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Demonstration of the 3-Methoxy Analog of Norepinephrine in Man

Recent studies by Armstrong, McMillan, and Shaw (*1*) have shown that a major metabolite of norepinephrine and epinephrine found in human urine is 3-methoxy-4-hydroxymandelic acid. Subsequently it was shown by Axelrod (*2*) that the 3-hydroxy position of both norepinephrine and epinephrine can be methylated by animal tissues to yield the corresponding 3-methoxy analogs. The 3-methoxy analog of epinephrine (ME) was also reported to be present in rat urine (*2*). Since the conversion of catecholamines to their methoxy analogs may play some role in the physiology of these agents, it seemed important to determine whether these substances exist in man.

Table 1. Chromatographic behavior of methoxy analogs of catecholamines and the compound extracted from four pheochromocytomas. Abbreviations correspond to the methoxy analogs of norepinephrine (MN), epinephrine (ME), and 3,4-dihydroxyphenylethylamine (MD). The chromatograms were sprayed for phenols with a freshly prepared solution of N, 2,6-tri-chloro-*p*-quinoneimine (0.05 percent weight/volume in ethanol) followed by borate buffer, *p*H 9.0 (*4*).

Compound	R_f		Color
	Isopropanol-5% NH_3 (8:2)	Butanol-acetic acid-water (8:1:1)	
1	0.62–0.66	0.31	Blue
MN	0.65	0.30	Blue
ME	0.80	0.28	Blue
MD	0.77	0.40	Tan-Pink

Since preliminary attempts to demonstrate the methoxy amines in human urine were unsuccessful, it was decided to look for them in tissues. A human tumor tissue which can be readily obtained and which contains large amounts of the parent catecholamines is pheochromocytoma. When several such tumors were extracted and the extracts subjected to paper chromatography, they were found to contain appreciable quantities of the 3-methoxy analog of norepinephrine (MN).

The tumors (*3*) were homogenized in 6 volumes of 0.01*N* HCl, adjusted to *p*H 10 by addition of a saturated solution of sodium carbonate, and extracted three times with 5 volumes of n-butanol. To the combined butanol extracts were added an equal volume of heptane and 0.2 volume of 0.5*N* HCl. After shaking, the aqueous layer was evaporated to dryness *in vacuo* and the residue taken up in 10 ml of methanol. The methanol was then evaporated to 0.4 ml, and portions were subjected to chromatography on Whatman No. 1 paper with two different solvents. The results of these studies, summarized in Table 1, indicate that all the tumors contained a butanol extractable base (compound 1) identical with MN, as shown by its R_f and the color obtained with the phenol reagent and ninhydrin. In several instances the areas corresponding to MN on the chromatograms obtained with isopropanol-NH_3 were eluted and rechromatographed with butanol-acetic acid. The R_f values obtained were again identical with those of authentic MN. The tumor containing epinephrine was found also to contain two additional phenolic substances, compounds 2 and 3. Compound 2 appeared on the chromatograms between MN and ME and gave a bluish-green color with the phenol reagent, unlike that of the three available standards. Compound 3 exhibited R_f values and color (tan-pink) identical with those of 3,4-dihydroxyphenylethylamine (MD).

The areas on the chromatogram corresponding to compounds 1, 2, and 3 were eluted with 0.01*N* HCl, and their fluorescence characteristics were compared with those of authentic MN, ME, and MD, by means of an Aminco-Bowman spectrophotofluorometer (Fig. 1). All three compounds and the reference standards exhibited maximal activation at 280 mμ and maximal fluorescence at 330 mμ.

Further evidence about the identity of compound 1 was obtained by eluting it from the chromatogram and subjecting it to distribution between equal volumes of 0.5*M* borate buffer, *p*H 10, and n-butanol. Analyses were carried out spectrophotofluorometrically. The distribution coefficients (concn. in butanol/concn. in aqueous) obtained for compound 1 and MN were 0.43 and 0.44, respectively,

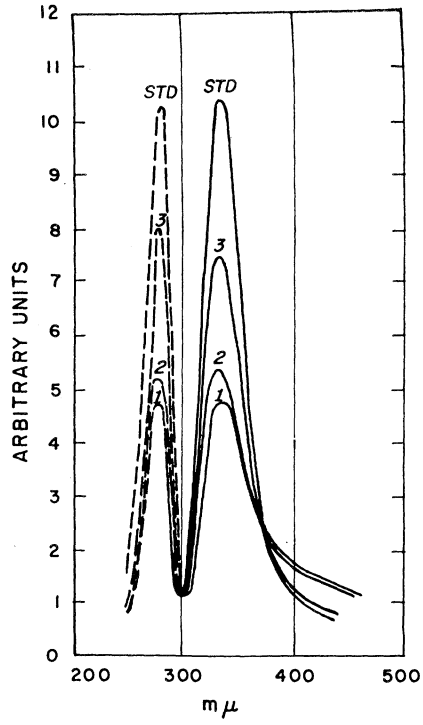

Fig. 1. Activation and fluorescence spectra in 0.01*N* HCl. Compound 1, apparent MN; compound 2, unknown; compound 3, apparent MD, Std., authentic MN; spectra obtained with authentic ME and MD were identical with those of MN.

whereas values for ME and MD were 4.0 and 6.1.

One tumor subjected to quantitative assay for MN by means of a combination of paper chromatography and spectrophotofluorometry was found to contain about 25 μg/g. Preliminary studies indicate that the tumors contain the enzyme which transfers the methyl group from S-adenosylmethionine to the 3-hydroxy group of catecholamines (*5, 6*).

ALBERT SJOERDSMA, WILLIAM M. KING
LEMUEL C. LEEPER
SIDNEY UDENFRIEND
National Heart Institute,
National Institutes of Health,
Bethesda, Maryland

References and Notes

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3. Four pheochromocytomas were frozen immediately after surgical removal and thawed just prior to chemical study. All the tumors were rich in norepinephrine (0.5 to 9.8 mg/g); only one tumor contained significant amounts of epinephrine (2 mg/g).
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5. The 3-methoxy compounds used in these studies were obtained through the generosity of Dr. B. Witkop, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. The advice of Dr. Julius Axelrod, National Institute of Mental Health, is gratefully acknowledged.
6. A more complete study of the biochemistry of catecholamines in pheochromocytoma is in preparation.

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