methyl amphetamine, or 3, 4-dichloroamphetamine (Table 2). The dextrorotatory α-methyl

TABLE 2. STERIC EFFECTS IN INHIBITION OF MAO BY α-METHYL AMINES

Compound	Molar concentration	Per cent inhibition	
		d-Isomer	*l*-Isomer
Amphetamine	10^{-3}	75	53
	10^{-4}	42	16
	10^{-5}	21	2
2,4-Dichloroamphetamine	10^{-3}	92	80
	10^{-4}	78	42
	10^{-5}	45	34
4-Chloro-N-methylamphetamine	10^{-3}	81	74
	10^{-4}	56	36
	10^{-5}	19	21
3,4-Dichloroamphetamine	10^{-3}	81	64
	10^{-4}	78	41
	10^{-5}	39	18

amines were in each case more active MAO inhibitors. This was true also if the inhibitors were preincubated with enzyme for 30 min at 37° prior to kynuramine addition. Pfeiffer[8] generalized that drugs of high potency have a tight geometric conformation to the receptor locus and should show relatively greater differences between optical isomers than do drugs of low potency. Thus the compounds reported in Table 2 are low potency MAO inhibitors *in vitro*, and the differences between stereoisomers are small.

Greater MAO inhibition by *d*- than by *l*-amphetamine has been reported previously.[2, 9] The absolute configuration of amphetamine was established by the work of Karrer and Ehrhardt,[10] who showed that D-phenylalanine (absolute configuration 2R)[11] was transformed, by catalytic reduction of the carboxyl group, into dextrorotatory amphetamine. Thus, *d*-amphetamine has the configuration 2S and would be

formed by decarboxylation without inversion of the corresponding L-α-amino acid (2S). Assuming that substitution of chlorine atoms on the phenyl ring does not change the sign of optical rotation, all the *d* compounds in Table 2 have the S configuration. The increased MAO inhibition by these isomers apparently reflects a better "fit" onto the enzyme molecule. α-Dimethyl-phenethylamine inhibited 58%, 8% and 0% at the concentrations shown in Table 2. Inhibition by this compound indicates than an α hydrogen is not essential for binding with MAO.[12] Stereospecificity of *in vivo* effects of amphetamine[13, 14] and chloroamphetamines[15] has been reported. Since MAO inhibition is probably not involved in these *in vivo* effects, the stereospecificity apparently relates to attachment to other "receptor" molecules.

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