

# Biosynthesis and Metabolism of Catecholamines

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THERE are three 3,4-dihydroxy (catechol) derivatives of phenylethylamine found in mammalian tissue (fig. 1). The first of these catecholamines to be identified was N-methyl-3,4-dihydroxyphenylethanolamine (adrenalin, epinephrine), which was found to be identical with the pressor substance of adrenal medullary extracts.<sup>1,2</sup> The similarity of responses to sympathetic-nerve stimulation to administration of epinephrine led Elliott<sup>3</sup> in 1905 to suggest that this substance might be the stimulant liberated at the nerve endings. Release of a sympathomimetic agent during sympathetic nerve stimulation was first demonstrated over two generations ago. These investigations and the role of the sympathetic nervous system in physiology are the subject of a classic monograph by Cannon and Rosenbleuth.<sup>4</sup>

The results of a series of investigations indicated that the transmitter released from sympathetic nerve endings was not epinephrine. It is now generally accepted that norepinephrine (3,4-dihydroxyphenylethanolamine) is the true sympathetic neurotransmitter.<sup>5</sup> This catecholamine is also present in brain, where it is localized to specific neurons. There is a great deal of evidence which indicates that its biosynthesis, role as a neurochemical transmitter and metabolism in brain are similar to those in the peripheral sympathetic nervous system.<sup>6</sup>

The third catecholamine, dopamine (3,4-dihydroxyphenylethylamine), initially was thought to be important only as an intermediate in the synthesis of the other catecholamines. More recently, however, evidence which suggests that this compound is also a neurochemical transmitter in certain areas of the brain has accumulated.<sup>7</sup>

It is the purpose of this review to outline briefly current concepts of the control of catecholamine biosynthesis, storage and metabolism, to serve as a basis for understanding the physiology and pharmacology of neurons which eject these amines as neurochemical transmitters.

## Synthesis

Tyrosine is the precursor of all the catecholamines. Funk<sup>8</sup> suggested that 3,4-dihydroxyphenylalanine (dopa) is an intermediate in epinephrine biosynthesis (fig. 1); but tyrosine hydroxylase, the enzyme responsible for conversion of tyrosine to dopa, has been demonstrated only recently.<sup>9</sup> This reaction is a branch point in the metabolism of tyrosine and is rate-limiting in catecholamine biosynthesis. Inhibitors of tyrosine hydroxylase (e.g.,  $\alpha$ -methyl-tyrosine) effectively lower levels of tissue catecholamines<sup>10</sup> and diminish the amounts of transmitter released during nerve stimulation.<sup>11</sup>

During sympathetic nerve stimulation, there is an increase in conversion of tyrosine to dopa<sup>12,13</sup> but no change in tyrosine hydroxylase activity *in vitro*.<sup>14</sup> Control of dopa synthesis is thought to be mediated by alterations in free intraneuronal catecholamines<sup>15</sup> which limit the availability of the tetrahydropteridine cofactor of tyrosine hydroxylase.<sup>9</sup> This provides a means for feedback inhibition of synthesis by the product. During nerve stimulation, intraneuronal cytoplasmic levels of catecholamine decrease because they are transferred to sites from which norepinephrine is discharged. This is accompanied by an increase in available tetrahydropteridine cofactor, with consequent activation of tyrosine hydroxylase and increased dopa formation.

The decarboxylase which converts dopa to dopamine was the first of the enzymes involved in catecholamine biosynthesis to be

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demonstrated.<sup>16</sup> This enzyme is relatively nonspecific and is probably best considered an aromatic amino acid decarboxylase.<sup>17</sup> There appears to be an abundance of decarboxylating enzyme; dopa is converted to dopamine as rapidly as it is formed, and inhibition of this enzyme is not an efficient means of diminishing catecholamine biosynthesis. As indicated above, at first dopamine was considered to be unimportant except as an intermediate in norepinephrine and epinephrine synthesis. This catecholamine, however, is present in significant quantities in basal ganglia, and disorders in dopamine metabolism have been reported in Parkinsonism.<sup>7</sup> Experimentally, brain lesions or drugs which produce movement disorders have also been shown to cause alterations in dopamine formation or metabolism.

The role of dopamine as a central neurotransmitter has recently been reviewed.<sup>7</sup>

Only small amounts of dopamine are present in the adrenal medulla or sympathetically-innervated tissues. This amine is rapidly converted to norepinephrine by dopamine- $\beta$ -hydroxylase. This enzyme appears to be located in storage granules<sup>18</sup> which are responsible for intraneuronal binding of norepinephrine. Drugs such as reserpine which interfere with catecholamine storage in these structures also retard conversion of dopamine to norepinephrine.

In the adrenal medulla of mammals, phenylethanolamine N-methyltransferase catalyzes the conversion to epinephrine as well as other phenylethanolamines to their N-methyl analogues using S-adenosylmethionine as a methyl

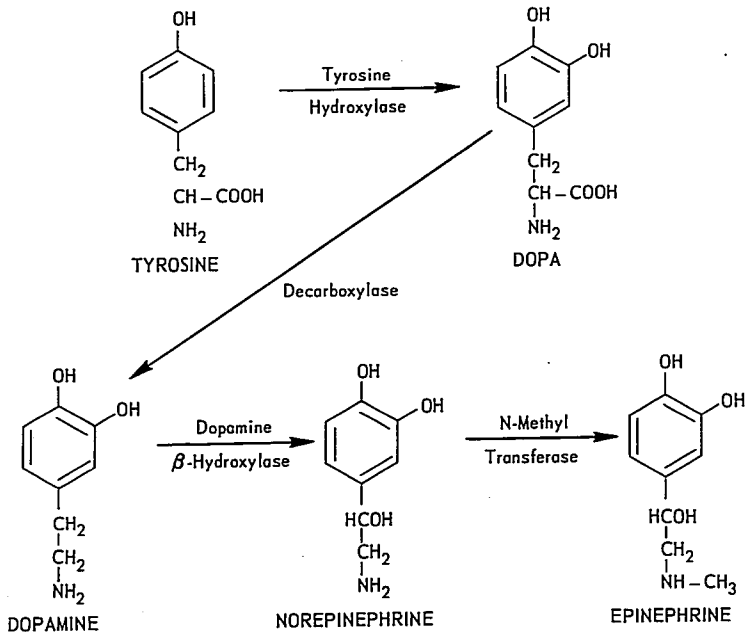


FIG. 1. Biosynthesis of catecholamines.

donor.<sup>19</sup> Levels of this enzyme appear to be influenced by adrenal cortical hormones,<sup>20</sup> and it has been suggested that part of the increased sensitivity to hypoglycemia in hypo-adrenal states is a result of an inability to produce epinephrine. In frogs and some other lower species, the N-methylating enzyme appears to be present in peripheral sympathetic nerves, and epinephrine is apparently the neurotransmitter.

### Storage and Release

In the adrenal medulla, catecholamine-containing granules contain a specific protein and one mol of adenosinetriphosphate (ATP) for every four mols of amine.<sup>21</sup> Uptake of catecholamines by these particles *in vitro* is stimulated by ATP and magnesium ions. Similar observations have been made using vesicles isolated from bovine splenic nerve or sympathetically-innervated tissues from a variety of species.<sup>22, 23</sup> Norepinephrine-H3 taken up into adrenergic neurons in brain or peripheral sympathetic nerves appears to be associated with dense-core synaptic vesicles seen in the electron microscope.<sup>24</sup> The results of such studies have led to the hypothesis that norepinephrine is stored in these vesicles as a complex with ATP, magnesium ion and a specific protein.

Release of catecholamines from the adrenal medulla is accompanied by release of both ATP<sup>25</sup> and the specific protein,<sup>26, 27</sup> in a ratio similar to that found in the granules. These findings suggest that the process of release involves extrusion of the contents of the vesicles. Since calcium ions are necessary for release of catecholamines from sympathetic nerves<sup>28</sup> as well as from the adrenal medulla,<sup>29</sup> this concept has been extended to include all adrenergic neurons. Calcium uptake is thought to be associated with the process of coupling excitation to release of the contents of the vesicles, but the mechanism of this process is unknown.

### Metabolism and Inactivation

Many amines, including the catecholamines, are substrates for monoamine oxidase (MAO), an enzyme which converts them to the corre-

sponding aldehydes.<sup>30</sup> This intermediate is rapidly metabolized, usually by oxidation to the acid but in some circumstances by reduction to the alcohol. The metabolic fate of the catecholamines, however, was largely unknown until Armstrong *et al.*<sup>31</sup> demonstrated that 3-methoxy-4-hydroxymandelic acid (vanillyl-mandelic acid, VMA) was the major urinary metabolite of norepinephrine. This compound could be formed by oxidative deamination and subsequent O-methylation of epinephrine as well as norepinephrine. The enzyme responsible for the latter reaction, catechol-O-methyl transferase (COMT), was found to transfer the methyl group of S-adenosylmethionine to a variety of catechols; catecholamines are excellent substrates.<sup>32</sup> Thus, catecholamines are subject to metabolic inactivation by deamination or O-methylation (Fig. 2). When catecholamines or their O-methylated derivatives are deaminated, an aldehyde intermediate is formed. In man, in most tissues, the aldehyde is further oxidized to the corresponding acid. If deamination precedes O-methylation, the catechol acid is O-methylated and the products (homovanillic acid from dopamine or VMA from the other catecholamines) are major urinary metabolites. In some species (*e.g.*, rat) and in the brain of man, however, the intermediate aldehyde derived from norepinephrine appears to be mainly reduced to the glycol. The 3-O-methyl derivative is conjugated with sulfate; in human spinal fluid, the sulfate of 3-methoxy-4-hydroxyphenylglycol is the major metabolite of norepinephrine. This compound is not a major urinary metabolite in man, representing only about 10 per cent of the excreted catecholamine products. It is not formed exclusively in brain, but may become an index of central nervous system norepinephrine metabolism in man.

The O-methylated amine derivatives of the catecholamines, 3-methoxydopamine, normetanephrine and metanephrine, are normally formed in the urine. As might be expected, these increase when MAO is inhibited. Similarly, the deaminated catechol products are excreted in relatively small amounts. The major urinary metabolites are both deaminated and O-methylated so that these provide little indication of which pathway, O-methylation or deamination, predominates as the means for

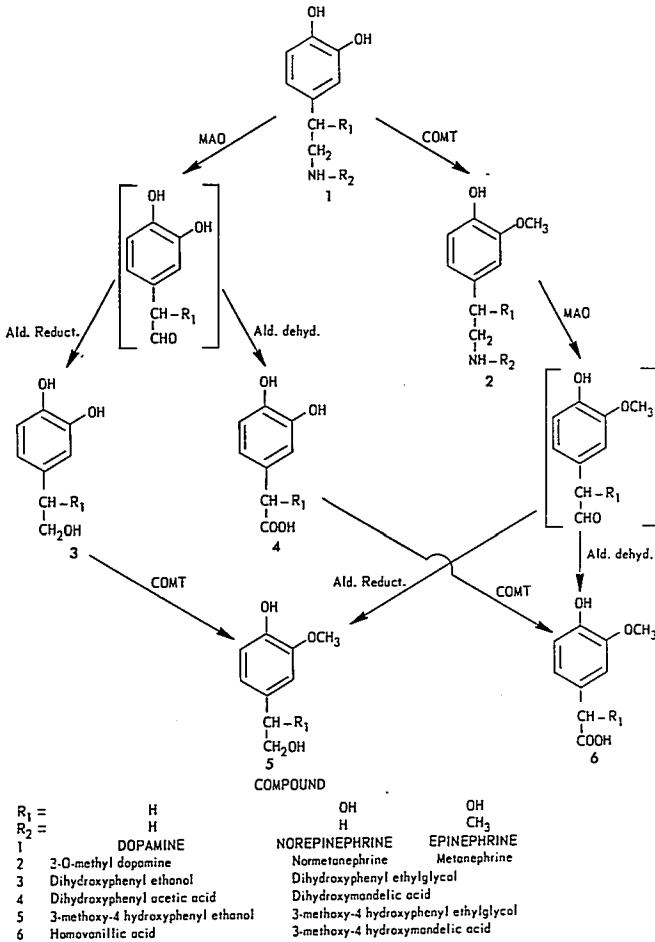


FIG. 2. Metabolic inactivation of catecholamines.

enzymatically inactivating the catecholamines. Axelrod, in his excellent review,<sup>33</sup> has summarized the evidence that O-methylation is the major route for metabolism of intravenously

administered catecholamines. Although COMT is ubiquitous, levels are highest in the liver and kidney. Since these organs receive a major fraction of the cardiac output and cate-

cholamines can be largely inactivated by COMT in a single passage through the liver, it is not surprising that O-methylation is important in inactivating circulating catecholamines. Most epinephrine is formed in the adrenal medulla and discharged into the circulation before being metabolized. The fate of intravenously administered epinephrine-H3 would be expected to approximate closely the fate of endogenous epinephrine. This assumption, however, may not be valid for norepinephrine, which is present in sympathetic nerves and in brain.

Demonstration of a predominant role for COMT in enzymatic inactivation of circulating catecholamines did not satisfactorily explain a number of other observations, particularly in relation to the effects of drugs which inhibit MAO. Inhibitors of MAO generally cause elevation of tissue levels of catecholamines, but COMT inhibitors do not. MAO inhibitors diminish the rate of reserpine-induced catecholamine depletion and reverse its sedative effects. Norepinephrine-H3 injected into the circulation is partially metabolized immediately, mostly by COMT; but a portion is taken up into sympathetic nerves and mixes with the endogenous catecholamine. This portion of injected norepinephrine is destroyed in the tissue, mostly by deamination. These observations suggest that MAO plays an important role in catecholamine metabolism.<sup>34</sup>

It is now generally believed that the route of metabolism is determined largely by the site of catecholamine release. MAO is a mitochondrial enzyme, present in the nerve ending, while COMT appears to be largely extraneuronal. Norepinephrine is protected from intraneuronal destruction by MAO since it is sequestered in the synaptic vesicles; but a portion reaches the cytoplasm and can be destroyed by this enzyme. When MAO is inhibited, destruction of cytoplasmic catecholamines (dopamine as well as norepinephrine) is prevented and stores of norepinephrine increase. The elevated levels of free intraneuronal catecholamines diminish catecholamine synthesis by decreasing tyrosine hydroxylase activity, as described above.

Interference with binding in the vesicles (*e.g.*, by reserpine) results in exposure of the catecholamines to destruction by MAO in the

neuron; little reaches the extraneuronal sites, and there is no great sympathetic response. The consequent lack of transmitter results in diminution of sympathetic responsiveness. When released extraneuronally by nerve stimulation or by drug action, norepinephrine reaches the receptor and a response is elicited. O-methylation, in the tissue or after entry into the circulation, is the principle route of metabolic inactivation of the extraneuronally liberated catecholamine.

Neither MAO nor COMT, however, seems to be important in terminating the activity of norepinephrine liberated at sympathetic nerve endings. Even simultaneous inhibition of both enzymes does not potentiate the effects of nerve stimulation greatly. In perfused organs such as the heart or spleen, uptake into sympathetic neurons is more important than metabolism in removing norepinephrine. Procedures which result in supersensitivity, such as administration of cocaine or chronic sympathetic denervation, are associated with a noticeable decrease in norepinephrine uptake.<sup>35</sup>

The process of uptake by transport across the neuronal membrane is distinct from the process of storage in synaptic vesicles. Drugs which interfere with one need have little or no effect upon the other. Reserpine, which interferes with norepinephrine storage, has little effect on uptake. Cocaine, and desmethyl-imipramine, for example, which interfere with uptake, do not influence tissue levels of the catecholamine. Inhibition of storage, unlike inhibition of uptake, is not associated with striking supersensitivity to norepinephrine.

#### False Adrenergic Transmitters

The various processes involved in the synthesis, storage, release and transport of norepinephrine are not specific. Phenylethylamine derivatives can compete with norepinephrine for uptake<sup>36</sup> into the neuron and can thereby potentiate the catecholamine. Neither dopamine- $\beta$ -hydroxylase nor the storage mechanism is specific. Various phenylethylamines are substrates for the enzyme,<sup>37</sup> and the  $\beta$ -hydroxylated products can replace norepinephrine at its storage sites.<sup>38</sup> Such compounds can be released by nerve stimulation and partially replace the physiologic transmitter. They

are relatively impotent, false transmitters of adrenergic impulses, and interfere with synaptic efficacy.

There are several ways in which false transmitters can be made to accumulate.<sup>39</sup> A drug may inhibit the enzyme that normally limits the population of the false transmitter and its precursor. Tyramine and its  $\beta$ -hydroxylated derivative, octopamine, are destroyed readily by MAO. Ordinarily, little octopamine is formed in the body because tyramine is destroyed by MAO as rapidly as it is formed and little of this amine comes into the presence of dopamine- $\beta$ -hydroxylase. Animals in which MAO is inhibited, however, show conspicuously elevated levels of octopamine in various peripheral tissues as well as brain. Since chronic sympathetic denervation prevents this accumulation, octopamine is presumed to be present in sympathetic neurons. Studies of subcellular distribution indicate that the amine is stored in vesicles similar to those which store norepinephrine. Octopamine is released by nerve stimulation, replacing a portion of the norepinephrine, but is a relatively impotent "false adrenergic transmitter."

The normal check on amine population may be upset by administration of quantities that overcome the enzyme's capacity. Tyramine, given in large, repeated doses even without MAO inhibition, results in octopamine accumulation. Initially, norepinephrine is displaced from its binding sites and released intraneuronally, ultimately eliciting a sympathetic response. Further doses of tyramine continue to deplete, and finally to exhaust, the norepinephrine stores. The octopamine which replaces the norepinephrine is also released by tyramine; but it is a weak effector of the norepinephrine-mediated responses, and tachyphylaxis to this indirectly-acting sympathomimetic amine develop. The presence of octopamine as a false transmitter interferes with sympathetic nerve responses.

False transmitters may accumulate following administration of relatively small doses of variants of phenylethylamine which are not destroyed by MAO;  $\alpha$ -methyl amines are not substrates for this enzyme. Metaraminol ( $\alpha$ -methyl-m-hydroxyphenylethanolamine, aramine) provides a well-known example of an amine which can replace norepinephrine.<sup>40</sup> It

is not a substrate for MAO nor COMT. When given intravenously, this amine displaces norepinephrine and thereby elicits sympathetic responses. Once norepinephrine stores are depleted, a larger dose of metaraminol can maintain blood pressure by its direct effect on the receptors. Withdrawal of the drug may present difficulties, however, because it is a relatively inactive false transmitter and cannot, in the quantities liberated by sympathetic nerve endings, maintain adequate sympathetic function. Norepinephrine infusion can, however, replenish transmitter stores in the sympathetic nerves and induce a return towards normal function. In man, metaraminol, when given orally and in low doses, replaces norepinephrine without releasing sufficient quantities of the catecholamine to produce a physiologic effect.<sup>41</sup> The efficacy of sympathetic nerve impulses is thereby reduced and orthostatic hypotension may result.

### Summary

Sympathetic neuronal function is dependent upon the ability of sympathetic nerves to discharge norepinephrine when excited by a nerve impulse. The processes for synthesis, storage, release and metabolism and for inactivation of norepinephrine have been discussed as necessary prerequisites to understanding how sympathetic neuronal function or central adrenergic synaptic efficacy may be altered. The role of false adrenergic transmitters in altering adrenergic neuronal efficacy also has been discussed.

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