BIOCHEMISTRY OF CATECHOLAMINES[1]

PERRY B. MOLINOFF[2] AND JULIUS AXELROD
Laboratory of Clinical Science, National Institute of Mental Health
Bethesda, Maryland

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INTRODUCTION

The catecholamines noradrenaline, adrenaline, and dopamine are 3,4-dihydroxy derivatives of phenylethylamine. They are widely distributed throughout the animal kingdom, most often in nerve cells. Catecholamines are found in the peripheral sympathetic nervous system (1), and in the adrenal medulla (2), and are unequally distributed in different areas of the central nervous system (3). Small amounts of catecholamines are also found in chromaffin cells scattered throughout the body.

Noradrenaline is highly localized in peripheral postganglionic sympathetic nerves (2). Its concentration in some sympathetic ganglia may be as high as 100 μg/g (4) while in sympathetically innervated tissues its concen-

[1] Abbreviations used are: dopa, 3,4-dihydroxyphenylalanine; dopamine, 3,4-dihydroxyphenylethylamine; COMT, catechol-O-methyltransferase; MAO, monoamine oxidase; PNMT, phenylethanolamine-N-methyltransferase.

[2] Presently Guggenheim Fellow, Department of Biophysics, University College, London.

tration ranges from 0.1 to 2 μg/g (2). The nerve endings make up only a small part of the total mass of the tissue, and thus the actual concentration of noradrenaline in the endings is probably between 100 and 500 μg/g (4). Postganglionic sympathetic denervation leads to an almost complete disappearance of noradrenaline from most tissues (5) which suggests that it is wholly contained within the sympathetic nerves.

Noradrenaline is released from sympathetic nerve terminals as a neurotransmitter (2) and exerts most of its effects locally on postsynaptic cells. Adrenaline functions mainly as a hormone, being released into the general circulation primarily from the adrenal medulla. Small amounts of adrenaline have been found in mammalian brain (3) and heart (6). A third catecholamine, dopamine, serves as a precursor of noradrenaline and adrenaline in both the peripheral and central nervous systems. Its most important function however may be in the brain, where high concentrations are found in the striatum (7, 8). Dopamine is thought to be a synaptic transmitter functioning to mediate inhibition in a motor-coordinating nigrostriatal pathway.

Elliott (9) in 1905 first postulated that an adrenaline-like substance might be released from sympathetic nerves and thus be responsible for chemical transmission at synapses. The first experimental evidence for neurochemical transmission was obtained in 1921 by Loewi (10), who described both an inhibitory and an excitatory substance released from frog or toad heart on stimulation of the vagus nerve. Similarities between the effects of sympathetic nerve stimulation and those of exogenously applied adrenaline led many workers over the next two decades to assume that the transmitter liberated at sympathetic nerve endings was adrenaline. Discrepancies between the effects of nerve stimulation and those of exogenously applied adrenaline had however been reported (11). That noradrenaline is the sympathetic neurotransmitter was finally established by von Euler (12), who identified noradrenaline in splenic nerves by chemical isolation. Direct evidence that noradrenaline is released by postganglionic sympathetic nerves comes from the work of Peart (13), who showed that it is liberated after stimulation of the splenic nerve of the cat.

It has recently become possible to visualize catecholamines in situ. A highly specific and sensitive fluorescence-histochemical method (14) has permitted the direct visualization at a cellular level of the biogenic amines dopamine, noradrenaline, adrenaline, and serotonin. The use of this technique has confirmed the conclusion that the noradrenaline present in peripheral mammalian tissues is localized almost exclusively in sympathetic nerves (14). More specifically, the use of gradient-centrifugation techniques (15), the autoradiographic localization of tritium-labeled noradrenaline (16), and electronmicroscopy (17) have combined to show that noradrenaline is localized not only within the sympathetic nerve endings, but also largely within specific vesicles contained in these nerve endings.

Catecholamines influence the actions of a wide variety of tissues such as vascular smooth muscle, adipose tissue, liver, heart, and brain (2). In many

cases they act by causing an increase in the activity or amount of a specific enzyme or enzymes. These effects are frequently mediated by adenosine 3′,5′-monophosphate (18, 254).

Our knowledge of the biochemistry of catecholamines is more precise in the peripheral nervous system than in the more biochemically and anatomically complex central nervous system. Most of this review will therefore refer to the peripheral sympathetic nervous system. It is likely that the general processes described will in the future be shown to exist in the central nervous system. We will be concerned with the synthesis and metabolism of the catecholamines as well as with the mechanisms of amine storage, release, and inactivation. The final section will discuss some of the mechanisms which operate to regulate the levels of catecholamines within the neuron and in the adrenal medulla. Several recent reviews on catecholamines are recommended (19–30).

Biosynthesis

The biosynthesis of catecholamines begins with tyrosine. This pathway was first suggested by Blaschko in 1939 (31) (Figure 1) and was finally confirmed in 1964 with the demonstration of the enzyme tyrosine hydroxylase (32). The four enzymes involved in catecholamine biosynthesis do not have the same subcellular distribution. Thus intracellular migrations of substrates for these enzymes take place as tyrosine is converted to noradrenaline in the sympathetic nerves and to adrenaline in the adrenal medulla.

Although the pathway proposed by Blaschko (31) is the major pathway of catecholamine biosynthesis, lack of specificity of several enzymes involved permits several alternative pathways. One such pathway from tyrosine to noradrenaline and adrenaline may exist via tyrosine, tyromine, octopamine, and synephrine (33–34). Indirect evidence of the conversion of tyramine to catecholamines in vivo has been obtained from the detection of radioactive C^{14}-noradrenaline in the urine of rats injected with C^{14}-tyramine or C^{14}-octopamine (33). There is also a pathway from tyrosine to adrenaline which bypasses noradrenaline. In this reaction sequence dopamine is converted to epinine by a nonspecific phenylalkylamine-N-methyltransferase which has been found in rabbit lung (35). Epinine can then be β-hydroxylated by dopamine-β-hydroxylase to form adrenaline (36). This later series of reactions was in fact proposed by Hallé (37) in 1906 for the biosynthesis of adrenaline.

Tyrosine hydroxylase.—Tyrosine hydroxylase (EC 1.14.3.-) is the enzyme responsible for the conversion of tyrosine to dopa, a reaction established by isotopic studies in the early 1950s (38–39). Direct demonstration of the enzymatic hydroxylation of tyrosine did not come, however, until 1964 (32). Udenfriend and his co-workers found enzyme activity in tissues that contained catecholamines such as spleen, brain, and adrenal medulla and noted only minimal activity in the adrenal cortex. Tyrosine hydroxylase

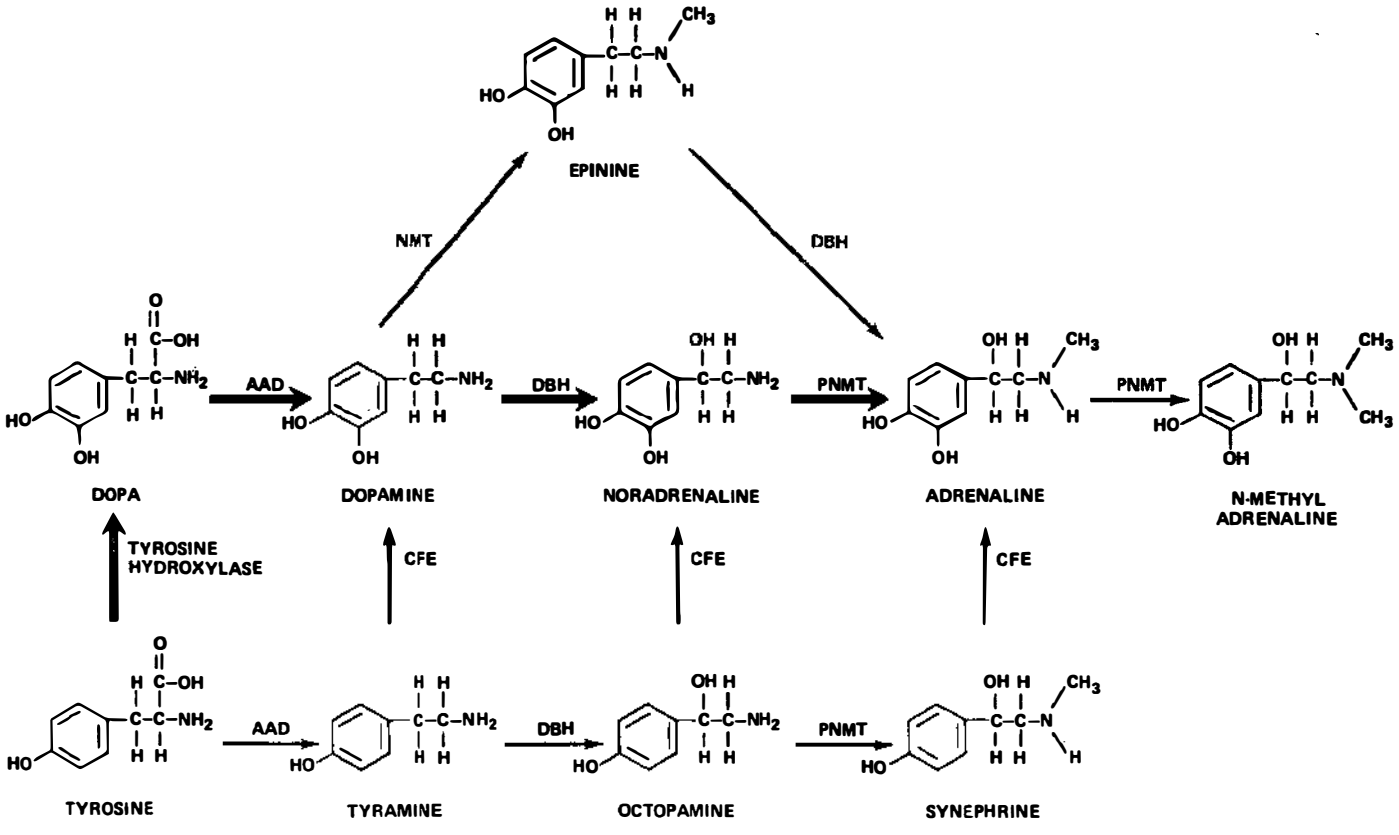

FIGURE 1. Biosynthesis of catecholamines. DBH—dopamine-β-hydroxylase; NMT—nonspecific methyltransferase; AAD—aromatic acid decarboxylase; PNMT—phenylethanolamine-N-methyltransferase; CFE—catecholamine-forming enzyme.

was purified from bovine adrenal medulla. The properties of the adrenal enzyme have been studied in great detail. Tetrahydropteridines such as 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine produce a marked stimulation of activity. In addition to the reduced pteridines (40), Fe^{++} (32) and O_2 are required for maximum activity. The K_m of the enzyme for tetrahydropteridine is about $5 \times 10^{-4} M$. At high concentrations pteridines inhibit the enzyme but this inhibition is reversed by Fe^{++} (41).

Tyrosine hydroxylase hydroxylates tyrosine to dopa but not tyramine to dopamine (32) and it does not hydroxylate D-*p*-tyrosine nor DL-*m*-tyrosine. α-Methyl-*p*-tyrosine, a competitive inhibitor of tyrosine hydroxylase, is converted to its 3-hydroxy derivative. Tissue catecholamines are depleted following the administration of α-methyl-*p*-tyrosine (42) while the concentration of serotonin is not decreased (43).

Working with enzyme preparations obtained from the supernatant fractions of the adrenal, it has been reported that double reciprocal plots of velocity against tyrosine concentration at different concentrations of tetrahydropteridine result in a series of parallel lines (44). A *ping-pong* mechanism has been postulated in which the substrates of the reaction (oxygen, tyrosine, and tetrahydropteridine) add to the enzyme in an obligatory sequential order (44). Recently Goldstein et al (45) have purified particulate tyrosine hydroxylase from the adrenal after solubilization by trypsin digestion (46). Experiments with this enzyme suggest a different mechanism of action from that described for the enzyme from the supernatant fraction. Double reciprocal plots against tyrosine or oxygen concentration at different concentrations of pteridine yield intersecting lines. A mechanism was suggested in which a quaternary complex is formed as an intermediate step with the conversion of this complex into products and enzyme being the rate-limiting step in the reaction. The different results obtained with tyrosine hydroxylase prepared from the supernatant after high-speed centrifugation from those obtained with enzyme solubilized by trypsin digestion of adrenal particles may reflect different species of enzyme molecule or may reflect alterations in the molecular form of the enzyme during one or both of the purification procedures. A molecular weight of 192,000 has been reported for the supernatant enzyme while the trypsin-digested enzyme has a molecular weight of only 50,500 (47).

It was initially reported that tyrosine hydroxylase in the bovine adrenal medulla is largely particle bound (32). However, incubation of radioactive tyrosine with particle fractions does not result in the formation of significant amounts of catecholamines (48). Laduron & Belpaire (49) studied the subcellular distribution of several enzymes involved in catecholamine synthesis and concluded that tyrosine hydroxylase is found mainly in the soluble fraction. They explained the original findings by reporting that the enzyme can be easily adsorbed to material which sediments in the nuclear fraction. Musacchio & Wurzburger also concluded that tyrosine hydroxylase is not usually membrane bound (50). They found that when freshly

prepared adrenal homogenates are layered onto density gradients, tyrosine hydroxylase activity remains in fractions at the top of the tube. If the homogenate is kept for a few hours before layering, much of the activity is adsorbed to particles and becomes sedimentable (51). Petrack has shown that tyrosine hydroxylase isolated from guinea pig brain is in the supernatant following centrifugation of homogenates at 150,000 × *g* for 1 hr (52).

Tyrosine hydroxylase is usually assayed (32) using 3,5-H^3-tyrosine. Tritium, released during the hydroxylation, is separated from the tyrosine and dopa by ion-exchange column chromatography.

Inhibitors of tyrosine hydroxylase have been described which act by competing with substrate, pteridine cofactor, or Fe^{++}. Some substituted tyrosines are substrates for the enzyme: α-methyl-*p*-tyrosine is, for example, converted to α-methyldopa (42). Halogenated compounds such as 3-iodotyrosine (53–54) are among the most potent inhibitors of the enzyme both in vitro and in vivo, where they cause a lowering of endogenous levels of catecholamines in many tissues (55). Many derivatives of phenylalanine are competitive inhibitors of tyrosine hydroxylase especially after halogenation or substitution of an α-methyl group (56). Nagatsu et al reported that tyrosine hydroxylase is inhibited by several catecholamines (32), and it is thought that this inhibition is involved in the regulation of catecholamine synthesis. Despite their obvious similarity to tyrosine, the inhibition by catecholamines is competitive not with substrate but with the pteridine cofactor in its reduced form (53, 57). A similar type of inhibition is also observed with 3,4-dihydroxyphenylacetamide (53), the most potent inhibitor of tyrosine hydroxylase yet reported. It also inhibits other pteridine-requiring enzymes (58). Tyrosine hydroxylase can also be inhibited by chelating agents such as αα-dipyridyl (32) and φ-phenanthroline (53, 58). These compounds probably act by chelating Fe^{++}, and their effect is offered as evidence that Fe^{++} has a specific role in the function of this enzyme (59). Tryptophan (60) as well as several 5-halotryptophans (61) can also inhibit tyrosine hydroxylase; α-methyl-5-hydroxytryptophan is especially potent.

Dopa decarboxylase.—Dopa decarboxylase (EC 4.1.1.26) is a widely distributed enzyme whose role in catecholamine synthesis is the formation of dopamine from dopa. This enzyme is optically specific for L isomers (62, 63) but is not specific for any particular aromatic amino acid. Thus, the decarboxylation steps in the synthesis of the biogenic amines (catecholamines, tyramine, serotonin, and histamine) are all catalyzed by this same enzyme. It also acts on amino acids such as *m*-tyrosine, α-methyldopa or α-methyl-*m*-tyrosine (64, 65). Its lack of substrate specificity has led to the suggestion that its name be changed to L-aromatic amino acid decarboxylase (66). This reaction was recognized in vitro as early as 1938 (62), and in vivo evidence of the transformation was obtained in 1942 when dopamine was detected in urine following the injection of dopa (67).

Dopa decarboxylase has been purified (66, 68). Most of the enzyme activity is found in the supernatant fluid after high-speed centrifugation (69). The activity of the enzyme with respect to different substrates is usually constant throughout the purification. The enzyme has a pH optimum of 7.2, a $s_{20,w}$ of 6.9 and molecular weight of 109,000. The K_m for either dopa or tyrosine is about $5 \times 10^{-4} M$ (68).

Pyridoxal phosphate is a cofactor of this enzyme, and is tightly bound to the apoenzyme as a Schiff's base. The absorption spectrum is similar to that of other pyridoxal phosphate enzymes with an absorption maximum at 415 mμ (70). However, if additional cofactor is added to a purified enzyme preparation the activity increases but the absorption spectrum is unchanged. The source of the enzyme, the choice of substrate, and the pH of the incubation all affect the degree of activation by exogenous pyridoxal phosphate (70).

Dopa decarboxylase is widely distributed in mammalian tissues and has been detected in human carcinoid and in phaeochromocytoma (71), in neural tissues such as sympathetic ganglia and postganglionic nerve trunks (72), in nerve terminals, and in noradrenaline-storage particles (73–74). In the central nervous system the distribution of enzyme activity is similar to that of the catecholamines, with the highest activity being found in the reticular formation, the hypothalamus, and portions of the thalamus and rhinencephalon (75).

The conversion of dopa to dopamine by dopa decarboxylase can be measured by manometric determination of carbon dioxide (72, 76), fluorometric determination of dopamine (75), or by use of radioactive substrates (77).

It was initially hoped that inhibition of dopa decarboxylase would permit therapeutic modification of catecholamine synthesis. The wide distribution and high activity of this enzyme in many tissues has made it difficult, however, to achieve a degree of inhibition sufficient to decrease catecholamine levels. Of the inhibitors studied the one with the widest clinical application is L-α-methyldopa. This compound is a potent inhibitor of dopa decarboxylase in vitro (78) and has catecholamine-depleting (79) and hypotensive actions (80) in vivo. These latter effects are not however related to decarboxylase inhibition. In fact, α-methyldopa is itself decarboxylated, leading to the eventual formation of α-methylated metabolites which displace noradrenaline and serve as false neurotransmitters (81). Several classes of compounds not structurally similar to dopa also inhibit this enzyme: these include hydrazino acids (82), hydrazines (83), and benzyloxyamines (84). Other inhibitors of dopa decarboxylase include the strongly reducing borohydride, decaborane (85), and sulfhydral reagents such as N-ethylmaleimide (66). Hepatic dopa decarboxylase can be induced by phenobarbital and α-hexachlorcyclohexane (86).

Dopamine-β-hydroxylase.—The enzyme which catalyzes the β hydroxylation of dopamine to noradrenaline is, like tyrosine hydroxylase, a mixed-

function oxidase. The reaction was first demonstrated to occur in vivo (87) in 1956 and in vitro the next year (88).

Dopamine-β-hydroxylase (EC 1.14.2.1) was solubilized from particles of beef adrenal and found to require ascorbic acid (89) for activity. It is inactive under anaerobic conditions or in the presence of KCN. The enzyme also catalyzes the conversion of several phenylethylamines other than dopamine to their β-hydroxylated products (90). The structural characteristics necessary for substrate activity are an aromatic ring with a side chain of two or three carbon atoms terminating in an amino group.

The enzyme has been purified to homogeneity and has been found to have a molecular weight of 290,000 and a $s_{20,w}$ of 8.9 in 0.1 *M* NaCl and 0.005 *M* potassium phosphate, pH 6.8 (91). Purified enzyme hydroxylates about 1000 μmoles of dopamine per μmole of enzyme per minute at 25° (91). Dopamine-β-hydroxylase contains approximately 2 μmoles of Cu^{++} per μmole of enzyme as well as variable amounts of Cu^{+}. The first step in the reaction is the reduction of the cupric copper by an external source of electrons such as ascorbic acid (92).

To obtain full activity in vitro, a number of cofactors are required in addition to ascorbate. These include fumarate or acetate, catalase, and ATP. Fumarate may accelerate the reoxidation of the enzyme Cu^{+} to Cu^{++}, or it may function to facilitate the formation of the reduced enzyme-oxygen complex (93). Both catalase and ATP seem to protect the enzyme from inactivation. The stimulatory effect of ATP becomes less marked during purification of the enzyme (89), and is absent if initial rates are measured (90). Further, in the presence of catalase, ATP has no additional effect. The catalase probably destroys peroxides which are formed by the autooxidation of either ascorbate or dopamine.

Most assays for dopamine-β-hydroxylase are suitable only for measuring the activity of relatively pure enzyme. Methods have been based on the fluorometric or chromatographic determination of noradrenaline formed from dopamine (94). Other procedures have taken advantage of the fact that periodate will remove the side chain of β-hydroxylated amines. For example, tyramine is converted to octopamine, which yields on periodate cleavage *p*-hydroxybenzaldehyde and formaldehyde (93, 95). In a recent coupled enzymatic assay (96) phenylethanolamine or octopamine, formed by dopamine-β-hydroxylase, is converted to its N-methyl derivative by phenylethanolamine-N-methyltransferase using C^{14}-S-adenosylmethionine as methyl donor. This assay has permitted dopamine-β-hydroxylase activity to be determined in homogenates of tissues such as heart, salivary gland, individual sympathetic ganglia, and brain.

Dopamine-β-hydroxylase is associated with the chromaffin granule in the adrenal medulla (97). These granules are saclike structures sensitive to osmotic pressure and surrounded by a semipermeable membrane. Approximately 60 percent of the enzyme is found bound to the chromaffin granule membrane while most of the remainder is contained in the soluble contents

of the granule (98). Similarly, it is associated with the catecholamine-containing granules in rat heart (99) and splenic nerves (100).

Endogenous inhibitors of dopamine-β-hydroxylase are present in most tissues which act by complexing with the Cu^{++} of the enzyme. The purified inhibitor from bovine heart is a heat-stable compound of low molecular weight which contains carbohydrate and organic phosphate (101). N-Ethylmaleimide blocks the activity of the purified adrenal inhibitor but is without effect on the purified inhibitor from bovine heart. When the enzyme is measured in vitro it is important to block the effects of the inhibitor by using Cu^{++}, N-ethylmaleimide, or *p*-chloromercuribenzoate.

The fact that dopamine-β-hydroxylase is a copper-containing enzyme has also made available a variety of pharmacologic inhibitors. Most contain thiol groups and act by binding enzyme Cu^{++}. Disulfiram (tetraethylthiuram) is a potent inhibitor of dopamine-β-hydroxylase both in vitro and in vivo (102–103), which decreases the noradrenaline content of the brain and heart. Disulfiram is probably reduced in vivo to diethyldithiocarbamate, which chelates $Cu.^{++}$. Certain tropolone derivatives (104) and a large variety of both aromatic and alkylthioureas (105) also inhibit dopamine-β-hydroxylase, probably by copper chelation.

Dopamine-β-hydroxylase also is inhibited by compounds structurally related to the phenylethlamines and their isosteres. Its most potent inhibitor yet reported is fusaric acid (106, 226).

Phenylethanolamine-N-methyltransferase.—The final step in the biosynthesis of adrenaline is the N methylation of noradrenaline (Figure 1). Early studies in vivo and in vitro showed that the methyl group of methionine (107) and S-adenosylmethionine (108) is used for the enzymatic formation of adrenaline. The partially purified enzyme from the bovine adrenal gland (109) N-methylates phenylethanolamine and its derivatives but not phenylethylamines, and was thus named phenylethanolamine-N-methyltransferase (PNMT) (EC 2.1.1.-). The normally occurring phenylethanolamine derivatives methylated by PNMT include noradrenaline, normetanephrine, octopamine, synephrine, adrenaline, and metanephrine. With compounds such as adrenaline and synephrine which already have an N-methyl group, PNMT can add a second methyl group to form N,N-dimethyl derivatives. Amines such as neosynephrine, norephedrine, and *p*-hydroxynorephedrine are also substrates for PNMT. The enzyme has been purified about sixtyfold from bovine adrenal medulla (110). It has a $s_{20,w}$ molecular weight of 30,000. On titration with *p*-hydroxymecuribenzoate 8.5 sulfhydryl groups are titrated per molecule of enzyme. Kinetic measurements suggest a random binding of substrates with a preference for S-adenosylmethionine as the first substrate bound.

Phenylethanolamine-N-methyltransferase is strongly inhibited by its substrate noradrenaline and its product adrenaline (111). The inhibition of PNMT by adrenaline occurs at concentrations normally present in the adre-

nal gland and suggests that this might be of physiologic significance in regulating PNMT activity. PNMT is also inhibited by a number of amines not structurally related to phenylethanolamine, including tranylcypromine and two related compounds: 2-cyclohexylcyclopropaneamine and 8-amino-1,2-methanoindane HCl (112). The inhibition by tranylcypromine appears to be competitive with substrate. PNMT, unlike the metabolizing enzyme catechol-O-methyltransferase, does not require Mg^{++}; 2-mercaptoethanol increases its activity while heavy metals such as Cd^{++}, Hg^{++}, and Cu^{++} are inhibitory (113).

PNMT is highly localized in the adrenal medulla (109). It is also present in small amounts in the heart and brain of mammals (114–115), in hen, turtle, snake, and frog adrenal (116–117) and in the parotid gland of *Bufo marinus* (118). In the adrenal gland PNMT is localized mainly in the soluble fraction of the cell (109). It is absent in the fetal adrenal gland, first appearing about 3 days before birth (119). Enzyme isolated from several species differs with regard to electrophoretic mobility on starch block, heat stability, and substrate specificity (120).

Phenylethanolamine-N-methyltransferase is assayed by incubating either normetanephrine or phenylethanolamine with C^{14}-S-adenosylmethionine. The radioactive N-methylphenylethanolamine or metanephrine is extracted into a mixture of toluene and isoamyl alcohol and the radioactivity determined (109).

The marked specificity of PNMT for phenylethanolamine derivatives has also been used in assays for octopamine (121), noradrenaline (122), and dopamine-β-oxidase (96); such assays are more sensitive than other existing methods. Removal of the pituitary causes a marked fall in PNMT activity in the rat adrenal (123). Administration of large amounts of glucucorticoids or ACTH restores enzyme activity.

Minor biosynthetic enzymes.—An enzyme that catalyzes the conversion of monophenolic phenylethylamines to catecholamines is present in liver microsomes (124). This enzyme requires TPNH and oxygen and is similar to the enzymes described for the metabolism of amphetamine and other drugs (125). The normally occurring substrates, tyramine, octopamine, and synephrine, yield dopamine, noradrenaline, and adrenaline respectively (124; Figure 1). The activity of this enzyme is measured by incubating monophenols, liver microsomes which contain the hydroxylating enzyme, and the soluble supernatant fraction which contains catechol-O-methyltransferase together with C^{14}-methyl-S-adenosylmethionine. Any catecholamine formed is immediately O-methylated to form a metabolite with a radioactive methyl group. The formation of metanephrine from octopamine has been demonstrated in vivo (33), indicating that this reaction is operating in the metabolism of normally occurring physiologically active compounds.

Nonspecific N-methyltransferase enzymes have been found in rabbit lung (35) and in parotid gland of *B. marinus* (118). These enzymes are

capable of N-methylating dopamine to epinine, noradrenaline to adrenaline, serotonin to N-methylserotonin, and tryptamine to the psychotomimetic dimethyltryptamine.

Metabolism

An important observation which led to our present knowledge of the reactions involved in catecholamine metabolism was made by Armstrong, McMillan & Shaw in 1957 (126). They reported that a 3-O-methylated β-hydroxylated catechol acid, 3-methoxy-4-hydroxymandelic acid (vanilmandelic acid, VMA) is a urinary metabolite of both adrenaline and noradrenaline. Soon thereafter the O-methylated amines normetanephrine and metanephrine were also found in urine (127, 128).

The metabolism of catecholamines involves primarily two enzymes: catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO) (Figure 2). Both act on a wide variety of amines and each is fully active on the product of the other. Thus an entire spectrum of metabolites of catecholamines can be identified in urine, some acted on by MAO, COMT, or both.

In addition to these two major enzymes of catecholamine metabolism, two other enzymes—an aldehyde oxidase and an aldehydase reductase—are present and act on the products of MAO and COMT (Figure 2). Aldehyde dehydrogenase has been demonstrated in a guinea pig kidney preparation containing NAD (129). In man vanilmandelic acid, an oxidized product, makes up about 40 percent of the total urinary metabolites (130) while in some other species 3-methoxy-4-hydroxyphenylglycol, a reduced metabolite, is predominant (131).

An analogous pathway to the one shown for noradrenaline metabolism in Figure 2 can be drawn for dopamine and for adrenaline.

About one third of the total catechol metabolites in human urine come from dopamine (132, 221). Some of these come from the brain where dopamine is a putative neurotransmitter but probably most arise from dopamine which has been acted on by MAO before it could be protected by uptake into storage granules. The principal metabolite of dopamine is homovanilic acid (HVA), a product of the action of MAO, COMT, and aldehyde dehydrogenase.

Monoamine oxidase.—Monoamine oxidase (MAO) (EC 1.4.3.4) was first described in 1928 by Hare (133) who showed that tyramine was deaminated to *p*-hydroxybenzaldehyde. MAO also deaminates catecholamines such as noradrenaline, adrenaline, and dopamine (134). The corresponding aldehyde is then, depending on the nature of the substrate, either oxidized to form an acid metabolite or reduced to an alcohol metabolite. MAO deaminates compounds in which the amine group is attached to the terminal carbon atom. N methylation and β-hydroxylation decrease the susceptibility of phenylethylamines to MAO (135). Thus, tyramine and dopamine are metabolized more readily than noradrenaline and adrenaline. Monoamine oxidase

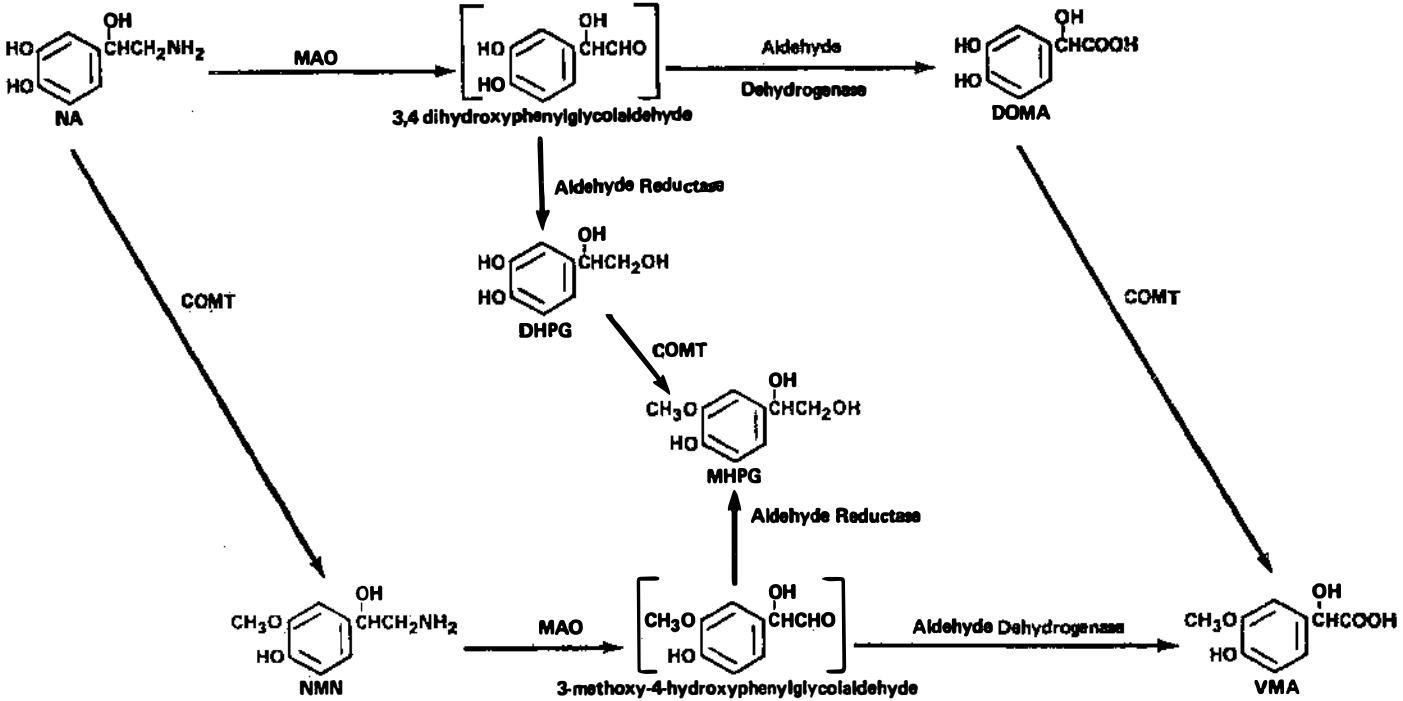

FIGURE 2. Metabolism of noradrenaline (NA). NMN—normetanephrine; DOMA—dihydroxymandelic acid; DHPG—dihydroxyphenylglycol; VMA—vanilmandelic acid; MAO—monoamine oxidase; COMT—catechol-O-methyltransferase; MHPG—3-methoxy-4-hydroxyphenylglycol.

is widely distributed in tissues of many vertebrate species (135) where it is present mainly in the outer membrane of the mitochondria (136). Subcellular fractionation studies indicate that in rat heart and salivary gland MAO is also associated with the microsomal fraction which also contains the noradrenaline storage vesicles (137–138). In tissues heavily innervated by sympathetic nerves, destruction of these nerves causes a marked reduction in MAO activity (139) which suggests that some MAO is present in these nerve terminals.

Monoamine oxidase has been purified and found to have a molecular weight of about 290,000 and to contain a flavoprotein and copper (140–141). The valence of copper does not change during the oxidative deamination of amines; thus it appears unlikely that it plays an active role in the reaction. Pharmacologic evidence that multiple forms of MAO exist (142–143) has been confirmed by the electrophoretic separation of several species of MAO from rat liver and brain (144–145).

The several species of MAO differ with respect to substrate specificity, heat stability, and the effect of different inhibitors. The electrophoretic pattern of MAO isolated from embryonic brain is different from that of MAO isolated from the brain of newborn animals (146).

Monoamine oxidase can be measured fluorometrically by deaminating kynuramine (147) or tryptamine (148) or radiometrically using tryptamine as substrate (149).

An increase in cardiac monoamine oxidase activity has been observed in rats made hypertensive by treatment with deoxycorticosterone and sodium chloride (150). Prolonged treatment of rats with isoproterenol also results in an increase in the activity of salivary gland MAO (151). This increase can be blocked by the prior administration of either the beta blocking agent, propranolol, or the inhibitor of protein synthesis, actinomycin D.

Many types of MAO inhibitor have been used (152) since the introduction of the hydrazine derivative iproniazid (153). Monoamine oxidase inhibition by blocking agents of the hydrazine type tends to be long lasting and irreversible. Other classes of MAO inhibitors are amines containing an acetylene group, cyclopropylamines, β-methylphenylethylamines (152), and harmaline and related carbolines (154). Administration of MAO inhibitors results in an elevation of tissue levels of noradrenaline, octopamine (121), dopamine, and serotonin (155). Inhibition of MAO in vivo decreases the excretion of deaminated metabolites of catecholamines such as 3-methoxy-4-hydroxymandelic acid, 3-methoxy-4-hydroxyphenylglycol, and homovanillic acid, while the excretion of normetanephrine, octopamine, and tyramine increases (156).

Inhibition of MAO does not prolong the physiological effects of noradrenaline (157). Monoamine oxidase has however been found to play an important role in the regulation of the intracellular level of this neurotransmitter. Any noradrenaline which leaks into the axoplasm from storage vesicles is metabolized to physiologically inactive metabolic products (158).

Catechol-O-methyltransferase.—The observation that noradrenaline and adrenaline are metabolized to the corresponding 3-O methylamines normetanephrine and metanephrine prompted a search for an O-methylating enzyme (127–128). Catechol-O-methyltransferase (EC 2.1.1.6), first described in rat liver (159), requires S-adenosylmethionine as a methyl donor, and also a divalent cation such as Mg^{++}. Ions which can substitute for Mg^{++} include Co^{++}, Ca^{++}, Zn^{++}, and Ni^{++}. The enzyme can O-methylate catechols but not monohydroxy derivatives of phenylethylamine. Normally occurring compounds O-methylated by the enzyme include noradrenaline, adrenaline, dopamine, dopa, 3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylacetic acid (159), 3-hydroxyestradiol (160), and ascorbic acid (161). The enzyme can also O-methylate exogenously administered catechols such as 3,4-dihydroxyephedrine, 3,4-dihydroxyamphetamine (159), and a variety of substituted catechols (162) and polyphenols (163).

In vivo, O methylation occurs exclusively on the meta position (128). With purified COMT O methylation can occur on both meta and para positions (164). The polarity of the aromatic substrate and the pH of the reaction mixture affect the ratio of para to meta O-methylated products formed in vitro (165). A novel type of interconversion of 4-O-methyl-3-hydroxyacetophenone to 3-O-methyl-4-hydroxyacetophenone occurs in vivo (166). This results from the O demethylation of the 4-O-methylated compound to form a catechol which is subsequently O-methylated on the 3 position by COMT.

Catechol-O-methyltransferase has been purified approximately 450-fold from rat liver (162, 167). When purified the enzyme is labile, requires dithiothreitol for stability, and has a molecular weight of about 24,000. With starch-block electrophoresis at least two separate forms of the enzyme have been found (120), which differ with respect to electrophoretic mobility, heat stability, and kinetic constants though both have the same substrate specificity. Catechol-O-methyltransferases from a variety of mammalian species show different electrophoretic mobilities (120). Antibodies to the purified COMT have been prepared (168).

Catechol-O-methyltransferase activity is inhibited by dichloromercuribenzoate, which suggests that the enzyme has a sulfhydryl group in the region of its active site (159). Polyphenols such as pyrogallol inhibit COMT in vitro but are less effective in vivo (169). Dihydroxyphenyl acetamides inhibit this enzyme (170) competitively and are themselves O-methylated. A normally occurring compound that blocks O methylation in vitro and in vivo is 3-hydroxyestradiol (160). Another class of COMT inhibitors are substituted 3,5-dihydroxy-4-methoxybenzoic acids (171), which act noncompetitively and are effective in vivo. Chelating agents such as tropolones can also inhibit COMT (172). Pyridoxal 5′-phosphate is also a competitive inhibitor of COMT in vitro (Black and Axelrod, unpublished).

Catechol-O-methyltransferase is measured by incubating a catechol substrate, usually noradrenaline, with Mg^{++} and C^{14}-S-adenosylmethionine. The

O-methylated product is extracted into an organic solvent and the radioactivity measured (173). Catechol-O-methyltransferase has been used in a highly sensitive assay for catecholamines (174).

Catechol-O-methyltransferase is found in many different mammalian species (159) and has also been identified in plants (175–176). In most animals the highest activity is found in the liver and kidney. There is an asymmetric distribution of COMT in the brain, activity being highest in the area postrema and lowest in the cerebellar cortex (177). Although most of the enzyme is found in the soluble fraction of the mammalian cell, there are also small amounts present in fat-cell membranes (178) and in microsomes (179). Catechol-O-methyltransferase is present mainly outside the sympathetic neuron (180), in contrast to MAO which is present within the neuron (139) (Figure 3). However, a small amount of enzyme activity occurs in the sympathetic nerves of the nictitating membrane and the vas deferens (181).

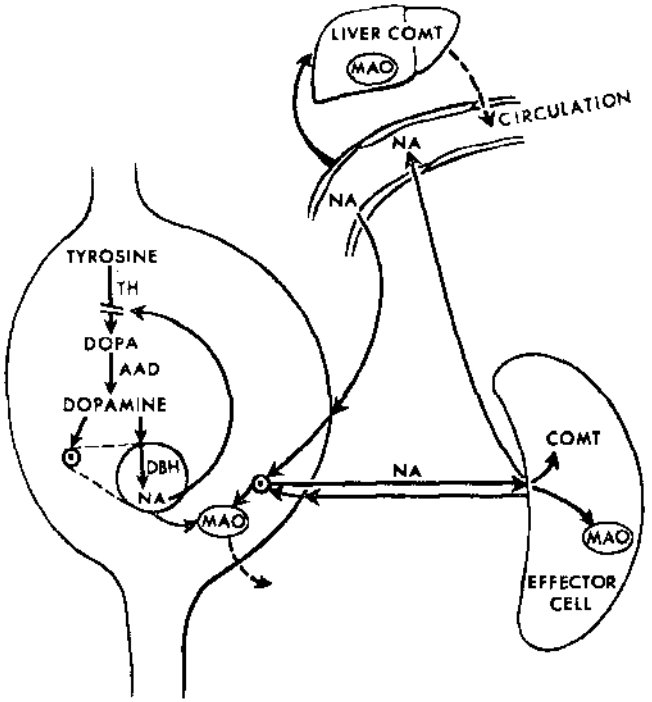

FIGURE 3. The formation and fate of noradrenaline (NA) at a varicosity of a sympathetic nerve terminal. TH—tyrosine hydroxylase; AAD—aromatic amino acid decarboxylase; DBH—dopamine-β-hydroxylase; MAO—monoamine oxidase; COMT—catechol-O-methyltransferase.

Physiologically, COMT is involved in the metabolism of catecholamines released into the circulation (158, 180) and in the inactivation of noradrenaline in tissues lacking an abundant adrenergic innervation (182). Catechol-O-methyltransferase may also be associated with an extra neuronal uptake mechanism (183).

Uptake and Inactivation

Catechol-O-methyltransferase and monoamine oxidase act on catecholamines to produce physiologically inactive metabolic products, but neither of these enzymes plays an important role in terminating the physiological actions of the neurotransmitter noradrenaline or of the hormone adrenaline. The rapid inactivation of catecholamines is due to uptake into the presynaptic nerve endings. This uptake is studied by injection of a tracer amount of radioactive noradrenaline. Some of the catecholamine injected is taken up and retained unchanged in adrenergically innervated tissues (184–185) where it remains long after the physiological effects have disappeared. When the sympathetic nerves are destroyed surgically (186), immunologically (187), or chemically (188), the denervated tissues no longer can take up and retain noradrenaline. Histochemical and autoradiographic studies indicate that the uptake sites are located in the postganglionic nerve terminals (189) where noradrenaline is stored in dense-core granules about 500 A in diameter (190–191). When both metabolizing enzymes for catecholamines are inhibited in vivo the actions of noradrenaline are only slightly prolonged (192). However, blocking the uptake of noradrenaline by drugs (193) markedly enhances the response. Thus, uptake across the neuronal membrane and subsequent retention in the storage vesicles are important mechanisms for rapid termination of the physiological effects of noradrenaline. Radioactive noradrenaline taken up from the circulation can be liberated from the sympathetic nerve terminal by nerve stimulation (194–195).

Reuptake is the major mechanism for inactivation particularly in tissues with rich adrenergic innervation. In sparsely innervated tissues (media of blood vessels, for example) COMT is also important in terminating the effects of noradrenaline (182). Noradrenaline and adrenaline discharged into the bloodstream either from the adrenal gland or by overflow after neuronal release are primarily inactivated by liver and kidney COMT and MAO or by reuptake by sympathetically innervated organs.

Figure 3 shows schematically the intraneuronal synthesis, storage, release, and uptake of noradrenaline and its extraneuronal disposition.

Experiments on the uptake of H^3-noradrenaline by heart and brain slices have suggested that it obeys saturation kinetics of the Michaelis-Menten type and involves active transport (21, 196). Energy derived from either glycolysis or oxidation is required for the transport of noradrenaline across the neuronal membrane (197) and sodium ions must be present in the external medium (198–199). Noradrenaline uptake is a temperature-dependent process with a Q_{10} of about 2 (200). From these observations it appears

that an active ion carrier mechanism is involved in the transport of noradrenaline across the neuronal membrane. A second Na^+-dependent neuronal carrier mechanism has been described which requires low [Na^+] and is optically specific (201). It may function intraneuronally where [Na^+] is low. Although both stereoisomers of noradrenaline and adrenaline are taken up by nerve terminals the naturally occurring *levo* forms are removed more rapidly (200). The process also shows molecular selectivity: the efficiency of uptake of noradrenaline is twice that of adrenaline while little if any isoproterenol is taken up by this mechanism. Many other amines structurally related to noradrenaline can be taken up and can accumulate in sympathetic nerves by a neuronal uptake process. These amines include tyramine (202), α-methylnoradrenaline (203), metaraminol (204), adrenaline (205), and dopamine. These compounds or their metabolites are then stored in nerve granules and can serve as false neurotransmitters (206). Cocaine and antidepressant drugs such as impramine and sympathiomimetic amines block the uptake of noradrenaline into the nerve (20–21, 193, 196, 207–209). Compounds that block uptake also prevent the inactivation of noradrenaline and thus prolong its physiologic actions.

An extraneuronal uptake process for noradrenaline, called uptake 2, has been found (183, 210–213) which is blocked by normetanephrine (a metabolite of noradrenaline) and by adrenergic blocking agents (183, 212) like phenoxybenzamine. Uptake 2 operates at all concentrations of catecholamines to transport the amines into nonneuronal tissues where they are subsequently metabolized (214). Compounds such as adrenaline and isoproterenol which have a relatively low affinity for intraneural uptake and a high affinity for uptake 2 may be inactivated, mainly by the latter process.

Storage

Catecholamines in sympathetic nerves or in the adrenal medulla are stored in membrane-bound granules. The ability to sequester noradrenaline and adrenaline in specific storage vesicles serves to inactivate the amines temporarily and to protect them from enzymatic destruction until they are released by an appropriate stimulus. Catecholamine storage vesicles were first isolated from the adrenal medulla by differential centrifugation techniques (215, 216). These medullary storage granules have a high concentration of ATP with a constant molar ratio of catecholamines to ATP of 4:1 (216), which suggests that ATP might function to form a salt complex with the amines (217). Specific proteins in the chromaffin granule have been identified as dopamine-β-hydroxylase (218) and at least eight species of chromogranin (219). The principal chromogranin, making up about 40 percent of the soluble protein, is called chromogranin A. It has a molecular weight of about 77,000, with an elevated content of glutamic acid (220). The chromaffin granule can be isolated in relatively pure form by discontinuous gradient-centrifugation techniques (222). Catecholamines are concentrated by isolated chromaffin granules via a temperature-dependent mechanism

which also requires Mg^{++} and ATP (223–224) and which can be blocked by reserpine. In addition to noradrenaline, the chromaffin granule takes up adrenaline, dopamine, and serotonin (224).

Storage granules for noradrenaline have been demonstrated in sympathetic nerve terminals (16–17). High-speed centrifugation of splenic nerve (225) or heart (15) homogenates results in the sedimentation of noradrenaline-containing particles. These granules contain ATP as well as noradrenaline-forming dopamine-β-hydroxylase (99). With radioautography and electronmicroscopy the peripheral storage granule was shown to have a dense core and a diameter of 400–600 Å (16), in contrast to the chromaffin granule which is about 1000 Å in diameter. Using antibodies prepared against soluble components of the chromaffin granule of the adrenal medulla, two proteins, dopamine-β-hydroxylase and chromagranin A, were shown to be present in the noradrenaline storage vesicle of sympathetic nerves (227). Monoamine oxidase is associated with these vesicles (137–138). Some larger granules are seen in adrenergic nerve endings and these may also contain noradrenaline (228).

Granules isolated from splenic nerves have an uptake mechanism similar to that of adrenal chromaffin granules. This process requires ATP and Mg^{++} (229), is temperature dependent, and is blocked by reserpine, which also prevents the retention of noradrenaline in the storage granule by an as yet unexplained mechanism. Catecholamines released by reserpine are deaminated and made physiologically inactive by intraneuronal monoamine oxidase before they leave the nerve (230). In aqueous solution, biogenic amines, including catecholamines and 5-hydroxytryptamine, form aggregates with ATP (231). Low concentrations of divalent metal ions increase this aggregation while monovalent cations cause disaggregation.

Noradrenaline is released from storage granules by many phenylethylamine derivatives, including tyramine and amphetamine (81, 232). In contrast to reserpine, these sympathomimetic amines usually release noradrenaline from the neuron in a physiologically active form (230). The noradrenaline storage vesicle can also retain other compounds, including adrenaline (205), dopamine, α-methylnoradrenaline (202), octopamine (233), and metaraminol (204). All of these amines can displace noradrenaline from the sympathetic nerve granules (234), and they or their metabolites are then released from the nerve terminal on nerve stimulation as false neurotransmitters (25, 205, 235). The neurotransmitters noradrenaline and serotonin in the pineal are located in the same storage granule (236). Octopamine is also present normally in sympathetic nerve terminals of several tissues (233) and is presumably stored in vesicles. This amine is released on stimulating splenic nerves in normal and monoamine oxidase-inhibited cats (Gewirtz, Molinoff and Kopin, unpublished observations).

5-Hydroxydopamine and 6-hydroxydopamine have been recently introduced as important experimental tools in studying the sympathetic nervous system. 5-Hydroxydopamine has a very high affinity for noradrenaline stor-

age granules and has proved useful as a marker for adrenergic nerve endings (237). 6-Hydroxydopamine causes a rapid degeneration of adrenergic nerve endings but leaves the cell body and axons unaffected (238). The effects of 6-hydroxydopamine are long lasting though not irreversible. Thus, some recovery of noradrenaline content is noted after about 4 weeks with full recovery as a result of neural regeneration taking about 3 months (239). 6-Hydroxydopamine does not cross the blood-brain barrier when given systemically, and thus has no effect on brain amines. When it is introduced directly into the brain by intraventricular or intracisternal injection it produces a rapid and long-lasting depletion of brain catecholamines (240) without affecting serotonin content (241). Unlike its effects in the periphery, the depletion of catecholamines in the brain appears to be irreversible. Chemical sympathectomy by 6-hydroxydopamine causes the disappearance of tyrosine hydroxylase and dopamine-β-hydroxylase (242–243) as well as noradrenaline from rat heart. The concentration of catecholamines in the adrenal gland is not affected by 6-hydroxydopamine (244), but the activities of tyrosine hydroxylase (243), phenylethanolamine-N-methyltransferase (243), and dopamine-β-hydroxylase (242) are all increased in this tissue.

Release

In the adrenal medulla adrenaline and noradrenaline are present almost exclusively in the chromaffin granules while the noradrenaline found in the peripheral sympathetic nervous system is largely localized in the granular vesicles. There are many similarities between the processes described for the relase of catecholamine from the adrenal medulla and from peripheral nerve terminals, but it is not yet certain that the same mechanisms are involved. Douglas & Rubin (245) using the adrenal gland perfused in a retrograde fashion found that the addition of acetylcholine to the perfusion media resulted in a marked increase in the output of catecholamines. Acetylcholine could release catecholamines in the absence of sodium but not in the absence of calcium. Excess potassium increases calcium uptake, probably as a result of a depolarizing action, and thus leads to an increased release (246).

The adrenal chromaffin granules are membrane-limited organelles ranging in size from 500 to 2000 Å. It now appears likely that catecholamines are released directly from these granules by a process called exocytosis. Whenever catecholamine secretion is induced, ATP and its metabolites are also released. The ratio of catecholamine to nucleotide in the perfusate is the same as in the intact granules (247). Moreover, under appropriate experimental conditions ATP can escape unhydrolyzed even though the cell and its organelles are rich in ATPases, which suggests that the nucleotide is extruded directly to the cell exterior. Another constituent of the chromaffin granules is the protein chromagranin. This substance would not be expected to pass through intact membranes but it also is found in the effluent of stimulated adrenal glands (248–249). On the other hand, stimulation produces

no significant increase in the efflux of phospholipid or cholesterol, the principal lipids of the chromaffin granule membrane (250). Electronmicrographs show the membrane of the chromaffin granule fused with that of the cell (251).

Using starch-gel electrophoresis it was found that chromagranin A is only one of eight soluble proteins of bovine chromaffin granules and that all eight are secreted on stimulation (252). On stimulation of the adrenal, dopamine-β-hydroxylase, which is found within and bound to chromaffin granules (253), is released together with catecholamines in the same proportion as they are found in the soluble fraction of lysed vesicles (218). Not only are all of the soluble contents of the chromaffin granule released, but this release occurs as an all-or-none phenomenon with respect to any single granule (218).

Dopamine-β-hydroxylase (99) and chromogranin A (255) are also present in the noradrenaline-containing vesicles of bovine splenic nerve. Stimulation of the calf splenic nerve leads to the simultaneous release of noradrenaline, chromogranin A, and dopamine-β-hydroxylase (256). Release of the latter has also been reported on stimulation of the splenic nerve of several other species (257–258). Omission of calcium from the perfusion fluid strongly inhibits the release of these three substances, which indicates another similarity to release from the adrenal medulla (256). Dopamine-β-hydroxylase is also found in circulating blood. Preliminary evidence indicates that this enzyme arises mainly from the sympathetic nerve terminals (Weinshilboum and Axelrod, unpublished).

Turnover

The tissue concentration of catecholamines remains constant in the face of major changes in sympathetic nerve activity. For example, exposure of animals to either high or low temperatures increases the rate of utilization of catecholamines without changing tissue levels (264).

Several experimental approaches have been used to measure the turnover and thus the rate of synthesis of catecholamines. In the most commonly used approach H^3-noradrenaline is administered to the animal, and the rate of disappearance of the labeled catecholamine is followed (259). It is assumed that the noradrenaline taken up (185) equilibrates uniformly with the endogenous catecholamine. Another approach involves administering labeled precursors such as tyrosine or dopa and then measuring the rate of formation of noradrenaline. The actual rate of synthesis of catecholamines is difficult to calculate, however, because the specific activity of the precursor is rapidly and continuously changing during the course of the experiment. The radioactive precursor can be infused at a constant rate which, assuming steady-state kinetics, allows for a more rigorous mathematical treatment (260). These methods assume that the noradrenaline formed from exogenous tyrosine (or dopa) is turning over at a rate identical to that of the endogenous stores. A third approach is to inhibit noradren-

aline synthesis with α-methyl-*p*-tyrosine (42) and to then follow the decline in endogenous amine content. In this approach it is assumed that neither the blockade in noradrenaline synthesis nor the changing level of amine affects the normal rate of turnover of the endogenous catecholamine (261). However, it is likely that catecholamine synthesis rates are in fact inversely related to catecholamine levels (268).

Turnover measurements are useful if the aim is to compare different pharmacological or physiological manipulations. However, it is doubtful if any of these methods will provide absolute values. The main difficulty is that these procedures assume that all of the tissue noradrenaline is in the same physiologic and anatomical pool, but there is considerable evidence that this is not the case. Thus, Kopin and his co-workers (262) have shown that newly synthesized noradrenaline is preferentially released from cat spleen on stimulation of the sympathetic nerves. A number of investigators have provided pharmacologic evidence that several pools of tissue catecholamines exist, (232, 263) which may be in identical storage particles and differ only in their distance from the nerve terminal membrane (232).

When sympathetic nerve tone is increased there is a concomitant increase in the rate of loss of catecholamines and in the rate of synthesis. Thus, exposure of animals to either increased or decreased environmental temperature results in an increase in the turnover rate of noradrenaline both in heart and brain (264). Similarly, electrical stimulation of sympathetic nerves also enhances the rate of synthesis of C^{14}-noradrenaline from C^{14}-tyrosine (265). The opposite result is seen in studies where sympathetic activity is inhibited. Thus, ganglionic blocking agents reduce the turnover rate of heart noradrenaline (266–267).

Administration of monoamine oxidase inhibitors increases the levels of noradrenaline in both brain and heart (268) and significantly decreases the turnover rates of these amines, while reserpine administration decreases the levels of noradrenaline and increases turnover rate.

Cardiac noradrenaline turnover is increased in thyroid-deficient rats and, to a lesser extent, in adrenalectomized rats (269). A generalized increase in turnover occurs in rats made hypertensive by steroid-induced salt loading (270). This increase is associated with a decrease in the noradrenaline content of the heart, spleen, and intestine.

Axoplasmic Transport

The large distance frequently separating nerve endings from their cell bodies is one of the unique features of the neuron. The axon which connects the cell body to the nerve ending is not only the part of the neuron which conducts nerve impulses; it also provides a system in which a continuous flow of intraneuronal material can take place. Most constituents of both axons and nerve terminals are believed to be synthesized in the cell body and then transported in a proximodistal direction by axoplasmic flow (28, 271–272). Materials must be transported for both the maintenance and (during

development) growth of the neuron, and to replace those constituents of the nerve cell lost during nerve firing. That axoplasmic flow does occur is shown by the accumulation above a constriction produced by a ligature around the nerve of mitochondria (273), neurosecretory granules (274–275), and enzymes such as dopamine-β-hydroxylase (276). Other studies have shown an accumulation of phospholipids (277) and radioactive proteins (278) proximal to a constriction. Axon transport is a highly specialized process and different constituents are transported at their own characteristic rates. Thus after H^3-leucine has been injected into the region of the seventh lumbar and first sacral horn, radioactive proteins are formed and transported down the axons (279) in two distinct waves of radioactivity: a fast phase with a rate of some 900 mm/day and a slow phase with a rate of 1 to 30 mm/day, depending on the method of calculation.

There is an accumulation of noradrenaline above but not below a ligature placed around a sympathetic nerve (274, 280, 281). When two constrictions are placed on the same nerve no accumulation is observed above the second, more distal, constriction (282). Geffen and his co-workers (283) labeled transmitter stores by the infusion of C^{14}-noradrenaline into a branch of the splenic artery. They found no evidence for a bidirectional transport of noradrenaline. The transport process is not dependent on nerve impulse traffic as the accumulation above a constriction is not affected by surgical separation from the central nervous system (decentralization) (284). The amount of noradrenaline which accumulates in 24 hr above a constriction placed around the splenic nerve of the cat is only 1 percent of that contained in the splenic nerve terminals. Since the half-life of noradrenaline in most axon terminals is less than 1 day this suggests that processes such as local synthesis and uptake are of greater importance in the economy of the sympathetic transmitter than is axonal transport. Assuming that the noradrenaline which accumulates above a constriction is stored within storage granules and that granules do not increase their content of noradrenaline during the accumulation period, it has been calculated that the rate of transport of the granules is 5 and 6 mm per hr in the rat and cat respectively and that their average life-span is 35 and 70 days (285). This value greatly exceeds the time required for renewal of the noradrenaline in the terminals (286), which suggests that much of the catecholamine in the terminals is made locally and that granules are reused many times during their life-span.

It has been proposed that neurotubules are involved in the rapid proximodistal transport system of nerves (287). These microtubules are composed of subunits of molecular weight of 60,000 (288), which bind and are precipitable by colchicine (289) and vinblastine (290). The rapid transport of amine storage vesicles (291) and of acetylcholinesterase (292) is prevented by the injection of colchicine under the epineurium of the sciatic nerve. Transport of diphosphopyridine nucleotide diaphorase is relatively less affected than that of acetylcholinesterase (292) which suggests that

rapid transport is based on neurotubules and that this high-speed flow is superimposed on a slow movement of the entire axonal contents.

It has been assumed that the noradrenaline which accumulates above a constriction is contained within granules which appear proximal to a constriction (293). Evidence has been presented suggesting that the vesicles formed proximal to a ligature arise from the disintegration of neurotubules (294). On the other hand, Geffen & Ostberg (295) have presented suggestive evidence that large dense-core vesicles such as are seen proximal to a constriction are transformed into smaller dense-core synaptic vesicles as they migrate down the axon.

With immunological techniques it has been demonstrated that antibodies prepared against adrenal catecholamine binding proteins and against adrenal dopamine-β-hydroxylase are immunologically cross-reactive with proteins involved in the synthesis and storage of noradrenaline in sympathetic nerves (296). By use of these antibodies it was shown that dopamine-β-hydroxylase and catecholamine binding proteins accumulate proximal to an axonal constriction. The activity of dopa decarboxylase is increased both above and below a constriction (297) while tyrosine hydroxylase activity decreases slightly proximal to a constriction (298).

Regulation

The catecholamine content of the adrenergic neuron remains constant despite marked fluctuations in the level of activity in the sympathetic nervous system (299). This results both from the presence of an efficient reuptake system (194), allowing for repeated reuse of the same molecules, and from close regulation of catecholamine synthesis (27). The efficiency of reuptake is not sufficient to maintain the concentration of catecholamines indefinitely without the aid of ongoing synthesis. Thus, if synthesis of noradrenaline is blocked by the administration of an inhibitor of either tyrosine hydroxylase (42) or of dopamine-β-hydroxylase (102) the concentration of noradrenaline falls rapidly. The precise control of catecholamine levels means that it is difficult to assess the level of activity in the adrenergic neuron accurately by measuring levels of catecholamines. More meaningful parameters are the rate of conversion of radioactive precursors into specific catecholamines and the rate of catecholamine turnover, whether assessed by the blockade of synthesis or by the disappearance of radioactive tracers.

Catecholamine levels or their physiological actions may be regulated at many sites. These include the sensitivity of the receptor, the ease of release of the transmitter, the avidity of the reuptake system, and the relative rates of synthesis and degradation of the four enzymes involved in catecholamine biosynthesis. Each enzyme has specific cofactor requirements, and they differ in their subcellular localization within the cell. Transport of these enzymes from their site of synthesis in the cell body to their site of action in the nerve endings may also be a regulated phenomenon. The availability of required cofactors such as tetrahydropteridine, pyridoxal phosphate, as-

corbic acid, and S-adenosylmethionine may also contribute to the regulation of catecholamine synthesis. Finally, the sympathetic neuron is itself under the influence of preganglionic neurons and thus transsynaptic influences are possible.

Several of these potential regulatory mechanisms are utilized. The rate of catecholamine synthesis varies from minute to minute with the level of activity in the sympathetic nervous system. Stimuli which lead to prolonged changes in the level of activity lead in addition to changes in the amounts of several of the enzymes involved in catecholamine biosynthesis. Further, the sympathetic nervous system is influenced by hormonal factors, and these regulate the activity of several of the enzymes involved. Bydgeman & von Euler (300) found that prolonged stimulation of the splanchnic nerve leads to the release of catecholamines into the adrenal vein. The sum of the amount released plus the amount remaining in the gland is greater than the amount initially present in the gland. This rapid increase in the rate of synthesis of catecholamines on nerve stimulation results from a decrease in the degree of inhibition of tyrosine hydroxylase. Tyrosine hydroxylase is inhibited by catecholamines such as dopamine and noradrenaline (53), apparently because of competition between the catechols and the pteridine cofactor required by the enzyme (44). Most of the noradrenaline in the sympathetic nerve endings is contained within vesicles and therefore would be expected to have only limited access to tyrosine hydroxylase which is outside the vesicles in the axoplasm. A small fraction of the noradrenaline outside the vesicles in the axoplasm may be the critical compound for the regulation of tyrosine hydroxylase. Alternatively, dopamine, normally found outside of the vesicle, may be responsible for regulating tyrosine hydroxylase activity.

Increases in adrenergic nervous activity whether due to physiological stress or electrical stimulation lead to increased synthesis of noradrenaline from tyrosine but not from DOPA (301–302). Working with the isolated hypogastric nerve–vas deferens preparation of the guinea pig, Weiner and his co-workers showed that the increased rate of synthesis of C^{14}-noradrenaline from C^{14}-tyrosine associated with nerve stimulation could be blocked by adding noradrenaline to the bath (303–304). Neither the total amount of noradrenaline nor the tyrosine hydroxylase activity measured in vitro was different in the stimulated preparation from the control. At the tyrosine hydroxylase step, noradrenaline synthesis might be controlled by tyrosine transport, by endogenous inhibitors or availability of cofactors, or by feedback inhibition of this enzyme. The fact that catecholamines inhibit tyrosine hydroxylase in vitro makes the last the most likely possibility. Support for this concept comes from other experiments in which the concentration of noradrenaline has been elevated by administering monoamine oxidase inhibitors or by injecting large amounts of noradrenaline. The increased concentration of noradrenaline leads to a decrease in the formation of noradrenaline from tyrosine but not from dopa (305).

The increased conversion of tyrosine to noradrenaline observed with

nerve stimulation occurs coincident with nerve activity. However, Weiner and co-workers have described an enhanced rate of noradrenaline synthesis occurring in the period immediately following a 1 hr period of stimulation (306). This increase, unlike that which occurs during nerve stimulation, is not abolished by noradrenaline but is inhibited by puromycin. No increase in the amount of tyrosine hydroxylase has been demonstrated in this period immediately after stimulation (Kopin, Weiner and Thoa, unpublished).

The changes in catecholamine synthesis which occur consequent to alterations in the feedback inhibition of tyrosine hydroxylase by catecholamines take place on a time scale of minutes. There are, in addition, long-term regulatory mechanisms, some of which are mediated by hormones and some by nerve impulses. Hypophysectomy leads to a marked decrease in the activity of phenylethanolamine-N-methyltransferase (123), a fall in the content of adrenaline in the adrenal medulla, and secretion of this catecholamine following induction of hypoglycemia by insulin (307). The activity of PNMT can be restored by ACTH or by administering very large doses of glucocorticoids (123). The amount of glucocorticoid required is far in excess of the normal replacement dose but may be consistent with the steroid concentrations normally delivered to the adrenal medulla from the cortex via the adrenal portal circulation (308). Inhibition of protein synthesis with puromycin or actinomycin D prevents the restoration of PNMT activity, which suggests that the steroid hormones may act by inducing the formation of additional enzyme protein (123). When fetal rats are hypophysectomized by decapitation neither adrenaline nor PNMT is present in the newborn rat adrenal (309). The administration of either corticoids or of ACTH to the mother of the decapitated rat fetuses results in normal levels of PNMT in the newborns. Fetal extrachromaffin tissue, mainly in the organ of Zuckerkandl, contains negligible amounts of PNMT but the enzyme can be induced in this tissue with ACTH (119).

Tyrosine hydroxylase (310) and dopamine-β-hydroxylase activity (311) also decline following hypophysectomy. The decrease in tyrosine hydroxylase activity is restored to normal by ACTH administration but not by amounts of glucocorticoids sufficient to completely restore PNMT activity (123), which raises the possibility of direct action by ACTH. Adrenocorticotropic hormone restores only about half of the decrease in dopamine-β-hydroxylase activity and again even very large amounts of glucocorticoids are without effect.

The administration of drugs such as reserpine which deplete catecholamines causes a fall in blood pressure and a reflex increase in activity in the sympathetic nervous system. After administration of reserpine, tyrosine hydroxylase and dopamine-β-hydroxylase activities increase in sympathetic ganglia (96, 312), the adrenal gland (313–315), and sympathetically innervated organs such as the heart (96) and salivary glands. In sympathetic ganglia such increases are usually apparent within 24 hr, but in the heart the increase in tyrosine hydroxylase activity occurs only after a 3 to 4 day lag

(314). The delayed increase can be blocked by administering cycloheximide on day 3 at the end of the lag period. This finding is consistent with the local synthesis of induced tyrosine hydroxylase in the nerve terminals rather than the peripheral movement of completed enzyme. The increase in dopamine-β-hydroxylase activity from sympathetic nerves in the heart (96) and the adrenal gland (315) is preceded by a fall, which most likely represents neuronal release of dopamine-β-hydroxylase contained in the adrenergic storage vesicles.

The drug-induced elevation of these enzymes is probably a function of increased neural activity since the increase in the activity of both tyrosine hydroxylase (312) and dopamine-β-hydroxylase (96) in the superior cervical ganglia is prevented by decentralizing this ganglion or by administering ganglionic blocking agents. Similarly, the increase in adrenal tyrosine hydroxylase can be prevented by adrenal denervation (244). Stimulation of the rat hypothalamus causes an increase in adrenal gland tyrosine hydroxylase and PNMT and this is blocked by denervation of the gland (316). Phenoxybenzamine, like reserpine, is thought to produce a reflex increase in activity in the sympathetic nervous system. Administering this drug leads to an increase in tyrosine hydroxylase activity as measured in vitro (244). This increase in synthetic capacity is reflected physiologically by an increase in the conversion of C^{14}-tyrosine to C^{14}-noradrenaline (317). In other experiments the catecholamine-synthesizing enzymes have been studied in rat sympathetic ganglia maintained in organ culture (Silberstein, Lemberger and Molinoff, unpublished). When the tissues are incubated in media with increased external potassium there is an increase in the activity of tyrosine hydroxylase and dopamine β-hydroxylase. These increases are blocked by the protein-synthesis inhibitor cycloheximide. These experiments strengthen the conclusion that the effects of drugs and of stress are due to transsynaptic neural regulation of catecholamine synthesis.

Phenylethanolamine-N-methyltransferase is also subject to neuronal regulation. Its activity increases following catecholamine depletion with reserpine (96) and after chemical sympathectomy with 6-hydroxydopamine (318) or stimulation of the hypothalamus (316). The increase after sympathectomy is blocked by adrenal denervation but is still demonstrable in hypophysectomized rats (318).

The increase in tyrosine hydroxylase (319) and dopamine-β-hydroxylase activities (Molinoff, Brimijoin and Axelrod, unpublished) following reserpine administration can be prevented by cycloheximide. Further, as measured by immunoabsorption, the rate of synthesis of dopamine-β-hydroxylase is 3–4 times faster in the adrenals of reserpine-treated rats than in control glands (320). This suggests that the increased activity in vitro represents the synthesis of new enzyme molecules as opposed to the activation of preexisting enzyme.

Repeated daily immobilization stress in rats leads to an increase in the activity of tyrosine hydroxylase (321), dopamine-β-hydroxylase (322), and

PNMT. If the animals are hypophysectomized before the first immobilization, the activity of all three enzymes is decreased. Immobilization then results in an increase in tyrosine hydroxylase activity but has no effect on the activity of PNMT (323). The increased activity of tyrosine hydroxylase and dopamine-β-hydroxylase in both hypophysectomized (323) and intact rats is abolished by adrenal denervation (322). It appears that the levels of these three synthetic enzymes are influenced by both neuronal and hormonal factors. The neuronal influence is predominant with tyrosine hydroxylase and the hormonal with phenylethanolamine-N-methyltransferase. Dopamine-β-hydroxylase seems to be intermediate between the two. Increased psychosocial stimulation of mice leads to a marked increase in the catecholamine content of the adrenal gland and in the activity of PNMT, MAO, and tyrosine hydroxylase (324). Mice raised in isolation had decreased PNMT and tyrosine hydroxylase activities though the concentration of catecholamines was not significantly decreased.

It has been suggested that hydroxylation of tyrosine is the rate-limiting step in catecholamine biosynthesis (325). This hypothesis is based on the relative rates of conversion of tyrosine, dopa, and dopamine to noradrenaline in vitro in the guinea pig heart, and on the fact that the concentration of tyrosine is well above the K_m of tryosine hydroxylase for tyrosine (44). It is probably true that the maximum rate of catecholamine synthesis is limited by the capacity of tyrosine hydroxylase to convert tyrosine to dopa, but it is still an oversimplification to consider tyrosine hydroxylase as the only controlling step in catecholamine synthesis. Not all of the steps in the conversion of tyrosine to adrenaline take place in the same compartment but rather some of the enzymes are found in the axoplasm of the neuron while β hydroxylation takes place in the granules. Further, the pathway of catecholamine synthesis is branched with a large fraction of the dopamine produced in the neuron being rapidly metabolized by monoamine oxidase, present in large amounts in the neuron. Dopamine and its metabolites in rats and in humans constitute about one third of the total catecholamine excretion (132, 221). A competition exists in which dopamine either can be deaminated or can be taken up into the granule and converted into noradrenaline. Either uptake into the granule or β hydroxylation must therefore be a second site at which control of catecholamine synthesis occurs (326). Finally, pharmacologically induced increases in nerve activity (96, 313–314), various types of stress (321–322), and psychosocial stimulation (324) all lead to an increase in the activity of several of the enzymes involved in catecholamine synthesis. This supports the hypothesis that several sites are involved in the regulation of catecholamine synthesis.

Cyclic adenosine 3′,5′-monophosphate has been implicated in many of the effects of catecholamines, whether acting as hormones or as neurotransmitters (18, 254). This is so in adipose tissue where catecholamines lead to increased lipolysis (327), and in several tissues where glycogenolysis is regulated by a mechanism which involves catecholamine stimulation of adenylcy-

clase (328). A similar mechanism leads as well to increased secretion of amylase from the parotid gland (329) and insulin from the pancreas (330). Experiments with the phrenic nerve-rat diaphragm preparation have suggested that the effect of adrenaline in promoting neuromuscular transmission is also mediated by cyclic adenosine 3′,5′-monophosphate (331). For recent reviews of this subject see (18, 254).

Many effects of catecholamines, whether or not exerted through adenylcyclase, involve the activation of specific enzymes. Most of these effects are examples of hormone action on enzyme activation, but several systems are now being described in which neurally released noradrenaline functions to increase enzyme activity. In the case of the control of lipolysis in adipose tissue it appears that neurally released noradrenaline can lead to the activation of triglyceride lipase by activating adenyl cyclase (332). Another example of a neural regulation exerted through catecholamines comes from work with tyrosine transaminase of liver, which is subject to regulation by a number of hormones and amino acids (333). Noradrenaline released from adrenergic neurons suppresses the synthesis of this enzyme, apparently by forming a complex with the pyridoxal 5′-phosphate cofactor (334). In addition to suppressing the daily rhythm of tyrosine transaminase it may lead under appropriate conditions to an induction of the enzyme (335).

Another example of neural regulation by noradrenaline stems from studies on melatonin synthesis in the pineal. Under normal lighting conditions the rat pineal gland exhibits a diurnal variation in its content of the amines serotonin and noradrenaline and the enzyme hydroxyindole-O-methyltransferase (336). The response of this enzyme to environmental light is initiated in the retina and is transmitted to the pineal by way of the central nervous system and the cervical sympathetic nerves (337).

Studies using pineal glands maintained in organ culture show that noradrenaline causes a marked increase in the conversion of tryptophan to melatonin (338). This stimulation depends on synthesis of new protein. One of the enzymes in the biosynthetic pathway leading to melatonin is N-acetyltransferase (339). The addition of noradrenaline or dibutyryladenosine 3′,5′-cyclic phosphate to pineal organ culture causes a tenfold increase in the activity of N-acetyltransferase (340). This increase is blocked by inhibitors of protein synthesis.

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