CATECHOLAMINE FORMATION IN INTACT TISSUES

LEMUEL C. LEEPER

Riverton Laboratories, Incorporated, Newark, New Jersey

A great part of our knowledge of the biosynthesis of the catecholamines, norepinephrine and epinephrine, has been obtained from studies in which various investigators used either the intact animal or intact tissues. In the past such studies were surrounded by special difficulties, the foremost of which was the demonstration of a net synthesis of the catecholamines (2). This was due in part to the very slow synthesis of these hormones which resulted in only a small increase in the already high levels of endogenous catecholamines. The availability of various radioactive precursors has contributed significantly towards overcoming this difficulty.

Although the catecholamines have been studied since the early part of the century, few significant results concerning their biosynthesis were obtained until 1947. At that time, Gurin and Delluva (3) demonstrated that after the administration to rats of phenylalanine labeled with C^{14} in the *alpha* carbon, radioactive epinephrine could be isolated from the adrenals. This suggested that decarboxylation was involved in some reaction in the formation of epinephrine. Later, using the same animal, Udenfriend and Wyngaarden (10) found that when tyrosine-2-C^{14} and 3,4-dihydroxyphenylalanine-3-C^{14} (DOPA-3-C^{14}) were injected, radioactive norepinephrine and epinephrine were found in the adrenals. In these studies phenylethylamine-2-C^{14} and tyramine-1-C^{14} did not give rise to labeled norepinephrine and epinephrine. This demonstrated that they were not involved in the biosynthetic pathway. Other studies by this author (6) demonstrated that carbon-labeled epinephrine appeared in rat adrenals after the injection of 3,4-dihydroxyphenylethylamine-1-C^{14} (dopamine-1-C^{14}). The specific activity of the isolated epinephrine in this experiment was greater than that obtained by Udenfriend and Wyngaarden when DOPA, their best precursor, was used. A specific activity of 15,000 to 26,000 counts per minute per micromole of epinephrine was obtained as compared to approximately 2,000 counts per minute per micromole when DOPA was used as a precursor. This was good evidence that dopamine was the more immediate precursor.

Keller, Boissonnas and du Vigneaud (4) reported that dietary C^{14}-methyl labeled methionine was a source of the methyl group of epinephrine. This has also been demonstrated in the intact rat by Masuoka *et al.* (7) from which they were able to isolate carbon-labeled epinephrine after the injection of norepinephrine-2-C^{14}.

All of the previously mentioned experiments show that the pathway proposed by Blaschko (1) exists in the intact animal. However, these experiments tell little about the organs involved. Udenfriend and Wyngaarden (10) observed that when epinephrine-C^{14} was injected only a very small portion of the circulating epinephrine was taken up by the adrenal medulla. This is interesting since it indicates

that the bulk of adrenal epinephrine is synthesized from some precursor within the gland.

Recently, some studies were performed in Dr. Udenfriend's laboratory by the author in collaboration with Dr. Rosenfeld (8) which were designed to study the ability of the adrenal gland to perform the various transformations in the biosynthesis of catecholamines. In these experiments intact calf adrenals, isolated from the metabolic influences of other tissues, were perfused with an artificial medium containing various C^{14}-labeled substrates. Catecholamines were isolated from the glands by a lengthy procedure involving adsorption on alumina, paper chromatography, isotopic dilution and recrystallization techniques. Since it was possible to perfuse as many as five glands simultaneously it was possible in most cases to form amounts of catecholamines which could not be formed using other techniques. Through the use of the isolation techniques milligram quantities of catecholamines were obtained. The homogeneity of the isolated compounds was determined by crystallization techniques. In some cases the site of the carbon label was determined by enzymatic degradation.

Some very significant results were obtained from the perfusion experiments. It was demonstrated that starting with dietary tyrosine the calf adrenal was able to perform all of the steps in the transformation. Perfusion of 200×10^6 counts per minute of DL-tyrosine-2-C^{14} resulted in the appearance of significant labeling in isolated norepinephrine, 66,000 counts per minute, but only an insignificant amount of labeling in the epinephrine (less than 1,900 total counts per minute).

In nonradioactive experiments it was easy to show the conversion of DOPA to dopamine. When dopamine-1-C^{14} was perfused there was an appreciable increase in the total activity of isolated norepinephrine (approximately 300,000 counts per minute) over that obtained from the perfusion of tyrosine. The total activity of epinephrine remained low until L-methionine was added to the perfusion medium. Then there was an increase in activity from less than 16,000 counts per minute to 133,000 counts per minute.

Several interesting observations were made in experiments involving the N-methylation of norepinephrine. The perfusion of 14×10^6 counts per minute of norepinephrine resulted in adrenal epinephrine with 14,000 total counts per minute. In an attempt to stimulate the system, several labeled methyl donors were added to the perfusion medium. Formaldehyde-C^{14} and sodium formate-C^{14} were both methyl donors but they were only one-tenth as active as L-methionine-methyl-C^{14} which produced 1.5×10^6 counts per minute in the isolated epinephrine. When choline-methyl-C^{14} was perfused no activity was found in the epinephrine. The epinephrine formed in each case was subjected to a combination of chemical and enzymatic degradation to show that the label was indeed present in the N-methyl group. One criticism of comparable experiments that have been reported with adrenal homogenates (5) is that the apparent epinephrine formed during incubation was not degraded to show that there was actually produced an N-methyl group. It is now known that O-methyl norepinephrine is produced in such an *in vitro* experiment. Unless the authors were aware of this it may well be that the radioactivity in 0-methyl norepinephrine traveled on their chromato-

graphic column with the epinephrine band. If this is true there is still doubt as to the demonstration of the formation of significant amounts of epinephrine from norepinephrine in homogenates.

In the perfusion experiments it was also possible to show that epinephrine is not demethylated by the adrenal gland to form norepinephrine. No activity was found in the norepinephrine after the perfusion of 20.5×10^6 counts per minute of *dl*-epinephrine-1-C^{14}.

These experiments demonstrate that the adrenal gland, isolated from other metabolic influences, can start with dietary tyrosine and form norepinephrine and epinephrine.

Another possible pathway for the biosynthesis of catecholamines involves the side-chain oxidation of 3,4-dihydroxyphenylalanine to 3,4-dihydroxyphenylserine and the decarboxylation of this compound to form norepinephrine. Schmiterlöw (9) reported an increase in the amount of norepinephrine in rabbit urine after the injection of 3,4-dihydroxyphenylserine. However, there is no evidence for the conversion of 3,4-dihydroxyphenylalanine to 3,4-dihydroxyphenylserine nor has this compound been demonstrated in animal tissues.

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