Enzymic conversion of *p*-tyramine to *p*-hydroxyphenylethanolamine (norsynephrin)

With the discovery of norsynephrin and synephrin (N-methylnorsynephrin) in urine[1-3] and of the former in animal tissues[1], it became of interest to study their biosynthesis. It has now been possible to demonstrate that the enzyme 3,4-dihydroxyphenylethylamine (dopamine) β-oxidase is not specific for dopamine but can catalyze the oxidation of *p*-tyramine to norsynephrin. In addition, the simplicity and specificity of the method for the determination of norsynephrin, in the presence of its precursor, make possible a simplified procedure for measuring dopamine β-oxidase activity.

In a typical experiment 0.5 g of slices were suspended in 3.0 ml Krebs–Ringer bicarbonate buffer, pH 7.4, and preincubated at 37° with 300 μg of the monoamine oxidase inhibitor, harmaline, for 5 min. 30 μmoles *p*-tyramine were then added and the incubation was continued for 30 min in an atmosphere of 95 % O_2–5 % CO_2. The reaction was stopped by adding 3.0 ml 12 % trichloroacetic acid and the slices were homogenized in the mixture before centrifugation.

Assay of norsynephrin. Periodate quantitatively oxidizes norsynephrin but not tyramine to *p*-hydroxybenzaldehyde. The latter compound, when dissolved in NH_4OH, has a sharp absorption peak at 330–333 mμ which serves as the basis for the determination of the enzymically formed norsynephrin. The deproteinized incubation mixture is passed through a 0.6 × 3 cm Dowex-50 H^+ column. After washing the column with 5–10 ml water, the adsorbed bases are eluted with 3 ml 3 *N* NH_4OH and the eluate is collected in a 40-ml extraction tube. Norsynephrin is then converted to *p*-hydroxybenzaldehyde by the addition of 0.3 ml 2 % $NaIO_4$. After 3 min, excess periodate is reduced with 0.3 ml 10 % $NaHSO_3$. The benzaldehyde is extracted from the solution with 30 ml toluene, and an aliquot of the solvent is re-extracted with 1 ml 3 *N* NH_4OH. Absorbancy is measured at 330 mμ and compared with a norsynephrin standard* carried through the entire procedure.

As shown in Table I β-oxidation of tyramine was found to be most active in adrenal medulla, less activity being present in brain and little in liver. To show that the enzymically formed material was norsynephrin, several incubation flasks with adrenal-medulla slices were pooled and aliquots were taken for various analyses.

TABLE I

HYDROXYLATION OF TYRAMINE BY TISSUE SLICES

Tissue	*Norsynephrine formed (μg/g/h)*
Beef adrenal medulla	39.7
Hog-brain hypothalamus	4.6
Hog-caudate nucleus	3.9
Hog cortex and cerebellum	1.7
Whole rat brain	1.3
Rat liver	0.7

Slices (0.5 g) were suspended in 3.0 ml Krebs–Ringer bicarbonate buffer, pH 7.4, and preincubated at 37° with 300 μg harmaline for 5 min. 30 μmoles *p*-tyramine were then added and the incubation was continued for 30 min in an atmosphere of 95 % O_2–5 % CO_2.

* Norsynephrin was generously supplied by Dr. SIDNEY ARCHER of Sterling Winthrop Research Institute.

The product obtained on treatment with periodate was found to have the identical spectrum of *p*-hydroxybenzaldehyde. Chromatography of the experimental incubation mixtures in *n*-butanol–acetic acid–water (50:12:50, butanol layer) and in ethanol–conc. NH_4OH–water (180:10:10) revealed a new spot having the same R_F values as authentic norsynephrin and yielding the same colors with spray reagents; pink with diazotized *p*-nitroaniline, yellow with diazotized sulfanilic acid, and ultraviolet adsorption with 1 % periodate in 5 % Na_2CO_3 (Fig. 1). In another experiment with similar amounts of adrenal-medulla slices and [^{14}C]tyramine, the norsynephrin was separated from the tyramine by one-dimensional chromatography in the butanol solvent and rechromatographed in two dimensions using the solvents isopropanol–conc. NH_4OH–water (200:10:20) and *n*-butanol–acetic acid–water (120:30:50).

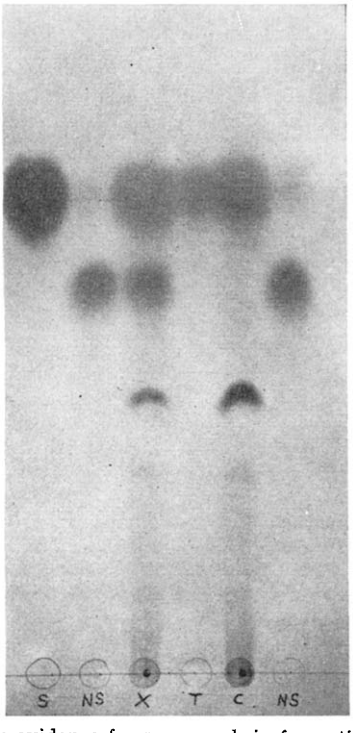

Fig. 1. Paper chromatographic evidence for norsynephrin formation with adrenal medulla slices. "S" signifies authentic synephrin; NS, norsynephrin; X, incubation mixture; T, tyramine; C, zero-time incubation. The solvent used was ethanol–conc. NH_4OH–water (180:10:10) and the spray reagent was diazotized *p*-nitroaniline.

The radioactivity and norsynephrin color reactions coincided in one spot, which migrated exactly as authentic norsynephrin. Furthermore, when the enzymically formed norsynephrin was treated with periodate, it yielded one detectable radioactive product which could be extracted with toluene. This was characterized* as *p*-hydroxybenzaldehyde by the use of gas chromatography, using a 6-ft. column

* We wish to thank Dr. ARTHUR KARMEN for these measurements.

composed of 12 % ethylene glycol–adipate polyester on Chromosorb-W coupled to a scintillation counter[4].

The following findings indicate that the enzyme responsible for norsynephrin formation is dopamine β-oxidase. First, adrenal-medulla particles prepared according to LEVIN *et al.*[5] were found to have similar requirements for norsynephrin formation as for norepinephrine formation. Ascorbate, fumarate and adenosine triphosphate all stimulated and KCN inhibited the hydroxylation. Secondly, dopamine was found to inhibit the oxidation of tyramine in a competitive manner. Finally, the localization of activity in adrenal medulla and brain-stem areas were also characteristic of dopamine β-oxidase[6].

Norsynephrin was first isolated from octopus salivary glands by ERSPAMER[7] who called it octopamine. Until its more recent finding in human and animal urine and in tissues, it was merely considered peculiar to the octopus. However, it is now apparent that a general aromatic L-amino acid decarboxylase in mammalian tissues[8] makes available tyramine (and other aromatic amines) for β-oxidation. The activity of the β-oxidase with a variety of amines is currently under investigation. The significance of synephrin and norsynephrin is not apparent, but it should be pointed out that they are fairly active pharmacologic agents[9]. Furthermore, as pointed out by ERSPAMER[7], they are readily oxidized by chemical means to epinephrine and norepinephrine respectively.

Laboratory of Clinical Biochemistry, National Heart Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Bethesda, Md. (U.S.A.)

JOHN J. PISANO
CYRUS R. CREVELING
SIDNEY UDENFRIEND

[1] Y. KAKIMOTO AND M. D. ARMSTRONG, *Federation Proc.*, 19 (1960) 295.
[2] J.J. PISANO, *Clin. Chim. Acta*, 5 (1960) 406.
[3] J. J. PISANO, J. OATES, A. KARMEN, A. SJOERDSMA AND S. UDENFRIEND, to be published.
[4] A. KARMEN AND H. R. TRITCH, *Nature*, 186 (1960) 150.
[5] E. R. LEVIN, B. LEVENBERG AND S. KAUFMAN, *J. Biol. Chem.*, 235 (1960) 2080.
[6] S. UDENFRIEND AND C. R. CREVELING, *J. Neurochem.*, 4 (1959) 350.
[7] V. ERSPAMER, *Nature*, 169 (1952) 375.
[8] S. UDENFRIEND, W. M. LOVENBERG AND H. WEISSBACH, *Federation Proc.*, 19 (1960) 7.
[9] A. M. LANDS AND J. I. GRANT, *J. Pharmacol. Exptl. Therap.*, 106 (1952) 341.

Received August 22nd, 1960

Biochim. Biophys. Acta, 43 (1960) 566–568

Biological activities of isomeric estriols

In a comprehensive study of the estrus-producing values of a large number of steroidal estrogens, we have employed in the St. Louis University laboratories both the Mather Modification[1] of the MARRIAN–PARKES[2] assay procedure using the adult castrate mouse and the CURTIS–DOISY[3] method using the 20-day-old intact female rat, as we believe that these two assay methods are suitable for the comparative study of estrogens of widely varying potencies. An average of about 50 animals was used for each dose level of each estrogen in obtaining dose-response curves.

As shown in Table I, the three isomeric estriols were surprisingly estrogenic. Especially noteworthy is the fact that 17-epiestriol in the CURTIS–DOISY assay is