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The inhibition of norepinephrine and epinephrine synthesis *in vitro*

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THE biogenesis of norepinephrine involves the oxidation of phenylalanine to tyrosine, the catalyzed conversion of tyrosine to 3,4-dihydroxyphenylalanine by tyrosinase, the decarboxylation of 3,4-dihydroxyphenylalanine to dopamine (3,4-dihydroxyphenylethylamine) by DOPA decarboxylase, and the β-hydroxylation of dopamine to norepinephrine by dopamine β-oxidase. Epinephrine is formed from norepinephrine by N-methylation of the latter. However, the β-hydroxylation of epinine (3,4-dihydroxyphenylethylmethylamine) may be considered as another possible route for the formation of epinephrine.[1]

The enzyme dopamine β-oxidase, which converts dopamine to norepinephrine, has been shown to be non-specific for dopamine.[5] Among other structural analogs of dopamine, epinine in high concentrations inhibits the conversion of dopamine to norepinephrine. The present communication will show that dopamine β-oxidase catalyzes the conversion of epinine to epinephrine, but at a much lower efficiency than at which dopamine is converted to norepinephrine. Under the same conditions in which dopamine is converted to norepinephrine to the extent of 40–50 per cent, only 5–10 per cent of epinephrine is formed from epinine. It will also be shown that amphetamine and *p*-hydroxyamphetamine inhibit both the conversion of dopamine to norepinephrine and the conversion of epinine to epinephrine.

The enzyme dopamine β-oxidase was prepared by the method of E. Y. Levin *et al.*[2], but the purification on calcium phosphate gel was omitted. Either dopamine or epinine were added in the same concentrations to a mixture which contained the following components (in μmoles): potassium phosphate buffer, pH 6·4, 100; (1-methyl-2-phenyl)-ethyl hydrazine hydrochloride, 1·3; ascorbic acid, 6; fumaric acid, 10; ATP, 12·5. To this mixture, 0·2 ml of the enzyme was added and the final volume was adjusted to 1 ml with phosphate buffer, pH 6·4. The reaction mixture was incubated for 20 min at 37 °C, using air as a gas phase. At the end of the period of incubation the reaction was stopped by the addition of 0·5 ml of 3% trichloroacetic acid, the precipitated proteins were removed by centrifugation, and the supernatant fluid was adjusted to pH 6 and diluted to 10 ml with water. Depending upon the precursor used, an aliquot was then analyzed either for epinephrine or norepinephrine, by a modification of the fluorometric method of von Euler.[3] The compounds used in the present experiments have not been found to interfere with the specificity of the trihydroxyindole fluorometric method. However, an excess of potassium ferricyanide was ued in order to oxidize the ascorbic acid which was present in the incubation mixture.[4] The relative inhibition rate of dopamine β-oxidase by amphetamine and *p*-hydroxyamphetamine was determined by a comparison of the

amount of norepinephrine or epinephrine formed in an incubation mixture which contained only the substrate (dopamine or epinine) and an incubation mixture which contained the compound to be tested and the substrate.

The accompanying table shows the effects of test compounds on the conversion of dopamine to norepinephrine and epinine to epinephrine. *p*-Hydroxyamphetamine is a more potent inhibitor than amphetamine. This is in agreement with the previous findings that tyramine is a more potent inhibitor

TABLE 1. INHIBITION OF NOREPINEPHRINE AND EPINEPHRINE SYNTHESIS *in vitro**

Inhibitor	Amount added in μmoles		Amount formed in μmoles		Percent inhibition	
	Substrate†	Inhibitor	Epinephrine from epinine	Norepinephrine from dopamine	Epinephrine from epinine	Norepinephrine from dopamine
None	1·05	none	0·075 ± 0·01	0·45 ± 0·05		
DL-Amphetamine	1·05	2·75	0·037 ± 0·004	0·315 ± 0·03	50	30
DL-Amphetamine	1·05	5·50	0·018 ± 0·002	0·180 ± 0·02	75	60
DL-*p*-Hydroxyamphetamine	1·05	2·37	0·022 ± 0·002	0·225 ± 0·02	70	50
DL-*p*-Hydroxyamphetamine	1·05	4·75	0·015 ± 0·002	0·135 ± 0·01	80	70

* Figures represent averages of 3 experiments in each series.
† Dopamine or epinine.

for dopamine to norepinephrine conversion than is phenylethylamine.[5]

Studies are now under way on the nature of the inhibition of epinephrine and norepinephrine synthesis by amphetamine and *p*-hydroxyamphetamine, as well as on the extent to which epinine is converted to epinephrine *in vivo*.

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Effect of colchicine on the intestinal xanthine oxidase

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COLCHICINE was shown to inhibit the enzyme xanthine oxidase (XO) of rat liver and to enhance the activity of this enzyme in blood serum.[1]