THE METABOLISM OF CATECHOLAMINES *IN VIVO* AND *IN VITRO*

JULIUS AXELROD

Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

The important role that epinephrine plays in mammalian physiology has been recognized for more than fifty years, yet the metabolic fate of this compound and related catecholamines is poorly understood. Many studies point to deamination (10), oxidation (16) and conjugation (21) as pathways for the metabolism of epinephrine. Until recently, the extent to which these various metabolic processes are involved in the inactivation of catecholamines has been conjectural. Several investigations have indicated that the direct deamination of epinephrine by monoamine oxidase is involved only to a minor extent in the inactivation of epinephrine (13, 15, 17, 18). Although oxidases have been shown to transform epinephrine to adenochrome *in vitro* (16), no evidence has been obtained for the formation of the latter in the intact animal (23, 24). After the oral ingestion of large amounts of epinephrine, Richter observed that a major fraction of the catecholamine appeared in the urine as a sulfoconjugate (21). However, Schayer *et al.*, using physiological doses of C^{14}-labeled epinephrine parenterally administered, found that only negligible quantities of epinephrine were excreted as a conjugate in the rat (22).

Recently Armstrong and co-workers have shown that a major metabolic product of norepinephrine in man is 3-methoxy-4-hydroxymandelic acid (1). Three possible pathways for the metabolism of catecholamines are suggested by these findings: 1) deamination of the amines followed by O-methylation; 2) O-methylation preceding deamination; 3) both reactions occurring simultaneously.

Studies in our laboratory have demonstrated the O-methylation of administered catecholamines (2, 6, 7) as well as the normal occurrence of metanephrine (3-O-methylepinephrine) and normetanephrine (3-O-methylnorepinephrine) in urine and certain tissues (7). In addition, an enzyme that catalyzes the O-methylation of catecholamines has been found (2, 9).

Metanephrine and normetanephrine in rat urine and tissues. Rat urine was examined for endogenous metanephrine and normetanephrine after treatment with β-glucuronidase, extraction with organic solvents and separation by paper chromatography (2, 7). The resulting extract showed the presence of compounds that had the same R_f values as normetanephrine and metanephrine in 3 solvent systems and the same color reactions as authentic samples of normetanephrine and metanephrine. The amount of normetanephrine in the urine was greater than that of metanephrine.

Adrenal glands and spleen, organ tissues which have high concentrations of epinephrine and norepinephrine were examined for the corresponding methoxy derivatives (7). Extracts of the adrenal glands showed the presence of two com-

pounds having the same R_f values on paper chromatograms and color reaction as metanephrine and normetanephrine. Chromatograms of spleen extracts had a single spot which gave the same color reactions and R_f values as normetanephrine.

O-Methylation of exogenous catecholamines in the rat. Epinephrine was administered to rats intraperitoneally and the urine was treated with β-glucuronidase, extracted into organic solvents and chromatographed (2, 6, 7). The resulting extract showed the presence of large amounts of material with the same ultraviolet fluorescent spectrum, R_f values in 3 solvent systems, color reactions and partition coefficients as synthetic metanephrine.

The extent to which epinephrine is O-methylated in the rat was studied using tritium-labeled epinephrine (4). *dl* β-H^3-Epinephrine (0.2 mg/kg) was administered intraperitoneally and the urine was collected for 24 hours. About 87% of the administered radioactivity appeared in the urine. Free metanephrine and metanephrine glucosiduronic acid accounted for about 55% of the radioactivity in the urine (Table 1). Approximately 12% of the radioactivity was present as 3-methoxy-4-hydroxymandelic acid. Only traces of 3 ,4-dihydroxymandelic acid could be detected.

To delineate further the metabolism of epinephrine, the fate of its major metabolite, metanephrine, was studied. β-H^3-Metanephrine (0.2 mg/kg) was administered intraperitoneally and the urine collected for 24 hours. Essentially the same amount of administered radioactivity was excreted as metanephrine (free and conjugated) and 3-methoxy-4-hydroxymandelic acid after β-H^3-metanephrine as was observed after β-H^3-epinephrine (Table 1). These results indicated that most if not all of the 3-methoxy-4-hydroxymandelic acid arises from the deamination of metanephrine in the rat.

To examine the relative importance of deamination in epinephrine metabolism, the effect of iproniazid, a monoamine oxidase inhibitor, was studied. When

TABLE 1

Metabolic fate of epinephrine in man and rat

Species	Compound Administered	Radioactivity in Urine	Urinary Radioactivity Present as Metanephrine			VMA†
			Free	Conjugated*	Total	
		%	%	%	%	%
Rat	H^3 Epinephrine	87	20	34	54	10
Rat	H^3 Epinephrine + Iproniazid	80	15	74	89	5
Rat	H^3 Metanephrine	80	11	48	59	13
Rat	H^3 Metanephrine + Iproniazid	73	38	60	98	4
Man	H^3 Epinephrine	88	12	43	55	35
Man	H^3 Metanephrine	90	14	36	50	25

* In rat, all of the conjugated metanephrine was present as a glucosiduronic acid, but in man only a small amount (6%) of metanephrine glucosiduronic acid was found in urine.

† 3-Methoxy-4-hydroxymandelic acid (Vanillyl mandelic acid).

rats were treated with iproniazid, almost all of the administered epinephrine or metanephrine was excreted as free and conjugated metanephrine (Table 1). In addition, the amount of 3-methoxy-4-hydroxymandelic acid was markedly reduced. These observations indicated that monoamine oxidase is primarily involved in the deamination of metanephrine.

Following the administration of norepinephrine to rats, normetanephrine was isolated from the urine (6, 7). Normetanephrine extracted from the urine and authentic normetanephrine had the same ultraviolet fluorescent spectra, R_f values, color reactions and distribution coefficients (7). The administration of norepinephrine resulted in the excretion of large amounts of normetanephrine and normetanephrine glucosiduronic acid (6). Pretreatment of rats with iproniazid led to a 2-fold increase in the excretion of normetanephrine (free and conjugated).

The administration of dopamine (3-hydroxytyramine) to rats resulted in the excretion of 3-methoxytyramine (free and conjugated) (7). In contrast to epinephrine and norepinephrine, the methoxylation of dopamine occurred only to a minor extent. Considerable quantities of homovanillic acid, a deaminated metabolite of 3-methoxytyramine was also found in the urine. Treatment with iproniazid produced a 5-fold elevation in the excretion of 3-methoxytyramine.

O-Methylation of epinephrine in man. Armstrong *et al.* found 3-methoxy-4-hydroxymandelic acid (VMA) as a normal constituent in human urine (1). These investigators found that the amount of this compound was markedly elevated in normal subjects after an infusion of norepinephrine, and also in patients with pheochromocytomas. In addition to 3-methoxy-4-hydroxy-mandelic acid we have found large amounts of normetanephrine predominantly as a conjugate in the urine of subjects with pheochromocytomas (20). These observations indicate that O-methylation is an important pathway in the metabolism of catecholamines in man.

The availability of radioactive epinephrine made possible a quantitative study of the metabolism of this hormone in man (19). One milligram of *dl* β-H^3-epinephrine bitartrate was infused intravenously over a period of 45 minutes to 5 human subjects. Over a period of 48 hours about 90% of the administered radioactivity appeared in the urine. Approximately 55% of the radioactivity found in the urine was free and conjugated metanephrine and 30% was 3-methoxy-4-hydroxymandelic acid (Table 1). Less than 3% of the radioactivity was present as 3,4-dihydroxymandelic acid.

To study the role of deamination in the metabolism of epinephrine in man, the metabolic fate of its major product, metanephrine, was investigated (19). About the same fraction of the β-H^3-metanephrine was excreted as metanephrine and 3-methoxy-4-hydroxymandelic acid as was observed after β-H^3-epinephrine administration (Table 1), indicating that the acid arises mainly from metanephrine. These results together with those obtained in the rat indicate that monoamine oxidase is primarily concerned with the deamination of metanephrine rather than epinephrine. This conclusion is compatible with the findings made by others that monoamine oxidase inhibitors do not prolong the physiologic actions of epinephrine *in vivo* (13, 15, 17, 18).

Physiological activity of normetanephrine and metanephrine. Evarts *et al.* (14) found that normetanephrine possesses relatively weak pressor activity. In contrast to the marked physiological and psychological actions of epinephrine, we have noted that the administration of metanephrine to man (19) produced no detectable effects. The observations suggest that O-methylation of catecholamines is an inactivation process.

From the findings reported here and elsewhere it can be concluded that the principal pathway of metabolism of epinephrine in rat and man is its O-methylation to metanephrine which is then deaminated and conjugated. Norepinephrine presumably follows the same pathway.

Enzymatic O-methylation of catecholamines. Studies described above have demonstrated the O-methylation of catecholamines *in vivo*. These observations suggested the presence of an enzyme that can methylate an oxygen group. Since enzymes have been described that can transfer the methyl group of S-adenosylmethionine to nitrogen (11, 12), an analogous reaction in which the methyl group is transferred to oxygen was considered. When a soluble supernatant fraction of rat liver was incubated with S-adenosylmethionine (AMe) and epinephrine, the catecholamine disappeared (2, 9); in the absence of AMe negligible metabolism occurred (Table 2). The product of this reaction was isolated and found to be identical with synthetic metanephrine with regard to its ultraviolet fluorescent spectra, R_f values, partition coefficients in a number of solvent systems, and color reactions (2, 9). These findings demonstrated the occurrence of an enzyme, catechol O-methyl transferase, that transfers the methyl group of AMe to the 3-hydroxy group of epinephrine.

Catechol O-methyl transferase was purified about 30-fold from rat liver and its properties studied (9). In the absence of AMe or Mg^{++} very little O-methylation took place (Table 2), but a number of divalent cations, including Co^{++}, Mn^{++}, Cd^{++}, Fe^{++} and Ni^{++} could be substituted for Mg^{++}. For every mole of epinephrine metabolized, 1 mole of metanephrine was formed (Table 2). All substrates examined having a catechol nucleus were O-methylated, including the following normally occurring compounds: norepinephrine; dopamine; 3,4-dihydroxyphenylalanine; 3,4-dihydroxymandelic acid, as well as many synthetic catechols. The enzyme showed no stereo-specificity toward the *d*- or the *l*-isomers of epinephrine, nor did it O-methylate monophenols.

TABLE 2

Enzymatic O-methylation of epinephrine

	Metanephrine Formed	Epinephrine Disappeared
	μmoles	*μmoles*
Complete system	0.059	0.055
S-Adenosylmethionine omitted	0	0
$MgCl_2$ omitted	0.004	0.006

Purified enzyme obtained from rat liver was incubated at 38° C with *l*-epinephrine, S-adenosylmethionine and $MgCl_2$.

In the presence of SH-binding agents such as *p*-chloromercuric benzoate and iodoacetic acid, the O-methylation of epinephrine was inhibited.

The specificity of the O-methyl transferase towards catechols and the requirement for a divalent cation suggest that the metal serves as a complexing agent to attach the substrate by means of the two adjacent hydroxy groups to the enzyme surface. The site of attachment is presumably to an SH-group, since SH-binding agents inhibit the reaction. It is likely that O-methylation results from a nucleophilic attack of the hydroxy group of the substrate on the electrophilic methyl carbon of S-adenosylmethionine.

All organs examined (liver, lung, kidney, spleen, small intestine, brain, heart, salivary gland, pituitary, pancreas, aorta, inferior vena cava) except skeletal muscle, can O-methylate catecholamines (9). The presence of catechol O-methyl transferase in those tissues where epinephrine and norepinephrine exert their effects suggests that it acts locally in the transformation of these hormones.

Catechol O-methyl transferase was found to be present in all mammalian species studied including man, monkey, cow, pig, rat, mouse, guinea pig, cat and rabbit (9).

The finding that the major pathway for the metabolism of epinephrine and norepinephrine involves the formation of physiologically inactive O-methylated derivatives indicates that catechol-O-methyl transferase is the principal enzyme in the activation of these hormones.

Catechol O-methyl transferase in the nervous system. Norepinephrine has been shown to play an important role as a neurohumoral agent in the sympathetic nervous system. In addition, studies have implied that catecholamines are involved in the activity of the central nervous system. However, the manner in which these amines are inactivated in the nervous system is not clear. We have found normetanephrine to be present in brain extracts of rats treated with iproniazid (3). Normetanephrine could not be detected in the brain extracts of untreated rats. These findings indicate that endogenous norepinephrine is O-methylated to normetanephrine in rat brain and that the latter compound is further metabolized by deamination. The enzymes in the nervous system concerned with these metabolic processes were studied (3). Incubation of norepinephrine with rat brain preparations fortified with S-adenosylmethionine resulted in the formation of normetanephrine. When incubated with brain mitochondria and aldehyde dehydrogenase norepinephrine underwent deamination and oxidation to 3-methoxy-4-hydroxymandelic acid. An enzyme that can form S-adenosylmethionine from adenosinetriphosphate and methionine was also present in brain (5). Catechol O-methyl transferase has been found to be present in the sympathetic as well as the parasympathetic nervous system (5). The observations described above indicate the following metabolic route for the neurohumoral agent, norepinephrine, in the central and peripheral nervous system: Norepinephrine → normetanephrine → 3-methoxy-4-hydroxymandelic acid.

Although conclusive evidence for the principal route for the metabolism of norepinephrine and epinephrine in the central and peripheral nervous system

is not presently available, it appears likely from our observations that O-methylation constitutes an important pathway for the metabolism of catecholamines in the nervous system.

Metabolism of metanephrine and normetanephrine. The enzymes concerned with the transformation of metanephrine and normetanephrine were studied. In the rat, a major route of metabolism of O-methylated metabolites of catecholamines involved conjugation with glucuronic acid. Incubation of metanephrine or normetanephrine with microsomes of rat liver and "active" glucuronic acid, uridine diphosphate glucuronic acid, resulted in the formation of the corresponding glucosiduronic acid of these amines (4).

Deamination and oxidation of metanephrine and normetanephrine to 3-methoxy-4-hydroxymandelic acid constitute an important pathway in the transformation of these compounds in the intact organism (2, 6, 7). When the O-methylated amines were incubated with liver or brain mitochondria and aldehyde dehydrogenase, 3-methoxy-4-hydroxymandelic acid was formed (3, 9). Iproniazid markedly inhibited this reaction *in vitro*, indicating that the deamination is catalyzed by monoamine oxidase.

An enzyme was found in the microsomes of rabbit liver that catalyzes the O-demethylation of metanephrine to epinephrine (8). The reaction required the presence of both reduced triphosphopyridine nucleotide and oxygen. The physiological significance of this enzymatic process is undetermined at present.

Summary

The principal pathway for the metabolism of epinephrine in rat and man is O-methylation to metanephrine, a physiologically inactive metabolite, which is then conjugated or deaminated and oxidized to 3-methoxy-4-hydroxymandelic acid. Norepinephrine undergoes the same transformation in the rat and presumably in man.

An enzyme, catechol O-methyl transferase, that catalyzes the O-methylation of epinephrine, norepinephrine and other normally occurring and synthetic catechols, is described. Catechol-O-methyl transferase is widely distributed in all organ tissues including the autonomic and central nervous system, and appears to be the enzyme mainly responsible for the metabolism of epinephrine and norepinephrine.

Evidence is presented which indicates that amine oxidase is concerned chiefly with the deamination of the O-methylated metabolites of the catecholamines.

Enzymes involved in the conjugation of metanephrine and normetanephrine with glucuronic acid and the conversion of metanephrine to epinephrine are briefly described.

Metanephrine and normetanephrine were found to be normal constituents in rat urine and certain tissues of the rat.

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