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3-Methoxy-4-hydroxyphenylglycol sulfate in brain and cerebrospinal fluid

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THE MAJOR urinary metabolites of catecholamines are the products of two enzymatic reactions, deamination and *O*-methylation. The major metabolite of dopamine, 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid), has been found in brain and in cerebrospinal fluid,[1, 2] but attempts to find the corresponding metabolite of norepinephrine (3-methoxy-4-hydroxymandelic acid) have failed.[3, 4]

We have recently demonstrated that the sulfate conjugate of 3-methoxy-4-hydroxyphenylglycol (MHPG) is the major metabolite formed in brain when norepinephrine-^{3}H or normetanephrine-H^3 is injected into the cisterna magna of rats.[5] These findings led us to examine brain and spinal fluid of several species for endogenous 3-methoxy-4-hydroxyphenylglycol sulfate.

Brains of animals killed by decapitation were rapidly removed and homogenized in 4 volumes of ice-cold 0·4 N perchloric acid. Proteins in cerebrospinal fluid obtained by lumbar or ventricular puncture were precipitated by addition of 0·04 vol. of perchloric acid (60 per cent). After centrifugation, aliquots of the clear supernatant equivalent to 1·5–2·0 g brain or 5 ml cerebrospinal fluid were adjusted to pH 5·5 with 1 N sodium hydroxide; 1 ml of 1 N sodium acetate buffer (pH 5·5) and 0·4 ml of a sulfatase (Glusulase, Endo Products, New York) were then added. Free MHPG was extracted and assayed without incubation, while total MHPG was determined after incubation at 37° for 24 hr. The procedures for isolation and assay by gas chromatography of MHPG have been described by Wilk *et al.*[6] When possible, samples were assayed in duplicate and samples with 1 or 2 μg MHPG added were assayed in parallel to assess recovery. In some experiments, a trace amount of MHPG-sulfate-^{3}H (previously isolated from the urine of rats treated with norepinephrine-7-^{3}H) was added to correct for completeness of hydrolysis as well as for recovery of the free MHPG. (Hydrolysis was 40–80 per cent effective, while recovery of hydrolyzed MHPG was 80–90 per cent.)

In other experiments, pooled ethyl acetate extracts of the products of hydrolysis were evaporated *in vacuo* and the dry residue was taken up in a small volume of ethyl acetate and applied to Whatman No. 3 mm filter paper. After chromatography in butanol:ethanol:water (4:1:1), elution and rechromatogramming on thin-layer silica gel (isopropanol:ammonia:water, 8:1:1), a compound was found which had the same R_f value and the same color after treatment with diazotized *p*-nitroaniline as authentic MHPG.

Free or conjugated MHPG was present in the brains of all species examined (Table 1). In the cat there appeared to be little if any conjugated MHPG, while in the rat and guinea pig only MHPG

sulfate was found. In the Green monkey most MHPG was conjugated, but in the Rhesus monkey almost all MHPG was free. MHPG was present mostly in the conjugated form in human spinal fluid. Levels of MHPG sulfate were higher in fluid (four samples) obtained from the cerebral ventricles (250–400 mμg/ml) than in lumbar (6 samples) spinal fluid (50–200 mμg/ml).

TABLE 1. FREE AND TOTAL 3-METHOXY-4-HYDROXYPHENYLGLYCOL IN BRAIN*

Species	Free	Total	N
Rat	0	40–60	(6)
Guinea pig	trace	59,60	(2)
Cat	207	247	(1)
Monkey (Rhesus)	352–627	412–627	(3)
Monkey (Green)	160–162	526–649	(3)

* Results are expressed as mμg/g brain. Number of animals is indicated in parentheses. All samples were assayed in triplicate.

Attempts to identify an acidic *O*-methylated deaminated metabolite of endogenous norepinephrine in brain or spinal fluid have not been successful.[3, 4] The reason for this became apparent when it was found that the sulfate conjugate of MHPG was the major metabolite of intracisternally administered norepinephrine-^{3}H or normetanephrine-^{3}H.[5] This compound is a urinary metabolite of intravenously administered epinephrine-^{3}H in man[7] as well as in experimental animals.[8] Since i.v. administered norepinephrine does not penetrate the blood–brain barrier, this product is not formed exclusively in brain.

The major metabolite of dopamine in brain is homovanillic acid, which is formed by *O*-methylation and deamination. The intermediate aldehyde product of deamination of this phenylethylamine apparently is preferentially oxidized to form the acid.[9] The corresponding α-hydroxyaldehyde metabolite of norepinephrine, however, is reduced to form the glycol.[5, 9] Preliminary studies[10] suggest that enzyme specificity leading to aldehyde reduction rather than to oxidation may depend upon the presence of the α-hydroxyl group.

Although MHPG is mainly present as the conjugate in most species, this is not true in the cat. The finding of free MHPG in this species is consistent with the observations of Mannarino *et al.*[11] that free MHPG is formed from norepinephrine-^{14}C injected into cerebral ventricles of cats. The Rhesus monkey appears to be similar to the cat in this respect.

The levels of MHPG and its conjugate are higher in the monkey and cat than in the rat or guinea pig. This might be due to differences in the rate of formation of the metabolite or in its rate of transport out of the brain. The finding of higher levels of MHPG sulfate in cerebral ventricles than in lumbar spinal fluid of man suggests that there may be transport of the metabolite out of the cerebrospinal fluid during its passage into and through the fourth ventricle. Such transport of substances through the choriod plexus out of brain has been previously described.[12, 13]

Demonstration of MHPG sulfate in brain and spinal fluid and the availability of a sensitive, specific method for its assay provide a new approach to the assessment of turnover and metabolism of endogenous norepinephrine in brain.

Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Md., U.S.A.

SAUL M. SCHANBERG*
GEORGE R. BREESE
JOSEPH J. SCHILDKRAUT†
EDNA K. GORDON
IRWIN J. KOPIN

* Present address: Department of Clinicial Pharmacology and Neurology, Duke University Medical Center, Durham, N.C.

† Present address: Department of Psychiatry, Harvard Medical School, Massachusetts Mental Health Center, Boston, Mass.

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Microsomal monoamine oxidase in sympathetically innervated tissues*

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MOST OF the monoamine oxidase (MAO) from rat liver homogenates appears to be predominantly associated with the mitochondrial fraction,[1–4] although a small portion of the enzyme activity has also been found in the "microsomal" fraction.[2] However, in rat,[5] human,[6] dog,[6] cat[7] and bovine[6] brain as well as in the bovine adrenal medulla[8,9] MAO has been reported to occur exclusively in the mitochondrial fraction. In fact, Rodriquez de Lores Arnaiz and de Robertis concluded that MAO is not present in synaptic vesicles or intact nerve endings.[5] In addition, no MAO has been found in the microsomal fraction of brain homogenates.[6] Recently, Roth and Stjärne[10] have reported that a large portion of the MAO in bovine splenic nerve appears to sediment not with the mitochondria, but rather in the fraction containing the amine storage granules. Further investigation revealed that during density gradient centrifugation the MAO in this preparation did not sediment any further than the amine storage granules in a linear sucrose gradient. In fact, the MAO activity peaked in a fraction less dense than that of the major amine peak.[11] The significance of this finding was unclear, but could be indicative of any of the following possibilities: (a) that the microsomal-like MAO may be contained in fragments of mitochondrial membrane sheared off during homogenization; (b) that splenic nerve tissue contains a specialized type of mitochondria with different sedimentation properties and electron microscopic morphology; (c) that some MAO in this tissue is located in a site other than mitochondria, perhaps in the amine storage granules. Therefore, an investigation was conducted to compare the distribution of MAO activity in rat liver with that in sympathetically innervated tissue such as the rat heart, vas deferens, salivary gland and also the bovine splenic nerve by means of techniques previously applied for the isolation of amine storage particles from bovine splenic nerve and rat heart.[12]

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