REGULATION OF NOREPINEPHRINE BIOSYNTHESIS[1]

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INTRODUCTION

In addition to its ability to release norepinephrine consequent to stimulation, the adrenergic neuron can carry out a variety of functions related to the metabolism of its neurotransmitter. Both the adrenal medulla chromaffin cells (1–3) and the adrenergic neuron (3–5) store norepinephrine within vesicles which exhibit a characteristic dense core as seen in electron micrographs which have been appropriately fixed prior to examination (6–14). Studies on isolated chromaffin granules and adrenergic vesicles have shown that the storage of catecholamines is associated with an active uptake process from the adjacent cytoplasm (15–17). This uptake process appears to require ATP and Mg^{++} and is selectively inhibited by catecholamine depleting agents of the reserpine type (15, 16). In addition to the uptake process into isolated vesicles, the neuron concentrates norepinephrine across the axonal membrane (18). This process is not inhibited by reserpine (19), but may be selectively blocked by other agents, among which are cocaine, imipramine, and desmethylimipramine (20–23). The uptake of the catecholamines and other phenylethylamines across the axonal membrane is a very rapid and efficient process. It is generally believed that this uptake is responsible for the termination of the biological actions of either released or administered catecholamines. Blockade of this uptake process leads to the potentiation of the actions of norepinephrine and epinephrine (24–26).

Norepinephrine is stored within chromaffin granules or adrenergic vesicles largely in a bound form (27–30). The catecholamines appear to interact with ATP (31–34), resulting in the formation of a tetracatecholamine-ATP complex (35). This salt complex presumably is further bound to soluble proteins, the chromogranins, within the storage particle, although there is no direct evidence for the formation of a quantitatively significant complex (30, 36–38). Nevertheless, the ability of labelled catecholamines to enter the storage particles without exchanging with the bulk of the endogenous stores

[1] Abbreviations used in this review are: dopa, (L-3,4-dihydroxyphenylalanine): dopamine, (3,4-dihydroxyphenylethylamine).

of catecholamines at 0°C suggests that the endogenous amines are combined with macromolecular components within the granules in a stable complex (27, 29).

Monoamine oxidase, an enzyme which appears to be localized in mitochondria (39, 40), is present within the adrenergic neuron (41, 42). The evidence that monoamine oxidase is localized in particles which sediment with microsomes, perhaps in synaptic vesicles (43, 44), is not conclusive, since the possibility that the enzyme is present in fragments of outer mitochondrial membrane which have been removed during homogenization, has not been excluded (40, 45). This enzyme, which catalyzes the oxidative deamination of a variety of catecholamines, is presumably responsible for the catabolism of free intraneuronal norepinephrine. Intraneuronal deamination of norepinephrine and other catecholamines readily occurs if uptake into and storage in the chromaffin granules or synaptic vesicles are blocked (46, 47).

Catechol-O-methyltransferase, the second enzyme responsible for the ultimate degradation of catecholamines, is apparently not important in metabolizing intraneuronal norepinephrine, but is more significantly involved in the catabolism of circulating catecholamines, chiefly in the liver and kidney (48). Because of its wide distribution, it is not certain whether this enzyme is present in the nerve ending and, if so, whether it is highly active in this structure. Recent evidence by L. L. Iversen and B. Jarrott (personal communication) suggests that catechol-O-methyltransferase is present in the adrenergic neuron. Its kinetic constants, however, differ somewhat from those of the extraneuronal enzyme. The affinity of norepinephrine to the intraneuronal enzyme is less than its affinity to the extraneuronal enzyme.

Under varying physiological conditions, the norepinephrine content of sympathetic tissues does not change. To a considerable extent, this is because of the conservation mechanism for norepinephrine, namely reuptake of released norepinephrine across the neuronal membrane and reincorporation of the amine into the storage vesicles. However, if norepinephrine synthesis is blocked, tissue levels of this amine fall rather rapidly (49). The rate of fall of the tissue levels of norepinephrine after inhibition of synthesis varies from organ to organ and among species. The rate of decline is also dependent on the physiological activity of the adrenergic neurons of the tissue. In hibernating animals, synthesis and turnover of norepinephrine are severely reduced (50). Conversely, stress will increase the turnover of norepinephrine rather markedly in several peripheral tissues (49, 51, 52). In man, physical exercise is associated with increased excretion of norepinephrine and norepinephrine metabolites, suggesting that increased turnover of norepinephrine occurs in the human during increased sympathetic activity (53). Unless inhibitors of norepinephrine synthesis are administered concomitantly, tissue norepinephrine levels are not drastically altered during periods of stress wherein adrenergic nervous activity is increased (52).

Since the turnover of norepinephrine varies with the activity of the adrenergic nervous system, and since the levels of norepinephrine within tis-

sues remain constant unless synthesis is inhibited, it must be concluded that the rate of synthesis of norepinephrine in normal tissue is variable and is responsive to changes in sympathetic nervous activity. The purpose of this review is to consider the pathway of biosynthesis of norepinephrine and to discuss the mechanisms by which synthesis is influenced during altered sympathetic nervous activity. Many studies indicate that norepinephrine synthesis is subject to several regulatory influences and that a variety of pharmacological inputs which affect adrenergic physiology can indirectly affect norepinephrine synthesis. The mechanisms by which norepinephrine synthesis is modified by these various pharmacological and physiological inputs will be discussed.

The Biosynthesis of Norepinephrine

Although norepinephrine can be synthesized from phenylalanine *in vivo* (54), this pathway is probably insignificant, since tyrosine from dietary sources is very abundant in animal tissues. Therefore, the first significant enzymatically catalyzed step in the synthesis of this catecholamine is the hy-

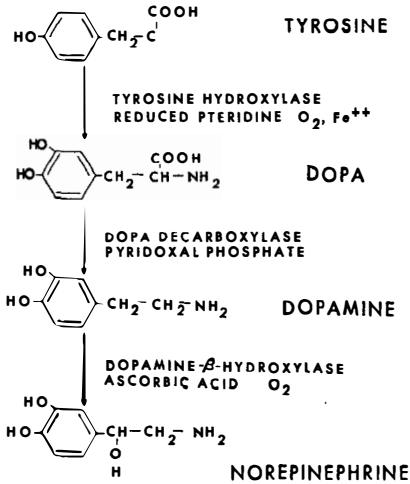

FIG. 1. Biosynthesis of norepinephrine from tyrosine.

droxylation of tyrosine to form dopa (Figure 1). Tyrosine hydroxylase, the enzyme which catalyzes this reaction, is present in the adrenal medulla (55), within adrenergic nervous tissue (56), and in brain (57–59). Chronic denervation of adrenergically innervated tissue is associated with complete disappearance of this enzyme, suggesting that the enzyme is exclusively localized within adrenergic nerves (60). The intracellular localization of the enzyme is not altogether clear. Musacchio has found that the enzyme is present in the soluble fraction of adrenal medulla tissue (61, 62). Petrack et

al. (63), on the other hand, have demonstrated that a large fraction of tyrosine hydroxylase activity can be recovered in the particulate fraction of adrenal homogenates, and the enzyme can be solubilized by treatment of the tissue with trypsin. In homogenates of sympathetic ganglia (64) and of bovine splenic nerves (65), the bulk of the enzyme is present in the supernatant fraction. In brain homogenates a significant fraction of the enzyme is associated with the particulate fraction of this tissue, although much or all of the enzyme found in the particulate fraction may be present in a soluble form in axoplasm which is entrapped within synaptosomal membranes (55, 58, 66). In peripheral adrenergic tissues, tyrosine hydroxylase does not appear to be localized within the storage vesicles in which the catecholamines are concentrated (62, 67). Although the results are by no means conclusive, there is some indication that two types of tyrosine hydroxylase may exist which demonstrate different kinetic properties and may have different subcellular distributions (68, 69).

Tyrosine hydroxylase is a mixed function oxidase which requires oxygen and a reduced pteridine cofactor for activity (55, 68, 70). Iron also appears to be required as a cofactor in the enzymatic hydroxylation of tyrosine to dopa (55, 63, 68). The pteridine cofactor has not been completely defined, but it appears to be chemically similar to or identical with biopterin (71), the cofactor which is also required for activity of liver phenylalanine hydroxylase (72, 73). The reduced pteridine combines with the inactive, oxidized form of the enzyme, converting the latter to the active, reduced form. In turn, the pteridine is oxidized. It must then be enzymatically reduced before it can again function to regenerate the active form of the enzyme (68). Since a pteridine cofactor is required for the aromatic hydroxylation of tyrosine, the quantity of this pteridine cofactor in adrenergic nervous tissues, its rate of formation, and the regeneration of the reduced form of the pteridine are factors which may be critical determinants of the rate of hydroxylation of tyrosine. Musacchio has demonstrated that a dihydropteridine reductase is present in adrenal medulla tissue (74). Much more information is required before the roles of the cofactor and its enzymes of synthesis and regeneration in the regulation of neurotransmitter synthesis are defined.

The decarboxylation of dopa to dopamine is catalyzed by a relatively nonspecific aromatic amino acid decarboxylase (75). This enzyme requires pyridoxal phosphate for activity. The enzyme appears to be localized in the cytoplasm of brain, adrenergic neurons, and the adrenal chromaffin cell, although there is some indication that at least a fraction of the dopa decarboxylase in the brain is particulate (76). It is possible, however, that this particulate fraction of the enzyme is in reality present in a soluble form within the pinched off nerve endings (synaptosomes) (66, 67).

Dopamine-β-hydroxylase, which catalyzes the hydroxylation of dopamine to norepinephrine, like tyrosine hydroxylase, is a mixed function oxidase (78–80). The enzyme appears to contain copper, and its activity is inhib-

ited markedly by sulfhydryl reagents, such as disulfiram. The activity of dopamine-β-hydroxylase is dependent upon the presence of molecular oxygen and a reducing agent as cosubstrate. Ascorbic acid is a highly effective cosubstrate in the hydroxylation reaction catalyzed by this enzyme. The abundance of ascorbic acid in tissues would indicate that it is the natural cosubstrate in this mixed function oxidase reaction. Dopamine-β-hydroxylase appears to be localized exclusively in the storage vesicles of adrenergic nervous tissue (65, 67, 81). A portion of the enzyme is relatively easily eluted from storage vesicles, but a significant fraction of the enzyme appears to be rather tightly bound to the vesicles (82). During the process of secretion, a portion of the enzyme, presumably the soluble fraction, is discharged along with the entire soluble contents of the chromaffin granule (30, 83–85).

The formation of norepinephrine from tyrosine thus involves three enzyme catalyzed chemical reactions (Figure 1). This biosynthetic sequence is complicated by the apparent localization of the enzymes in different regions of the cell. Thus, tyrosine must be taken up from the extracellular fluid into the adrenergic neuron prior to aromatic hydroxylation to dopa. Because the adrenergic nerves comprise such a small fraction of the adrenergically innervated tissues which have been studied, the nature and the kinetics of tyrosine uptake into neurons are virtually unknown. The aromatic hydroxylation of tyrosine occurs within the adrenergic neuron. The exact intracellular site of this reaction is unknown, but it apparently does not occur in the storage vesicle. The dopa which is formed is decarboxylated within the axoplasm of the neuron (67, 77, 86), and the product, dopamine, must be taken up into the storage vesicle before it can be converted to norepinephrine by the enzyme dopamine-β-hydroxylase, which is located within this particle (81, 87). The newly synthesized norepinephrine is probably present in an unbound, labile form within the storage vesicle for at least a brief period of time (88). If it does not escape from the storage vesicle either by spontaneous leakage, or as a consequence of nerve stimulation, or as a result of the action of some drug, it is ultimately incorporated into the storage complex of the vesicle. The biosynthetic pathway within the adrenergic neuron is complicated even further by the presence of an enzyme which is able to degrade both norepinephrine and its immediate precursor, dopamine. Thus, prior to uptake into the storage vesicle the dopamine may be oxidatively deaminated to dihydroxyphenylacetic acid. Inhibitors of the vesicle uptake mechanism, such as reserpine, can in this manner markedly enhance the rate of oxidative deamination of dopamine (15, 47, 89). Norepinephrine, which may either leak or be discharged from the storage vesicle into the axoplasm, may also be deaminated by monoamine oxidase, and reserpine, by preventing the reuptake of norepinephrine into the storage vesicle, will facilitate the degradation of this amine (47, 90, 91). Thus, the net and total formation of norepinephrine is dependent not simply on the amounts and activities of the biosynthetic enzymes. It is also dependent on the kinetics of the uptake process of catecholamines into the storage vesicles, on the rate of release of

catecholamines from the vesicle, on the rate of uptake of these substances into the mitochondria where the monoamine oxidase can degrade the amine,

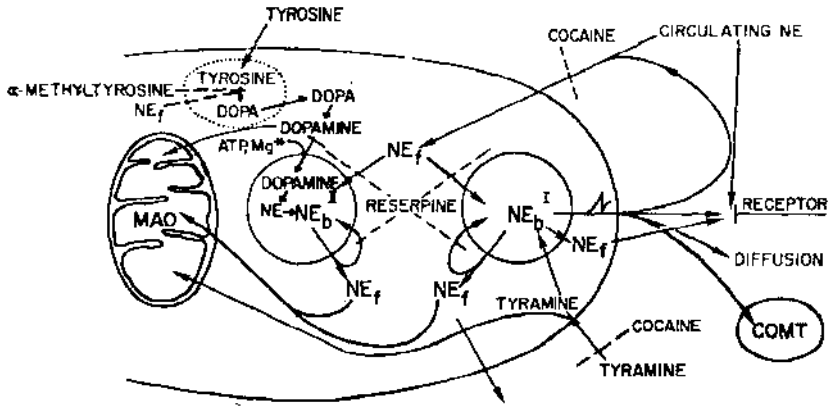

FIG. 2. Diagram of the adrenergic nerve terminal depicting norepinephrine metabolism and the effects of cocaine, reserpine, and tyramine on nerve terminal function. NE, norepinephrine; NE_f, norepinephrine which is not particle-bound; NE_b, norepinephrine bound in a storage complex; DOPA, 3,4-dihydroxyphenylalanine; DOPAMINE, 3,4-dihydroxyphenylethylamine; MAO, monoamine oxidase (in mitochondrion); COMT, catechol-O-methyl transferase; I, storage particle close to synaptic cleft whose bound norepinephrine is most susceptible to release by tyramine and other indirect sympathomimetic amines; II, more deeply located synaptic vesicle, relatively inaccessible to tyramine; ---= inhibition. Cocaine blocks axonal membrane uptake of tyramine and norepinephrine; reserpine blocks the Mg^{++}- and ATP-dependent uptake of dopamine and norepinephrine into the storage vesicle; and α-methyl-p-tyrosine and free norepinephrine inhibit the conversion of tyrosine to dopa.

and on the neuronal release and reuptake of both norepinephrine and dopamine (Figure 2). Many studies indicate that the rate of norepinephrine synthesis is critically dependent on all of these factors. The relative importance of each may vary considerably, depending on the physiological or pharmacological stress applied to the system.

THE REGULATION OF NOREPINEPHRINE BIOSYNTHESIS

In their extensive studies on the tyrosine hydroxylase enzyme of beef adrenal medulla, Udenfriend and coworkers demonstrated that catecholamines and other catechols are able to inhibit the activity of tyrosine hydroxylase (68, 92, 93). Although Ikeda et al. (68), present evidence that this inhibition represents competition between the catechol and the pteridine cofactor for the oxidized form of the enzyme, a direct chemical oxidation of the pteridine to an inactive form; e.g., by the *o*-quinone form of the catechol, has not been excluded. The kinetics of the inhibition would, at least

superficially, be similar (94). Approximately 50 percent inhibition of tyrosine hydroxylase activity was obtained with 1×10^{-3}M norepinephrine, when a similar concentration of synthetic pteridine cofactor was employed in the assay (92). It is quite likely that much smaller amounts of catecholamine in the axoplasm of the neuron would severely inhibit tyrosine hydroxylase activity, since the concentration of the cofactor within the neuron may be considerably less than 1×10^{-3}M. Thus, that fraction of the norepinephrine in the adrenergic neuron which is able to antagonize the action of the reduced pteridine cofactor either by a direct chemical reaction or by competitive binding to tyrosine hydroxylase may profoundly influence the activity of the enzyme, which, according to Udenfriend, is the rate limiting step in the biosynthesis of norepinephrine (95).

Increased adrenergic nervous activity is associated with enhanced synthesis of norepinephrine from tyrosine both *in vivo* (51, 52, 96, 97) and *in vitro* (98–100). In the isolated hypogastric nerve-vas deferens preparation of the guinea-pig, the enhanced synthesis of norepinephrine from tyrosine associated with nerve stimulation can be partially or completely prevented by the addition of norepinephrine to the bath (98, 100). Furthermore, norepinephrine is able to inhibit markedly its own synthesis from tyrosine in this preparation. Since the total amount of norepinephrine in the stimulated preparation is not different from that in the contralateral control, it is presumed that a small, chemically undetectable, compartment of norepinephrine is critical in the regulation of norepinephrine synthesis (98). Presumably this small compartment is depleted during nerve stimulation. The small compartment which appears to play a critical regulatory role in the activity of tyrosine hydroxylase is presumed to be free intraneuronal norepinephrine. Drugs which are known to block the storage or accelerate the release of norepinephrine within the axon are able to inhibit tyrosine hydroxylase by this indirect mechanism (see below). The synthesis of norepinephrine from dopa is not influenced by nerve stimulation, suggesting that the enzyme in the biosynthesis of norepinephrine which is enhanced by nerve stimulation is that catalyzing the first step in the reaction, tyrosine hydroxylase (100).

In addition to this end-product feedback regulation of tyrosine hydroxylase, other mechanisms appear to exist for the regulation of the biosynthesis of this transmitter. In the hypogastric nerve-vas deferens preparation of the guinea-pig, enhanced norepinephrine synthesis occurs during the period immediately following a program of stimulation of one hour duration (101). Unlike the enhanced synthesis which occurs during nerve stimulation, the post-stimulation increase in norepinephrine synthesis is not abolished by norepinephrine. The enhanced synthesis in the post-stimulation period is inhibited by puromycin, an inhibitor of protein synthesis. However, no increase in tyrosine hydroxylase enzyme has been demonstrated during the period immediately following stimulation (N. B. Thoa, I. J. Kopin, N. Weiner, unpublished). The mechanism for this enhanced synthesis of norepinephrine during the post-stimulation period, which is puromycin sensitive, is

unclear, but may be related to synthesis of another protein required in the hydroxylation of tyrosine; for example, pteridine reductase.

Enhanced adrenergic nervous activity for more prolonged periods is associated with an increase in the content of tyrosine hydroxylase within the tissue (64, 102–104). A wide variety of pharmacological agents which, either directly or indirectly, bring about enhanced frequency of adrenergic nerve stimulation, can produce this increase in tyrosine hydroxylase activity in the adrenal medulla and in adrenergic tissues. The time required to produce this increase appears to vary with the stimulus employed. Insulin hypoglycemia is associated with an increase in tyrosine hydroxylase activity in the adrenal gland within 12 hours (105). The administration of either reserpine or phenoxybenzamine, each of which reflexly increases adrenergic nervous activity as a result of diminished adrenergic tone, is associated with increases in the enzyme only after 24–48 hours (103, 106). Since this increase in tyrosine hydroxylase content can be blocked at least partially by inhibitors of protein synthesis, it is presumed that a chronic increase in adrenergic nervous activity in some manner leads to the induction of tyrosine hydroxylase (105, 107). The mechanism by which this enzyme is induced is unknown.

Studies of Norepinephrine Turnover: The Concept of Multiple Norepinephrine Pools

A considerable number of investigations have been performed in an attempt to determine the turnover of norepinephrine in various tissues of the body. It is presumed that, at steady state conditions, estimates of turnover of norepinephrine will yield information regarding the rate of synthesis of the catecholamine (108). Methods to determine turnover have included: (*a*) Administration of ^{3}H-norepinephrine to an animal and the subsequent determination of the rate of decay of ^{3}H-norepinephrine in the tissues. In these experiments, it is presumed that the labelled norepinephrine equilibrates with the endogenous norepinephrine, and that the rate of decline of labelled norepinephrine accurately reflects the rate of decline of the endogenous catecholamine (109). (*b*) Studies of the rate of decline of tissue norepinephrine after blockade of synthesis, for example, with α-methyl-p-tyrosine. For these experiments to be valid, it must be presumed that the administration of the α-methyl-p-tyrosine and the blockade of norepinephrine synthesis do not influence the normal rate of turnover of the endogenous catecholamine (110). (*c*) Estimates of the rate of synthesis of norepinephrine from labelled tyrosine when the precursor is continuously infused into the animal or is perfused through an isolated tissue (111). For these experiments to be valid, it must be assumed that the norepinephrine formed from tyrosine is turning over at a rate identical to that of the endogenous stores. In addition, all of these procedures to estimate norepinephrine synthesis and turnover assume that the norepinephrine being formed rapidly equilibrates with the bulk of the norepinephrine pool. If true, these procedures may accurately

reflect the turnover of norepinephrine and therefore, at steady state, its rate of biosynthesis. However, considerable evidence suggests that there are at least two, and perhaps several, norepinephrine pools in the body and that the turnovers of these pools of norepinephrine differ considerably. The use of radioactive precursors, such as ^{14}C-tyrosine or ^{3}H-dopa, to label the endogenous stores and subsequently follow the rate of decline of labelled catecholamine in various tissues, enables one to examine the turnover of only the quantitatively significant catecholamine pools (112). Burack & Draskóczy observed that the decay curves of ^{3}H-norepinephrine formed from ^{3}H-dopa were not linear with time, suggesting that more than one pool of norepinephrine exists (113). Sedvall et al. (114) and Kopin et al. (115) have shown that newly synthesized norepinephrine is preferentially discharged from the isolated perfused spleen on stimulation of the splenic nerve. Numerous studies using indirectly acting amines have demonstrated that all of the norepinephrine present in adrenergically innervated tissues is not equally susceptible to release by these agents (116–120). It is not known whether these pools are in compartments which are structurally different or whether they reside in identical storage particles which differ only in relation to their distance from the nerve terminal membrane or synapse (121). In either event, if these pools turn over at differing rates, the overall estimates of turnover will not reflect accurately the synthesis rates in either pool. It will more closely indicate the turnover of the larger, more stable pool. It appears likely that the pool of norepinephrine which is proximal to the synaptic cleft and either in or adjacent to the axonal membrane is preferentially released. If it is presumed that these sites are also capable of synthesizing norepinephrine, it seems likely that turnover of norepinephrine will be highest in the region adjacent to the synaptic cleft. The major pools of norepinephrine, located more deeply within the adrenergic neuronal structures, will turn over more slowly, and perhaps provide a storage pool or a reserve pool of neurotransmitter which is utilized at variable rates, depending upon the demands of the neuron. Studies of turnover, where the major pool of norepinephrine is being examined, as in the methods mentioned previously, will give an indication of the minimal rate of turnover of the catecholamine stores. In fact, turnover of a smaller pool may be very much more rapid. This small, metabolically labile pool under normal conditions may be the more significant pool in the physiology of the adrenergic neuron.

The Origin of the Synaptic Vesicles and the Enzymes Involved in Synthesis of Norepinephrine

It has long been assumed that the constituents of axons and nerve terminals are to a large extent synthesized in the cell body of the neuron and transported down the axon by proximo-distal axoplasmic flow (122–124). Recent studies indicate that the rate of somatofugal transport of different neuronal constituents by axoplasmic flow may differ considerably. Livett et

al. (125) have shown that labelled norepinephrine is transported down adrenergic neurons at approximately 5 mm per hour and a fraction of the neuronal protein is transported at a similar rate. A second protein component migrates down the nerve at a much slower rate, approximately 1 mm per day. Dahlström (126) and Dahlström & Häggendal (127), on the basis of nerve ligation studies, have suggested that granular vesicles migrate down adrenergic neurons at a rate similar to the rate of migration of norepinephrine reported by Livett et al. Based upon this migration rate, these workers estimate that the life span of adrenergic synaptic vesicles in the sciatic nerve is approximately 25 days for the rat and 70 days for the cat. Laduron & Belpaire have made similar estimations of axoplasmic flow of synaptic vesicles, based upon the accumulation of dopamine-β-hydroxylase proximal to a nerve ligation. They have determined the rate of transport of the enzyme to be 3 mm/hr, approximately twice as fast as that of norepinephrine. They suggest that the apparently slower rate of flow of norepinephrine is due to some degradation of the amine (128). A protein which is immunologically cross-reactive with the soluble protein of adrenal chromaffin granules has been detected in sympathetic nerves (129). This cross-reactive protein, presumably in synaptic vesicles, also accumulates proximal to the stricture after ligation of a sympathetic nerve (130, 131).

Mayor & Kapeller, using similar techniques of nerve ligation, have observed an accumulation of electron-dense vesicles proximal to the constriction. Presumably these are norepinephrine-containing vesicles accumulating at the site of the constriction (132). However, Pellegrino de Iraldi & De Robertis have recently presented evidence to suggest that the vesicles formed proximal to a nerve ligation may arise from the disintegration of neurotubules (133). It is conceivable that vesicles normally arise from neurotubules, perhaps at the more distal portions of the processes of various neurons. Damage to the neuron, for example by ligation at any site along its length, could lead to the production of vesicles as the neurotubules degenerate. Geffen & Ostberg, on the other hand, have provided suggestive evidence that larger dense core vesicles may migrate down the axon and ultimately may be transformed into the typical, small, dense-core synaptic vesicles (134).

The estimated half-life of adrenergic synaptic vesicles (35–100 days) is considerably longer than the estimated turnover rate of norepinephrine in adrenergic neurons of most peripheral tissues (less than 24 hr). This discrepancy might indicate that the synaptic vesicles are able to discharge their catecholamine contents while remaining sufficiently intact so that they can continue to synthesize catecholamines for subsequent discharge on stimulation. Alternatively, synaptic vesicles may continuously and spontaneously leak catecholamines into the axoplasm and continuously replenish their complement of catecholamines by reuptake and synthesis. In view of the demonstrated leakiness of isolated synaptic vesicles and their ability to synthesize norepinephrine from dopamine, it would appear that the latter explanation is

highly plausible. Recent evidence suggests that the chromogranins and dopamine-β-hydroxylase present in adrenergic vesicles are secreted during release of catecholamines (84), in a manner analogous to the secretion of these substances from isolated adrenal glands (83, 135–138). This would suggest that the process of exocytosis occurs during discharge of catecholamines from adrenergic nerve terminals, and would thus be analogous to the concept of neurosecretion which is believed to occur at cholinergic nerve terminals (139).

The Effect of Various Drugs on the Biosynthesis of Catecholamines

Tyramine.—Tyramine is a sympathomimetic amine which is presumed to act indirectly by release of norepinephrine from the nerve ending (140). After reserpine treatment and depletion of tissue norepinephrine, tyramine is unable to exert a sympathomimetic effect (141, 142). Perfusion of the tissue with norepinephrine in small amounts leads to a restoration of tyramine sensitivity, although no significant increase in the norepinephrine content of the tissue is detectable. When isolated tissues are repeatedly exposed to tyramine, tachyphylaxis to tyramine ensues. At a time when the tissue is tachyphylactic to tyramine, the norepinephrine stores are very little if at all depleted (143–145). Axelrod and coworkers have shown that ^{3}H-norepinephrine taken up by the isolated heart is released by tyramine, and the amount of labelled norepinephrine which is released is directly related to the sympathomimetic effect. After several administrations of tyramine, however, little ^{3}H-norepinephrine is released by subsequent injections. At this time, a considerable amount of labelled norepinephrine is still present in the heart (143). Chidsey & Harrison (119) have shown that labelled norepinephrine which has been taken up most recently is most susceptible to release by tyramine. Several hours or longer after the administration of ^{3}H-norepinephrine, tyramine is much less able to release the labelled norepinephrine which remains in the tissue. These studies suggest that the bulk of norepinephrine in the tissues is refractory to the releasing action of this agent.

Tyramine is taken up into the tissues and is converted to octopamine by dopamine-β-hydroxylase (145–147). Since this enzyme is in the adrenergic vesicles it implies that tyramine can be taken up into the vesicles (146). Octopamine is retained in the storage vesicles for considerably longer periods than is its precursor, which lacks a β-hydroxyl group (145, 146).

Although *in vitro* studies indicate that tyramine is able to release from tissues only a small fraction of the norepinephrine pool (145), either a large single injection (148) or multiple large injections (149, 150) of tyramine into animals will cause a rather marked depletion of norepinephrine. These observations appear to be inconsistent with the current theories of tyramine tachyphylaxis and the concept that tyramine releases norepinephrine from only a small susceptible pool. Neff et al. have challenged the theory of tyramine tachyphylaxis and, on the basis of kinetic studies on the depletion of tissue norepinephrine, they have suggested that the entire norepinephrine

pool in the tissues is equally susceptible to release by tyramine (149). However, large doses of tyramine inhibit the synthesis of norepinephrine by two mechanisms: (*a*) releasing norepinephrine from storage vesicles resulting in increased free intraneuronal norepinephrine and consequent end-product feedback inhibition of tyrosine hydroxylase (151); (*b*) inhibition of dopamine-β-hydroxylase by competing with dopamine for both the uptake site into the storage vesicle and for the available dopamine-β-hydroxylase (151, 152). Inhibition of norepinephrine synthesis could explain at least in part the profound reduction of all the norepinephrine stores in heart and in other adrenergic tissues as a consequence of *in vivo* administration of this and other indirectly acting amines. It might be expected that any agent which releases norepinephrine from storage sites into the intraneuronal cytoplasm would exert an inhibitory effect on norepinephrine synthesis indirectly through end-product feedback inhibition (153).

Reserpine.—Reserpine is a potent depletor of the major stores of norepinephrine in adrenergic tissues (154, 155). It appears to exert this action primarily through blockade of uptake of catecholamines into the storage particles (15, 16, 156). The unbound catecholamine is therefore more readily available to oxidative deamination by intraneuronal monoamine oxidase (46, 47). In addition to blocking the uptake of norepinephrine into the storage vesicle, dopamine uptake, which apparently occurs by the same mechanism as that of norepinephrine, is also profoundly inhibited by reserpine (47, 89). As a consequence of this inhibition by reserpine of dopamine uptake into the site where the enzyme dopamine-β-hydroxylase is localized, the synthesis of norepinephrine from dopamine is impaired. This inhibition of norepinephrine synthesis from dopamine has been demonstrated both in adrenal slices and in the isolated heart. The inhibition of the synthesis of norepinephrine from dopamine is reflected in a reduced urinary excretion of vanilmandelic acid and an enhanced excretion of homovanillic acid in patients on chronic reserpine therapy (157). Similar results have been reported in rats treated with reserpine, where urinary excretion of 3-methoxy-4-hydroxyphenylglycol, the major norepinephrine metabolite in this species, is markedly reduced (158). In isolated atria from rabbits pretreated with reserpine, the conversion of dopamine to norepinephrine is markedly inhibited, the production of norepinephrine metabolites from dopamine is markedly reduced, and the formation of dopamine metabolites is increased (47).

Since reserpine blocks the uptake of catecholamines into the storage vesicles, the administration of this drug would be expected to be associated with an increase in the free intraneuronal norepinephrine pool. Increased end-product feedback inhibition of tyrosine hydroxylase would be expected and the formation of dopa from tyrosine should be reduced. Marked inhibition of the activity of tyrosine hydroxylase in intact tissue shortly after the administration of reserpine has been demonstrated (N. Weiner, in preparation). With more chronic treatment, however, the total norepinephrine pool is profoundly depleted, and end-product feedback inhibition of tyrosine hy-

droxylase diminishes. Furthermore, the impaired sympathetic nervous activity as a consequence of chronic administration of reserpine results in a compensatory increase of adrenergic nervous activity in an attempt to restore sympathetic tone. This chronic increase in sympathetic nervous activity after reserpine treatment leads to an increase in the content of tyrosine hydroxylase in adrenergic nervous tissue (64). Although this increase can be antagonized by inhibitors of protein synthesis or actinomycin D (107), it cannot be concluded with certainty that the mechanism for the increase in the enzyme activity involves increased synthesis of the enzyme. These potent protein and RNA synthesis inhibitors are highly toxic and nonspecific, and their effects may be related to actions on other systems; e.g., on preganglionic or central cholinergic neurons (159).

The increase in the content of tyrosine hydroxylase as a result of prolonged stimulation of the sympathetic nervous system can be produced by a wide variety of pharmacological and other stimuli. This effect can be antagonized either by the administration of ganglionic blocking agents or by preganglionic denervation (103, 105). After several days of reserpine treatment, the turnover of norepinephrine, as determined by either the decline of ^{3}H-norepinephrine in tissues or the decline of endogenous norepinephrine after α-methyl-p-tyrosine, is approximately twice the control rate (160). The increase in the levels of tyrosine hydroxylase which develops in chronically stimulated sympathetic nerves appears to be mediated by some effect of either acetylcholine or another substance liberated from preganglionic nerve terminals or by the events which occur as a consequence of enhanced frequency of depolarization of the cell bodies of adrenergic neurons.

Thus, reserpine, through the single action of inhibiting the uptake of catecholamines into synaptic vesicles, can produce a multiplicity of indirect actions. In addition to its well known depleting effect, reserpine blocks the synthesis of norepinephrine at two steps in the biosynthetic pathway and also initiates a series of events which leads to increased levels of tyrosine hydroxylase, the enzyme which catalyzes the initial reaction in the formation of the neurotransmitter.

Conclusion

The above discussion is intended to indicate the complex effects various physiological and pharmacological inputs may have on the metabolism of the adrenergic neurotransmitter, norepinephrine. Although the rate and regulation of norepinephrine synthesis has been emphasized, other aspects of the metabolism of norepinephrine (storage, release, catabolism) must be analyzed with the realization that analogous complex interactions are virtually inevitable. An appreciation of this may be acquired either in general reviews of this subject (161–166), or in reviews which focus on special features of adrenergic nervous system physiology; such as false transmitters (167), uptake processes (168), release mechanisms (169), and the storage of catecholamines (30, 170).

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