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Substrate specificity of phenethanolamine N-methyl transferase

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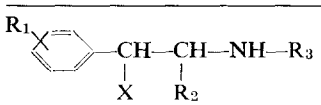
THE enzyme that transfers a methyl group from S-adenosylmethionine to norepinephrine and thus forms epinephrine was described by Kirshner and Goodall.[1] Axelrod characterized the enzyme further and called it phenethanolamine N-methyl transferase.[2] This enzyme is found primarily in the adrenals, with some activity also present in the heart and brain.[2, 3] Axelrod tested several phenethanolamines at $3{\cdot}3 \times 10^{-3}$ M and reported that normetanephrine and norparanephrine were the most active substrates, and that norepinephrine was methylated at a slower rate by a monkey enzyme.[2] Since the physiological role of the enzyme is presumably to form epinephrine from norepinephrine, it was surprising that the enzyme should preferentially methylate other naturally occurring substrates.

We have used the methods of Axelrod[2] to study this enzyme. Enzyme preparations were purified from whole adrenals of rabbits or bovine adrenal medullae through the first ammonium sulfate precipitation stage, and the dialyzed 35–55% ammonium sulfate precipitate resuspended in buffer was used. In our experiments assay mixtures contained $1{\cdot}33 \times 10^{-5}$ M S-adenosylmethionine-methyl-^{14}C (New England Nuclear Corp.), sodium phosphate buffer (0·083 M, pH 7·9) rabbit or bovine enzyme equivalent to 240 or 300 μg protein, respectively, and substrate (as indicated) in a total volume of 0·3 ml. The concentration of S-adenosylmethionine was lower than that used by Axelrod, but was not rate-limiting.

In our initial studies on the properties of this enzyme, the inhibition of normetanephrine methylation by other substrates or by structurally related compounds was measured. These results are summarized in Table 1. In general, phenethylamine and its derivatives were more effective inhibitors than

TABLE 1. PER CENT INHIBITION OF PHENETHANOLAMINE N-METHYL TRANSFERASE BY PHENETHYLAMINE AND PHENETHANOLAMINE DERIVATIVES



R_1	R_2	R_3	Phenethylamine X = H	Phenethanolamine X = OH
H	H	H	48	17*
H	CH_3	H	78	37
H	CH_3	CH_3	66	23
H	H	◁	68	36*
3,4 OH	H	H	61	99
3,4 OH	CH_3	H	65	14

Substrate was 1×10^{-3} M DL-normetanephrine with the rabbit enzyme. The above compounds were added at 1×10^{-3} M. Those marked with an asterisk were DL mixtures; the other phenethanolamines were L-isomers.

were phenethanolamines. L-Norepinephrine, however, was unique in its potency as an inhibitor in this sytem. At 10^{-4} M L-norepinephrine, a concentration one-tenth that of the DL-normetanephrine, 83% inhibition of normetanephrine methylation occurred. Therefore, norepinephrine and normetanephrine were compared as substrates.

With the rabbit or the bovine enzyme, L-norepinephrine was the most active substrate at low concentrations. This is shown for the bovine enzyme in Fig. 1. D-Norepinephrine was methylated at a slower rate, showing that the enzyme has stereospecificity. DL-Normetanephrine concentrations required for maximum activity were higher. Excess substrate was inhibitory in all three cases. At the highest substrate concentration studied, $3{\cdot}3 \times 10^{-3}$ M, D-norepinephrine and L-norepinephrine were methylated at 16% and 23% of the rate for DL-normetanephrine; Axelrod reported 15% and 21%

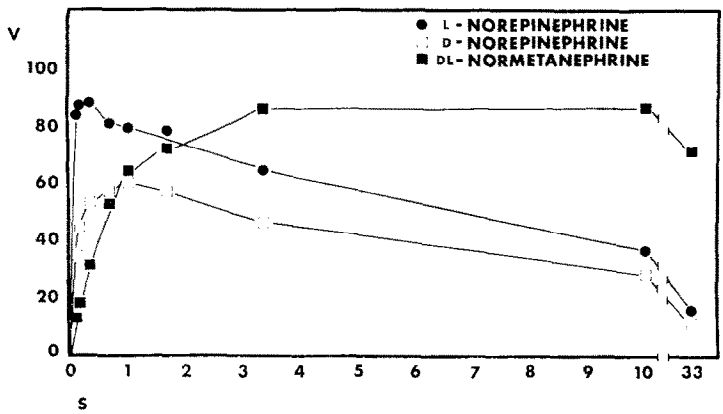


FIG. 1. N-Methylation of L-norepinephrine, D-norepinephrine, and DL-normetanephrine by the bovine enzyme. Velocity (V) is expressed in millimoles ($\times 10^9$) of substrate methylated during 30-min incubation. Substrate concentration (S) is expressed as M $\times 10^4$.

TABLE 2. MICHAELIS-MENTEN CONSTANTS FOR PHENETHANOLAMINE N-METHYL TRANSFERASE

Substrate	K_m, μM	
	Rabbit	Bovine
L-Norepinephrine	10	5
D-Norepinephrine	25	8
DL-Normetanephrine	330	73

K_m values were determined graphically from three plots: 1/V vs. 1/S, V vs. V/S, and S/V vs. S. Average values are shown.

with these substrates compared to L-normetanephrine, using the monkey enzyme at this substrate concentration.[2] Michaelis-Menten constants are shown in Table 2. The substrate with the lowest K_m is often considered to be the "natural" substrate for an enzyme. Therefore, these studies would lend support to the idea that this enzyme has the physiological role of forming epinephrine in the adrenal gland.

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