BIOSYNTHESIS OF ADRENALINE AND NORADRENALINE

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Starting with tyrosine, one can devise a number of pathways for the formation of adrenaline and noradrenaline. In the past several years a considerable body of information has been obtained which confirms Blaschko's hypothesis (3) on the metabolic route for the biogenesis of these hormones. The formation of adrenaline and noradrenaline from tyrosine in isolated adrenal medulla (19, 35, 47), in sympathetic nerves and ganglia (20, 21) and in intact animals (55, 56) has been unequivocally demonstrated and the intermediate compounds isolated (15, 21).

Early attempts (14, 27, 50, 51) to demonstrate the formation of adrenaline and noradrenaline produced conflicting and equivocal results mainly because of the difficulty in detecting small amounts of newly formed adrenaline and noradrenaline in the presence of large amounts of the preformed hormones. With the advent of radioactive tracers and the techniques of ion exchange and filter-paper chromatography the problem was greatly simplified.

In 1955, Demis *et al.* (12, 13) incubated 3,4-dihydroxyphenylalanine (DOPA) with homogenates of bovine adrenal medulla and isolated small amounts of radioactive noradrenaline by paper chromatography. Subsequently, Hagen (23) and Hagen and Welch (24) prepared hydroxytyramine-2-C^{14} by incubating DOPA-2-C^{14} with a particle-free supernatant fraction from ox adrenal medulla. The hydroxytyramine-2-C^{14} thus formed was isolated by ion exchange chromatography (37) and then converted to noradrenaline by incubation with a homogenate of chicken adrenal glands. More recently, Pellerin and D'Iorio (45) incubated DOPA-2-C^{14} with adrenal homogenates and, after chromatographing an extract of the reaction mixture on paper, found 7 radioactive zones among which were hydroxytyramine and noradrenaline. These experiments show unequivocally that the adrenal medulla can form adrenaline and noradrenaline from DOPA and hydroxytyramine but give little information about the metabolic pathway.

The first clear-cut demonstration that the adrenal medulla could convert tyrosine to hydroxytyramine, noradrenaline and adrenaline was made by Kirshner and Goodall (19, 35). When uniformly labeled *l*-tyrosine-C^{14} was incubated with slices of adrenal medulla, hydroxytyramine-C^{14}, noradrenaline-C^{14} and adrenaline-C^{14} were isolated by ion exchange chromatography (37). It was also shown that DOPA-C^{14} was converted to noradrenaline-C^{14} at 70 to 100 times the rate of conversion of tyrosine. Under identical conditions tyramine-C^{14} did not serve as a precursor of noradrenaline. Thus, one of the alternative pathways in which tyrosine is first decarboxylated to form tyramine does not function in beef adrenal medulla, and, indeed, tyrosine decarboxylase has not been found in any mammalian tissue. Udenfriend and Wyngaarden (56) found that intact

rats utilized phenylalanine, tyrosine and dopa but not tyramine or phenethylamine as precursors of adrenal noradrenaline and adrenaline. Leeper and Udenfriend (39) subsequently showed that the intact rat incorporated hydroxytyramine into epinephrine at 5 to 10 times the rate at which it utilized DOPA.

Further evidence that DOPA and hydroxytyramine lie on the metabolic route of noradrenaline formation was obtained by isotope dilution experiments (19). When unlabeled DOPA or hydroxytyramine was added to adrenal tissue slices synthesizing noradrenaline from tyrosine-C^{14}, the amounts of radioactivity incorporated into the noradrenaline fractions were greatly reduced; the addition of unlabeled tyramine did not decrease the amount of radioactivity in the noradrenaline fractions.

Although the evidence obtained above strongly supports the biosynthetic pathway proposed by Blaschko (3), it does not exclude the possibility of an alternative pathway (48) in which the side chain of DOPA is hydroxylated to form dihydroxyphenylserine (DOPS), which is then decarboxylated to form noradrenaline. Evidence shows that DOPS can be converted to noradrenaline by intact animals and isolated tissues. Dihydroxyphenylserine is decarboxylated at a slower rate than DOPA by extracts of guinea pig kidney and liver (1, 5) and by extracts of beef adrenal medulla (53). When dihydroxyphenylserine is injected into rabbits (49) an increased amount of noradrenaline is found in the urine. Drell, Eshleman and Clark (15) found noradrenaline-C^{14} in the urine of rats after injection of threo-DOPS-C^{14}. The decarboxylation of DOPA and DOPS were presumed to be mediated by the same enzyme, DOPA decarboxylase, but Werle and Jüngten-Sell (57) claim there is a specific DOPS decarboxylase in the adrenal medulla and sympathetic nerves, and propose that DOPS is the intermediate in the formation of noradrenaline. The evidence presented by these authors does not seem sufficient to justify their conclusions.

The fact that DOPA decarboxylase is found in the supernatant fractions (7, 34) of adrenal homogenates prepared in 0.25 M sucrose and that the enzymic activity required for the hydroxylation of the side chain of hydroxytyramine is found in the particulate fraction (24, 34) made it possible to demonstrate that DOPS is not an obligatory intermediate in the formation of noradrenaline and that it is probably not formed at all by the adrenal medulla (34). Aerobic incubation of DOPA-2-C^{14} with the particulate fraction of an adrenal medullary homogenate followed by anaerobic incubation with the supernatant fraction containing DOPA decarboxylase produced only hydroxytyramine-C^{14}. If DOPS were formed, then noradrenaline-C^{14} would have been obtained. Conversely, when DOPA was first incubated anaerobically with the supernatant fraction and this reaction mixture was then incubated aerobically with the particulate fraction, both hydroxytyramine-2-C^{14} and large amounts of noradrenaline-2-C^{14} were isolated. After incubation of adrenal slices with tyrosine-C^{14} in the presence of DOPA decarboxylase inhibitors Drell *et al.* (15) isolated labeled DOPA but could not detect any radioactivity in the isolated DOPS. Thus sufficient evidence is available from several different sources to confirm Blaschko's hypothesis (3).

The formation of noradrenaline and adrenaline in sympathetic nerves and

ganglia of the dog and cow has been reported by Goodall and Kirshner (20, 21). It was found that minced sympathetic nerves and sympathetic ganglia incubated with either DOPA-C^{14} or tyrosine-C^{14} formed hydroxytyramine-C^{14} and noradrenaline-C^{14}. The amounts of radioactivity found in the adrenaline fraction were quite low and could have been due to contamination. It is interesting to note that the stellate and thoracic sympathetic ganglia formed noradrenaline at the same rate as the thoracic sympathetic nerves and the splenic nerve. The cervical vagus formed small amounts of hydroxytyramine-C^{14} when incubated with DOPA-C^{14} but did not form noradrenaline. Holtz and Westermann (28) report rather high DOPA decarboxylase activity in sympathetic nerves and ganglia and very low activity in the preganglionic and vagus nerve fibers. DOPA was only slightly decarboxylated by these preparations. Schümann (52) found that hydroxytyramine constituted nearly 50% of the total catecholamines in sympathetic nerves and ganglia. From these reports it would seem that the pathway of noradrenaline formation in sympathetic nerves and ganglia is the same as that in the adrenal medulla.

A third alternative pathway for the formation of adrenaline and noradrenaline in which the side chain of tyrosine is hydroxylated to form *p*-hydroxyphenylserine and then decarboxylated to form *p*-hydroxyphenylethanolamine (octopamine) does not appear likely. Although octopamine has been found in the salivary gland of an octopus (16) it has not been found in mammalian tissues.

The reactions involved in the formation of adrenaline from noradrenaline have now been established. In 1949 Bülbring (8) reported that dog and cat adrenal homogenates form adrenaline in the presence of ATP. More recently von Euler and Floding (17) demonstrated that noradrenaline added to suspensions of medullary homogenates causes a small but significant increase of adrenaline in the absence of added ATP. Since the adrenal medulla is especially rich in ATP, further additions are not required. Keller *et al.* (32) have shown that the methyl group of adrenaline can come from methionine in the intact animal and Masuoka (42) and co-workers isolated C^{14} adrenaline from the adrenal gland of the rat after injections of noradrenaline-C^{14}.

The supernatant fraction of beef adrenal medullary homogenates has been shown to form adrenaline-C^{14}, from noradrenaline, ATP and methionine-methyl-C^{14} (36); this enzymatic reaction is similar to the methyl transferring systems described by Cantoni (10). The first step involves the reaction between ATP and methionine to form S-adenosylmethionine (11). The second step is the transfer of the methyl group of S-adenosylmethionine to the amino group of noradrenaline. The enzymes were partially purified by ammonium sulfate fractionation. When methionine is used as the methyl donor, ATP and Mg^{++} are absolutely required; when S-adenosylmethionine is the methyl donor, Mg^{++} and glutathione stimulate the formation of adrenaline but are not absolutely required. S-adenosylmethionine is utilized 20 to 30 times as rapidly as ATP and methionine by freshly prepared supernatant fractions in the formation of adrenaline. Masuoka, Clark and Schott (41) have obtained similar results with noradrenaline-C^{14}.

Although noradrenaline is generally accepted as the immediate physiological precursor of adrenaline, an alternative pathway in which hydroxytyramine is

first N-methylated and then hydroxylated suggests itself. The fact that relatively large amounts of noradrenaline and only small amounts of adrenaline are formed by tissue slices (19) indicates that the second pathway is of only minor importance. In addition, when hydroxytyramine is added to the enzyme system which utilized ATP and methionine-methyl-C^{14} to N-methylate noradrenaline, no radioactive fraction could be isolated by ion exchange chromatography which corresponded to N-methyl-hydroxytyramine (33). Thus it appears that noradrenaline is the only immediate physiological precursor of adrenaline. Since N-methyldopa is not decarboxylated by dopa decarboxylase (2) it is improbable that it is an intermediate in adrenaline synthesis.

Little information is available concerning the details of the reaction in which hydroxytyramine is converted to noradrenaline. Neri *et al.* (44) report that acetone powders of beef adrenals and certain molds convert hydroxytyramine to a norepinephrine-like material in the presence of ATP and either DPN or TPN. This problem is currently being investigated in this laboratory and the reaction appears to be quite complex (33). Dialyzed aqueous extracts of beef adrenal medulla acetone powder convert hydroxytyramine-C^{14} into noradrenaline-C^{14}. The noradrenaline-C^{14} was isolated by ion exchange chromatography and identified by paper chromatography in two different solvent systems. ATP alone increases the amounts of noradrenaline-C^{14} formed by 5- to 10-fold; DPN, TPN and Mg^{++} do not cause any further stimulation; ADP, AMP, adenosine and pyrophosphate only slightly stimulate the reaction; GTP, ITP, CTP and UTP stimulate about as effectively as ATP; Versene (3×10^{-3} M) inhibits the reaction but CN^- (3×10^{-3} M) does not. From these observations it appears that a nucleoside triphosphate and a metal ion are required for the reaction. The lack of specificity for nucleoside triphosphates may be due to the presence of an active nucleosidediphosphokinase in the acetone powders. This point and the elucidation of the role of the nucleoside triphosphate in the hydroxylation of hydroxytyramine await further purification of the involved enzymes.

The decarboxylation of DOPA to form hydroxytyramine is apparently the fastest reaction of the entire sequence in the conversion of tyrosine to adrenaline. The enzyme, DOPA decarboxylase, was first discovered in guinea pig and swine kidney (26) and subsequently in the adrenal medulla (38). The role of this enzyme is discussed elsewhere in THIS SYMPOSIUM.

At present one can only speculate about the reaction in which tyrosine is converted to DOPA in the adrenal medulla. One likely possibility is that the reaction is catalyzed by tyrosinase. Raper (46) isolated DOPA after treating tyrosine with tyrosinase obtained from the meal worm *Tenebrio molitor*. Hogeboom and Adams (25) found tyrosinase activity in the Harding-Passey mouse melanoma and Lerner *et al.* (40) showed that DOPA was one of the reaction products of this mammalian tyrosinase acting upon tyrosine. That this specific reaction occurs in the adrenal medulla at a sufficient rate to account for noradrenaline formation remains to be demonstrated.

Gurin and Delluva (22) and Udenfriend *et al.* (55, 56) have shown that phenylalanine is utilized by the intact rat and rabbit for the formation of adrenaline. The conversion of phenylalanine to noradrenaline has not been demonstrated

in isolated tissues but Fellman and Devlin (18) have shown that adrenal medullary slices form tyrosine and *ortho*-tyrosine from phenylalanine. Thus the adrenal medulla itself is able to carry out all of the reactions necessary for the conversion of phenylalanine to noradrenaline and adrenaline. The enzyme system of rat liver catalyzing the oxidation of phenylalanine to tyrosine is complex. At least two different protein fractions (30, 43), TPNH (29) and a third unidentified co-factor (31) are involved.

The distribution of the enzymes involved in the synthesis of adrenaline and noradrenaline tempts one to speculate on the site or sites of the individual reactions. The intracellular localization of the phenylalanine oxidase systems in the adrenal medulla is not known; however it has been found (30, 43, 54) in the soluble fraction of rat liver homogenized in isotonic KCl. The location of the enzyme which oxidizes tyrosine to DOPA again is not known. Tyrosine is poorly utilized by homogenates of adrenal medulla in the formation of noradrenaline —the reaction proceeds at about one-tenth the rate found in adrenal slices (33). No hydroxytyramine-C^{14} could be isolated after incubating tyrosine-C^{14} (33) with the supernatant fraction of adrenal medullary homogenates. It would appear, therefore, that the enzyme is associated with the particulate fraction of the homogenate similar to the tyrosinase of the Harding-Passey mouse melanoma (40). Little that is supported by evidence can be said about the site or the enzymes involved in the first two reactions of the sequence.

The distribution in homogenates of the enzymes for the remainder of the reactions is well established. DOPA decarboxylase (7) and the N-methylating enzymes (36) are found in the supernatant fractions, while the enzyme system which hydroxylates hydroxytyramine is found in the particulate fractions (24, 34). Although all of the enzyme catalyzing a specific reaction may be found in the supernatant fraction of a homogenate, one cannot categorically state that that enzyme is not associated with particulate matter in the cell.

Recent evidence indicates that the chromaffin granules of the adrenal medulla are the actual site of noradrenaline synthesis from hydroxytyramine. By centrifuging the granular fraction of adrenal medullary homogenates prepared in 0.3 M sucrose over a 1.5 M sucrose solution, granules were retained at the boundary region of the two sucrose solutions which had a very low catecholamine content, and a sediment was obtained which contained almost all of the activity of the original suspension (4). Blaschko *et al.* (6) modified this procedure somewhat and showed that the granular fraction retained at the boundary had several different enzymatic activities normally present in mitochondria, and that these activities were absent in the sedimented fraction. When these fractions were prepared as described by Blaschko *et al.* (4) and tested for the ability to form noradrenaline from hydroxytyramine (33) all of the activity was found to be associated with the sedimented granular fraction containing noradrenaline and adrenaline. Thus it appears that the chromaffin granules in the adrenal medulla not only serve as a storehouse for adrenaline and noradrenaline but are actually the site of noradrenaline formation from hydroxytyramine.

The distribution of the enzymes involved in the last three reactions in the

formation of adrenaline, namely decarboxylation of DOPA, hydroxylation of hydroxytyramine and methylation of noradrenaline leads one to construct a dynamic picture of the events occurring in the adrenal medullary cell. DOPA is decarboxylated in the extragranular portion of the cell. The hydroxytyramine thus formed has several pathways: it may pass into the blood stream and be excreted in the urine or be further metabolized by the liver and other organs; it may be further metabolized by enzymes in the extragranular portion of the cell or by the mitochondria; it may be converted to noradrenaline either on the surface or in the interior of the chromaffin granules. If noradrenaline is formed on the surface of the granules it may remain there until methylated to form adrenaline and then diffuse into the granules. If noradrenaline is formed in the interior of the granules it would have to diffuse into the clear cytoplasm, there be methylated, and then re-enter the chromaffin granules. This latter sequence of reactions would presumably be slow and is supported by the observations of Butterworth and Mann (9). These investigators found that after depleting the cat adrenal gland of its catecholamines it took 6 to 7 days for the total catecholamine content to recover its normal level. However, at this stage, the noradrenaline level was several times its normal value while the adrenaline was still well below its resting level. In the succeeding days the noradrenaline decreased and the adrenaline increased until the initial levels and distribution were attained at the end of one month.

Summary

The metabolic pathway for the formation of adrenaline and noradrenaline has been established, but much remains to be done on the individual reactions. Only two of the reactions in the sequence, the decarboxylation of DOPA and the N-methylation of noradrenaline, have been demonstrated with any clarity of detail. The oxidation of phenylalanine to tyrosine has been studied in other tissues and it is highly probable that the same system applies to the adrenal medulla. The formation of DOPA from tyrosine has been found to be catalyzed by tyrosinase both in invertebrates and in mammalian tissues and it is likely that this enzyme may also be found in the adrenal medulla. The reaction in which noradrenaline is formed from hydroxytyramine is the least understood of the entire sequence. Initial studies indicate that this reaction is complex but show promise of elucidation.

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