

Formation of Norepinephrine from Tyrosine in Isolated Rabbit Heart

The biogenesis of norepinephrine involves the oxidation of phenylalanine to tyrosine, the catalyzed conversion of tyrosine to 3,4-dihydroxyphenylalanine, 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. Virtually nothing is known about the formation of dopa from tyrosine in mammalian enzyme systems, except that this reaction occurs in medulla and that it is not catalyzed by tyrosinase¹. It remains, therefore, to be elucidated whether organs innervated by sympathetic nerves such as the heart are able to perform all the enzymatic reaction steps from tyrosine leading to norepinephrine.

The rabbit heart was perfused with dopamine- C^{14} , tyramine- C^{14} , and tyrosine- C^{14} and the formation of catechols and β -hydroxylated compounds were investigated.

Rabbits were pretreated with 100 mg/kg iproniazid and killed 16 h later. The thorax was immediately opened and the heart removed and perfused by the Longendorff technique with oxygenated (95% O_2 , 5% CO_2) Krebs-Ringer bicarbonate solution at 38°C. A stopcock on the cannula allowed continuous infusion of radioactive compounds into the perfusing fluid. The outflow from the hearts was collected during the perfusion time. The radioactive compounds in the heart and perfusate were isolated and analyzed by a method which involves extraction into organic solvents, ion exchange, alumina and paper chromatography as well as acetylation procedures².

After perfusion with dopamine- C^{14} , 1–3% was converted to norepinephrine which was accumulated in the heart tissue. In the perfusate, unchanged dopamine- C^{14} and two *O*-methylated metabolites, 3-methoxy dopamine and 3-methoxy norepinephrine were found. No norepinephrine was detected in the perfusate.

Perfusion with tyramine- C^{14} yielded norsynephrin as a major radioactive product in the heart tissue (Table). Only negligible amounts of unchanged tyramine- C^{14} were found. The perfusate contained tyramine- C^{14} and other metabolites which have not been identified at the present time. Neither dopamine- C^{14} nor norepinephrine- C^{14} have been detected after perfusion with tyramine- C^{14} . When tyrosine- C^{14} was perfused, norepinephrine- C^{14} as the main catechol, and dopamine- C^{14} as the minor, were isolated from the heart. In the perfusate a catechol was isolated with chromatographic characteristics of dopa- C^{14} . Whether tyramine or norsynephrin is formed after perfusion with tyrosine is now under investigation.

The present study shows that the heart is capable of performing all the enzymatic reactions necessary for the biosynthesis of norepinephrine. The finding that tyrosine is hydroxylated to dopa while tyramine is not converted to dopamine in the isolated heart shows the specificity of

this reaction. Of considerable interest now is to investigate whether other phenolic amino acids are also converted by this system to the corresponding catechols. Attempts are now being made to isolate the enzyme from the heart in order to study the co-factor requirements and the substrate specificity.

Previously it was shown that the enzyme which converts dopamine to norepinephrine is not specific and that tyramine is also a substrate of the same enzyme³. It may therefore be assumed that tyramine was converted in the isolated heart to norsynephrin by the same enzyme which formed norepinephrine from dopamine.

The formation of norepinephrine from tyrosine and norsynephrin from tyramine

Perfusion time in min	Perfused precursor			Product ^a	
	Compound	Weight in mg	c.p.m. $\times 10^6$	Compound	c.p.m. $\times 10^3$ per 10 g tissue
45 ^c	Tyramine	0.04	1.6	Norsynephrin	43.00
120	Tyrosine	0.85	3.5	Norepinephrine	9.6

^a The radiochemical purity of the products was established by paper chromatography in two different solvent systems of the free compounds and acetylated derivatives.

^b Figures represent averages of 3 experiments in each series.

^c The radioactive compound was perfused for 15 min and the perfusion was continued for the next 30 min.

Zusammenfassung. Die Bildung des Noradrenalins aus Tyrosin konnte im isolierten Kaninchenherzen nachgewiesen werden. Die Reaktion scheint für Tyrosin spezifisch zu sein. Aus Tyramin wurde kein Noradrenalin gebildet. Ebenfalls wurde die Umwandlung von Tyramin in Norsynephrin und von Dopamin in Noradrenalin nachgewiesen.

M. GOLDSTEIN and J. M. MUSACCHIO

New York University College of Medicine, Department of Psychiatry and Neurology, Neurochemistry Laboratory, New York (U.S.A.), April 1, 1963.

¹ N. KIRSHNER, *Adrenergic Mechanisms*. Ciba Foundation Symposium (Little, Brown & Co., Boston 1960), p. 17.

² M. GOLDSTEIN and H. GERBER, *Life Sciences* 4, 97 (1963).

³ M. GOLDSTEIN and J. F. CONTRERA, *J. biol. Chem.* 237, 1898 (1962).

PRO EXPERIMENTIS

A Method of Measuring the Intensity of Electron Spin Resonance Absorption

In this brief note a new quantitative approach to the study of electron spin resonance (ESR) absorption is described. In its essence, it is a method of *comparing* the intensity of ESR signals. Thus, it makes possible the

relative measurements of the concentration of paramagnetic centres, but not the *absolute* determinations.

The method has been elaborated in the course of biophysical investigations¹ and is adapted to the range of concentrations in which free radicals are shown to be present in biological materials (i.e. 10^{-6} – 10^{-8} mole/g of dry weight²). It may, however, be applied as well to the measurements of other paramagnetic species in a wider range of concentrations, provided that the investigated substance is isotropic, powdered, or dissolved.