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## Phenylethanolamine-*N*-methyltransferase in human adrenal gland*

Phenylethanolamine *N*-methyltransferase, which converts norepinephrine to epinephrine in the adrenal medulla, has been investigated in various mammalian species[1,2], but biochemical studies of this enzyme have not been reported in human adrenal glands.

The present paper describes a procedure for purification of this enzyme from human adrenal glands and also reports on some of the inhibitors and stimulators of this enzyme which have been hitherto unknown for the mammalian enzyme.

Adrenals were obtained from male accident victims within 2–4 h after death and were immediately frozen for future enzyme preparation. The "crude" enzyme preparation consisted of 100 000 × *g* supernatant solution of 10% (w/v) whole adrenal homogenate in isotonic KCl.

The method of enzyme assay was a modified method[3] of WURTMAN AND AXELROD[4]. The tubes were incubated at 37° for various lengths of time, in air. The methylated product was then extracted either with 6 ml of isoamyl alcohol–toluene (3:2, v/v) (for metanephrine), isoamyl alcohol–toluene (30:1, v/v) (for methyl phenylethanolamine), or *n*-butanol (for epinephrine). A 4-ml aliquot of the solvent was added to the phosphor containing 1 ml of ethanol. The radioactivity was measured in a Packard scintillation spectrometer. The amount of unreacted [$^{14}$C]*S*-adenosylmethionine extracted in the solvent was negligible. The appropriate correction was made for the complete extraction of $^{14}$C-labeled methylated products into the solvent. To demonstrate that the radioactivity counted represented the methylated product, the following procedure was performed: In the experiments where normetanephrine was used as a substrate, an aliquot of the solvent extract from the incubation mixture was placed on a paper chromatogram. The substrate and the product were then identified by the two-dimensional paper chromatography method of SMITH[5]. The activity on the areas corresponding to the substrate and the product were then eluted with ethanol, and the radioactivity was measured as before. More than 95% of the total radioactivity was in the area corresponding to the product. One unit of enzyme is defined as the amount that converts 1 nmole of the substrate to its methylated product in a designated time.

For further enzyme purification, the "crude" enzyme preparation was first purified by ammonium sulfate fractionation according to FULLER AND HUNT[2]. The 35–50% ammonium sulfate precipitated enzyme was designated as "40 enzyme".

For still further purification of the enzyme, 1-ml aliquots of the "40 enzyme" were applied to a 1.5 cm × 25 cm Sephadex G-200 column, and the enzyme eluted from the columns by 0.001 M phosphate (pH 7.0) in 1.5-ml aliquots.

Table I demonstrates the purification results from an average of two pooled enzyme preparations. Phenylethanolamine *N*-methyltransferase activity was also measured in the "crude" enzyme preparation from other freshly autopsied organs.

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Abbreviations: EDTA, ethylene diamine tetracetic acid; EGTA, ethylene glycol-bis[(β-amino ethyl ether)]*N*,*N*′-tetracetic acid.

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TABLE I

PURIFICATION OF HUMAN PHENYLETHANOLAMINE *N*-METHYLTRANSFERASE

Substrate: Normetanephrine, $0.7 \cdot 10^{-4}$ M. For details of experiment see the text.

| *Fraction* | *Specific activity (units/mg protein per 30 min)* | *Yield (%)* | *Purification (-fold)* |
|---|---|---|---|
| Crude | 0.45 | 100 | 0 |
| "40 Enzyme" | 1.75 | 100 | 3.9 |
| Sephadex G-200 eluate | 5.50 | 90 | 12.5 |

These studies (Table II) demonstrate the presence of a considerable amount of enzyme activity in the brain with some enzyme activity in the liver and heart. The adrenal, however, shows the highest enzyme activity.

Using the "40 enzyme" preparation, some of the basic properties of this enzyme were studied. The rate of product formation was time dependent up to 2 h but plateaued after this time. The enzyme activity was linear with increasing enzyme concentration. The effect of pH was also studied with potassium phosphate and Tris buffers. In Tris buffer the enzyme had pH optima of 8.2–8.6, whereas in phosphate the optimum was 7.9. Phosphate buffer inhibited the enzyme by about 50% at 40 mM.

Of the three substrates used, norepinephrine showed the lowest $K_m$ ($8 \cdot 10^{-5}$ M), and phenylethanolamine the highest ($2.0 \cdot 10^{-3}$ M), with $K_m$ of DL-normetanephrine in the intermediate range ($2.5 \cdot 10^{-4}$ M). Norepinephrine, however, inhibits the enzyme activity by about 50% at $7 \cdot 10^{-4}$ M concentration.

Although *p*-chloromercuribenzoate has been stated to inhibit phenylethanolamine *N*-methyltransferase activity in animals[1], no detailed studies on the possibility of involvement of sulfhydryl groups at the active site of the enzyme have been reported.

The present studies showed that the enzyme was inhibited by *p*-hydroxymercuribenzoate and *N*-ethylmaleimide at 0.001 M. The effect of the latter two inhibitors could be reversed by addition of 0.01 M 2-mercaptoethanol and dithiothreitol.

In another study the effect of heavy metals and other cations was investigated. These studies demonstrated that heavy metals such as $Cd^{2+}$, $Hg^{2+}$, $Zn^{2+}$, and $Cu^{2+}$ inhibited the enzyme at $10^{-5}$ M concentration by more than 50%, whereas other

TABLE II

PHENYLETHANOLAMINE *N*-METHYLTRANSFERASE ACTIVITY IN VARIOUS HUMAN TISSUES

Substrate: Normetanephrine, $0.7 \cdot 10^{-4}$ M.

| *Organ* | *Specific activity (units/mg protein per 30 min)* |
|---|---|
| Adrenal | 0.45 |
| Hypothalamus | 0.16 |
| Cerebrum | 0.10 |
| Heart | 0.025 |
| Liver | 0.021 |
| Plasma | 0.010 |

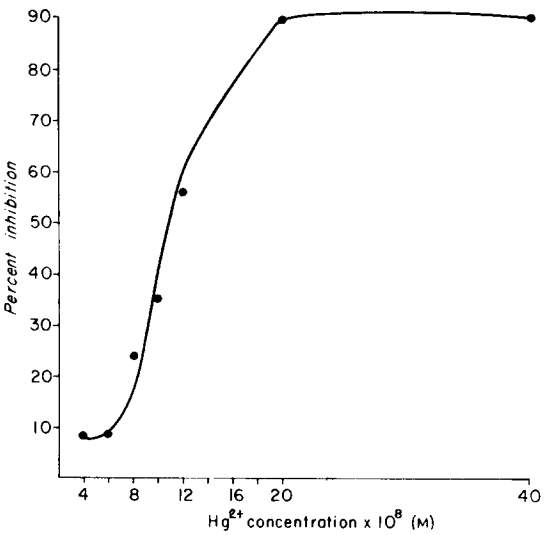


Fig. 1. Effect of various concentrations of $HgCl_2$ on phenylethanolamine *N*-methyltransferase activity.

cations such as $Fe^{2+}$, $Mn^{2+}$, $Co^{2+}$, and $Cr^{3+}$ had no inhibitory effect on this enzyme, even at higher concentrations. Furthermore, the inhibitory effect of $Cd^{2+}$ or $Hg^{2+}$ could be reversed by addition of 2-mercaptoethanol. To substantiate further that the inhibitory effects of the metal were specifically on the sulfhydryl grouping rather than due to non-specific denaturation of the enzyme, the activity of the enzyme was measured at various concentrations of mercury. The results (Fig. 1) showed a sigmoid type of dose–response curve which is more compatible with sulfhydryl group involvement in mercury inhibition of the enzyme than non-specific denaturation of the enzyme[6].

Table III illustrates additional agents which affect phenylethanolamine *N*-methyltransferase activity. Dithiothreitol and 2-mercaptoethanol stimulate the enzyme activity at 10 mM concentrations. Chelators such as EDTA and EGTA

TABLE III

EFFECT OF VARIOUS AGENTS ON HUMAN PHENYLETHANOLAMINE *N*-METHYLTRANSFERASE ACTIVITY

Substrate: Normetanephrine, $0.7 \cdot 10^{-4}$ M.

| *Addition* | *Concn. (mM)* | *Phenylethanolamine N-methyltransferase activity (units/mg protein per 30 min)* |
|---|---|---|
| Control | — | 1.41 |
| Dithiothreitol | 10 | 2.24 |
| 2-Mercaptoethanol | 10 | 2.00 |
| Tris buffer | 40 | 2.14 |
| EDTA | 4 | 2.24 |
| EGTA | 4 | 1.69 |
| Phosphate | 40 | 0.60 |
| ATP | 40 | 0.58 |
| ADP | 40 | 0.43 |

stimulate the enzyme both in the presence of 40 mM phosphate and 40 mM Tris. Tris buffer is stimulatory to the enzyme, but it is further stimulated by chelators. Other phosphorus-containing compounds such as ATP and ADP also showed some inhibitory effect on the enzyme activity.

To study the mechanism of inhibition by epinephrine and norepinephrine, these catecholamines were used with normetanephrine as the substrate for phenylethanolamine *N*-methyltransferase activity. $K_i$ was then calculated: epinephrine $1.7 \cdot 10^{-4}$ M, norepinephrine $5.6 \cdot 10^{-5}$ M. Studies of a Lineweaver–Burk plot for epinephrine showed a pattern of enzyme inhibition suggestive of non-competitive type of inhibition.

The present studies demonstrate in human adrenal an enzyme reaction which catalyzes the methylation of certain catecholamines. The enzyme properties are similar to those of other species. Furthermore, these studies report on hitherto unknown effects of heavy metals, chelators, Tris buffer, and thiol groups on the enzyme. The present studies imply that the existence of the thiol group is necessary for optimal enzyme activity, and that inhibition of the enzyme by heavy metals is probably through involvement of sulfhydryl groups on the active site of the enzyme.

Of the three substrates tested, norepinephrine has the lowest $K_m$, suggesting that it is probably a more natural substrate for the human phenylethanolamine *N*-methyltransferase than normetanephrine or phenylethanolamine. The $K_m$ for rabbit phenylethanolamine *N*-methyltransferase was also found to be lower for norepinephrine than normetanephrine[7].

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