O-Methylation of Catechol Amines *in Vivo**

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(Received for publication, February 24, 1958)

Although the metabolism of catechol amines, such as epinephrine, norepinephrine, and 3-hydroxytyramine (dopamine) was thought at one time to proceed mainly by deamination (3, 4), the possibility of other pathways has been recognized (5). Recently, Armstrong and McMillan (6) have found 3-methoxy-4-hydroxymandelic acid to be a major metabolite of epinephrine and norepinephrine. Three possible pathways are suggested by these findings: (*a*) deamination of amines is followed by O-methylation; (*b*) O-methylation precedes deamination; (*c*) both pathways operate concurrently. The following study demonstrates the normal occurrence of O-methyl metabolites of catechol amines in urine and certain tissues as well as the O-methylation of administered catechol amines. These naturally occurring derivatives of epinephrine and norepinephrine which have a methyl ether group on the phenolic hydroxyl in a position *meta* to the side chain, will be called *metanephrine* and *normetanephrine.*

Materials and Methods

Synthesis of 3-O-Methylcatechol Amines—Metanephrine (3-O-methylepinephrine) is formed in the reductive condensation (7) of methylamine with 3-methoxy-4-hydroxyphenylglyoxal available from 3-methoxy-4-hydroxyacetophenone by oxidation with selenium dioxide[1] (8).

In order to utilize a common intermediate in the synthesis of both 3-methoxytyramine as well as normetanephrine (3-O-methylnorepinephrine), a nitroalcohol was prepared by condensation of O-benzylvanillin with nitromethane (11–13) under special precautions in the cold. Dehydration of the nitroalcohol to give the nitrostyrene (14) occurs with great ease. The reduction of the nitrostyrene with zinc in acetic acid has been reported to lead to 3-methoxy-4-benzyloxyphenylacetaldoxime (12). With 10 per cent palladium on carbon in ethanol containing hydrochloric acid, 3-methoxytyramine was obtained in excellent yield.

When palladium was used in the reduction of the nitroalcohol, debenzylation and partial reduction of the nitro group was observed. The reduction mixture contained normetanephrine as well as the debenzylated nitroalcohol which in the course of isolation dehydrated to the known ω-nitro-3-methoxy-4-hydroxystyrene (15). When in the reduction of the nitroalcohol platinum was used first, the benzyl ether of normetanephrine was obtained which yielded normetanephrine on debenzylation in an over-all yield of 60 per cent.

Synthesis of DL-*Metanephrine*—In a 500 ml., three neck flask, equipped with a ground glass mechanical stirrer, a dropping funnel, and a gas inlet tube, 1.5 gm. of 10 per cent palladium charcoal and 0.5 gm. of palladium oxide in 50 ml. of ethanol was saturated with hydrogen. To this was added the solution of 0.31 gm. of methylamine in 5 ml. of ethanol, and then dropwise at the rate of 12 drops/minute a solution of 2 gm. of 3-methoxy-4-hydroxyphenylglyoxal (m.p. 84–86°, crystallized as as hydrate, $C_9H_8O_4 \cdot H_2O$, from ether) in 50 ml. of ethanol with vigorous agitation. In the course of 5 hours the mixture absorbed 470 ml. of hydrogen in accordance with theory. After filtration the clear solution was concentrated and the residue was dissolved in a small quantity of ethanol and saturated with dry hydrogen chloride. With the addition of ether, the oily hydrochloride deposited and solidified gradually. The hydrochloride (about 350 mg.) was dissolved in water, decolorized with charcoal, and concentrated to dryness. The viscous hydrochloride was recrystallized from water-acetone to yield 240 mg. of DL-metanephrine hydrochloride, identical in all respects with a sample synthesized via Friedel-Crafts acylation[1] (m.p. 175°). $\lambda_{max}^{CH_2H_5OH}$: 231 mμ (ε 7600); 280 mμ (ε 3100).

DL-*Normetanephrine*—11 gm. of vanillin benzylether (14) was dissolved in 180 ml. of hot absolute ethanol and then cooled in an ice-salt bath with stirring; the aldehyde separated as a finely divided crystalline powder. To this suspension was added 3.7 ml. of nitromethane and then, after cooling to −12°, an ice-cold solution of 5.1 gm. of potassium hydroxide in 100 ml. of 90 per cent ethanol was added dropwise with stirring in the course of 25 minutes. The temperature was kept at −15° to −12° throughout the time of addition, during which the aldehyde dissolved. The reaction mixture soon became a thick mush of fine colorless crystals. Agitation was continued for 2 hours while the temperature was kept at −10° to −5°, and the mixture was then acidified by the careful addition of 8 ml. of concentrated hydrochloric acid diluted with 100 ml. of ice water. The crystallization of the nitroalcohol was rendered complete by the addition of 300 ml. of cold water. The product (13.6 gm. yield > 90 per cent) was collected, washed with water, and dried (m.p. 97–105°). For purification, 10 gm. of the above crude product were dissolved in 120 ml. of ethanol by slight warming. To the light yellow solution were added 15 ml. of water. After several minutes in the ice bath 1.7 gm. of yellow crystals (m.p. 97–120°), largely the nitrostyrene derivative, separated. To the filtrate was added 100 ml. of water and the

* A preliminary report of this work has been published (1, 2).

[1] For preparations of metanephrine on a larger scale the Friedel-Crafts acylation of substituted guaiacols (9) proved to be more convenient. We are greatly indebted to Dr. B. F. Tullar, Sterling-Winthrop Research Institute, for placing DL-metanephrine hydrochloride, synthesized by this method, as well as the resolved compound, L-metanephrine-D-bitartrate (10) at our disposal.

solution was cooled in an ice bath for 1 hour. There was obtained 8.03 gm. of light yellow leaflets of α-(3-methoxy-4-benzyloxyphenyl)-β-nitroethyl alcohol, m.p. 107–109°.

$C_{16}H_{17}NO_5$

Calculated: C 63.36, H 5.65, N 4.62

Found: C 63.87, H 5.62, N 4.44

$\lambda_{max}^{CHCl_3}$: 2.81 μ (m); 6.25 μ (s); 6.45 μ (s); 6.65 μ (s); 6.87 μ (s); 7.09 μ (m); 7.28 μ (s); 7.52 μ (s). $\lambda_{max}^{CHCl_3}$: 281 mμ (ε 3400); 372 mμ (ε 640).

To a suspension of platinum black prepared from 500 mg. of platinum dioxide in 50 ml. of absolute ethanol saturated with hydrogen, was added a solution of 2 gm. of the nitroalcohol in 100 ml. of absolute ethanol. During 2 hours 374 ml. of hydrogen was taken up (calculated for the uptake of 3 moles, 369 ml. at 26°). After the catalyst was removed by filtration and washed with hot ethanol, a suspension of 400 mg. of 10 per cent palladium-charcoal and 50 ml. of ethanol containing 1 ml. of concentrated hydrochloric acid was added to the combined filtrates. During 3 minutes 141 ml. of hydrogen was rapidly taken up. The catalyst was filtered and washed with hot ethanol, the combined filtrate was concentrated to a small volume *in vacuo* and allowed to stand in the cold room overnight. The colorless crystalline product (680 mg.) was collected, m.p. 192–195°, reported 192–193° (7). A second crop (150 mg.) was obtained from mother liquors (total yield 58 per cent). $\lambda_{max}^{C_2H_5OH}$: 232 mμ (ε 7100); 282 mμ (ε 2970).

3-Methoxytyramine—3 gm. of α-(3-methoxy-4-benzyloxyphenyl)-β-nitro-ethyl alcohol were mixed with 3 gm. of fused sodium acetate and treated with 10 ml. of acetic anhydride. The mixture was kept close to the boiling point for 5 minutes and then poured into 60 ml. of cold water. An oil separated and quickly crystallized. After standing for 1 hour, the yellow-brown crystals (2.7 gm.) of 3-methoxy-4-benzyloxy-ω-nitrostyrene were collected, washed with water, and dried. Recrystallization from ethanol yielded yellow crystals (2.6 gm.), m.p. 124.5–125°, reported m.p. 122–123° (14).

The mixture of 0.5 gm. of 10 per cent palladium on charcoal and 1.4 gm. of the nitrostyrene in 100 ml. of absolute ethanol containing 1 ml. of concentrated hydrochloric acid and 2 drops of perchloric acid was hydrogenated catalytically. During 2 hours 620 ml. of hydrogen were taken up and the reduction was stopped (calculated for 4 moles, 615 ml. at 25°). The catalyst was filtered off and washed with hot ethanol, the filtrate was concentrated to a small volume and stored in the cold room. The deposited colorless crystals were collected and recrystallized from ethanol (870 mg.) (m.p. 208–212°). An additional crop (110 mg.) from mother liquors raised the total yield to 97 per cent.

$C_9H_{13}NO_2 \cdot HCl$

Calculated: C 53.02, H 6.93, N 6.88

Found: C 52.99, H 7.06, N 6.91

$\lambda_{max}^{C_2H_5OH}$: 228 mμ (ε 6100), 281 mμ (ε 2880).

3-Methoxytyramine was determined by the same procedure described for other methoxyamine metabolites (2).

TABLE I

*R_F values of methoxy metabolites of catechol amines**

Solvent system	Metanephrine		Normetanephrine		3-Methoxytyramine	
	From urine	Synthetic	From urine	Synthetic	From urine	Synthetic
n-Butanol-acetic acid-H_2O, 8:2:2	0.48	0.48	0.36	0.37	0.48	0.48
Isopropyl-alcohol-NH_3 (5 per cent), 8:2	0.80	0.80	0.55.	0.55	0.78	0.78
20 per cent KCl solution.	0.75	0.75	0.72	0.74	0.69	0.70

* Ascending technique with Whatman No. 1 filter paper was used. When chromatograms were sprayed with 0.1 per cent dichloroquinone chlorimide in alcohol followed by 0.1 M pH 10 borate buffer metanephrine and normetanephrine gave blue spots and 3-methoxytyramine gave a brown spot. With diazotized *p*-nitroaniline followed by 1 N ethanolic NaOH, metanephrine and normetanephrine yielded purple colors, whereas 3-methoxytyramine gave an olive spot.

RESULTS

Normetanephrine and Metanephrine in Rat Urine—Urine of six rats (N.I.H. stock) was collected for 24 hours and incubated with 100,000 units of bacterial β-glucuronidase (Sigma) at pH 6.5 for 4 hours at 37°. After incubation the mixture was adjusted to pH 10 with sodium hydroxide and borate buffer and extracted three times with 5 volumes of ethylene dichloride[2] containing 2 per cent isoamyl alcohol.[2] The organic phase was then shaken with 0.04 volume of 0.1 N HCl and the acid extract evaporated to dryness *in vacuo*. The residue was taken up in methanol and evaporated. The concentrated methanol extract was distributed along the starting line of Whatman No. 1 filter paper. After completion of the chromatographic process (ascending technique with butanol-acetic acid-water mixture, 8:2:2) the filter paper was dried and a strip of an area corresponding to an R_F value of 0.35 to 0.55 was cut out and eluted with methanol. The resulting extract had compounds that gave the same R_F values in three solvent systems and the same color reactions as authentic samples of normetanephrine and metanephrine (Table I). The intensity of the color reaction of the normetanephrine spot was greater than that of metanephrine, indicating the excretion of higher amounts of the former compound.

Metanephrine from Epinephrine—Six male rats were given 5 mg. of L-epinephrine-D-bitartrate per kilo of weight intraperitoneally and the urine was collected for 24 hours. To reduce the toxic effects of the catechol amine, 10 mg. of Dibenamine hydrochloride (per kilo of weight) was administered intraperitoneally 30 minutes prior to the injection of epinephrine. An aliquot of the urine was treated with β-glucuronidase, extracted and chromatographed as described above. The resulting extract was the same as synthetic metanephrine with regard to ultraviolet fluorescence spectrum (λ_{max} of fluorescence 335 mμ, λ_{max} of exciting beam 285 mμ), R_F values in three solvent systems (Table I) and distributions in two-phase systems (Table II).

About 21 per cent of the administered epinephrine was excreted as metanephrine glucuronide and 4 per cent as the free methoxy metabolite (*cf.* (2), Table 1). The excretion of total metanephrine was increased 2-fold when the rats were pretreated with iproniazid, a monamine oxidase inhibitor.

[2] All solvents were purified by successive washings with 1 N NaOH, 1 N HCl, and water.

Normetanephrine from Norepinephrine—The isolation of normetanephrine from urine after the intraperitoneal administration of L-normetanephrine-D-bitartrate (5 mg./kilo) followed the same procedure as described above. Normetanephrine isolated from the urine and authentic normetanephrine had the same ultraviolet fluorescence spectra (λ_{max} of fluorescence 335, λ_{max} of exciting beam 285 mμ), R_F values (Table I), color reactions and distribution coefficients (Table II).

The administration of norepinephrine to rats resulted in the excretion of 17 per cent normetanephrine, predominantly as glucuronide, (*cf.* (2), Table 2). After pretreatment with iproniazid the excretion of total normetanephrine was markedly elevated.

3-Methoxytyramine from 3-Hydroxytyramine—Six rats received 10 mg. of 3-hydroxytyramine hydrochloride (per kilo of weight) intraperitoneally and the urine was collected for 24 hours. An aliquot of the urine was incubated with β-glucuronidase and 3-methoxytyramine extracted and chromatographed as previously described. 3-Methoxytyramine isolated from the urine had the same ultraviolet fluorescence spectra (λ_{max} of emitting beam 335 mμ, λ_{max} of exciting beam 285 mμ), R_F values (Table I), color reactions, and distributions (Table II) as an authentic sample of the compound.

About 3 per cent of the administered 3-hydroxytyramine was excreted as 3-methoxytyramine, half of which was conjugated with glucuronic acid. Pretreating rats with iproniazid resulted in a 5-fold elevation in the excretion of total 3-methoxytyramine.

Urine, after treatment with β-glucuronidase, was also examined for homovanillic acid, the deaminated metabolite of 3-methoxytyramine. After acidification with hydrochloric acid, the urine was extracted with 10 volumes of *n*-butanol. The butanol layer was reextracted with 3 per cent sodium bicarbonate solution and the aqueous layer reextracted with *n*-butanol at pH 1. The butanol extract was reduced to a small volume and subjected to paper chromatography with the solvent systems described in Table I. Spots appearing on the chromatograms had the same color reactions and R_F values as those reported for homovanillic acid (16).

Normetanephrine and Metanephrine in Tissues—Several organ tissues which have high concentrations of epinephrine and norepinephrine were examined for the corresponding methoxy derivatives. 10 adult male rats (Osborne-Mendel stock) were decapitated. Adrenal glands, spleens, and brain stems were removed, chilled, and immediately homogenized with an equal volume of 0.1 N HCl. Another group of 10 rats was given 50 mg. of iproniazid phosphate (per kilo of weight) intraperitoneally twice daily for 3 days, and adrenal glands, spleens, and brain stems were prepared as above. The homogenates were adjusted to pH 10.0 with 1 N NaOH and borate buffer and the methoxy compounds extracted as described above except that isoamyl alcohol was used as the solvent. Methanol extracts of the tissues were subjected to two-dimensional chromatography (ascending technique) with Whatman No. 1 filter paper, isopropanol-ammonia (5 per cent), 8:2, being used as the first solvent system and *n*-butanol-acetic acid-water, 8:2:2, as the second. After the chromatogram was sprayed with 0.1 per cent 2,6-dichloroquinone chlorimide in alcohol (17) followed by 0.2 M borate buffer at pH 10.0, adrenal tissue extracts showed the presence of two compounds having the same R_F values and color reaction as metanephrine and normetanephrine (Table III). Chromatograms of spleen extracts had a single blue spot with the same R_F values as normetanephrine. In iproniazid-treated animals the amounts of the methoxy compounds were elevated as indicated by the increased intensity of the blue spots. Neither normetanephrine nor metanephrine was found in brain extracts of untreated rats. However, normetanephrine was present in brain stem extracts of rats that were treated with iproniazid.

TABLE II

Distribution of methoxy metabolites of catechol amines between ethylene dichloride and various buffers

Distributions were carried out in 10 volumes of ethylene dichloride containing 2 per cent isoamyl alcohol and 1 volume of 0.5 M borate buffer. An aliquot of the organic phase was extracted with 0.1 N HCl and the relative concentrations of the compounds were determined by measuring the fluorescence at 335 mμ after activation at 285 mμ. Results are expressed as per cent of compound extracted into the organic phase.

pH	Metanephrine		Normetanephrine		3-Methoxytyramine	
	From urine	Synthetic	From urine	Synthetic	From urine	Synthetic
8	10	12	8	6	20	22
10	52	52	33	33	72	72
11.3	29	28	12	11	56	56
11.7	22	21	10	8	50	51

TABLE III

Methoxy metabolites in tissues

Tissue	Untreated rats		Iproniazid-treated rats	
	Normetanephrine	Metanephrine	Normetanephrine	Metanephrine
Adrenal gland	+	+	++	++
Spleen	+	−	++	−
Brain	−	−	+	−

+ = present; − = absent.

DISCUSSION

These observations, together with related results (2) demonstrate the O-methylation of catechol amines prior to oxidative deamination and indicate that this pathway is a principal route of epinephrine and norepinephrine metabolism. The metabolic interrelationships of catechol amines *in vivo* are summarized in Fig. 1.

The endogenous catechol amine hormones undergo O-methylation in the same fashion. This is demonstrated by their presence in urine, adrenal glands and spleen. The active enzyme is an O-methyl transferase requiring the presence of S-adenosylmethionine and divalent cations and is described in another report (18).

In the light of these facts, the two major metabolites of C^{14}-labeled epinephrine and norepinephrine in rats (19) are best interpreted as the glucuronide of metanephrine (Peak 1 on radiogram of Fig. 1) (19), and 3-methoxy-4-hydroxymandelic acid glucosiduronic acid (Peak 2). The lesser peaks presumably are the unconjugated metabolites. The administration of a monoamine oxidase inhibitor (iproniazid) markedly increases Peak 1 at the expense of Peak 2. This is in agreement with

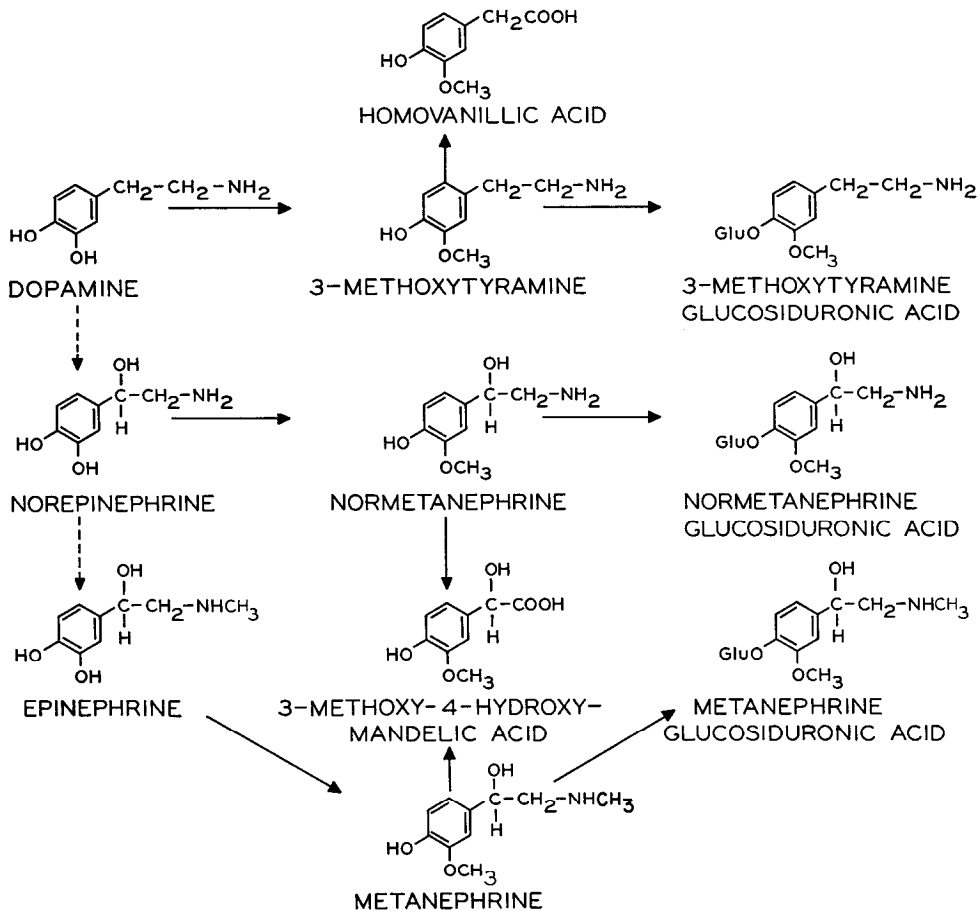

FIG. 1. Metabolism of catechol amines. Although the formation of 3,4-dihydroxymandelic acid from catechol amines *in vivo* has not been demonstrated such a metabolic sequence cannot be disregarded. The compound may arise from epinephrine and norepinephrine if deamination preceded O-methylation.

the observation that the excretion of the glucuronides of metanephrine and normetanephrine is increased more than 2-fold after the administration of epinephrine or norepinephrine together with iproniazid (2).

The successive replacement of phenolic hydroxyl groups of sympathomimetic amines is known to shift the pharmacological activity from the peripheral to the central nervous system with the concomitant loss of pressor action (9). In between extremes, such as the pressor-active epinephrine and the hallucinogenic mescaline, partially methylated analogues still carrying free phenolic hydroxyl groups are now acquiring interest. Normetanephrine which is present in brain after iproniazid deserves special attention with regard to possible psychopharmacological effects. It is conceivable that many of the physiological actions of epinephrine and norepinephrine are mediated through O-methylated metabolites.

SUMMARY

1. Methods for the synthesis of O-methylated metabolites of catechol amines, metanephrine, normetanephrine, and 3-methoxytyramine are described.

2. Normetanephrine (3-O-methyl-normetanephrine) and metanephrine (3-O-methylepinephrine) were present in rat urine as glucosiduronic acids.

3. Normetanephrine was found to be present in rat adrenal glands and spleen, metanephrine in adrenal glands and normetanephrine in brain after pretreatment with iproniazid.

4. After the administration of epinephrine, norepinephrine and 3-hydroxytyramine, the corresponding 3-O-methyl ether metabolites were identified in the urine.

Acknowledgment—It is a pleasure to thank Dr. B. F. Tullar, Sterling-Winthrop Research Institute, for his interest and active cooperation.

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