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J. METHYLATION REACTIONS IN THE FORMATION AND METABOLISM OF CATECHOLAMINES AND OTHER BIOGENIC AMINES

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THE ENZYMATIC CONVERSION OF NOREPINEPHRINE (NE) TO EPINEPHRINE (E)

The final step in the biosynthesis of catecholamines is the N-methylation of NE to E. This reaction was first described by Bülbring (32) after the incubation of adrenal glands and ATP. Keller *et al.* (58) subsequently demonstrated that the methyl group of methionine is incorporated into E. Studies *in vitro* then showed that the methyl donor for the N-methylation of NE was S-adenosylmethionine (59).

The enzyme that converts NE to E was studied in adrenal glands of monkeys after partial purification (5). This enzyme was found to have an absolute requirement for S-adenosylmethionine. Maximal enzyme activity occurred from pH 7.5 to 8.2 with phosphate buffers and between 8 and 9 with Tris buffers. Unlike catechol-O-methyl transferase, there was no stimulation with divalent cations. The fact that enzyme activity was completely inhibited by *p*-chloromercuribenzoate indicated that a sulfydryl group was essential for enzyme activity.

The substrate specificity was assayed by incubating a purified enzyme preparation obtained from monkey adrenal gland with a number of phenylethylamine derivatives and C^{14}-methyl-S-adenosylmethionine (5). Enzyme activity with respect to a particular substrate was measured by extracting the incubation mixture with an organic solvent that extracts the radioactive C^{14}-N-methylated amine. In addition to NE, this enzyme could N-methylate a number of structurally related compounds (table 1). All the phenylethanolamine derivatives examined were N-methylated. These included many naturally occurring compounds such as NE, E, normetanephrine, metanephrine, and octopamine as well as many drugs such as neosynephrine and norephedrine. The enzyme shows an absolute specificity towards phenylethanolamine derivatives, none of the phenylethylamine derivatives being N-methylated. Because of its substrate specificity this enzyme was named *phenylethanolamine N-methyl transferase.* Phenylisopropanolamines were also methylated but phenylisopropylamines and indoleamines were not. Both the *dextro* and *levo* phenylethanolamines were N-methylated but the levo isomers were better substrates.

Phenylethanolamine N-methyl transferase was also found to add an additional methyl group to secondary amines such as E, metanephrine, and neosynephrine. This latter finding led to an investigation of the normal occurrence of N-methylepinephrine in the adrenal gland (4). With column and paper chromatography, N-methylepinephrine was found in the adrenal glands of cattle, rats, rabbits, and monkeys. When N-methylepinephrine was administered intravenously to a rat, large amounts of N-methylmetanephrine were found in the

TABLE 1

Substrate specificity of phenylethanolamine N-methyl transferase

Substrate	Relative Activity
	%
l-Normetanephrine	100
dl-Phenylethanolamine	72
dl-m-Hydroxyphenylethanolamine	60
l-Norepinephrine	21
d-Norepinephrine	15
dl-Octopamine	18
dl-Neosynephrine	20
l-Norephedrine	14
l-Epinephrine	4
dl-Metanephrine	4
3-Methoxytyramine	0
Phenylethylamine	0
Dopamine	0
Tyramine	0
dl-Amphetamine	0

Phenylethanolamine N-methyl transferase was partially purified from monkey adrenal glands.

urine. N-Methylmetanephrine was found to occur normally in the urine and to be elevated in people with pheochromocytoma (55).

The fact that normetanephrine is the best substrate for phenylethanolamine N-methyl transferase is used to measure this enzyme. In this assay normetanephrine is incubated with enzyme, C^{14}-methyl-S-adenosylmethionine and buffer; the metanephrine-methyl-C^{14} formed is then extracted into a mixture of toluene and isoamyl alcohol at pH 10, and the radioactivity in the solvent is measured after the addition of phosphor (table 2).

Phenylethanolamine N-methyl transferase was present in the soluble supernatant fraction of the adrenal medulla (5). The formation of E would require NE to leave the chromaffin granule and enter the cytoplasm, where it is N-methylated. E then returns to the chromaffin granules to be stored until it is liberated.

Phenylethanolamine N-methyl transferase activity was found in the soluble supernatant fraction obtained from the adrenal glands of all species examined (rabbit, rat, monkey, cow, guinea pig, cat); highest activity was found in the rabbit adrenal gland and lowest in cat adrenal gland. There was little difference in enzyme activity between newborn and adult rats. A small amount of enzyme activity was found in rabbit heart. Measurable amounts of phenylethanolamine N-methyl transferase activity were also found in rabbit (5) and rat brain (75). Phenylethanolamine N-methyl transferase could not be found in any other mammalian tissue, but it has been found in several nonmammalian tissues. The parotid gland of *Bufo marinus* is rich in the enzyme and it is also present in the heart and brain of frogs (*Rana pipiens*). It was Loewi (69) who first sug-

TABLE 2

Assay of phenylethanolamine N-methyl transferase

Conditions	Radioactivity	Metanephrine Formed
	cpm	*mμmoles*
Complete system	1620	3.6
Normetanephrine omitted	90	0.2

Enzyme preparation from purified monkey adrenal gland (40 μg protein) was incubated in 15 ml glass-stoppered centrifuge tube with pH 7.9 phosphate buffer, C^{14}-methyl-S-adenosylmethionine (5,000 cpm) and normetanephrine. After 1 hr incubation, 0.5 ml pH 10 borate buffer was added to the incubation mixture and the C^{14}-methylmetanephrine was extracted into a mixture of toluene-isoamyl alcohol (3:2).

gested that E might serve as an adrenergic transmitter in frogs. E has recently been found in the adrenergic tracts of the frog heart and other frog organs such as the kidney, bladder and mesentery (41). The presence of this catecholamine in the cardiac adrenergic neurones and the ability of the frog heart to synthesize E provide strong evidence to support Loewi's original conclusion. Phenylethanolamine N-methyl transferase was also found in the brain and eyes of birds. Most interesting was the observation that the quail pineal gland has more phenylethanol N-methyl transferase activity than does the quail adrenal gland.

It has recently been found (90a) that the level of phenylethanolamine N-methyltransferase in the adrenal of the rat is regulated by glucocorticoids secreted by the adrenal cortex. The ability to form epinephrine is markedly depressed in hypophysectomized animals, and enzyme activity is fully restored by replacement doses of ACTH or by large doses of glucocorticoids.

Another enzyme has been found that can N-methylate NE to E (6). This enzyme has been found in the rabbit lung (16) and parotid gland of *Bufo marinus* (73). After purification from rabbit lung, the properties of this enzyme were studied. As with all other methyl transferase enzymes, S-adenosylmethionine served as the methyl donor. The most striking feature of this enzyme was its lack of specificity. Unlike phenylethanolamine N-methyl transferase, the enzyme in rabbit lungs can N-methylate phenylethylamine derivatives such as dopamine, tyramine, and phenylethylamine as well as NE and other phenylethanolamines (6). In addition, the rabbit lung enzyme can methylate normally occurring indoleamines such as serotonin (which is the best substrate for the enzyme) as well as compounds of such diverse structures as phenylisopropylamines (amphetamine, ephedrine), aromatic amines (aniline, *p*-aminophenol), piperidines (normeperidine), phenanthrenes (normorphine, norcodeine) and pyrrolidines (nornicotine). It can also convert normally occurring amines such as serotonin and tryptamine to N-methylated metabolites with psychotomimetic properties, *i.e.*, bufotenine, N,N-dimethyltryptamine.

It has been shown that dopamine-β-oxidase can hydroxylate epinine to E (30). Epinine occurs normally in the parotid gland of *Bufo marinus* (73). The observation that the rabbit lung enzyme can N-methylate dopamine to epinine

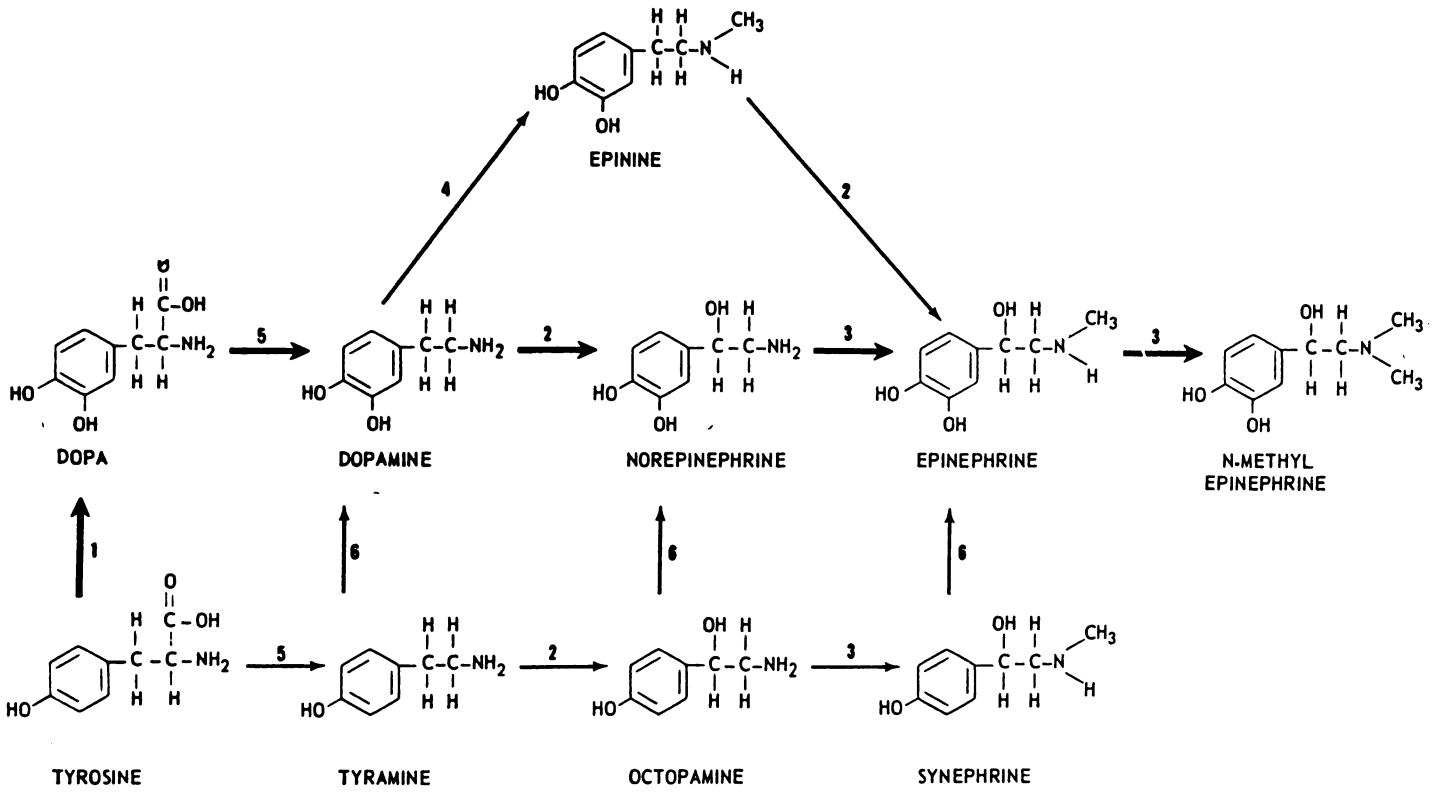

FIG. 1. Main and alternate pathways in the formation of catecholamines: 1) tyrosine hydroxylase, 2) dopamine β-oxidase, 3) phenylethanolamine N-methyl transferase, 4) nonspecific N-methyl transferase in lung, 5) aromatic amino acid decarboxylase, 6) catechol-forming enzyme.

establishes an alternate but minor pathway for the formation of E as follows: dopamine → epinine → epinephrine. The lack of specificity of the enzymes involved in the formation of catecholamines suggests several alternate pathways for the metabolism of these compounds (fig. 1).

THE ENZYMATIC O-METHYLATION OF CATECHOLAMINES

A search for an enzyme that O-methylates catecholamines was prompted by the finding of Armstrong *et al.* (1) that large amounts of an O-methylated product 3-methoxy-4-hydroxymandelic acid (VMA) was excreted in subjects with pheochromocytoma. It had already been shown that enzymes could transfer the methyl group of S-adenosylmethionine to the nitrogen of nicotinamide and guanidoacetic acid (33, 34). Thus it appeared probable that S-adenosylmethionine might transfer its methyl group to oxygen by a similar mechanism. When an extract of rat liver was incubated with E and S-adenosylmethionine, the catecholamine was metabolized (2). The metabolic product was identified as the *meta*-O-methylated product of E, 3-methoxy-4-hydroxyphenyl 2-methylaminoethanol (metanephrine).

The enzyme that O-methylates E was purified about 30-fold from rat liver (21). Enzyme activity was determined by incubating enzyme with E and S-adenosylmethionine and measuring the metanephrine formed. Metanephrine was isolated by extraction into a mixture of toluene and isoamyl alcohol at pH 10 and returned to 0.1 N HCl. The extracted metanephrine was measured fluorometrically. For a more sensitive assay, C^{14}-methyl S-adenosylmethionine can be used and the C^{14}-methylmetanephrine formed enzymatically can be measured after extraction into a mixture of toluene and isoamyl alcohol (71). When the purified enzyme was incubated with E, S-adenosylmethionine, and Mg^{++}, 1 mole of metanephrine was formed for each mole of epinephrine metabolized. The enzyme had an absolute requirement for Mg^{++} or other divalent cations such as Mn^{++}, Co^{++}, Ca^{++}, Zn^{++}, Fe^{++} or Ni^{++}. All catechols were O-methylated regardless of the substituent on the aromatic nucleus (21), including normally occurring compounds such as NE, dopamine, dopa, 3,4-dihydroxymandelic acid, and 3,4-dihydroxyphenylacetic acid. The enzyme did not show any specificity toward the *d* or *l* isomers. A number of monophenols were examined for their ability to be O-methylated by the enzyme and none served as substrates. Because the enzyme specifically O-methylated catechols, it has been named catechol-O-methyl transferase (COMT).

O-Methylation occurs mainly on the *meta* position, but also on the phenolic group *para* to electron-deficient as well as nondeficient side chains (81). When 3,4-dihydroxy acetophenone was incubated with catechol-O-methyl transferase at pH 7.4 about 50% each of *para* and *meta* O-methylated products were formed (81). Dopamine on the other hand formed 90% *meta* O-methylated products and 10% *para* O-methylated metabolites. 3,4-Dihydroxy acetophenone has a relatively stronger nucleophilic *para* hydroxy group and this may explain the preferential *para* O-methylation of this compound. When Zn^{++} was substituted for Mg^{++} or when incubations were carried out at a lower pH, there was less

para O-methylation of acetophenones (82). Phenolic substrates having three adjacent hydroxy groups (gallic acid, pyrogallol) are methylated on the middle hydroxy group regardless of whether the hydroxy group occupies a *para* or a *meta* position (74). When 3-methoxy-4-hydroxyphenylethylamine or 3-hydroxy-4-methoxyphenylethylamine were incubated with purified catechol-O-methyl transferase and S-adenosylmethionine, a partial interconversion of each isomer took place (36, 61).

The specificity for catechols and the requirement for divalent cations suggests that the cation may serve as a chelating agent to bring the enzyme, S-adenosylmethionine, and substrate together in a bridge complex. The methyl group of S-adenosylmethionine, which is electrophilic, would then be transferred to the hydroxy group by a nucleophilic displacement reaction. Since in the case of most catechols the *meta* hydroxy group is strongly nucleophilic, the transfer takes place on the 3 position.

The ability of catechol-O-methyl transferase to specifically O-methylate catechols has been found to be useful in studying the group of catechol-forming enzymes (8). These enzymes hydroxylate monophenols to catechols and are localized in the microsomes of the liver. Since the catechol metabolites formed would be unstable and the amounts formed are too small to measure, advantage was taken of the unique property of these compounds to be O-methylated with catechol-O-methyl transferase. In the measurement of the catechol-forming enzyme, monophenols were incubated with liver microsomes, which contained the hydroxylating enzyme, and soluble supernatant fraction, which contained catechol-O-methyl transferase, together with C^{14}-methyl S-adenosylmethionine. Any catechol formed would be immediately O-methylated to form a product with a radioactive methyl group (12). These metabolites are relatively stable and easily measured. A number of monophenolic biogenic amines were found to be hydroxylated and O-methylated by these enzymes (table 3). In addition, the enzyme system can form O-methylated metabolites from such phenolic

TABLE 3

Enzymatic formation of methylated catecholamines from monophenols

Substrate	Product
Tyramine	3-Methoxy-4-hydroxyphenylethylamine
N-Methyltyramine	3-Methoxyepinine
Octopamine	Normetanephrine
Synephrine	Metanephrine
p-Hydroxyamphetamine	3-Methoxy-4-hydroxyamphetamine
p-Hydroxyephedrine	3-Methoxy-4-hydroxyephedrine
N-Methylserotonin	6-Methoxy-N-methylserotonin
N-Acetylserotonin	6-Methoxy-N-acetylserotonin

Microsomes and soluble supernatant from rabbit liver were incubated with substrate, NADP, glucose-6-phosphate, C^{14}-methyl-S-adenosylmethionine and phosphate buffer pH 7.5. The radioactive O-methylated catechol was extracted into a mixture of toluene and isoamyl alcohol.

substrates as morphine, levorphanol, pentolamine, estradiol, and diethylstilbesterol (37).

Catechol-O-methyl transferase can be inhibited by pyrogallol and other catechols (15, 28). These compounds not only inhibit catechol-O-methyl transferase competitively, but also serve as substrates for this enzyme. The seven-membered-ring tropolones are also effective inhibitors of catechol-O-methyl transferase (9). The almost perfect isosterism between catechols and tropolones suggests that these inhibitors might displace catechols from the enzyme surface. The inhibition of O-methylation by *p*-chloromercuribenzoate and iodoacetate suggests that a sulfhydryl group is an active site on the enzyme surface.

The distribution of catechol-O-methyl transferase is widespread. It is present in various tissues, glands, blood vessels, sympathetic and parasympathetic nerves and ganglia, and all areas of the brain (9). There is an unequal distribution of the enzyme in the brain, highest activity being present in the area postrema and lowest in the cerebellar cortex. Catechol-O-methyl transferase activity has been found in fish and in amphibian and avian tissues.

Catechol-O-methyl transferase is confined mainly to the soluble supernatant fraction (21). A small amount of this enzyme is also present in the microsomal fraction of the liver (54). The microsomal O-methylating enzyme appears to be different from the soluble enzyme with respect to pH optima, and species distribution. Enzyme activities in rat and rabbit microsomes are approximately equal while the soluble catechol-O-methyl transferase is 50 times greater in the rat than in the rabbit. Exposure of rats to cold or treatment with benzpyrene increases microsomal catechol-O-methyl transferase but has no effect on the soluble enzyme (54). Another O-methylating enzyme found in rats (87) differs from catechol-O-methyl transferase with respect to substrate specificity (it O-methylates diiodophenols) and its subcellular distribution.

There is a 2-fold increase in catechol-O-methyl transferase in the rat uterus when pregnancy occurs (93). Large doses of thyroxine decrease catechol-O-methyl transferase in the liver (39), but the administration of thyroxine in amounts which produce hyperresponsiveness to E do not affect the enzyme (94). Catechol-O-methyl transferase activity is markedly deficient in the newborn rat and the developing chick embryo (53).

A catechol-O-methyl transferase has been found in plants. Like the animal transferase, it O-methylates catechols mainly on the *meta* position (42). Another methyl transferase had been reported in flowering bulbs of *Nerine bowdenii*, which O-methylates the catechol norbelladine on the *para* position (71). This enzyme is nonspecific and can O-methylate a variety of normal and foreign catecholamines.

O-Methylation of catecholamines in vivo. The formation of O-methylated metabolites *in vivo* was first discovered by Maclaglan and Wilkinson (70), who found that phenolic compounds were O-methylated in the body. It was then demonstrated that catechol flavanoids and dopa were excreted as a 3-methoxy-4-hydroxy phenylacetic acid (homovanillic acid) (38). The latter compound was also present as a normal constituent in human urine (1). The excretion of large

amounts of VMA in the urine of subjects with pheochromocytoma suggested the presence of the corresponding O-methylated amines. These compounds were soon found to be excreted normally (2, 20). After the injection of NE or E, large amounts of normetanephrine or metanephrine were excreted in the urine (2). This suggested that O-methylation might be an initial step in the metabolism of catecholamines. Other O-methylated metabolites of catecholamines such as 3-methoxytyramine (20), 3-methoxyphenylglycol (14), N-acetylnormetanephrine (83), N-methylmetanephrine (55), vanillic acid (19), and 3-methoxydopa were found in the urine (fig. 2).

The relative importance of O-methylation in the metabolism of catecholamines *in vivo* was determined in rats after the administration of NE or its metabolite normetanephrine (13). When either of these compounds was administered, about the same amounts of normetanephrine and VMA were excreted. After the administration of monoamine oxidase (MAO) inhibitor the excretion of VMA was reduced and that of normetanephrine was elevated. These findings suggested that O-methylation was an important step in the metabolism of NE.

The relative importance of O-methylation and deamination in the metabolism of catecholamines was studied after the intravenous administration of physiologic amounts of radioactive E to human subjects by examining the metabolic products in the urine (65). This study provided information regarding the fate of circulating catecholamines discharged from the adrenal medulla into the blood stream. The urine was collected for 24 hr and the metabolic products were separated and measured by specific methods involving column chromatography and solvent extraction. More than 80% of the administered E was excreted as O-methylated products: VMA (40%); metanephrine (free and conjugated) (40%); 3-methoxy-4-hydroxyphenylglycol sulfate (7%). Small amounts of E were excreted unchanged or as 3,4-dihydroxymandelic acid. By a double labeling technique involving the simultaneous administration of E-H^3 and C^{14}-metanephrine, it was estimated that about 20% of the circulating E was O-methylated and 25% was deaminated (16). The intravenous administration of NE also resulted in the excretion of large amounts of O-methylated products.

The O-methylation of catecholamines is an inactivation process since the physiological activity of the O-methylated products are much weaker than the parent compound. Inhibition of catechol-O-methyl transferase results in a prolongation of the pressor actions of catecholamines (27, 96). The relative importance of inactivation by catechol-O-methyl transferase was found to depend on whether the catecholamines are circulating or bound and on the tissue and species involved.

Almost all of the NE or E normally formed in the body is metabolized and excreted as O-methylated deaminated products. The daily excretion of endogenous O-methylated metabolites ranges from 2 to 4 mg for VMA (1), 100 to 300 μg for normetanephrine, and 100 to 200 μg for metanephrine. Most of the VMA presumably arises from the deamination of NE within the sympathetic nerves followed by O-methylation, probably outside the nerves. The VMA in the urine probably represents the amount of NE produced and metabolized before it had

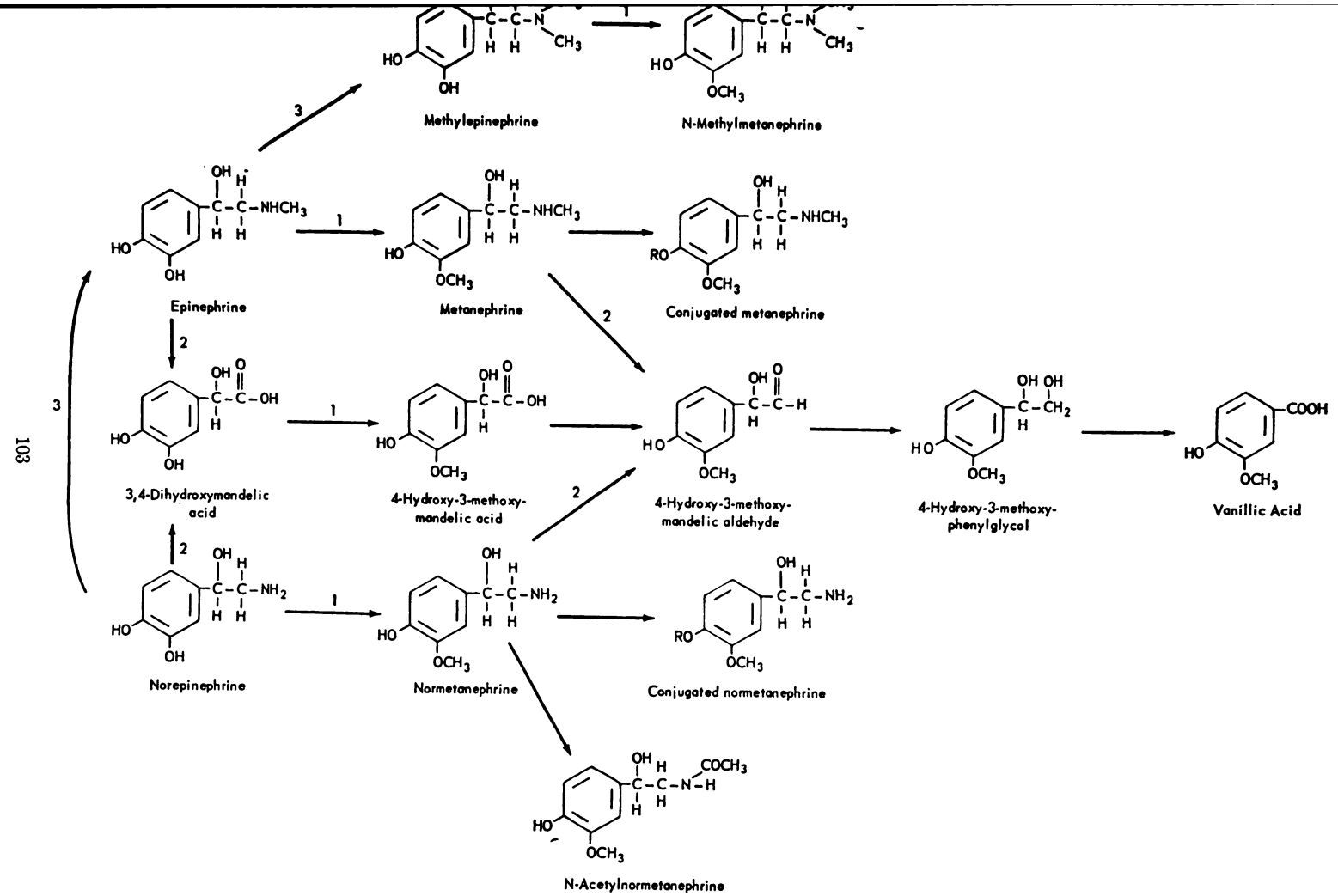

FIG. 2. Metabolism of norepinephrine and epinephrine: 1) catechol O-methyl transferase, 2) monoamine oxidase, 3) phenylethanolamine N-methyl transferase.

a chance to produce a physiological effect. The normetanephrine largely represents the amount of physiologically active NE that was discharged from the sympathetic nerves. In subjects with pheochromocytoma there is a marked elevation in the excretion of VMA (1), normetanephrine, metanephrine (65), and N-methylmetanephrine (55). There is an elevated excretion of homovanillic acid, VMA, methoxytyramine, methoxydopa, normetanephrine, and metanephrine in subjects with neuroblastoma (86). There is also an increased excretion of O-methylated catecholamines in subjects with certain types of mental disease. In periodic catatonia and schizophrenia, large amounts of normetanephrine (44) and 3,4-dimethoxyphenylethylamine (43) in the urine have been reported.

In rats and other species, but not in man, part of the metanephrine formed is O-demethylated to E and then remethylated again (61). *Para* and *meta* O-methylated derivatives of 3,4-dihydroxyacetophenone have been shown to undergo a novel type of transmethylation in rats (36). After the administration of the *para* methylether of 3,4-dihydroxyacetophenone, small amounts of the *meta* methylether of this compound were found in the urine, while the administration of the *meta* methylether of acetophenone results in the excretion of the *para* methylether of 3,4-dihydroxyacetophenone. This transfer of the methyl group of the same molecule is due to two enzymatic processes. Both O-methyl ethers are demethylated to catechols by an enzyme in the microsomes in the liver. The catechol is then O-methylated by catechol-O-methyl transferase on either the *para* or *meta* oxygen.

The extent of O-methylation of catecholamines in the whole animal was examined after the intravenous administration of radioactive NE to mice (89). Five minutes after the intravenous administration of the radioactive catecholamine the whole mouse carcass was assayed for the unchanged catecholamine or its metabolites. At that time about 50 % of the NE was metabolized, almost all of it (90 %) as normetanephrine, and the remaining 10 % as deaminated products. Essentially the same results were obtained with E (22).

Metabolic studies after the administration of radioactive E or NE into the portal vein indicated that the main site of O-methylation of circulating catecholamines is in the liver (52), but these compounds can also be O-methylated locally in other tissues *in vivo*. To simulate a sudden discharge of NE into the circulation, cats were given a rapid injection of NE-H^3 and the tissues examined 2 min later for the catecholamine and its O-methylated product normetanephrine (89). Within 2 min, at a time when almost all of the physiologic effects of the catecholamine were dissipated, H^3-normetanephrine was found in all tissues examined. In the liver the concentration or normetanephrine exceeded that of NE. There were relatively large amounts of NE-H^3 in the heart, spleen, blood vessels, and the salivary and adrenal glands. These observations indicate that O-methylation is an important step in the inactivation of circulating NE both in liver and in other peripheral tissues. Studies on the uptake and metabolism of H^3-catecholamines have also provided evidence that tissue binding is probably the most important mechanism for the inactivation of circulating and locally

released NE. The site of inactivation by binding has been shown to be the sympathetic neurons (50).

Since NE cannot cross the blood-brain barrier (88), the metabolism of this hormone was examined after its intraventricular injection into the brain of cats and rats (46, 72). The large amounts of H^3-normetanephrine and O-methylated deaminated products present in brain indicated that extensive O-methylation takes place in this tissue.

The metabolism of NE was also examined after local perfusion of the H^3-catecholamine in the heart and muscle. In the heart the major fraction of the perfused catecholamine was present as unchanged NE. The major metabolic products were either normetanephrine or O-methylated deaminated products (63). In another experiment the vascular bed of the skeletal muscles of the dog's leg was perfused with NE-H^3 at a constant rate (79) and metabolites present in the venous outflow were measured. NE-H^3 represented about 25% of the total radioactivity. The remainder could be accounted for as normetanephrine (22%) and O-methylated products 45%. This shows that O-methylation can be a major metabolic pathway locally in tissues.

The metabolic fate of NE-H^3 that was discharged from sympathetic nerves was also examined (49). In this experiment NE-H^3 was first injected intravenously into a cat and the spleen, which contained radioactive NE-H^3, was isolated and perfused with nonradioactive blood from a donor cat. The splenic nerve was then stimulated and the radioactivity in the blood was measured before and after stimulation. After each stimulation there was a marked increase in the concentration of NE-H^3 in the venous outflow (fig. 3). There was also an in-

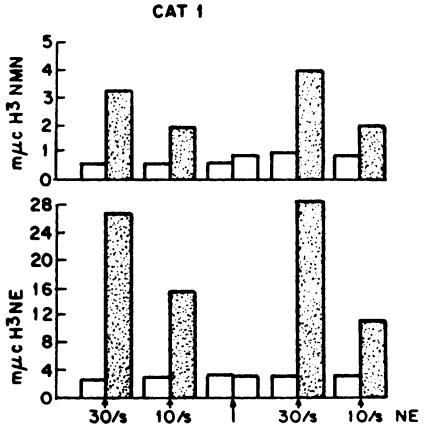

FIG. 3. Liberation and O-methylation of H^3-NE after stimulation of the splenic nerve. Cats were given H^3-NE, and the spleen was then isolated and perfused with blood obtained from a donor cat that was free of radioactive material. Splenic nerves were stimulated at a frequency of 10 or 30/sec. Nonradioactive norepinephrine was injected into splenic artery (middle arrow). Blood was collected for 2 min before and 2 min after stimulation and assayed for H^3-NE and H^3-normetanephrine (H^3-NMN). Empty bars are H^3-NE and H^3-NMN measured before, and stippled bar are the H^3-NE and H^3-NMN measured after stimulation. (From Nature, Lond. **192**: 172, 1961.)

crease in the amount of H^3-normetanephrine appearing in the venous outflow after each stimulation. H^3-Normetanephrine found was directly related to the amount of NE-H^3 released. Deaminated products were not elevated during stimulation. These results show that the NE discharged from sympathetic nerves is inactivated by several mechanisms: re-uptake into sympathetic nerves, diffusion and O-methylation. Similar experiments carried out in the vascular bed of the muscle of the dog showed that inactivation of the liberated transmitter occurs by rebinding or diffusion with little or no metabolism (79). However, when E-H^3 was introduced into sympathetic nerves of the leg muscle there was an elevated discharge of metanephrine after stimulation.

Many drugs can affect the activity of catechol-O-methyl transferase *in vivo*. Compounds that inhibit this enzyme can prolong the physiological effects (95) of and elevate the levels of administered catecholamine in tissue (51). Many years ago Bacq (27) demonstrated that pyrogallol and other catechols increased the duration of response to E and to sympathetic nerve stimulation. This suggested that pyrogallol might prolong the actions of catecholamines by competing for this enzyme. Incubation of catechol-O-methyl transferase in the presence of pyrogallol or other catechols inhibited the enzymatic O-methylation of catecholamines. This inhibition is competitive, as catechols are themselves O-methylated. When mice were pretreated with pyrogallol, the disappearance of administered NE and E were markedly slowed (15). In addition the formation of the O-methylated metabolites normetanephrine and metanephrine was reduced.

Treatment of rats with the catechol-O-methyl transferase inhibitor pyrogallol elevated the levels of injected NE-H^3 accumulated in heart, spleen, liver and skeletal muscle and reduced the concentrations of H^3-normetanephrine (51). Pyrogallol increased the excretion of administered catecholamines and catechol acids and decreased the formation of O-methylated metabolites, while MAO inhibitors elevated the excretion of the O-methylated amines metanephrine and normetanephrine (61). Catechol-O-methyl transferase inhibitors had little effect on the levels of endogenous catecholamines in heart and brain (35), but pyrogallol increased the endogenous levels of NE in certain areas of the brain of rats and mice (57). MAO inhibitors increased the concentration of endogenous normetanephrine in the brain (3). The pressor actions of guanethidine, a compound that releases norepinephrine from nerves, are prolonged after the administration of pyrogallol (95).

In an isolated perfused heart, adrenergic blocking agents such as phenoxybenzamine or dichloroisoproterenol (DCI) block the extraneuronal O-methylation of H^3-catecholamines; when the cells in the heart are broken these adrenergic blocking agents no longer inhibit O-methylation (40). These results suggest that the intact cell is necessary for adrenergic blocking agents to inhibit O-methylation and that catechol-O-methyl transferase is closely associated with the adrenergic receptor.

NE is stored in sympathetic nerves in granulated vesicles (90). The intraneural NE undergoes a different fate depending on how it is discharged from the nerve. A small fraction of the NE is released by drugs such as tyramine (11)

TABLE 4

Effects of sympathetic nerve stimulation, denervation and drug treatment on the O-methylation of catecholamines

Treatment	Normetanephrine in Tissues
COMT inhibitors	Decreased
MAO inhibitors	Increased
Sympathomimetic amines	Increased
NE uptake inhibitors	Increased
Phenoxybenzamine	Decreased
DCI	Decreased
Sympathetic nerve stimulation	Increased
Sympathetic nerve denervation	Increased
Immunosympathectomy	Increased

or by nerve stimulation (49) in a physiologically active form. The released NE either is O-methylated locally or it enters the circulation, where it is ultimately metabolized by catechol-O-methyl transferase in the liver. The NE released by nerve impulses undergoes a similar metabolic fate. There is also a larger, more firmly bound store, which is slowly released either spontaneously or by drugs such as reserpine. The NE released from the firmly bound store is deaminated by mitochondrial MAO in the sympathetic nerves (62). Thus NE leaves the nerve as a physiologically inactive deaminated catechol. This catechol is then O-methylated outside the nerve either locally or by the liver. Procedures that destroy the sympathetic nerves, such as denervation (77) or immunosympathectomy (56), give rise to an increase of O-methylated amines in these tissues after the administration of NE. In general drugs or physiological conditions that prevent entry of NE into adrenergic neurons increase the degree of O-methylation of the hormone (table 4). The role of catechol-O-methyl transferase in the metabolism of the NE released from nerves and in the circulation is shown in figure 4 (p. 108) and table 4.

In normal hearts, inactivation of NE occurs mainly by uptake into nerves (50) and only a small fraction is methylated. The increase in O-methylated metabolites of NE together with a decrease in uptake of the neurohumor in heart preparations devoid of sympathetic nerves suggests that catechol-O-methyl transferase is extraneural and close to sympathetic nerve terminals. The inhibitory action of adrenergic blocking agents on O-methylation further suggests that catechol-O-methyl transferase is in close juxtaposition to adrenergic receptors.

THE FORMATION AND METABOLISM OF BIOGENIC AMINES BY O- AND N-METHYL TRANSFERASES

In addition to catechol-O-methyl transferase, other methyl transferases have been found in animal tissues that are involved in the formation and metabolism of physiologically active amines. S-Adenosylmethionine serves as a methyl donor for all of these enzymes. The isolation of the O-methylated indole, mela-

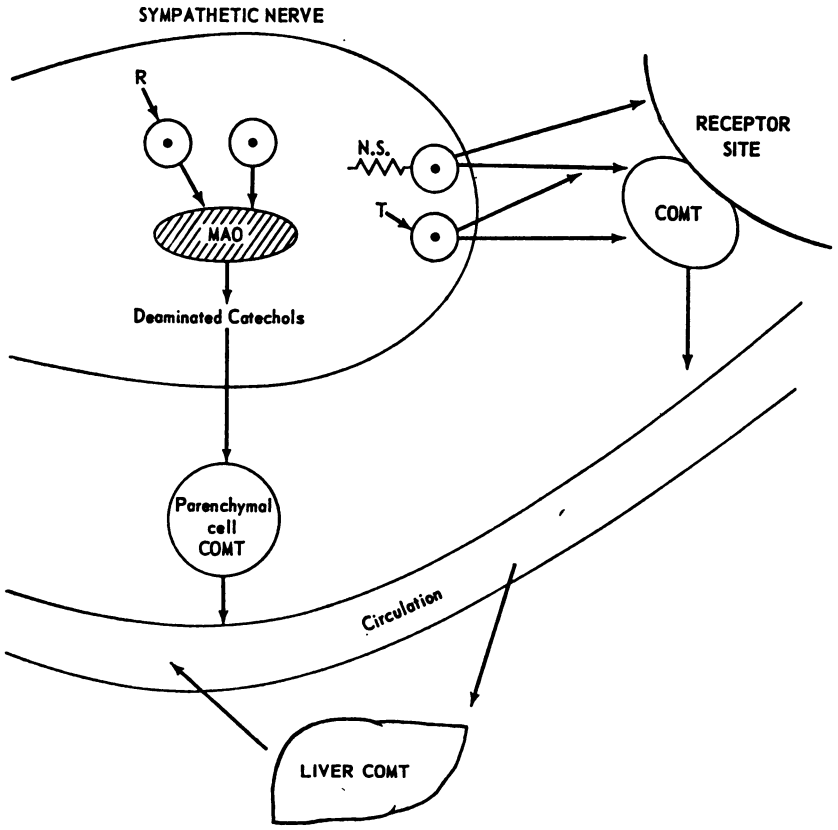

FIG. 4. Sites of action of COMT in the body. NS is nerve stimulation. T is tyramine. R is reserpine.

tonin (N-acetyl, 5-methoxytryptamine) (23, 24, 67) from the pineal gland prompted a search for the enzyme that makes this compound. Catechol-O-methyl transferase from rat liver was found to O-methylate these catecholindoles predominantly on the 6 position while hydroxyindole-O-methyl transferase from bovine pineal glands hydroxylated them mainly on the 5 position (16).

Hydroxyindole-O-methyl transferase was found to be present in pineal glands of mammals (18), birds (26), reptiles, amphibians (19), and fish (78). Small amounts of the enzyme are found in the brain of frogs and eyes of fish, amphibians, and birds (19, 78). Melatonin is physiologically active and is considered to be a pineal hormone (92). It lightens the frog skin and inhibits the estrous phase of the estrous cycle in rats. Consequently this O-methylation, unlike the O-methylation of catecholamines, can be considered an activation process. Environmental lighting produces a 3-fold increase in hydroxyindole-O-methyl transferase activity in pineal glands, and there is also a circadian rhythm in the activity of the enzyme (25). The effects of environmental lighting were abolished when rats were blinded or when sympathetic nerves to the pineal

TABLE 5

Properties of methyltransferase enzymes

Methyltransferase	Substrate	Product	Physiologic Activity of Product	Localization of Enzyme
Catechol-O	Epinephrine and other catechols, dihydroxyindoles	Metanephrine and other O-methylated metabolites	Decreased	Widespread
Hydroxyindole-O	N - Acetylserotonin, 5 - hydroxyindoles	Melatonin and other methoxyindoles	Increased	Pineal gland
Histamine-N	Histamine	Methylhistamine	Decreased	Widespread, highest in brain
Phenylethanolamine-N	Norepinephrine and other phenylethanolamines	Epinephrine and other N-methylated phenylethanolamines	Increased	Adrenal medulla, avian pineal, brain, amphibian heart, brain
Lung enzyme	Serotonin, phenylethylamines, catecholamines, normorphine, nornicotine, aniline, purines	N,N-Dimethylserotonin, N-methylated phenylethylamine, N-methylated catecholamines, morphine, nicotine, methylated purines	Increased	Lung
Methanol-forming enzyme	Water	Methanol	Increased	Pituitary

gland were cut (91). These results indicated that information about lighting reached the pineal gland enzyme by means of the eyes and the sympathetic nervous system. Preliminary observations suggest that a liberated neurotransmitter (NE) may be involved in controlling hydroyxindole-O-methyl transferase activity. Puromycin, a compound that inhibits protein synthesis also abolishes the effect of lighting on hydroxyindole-O-methyl transferase (25).

A major pathway in the metabolism of histamine in the body is ring N-methylation to form methylhistamine. Like the other N-methyl transferase enzymes this enzyme requires S-adenosylmethionine as the methyl donor and it is highly specific for histamine (31, 47, 48, 76, 84, 84a).

An interesting methylating enzyme was found in the pituitary gland. When C^{14} methyl S-adenosylmethionine was incubated with bovine pituitary gland, C^{14}-methanol and S-adenosylhomocysteine were formed. This reaction appears to proceed by the methylation of water to form methanol or by a hydrolytic cleavage of S-adenosylmethionine. The methanol-forming enzyme is highly localized in the soluble fraction of the posterior pituitary gland. Negligible activity was found in other mammalian tissues. All mammalian pituitary glands examined were found to convert S-adenosylmethionine to methanol.

The distribution of various transferases, the substrate specificity, and the physiological activity of the product, are shown in table 5.

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