Biochemical Pharmacology, Vol. 20, pp. 2095–2096. Pergamon Press, 1971. Printed in Great Britain

Studies on the detection of 3-methoxy-4-hydroxyphenylethanol in human cerebrospinal fluid*

(*Received* 12 *September* 1970; *accepted* 9 *November* 1970)

DOPAMINE is metabolized by catechol-*O*-methyl transferase and monoamine oxidase to an unstable aldehyde intermediate, 3-methoxy-4-hydroxyphenyl acetaldehyde. The aldehyde is then either oxidized to homovanillic acid (HVA)[1] or reduced to the corresponding alcohol, 3-methoxy-4-hydroxyphenyl-ethanol (MHPE).[2] In humans, the major metabolite of dopamine is HVA. Recent studies using rabbit cerebral cortex slices[3] and guinea-pig brain slices[4] indicate that the enzyme catalyzing reduction to MHPE possesses specificity for aldehydes containing an α-hydroxyl group, whereas substituted phenylacetaldehydes are preferentially oxidized to the acid. In the brain, norepinephrine may be preferentially metabolized to the alcohol, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG).[5,6] Increased metabolism to the alcohol can be induced by aldehyde dehydrogenase inhibitors such as disulfiram[7] and carbodiimide.[2] Ethanol ingestion has also been shown to favor production of alcohol metabolites of norepinephrine,[8] serotonin[9] and tyramine.[10]

The metabolism of dopamine in humans was studied by Goodall and Alton[11] by i.v. administration of [^{14}C]dopamine followed by sequential collection of urine specimens. Alcohol metabolites accounted for only 1 per cent of the total metabolites isolated. This study, however, only reflects peripheral dopamine metabolism. Demonstration of successful treatment of Parkinsonism with L-DOPA[12] has stimulated investigators to find means of assessing cerebral dopamine metabolism. If the dopamine metabolite, MHPE, is formed to a significant extent in man, it would seem likely that this would occur in brain and possibly be reflected in cerebrospinal fluid. A simplified gas–liquid chromatographic procedure for the detection of MHPE in cerebrospinal fluid has been developed and is described here. The principles of the assay are similar to those utilized in the gas–liquid chromatographic determination of urinary MHPG,[13] and in quantitative analysis of MHPG in cerebrospinal fluid.[14,15]

Human lumbar spinal fluid was obtained from patients at Mount Sinai Hospital, in some instances pooled, and frozen. To a 2-ml sample of cerebrospinal fluid was added 0·5 ml of 0·05 M citrate buffer, pH 5·2, and 0·05 ml glusulase (Endo Corp.). After incubation for 16 hr at 37°, the MHPE was extracted by shaking with two 6-ml portions of ethyl acetate. The pooled ethyl acetate extracts were evaporated to dryness and the residue was reacted with 0·25 ml trifluoroacetic anhydride in the presence of 1 ml ethyl acetate (pesticide grade) for 30 min at room temperature. The reaction mixture was then evaporated to dryness under nitrogen and the residue dissolved in 1 ml ethyl acetate for chromatography. Chromatography was carried out using a Packard gas chromatograph equipped with an electron capture detector. The instrument was operated at 50 V and at a sensitivity setting of 1×10^{-9} amps. Authentic MHPE yielded a single peak after reaction with trifluoroacetic anhydride, presumably that of the bis-trifluoroacetate. The resultant derivative possessed excellent electron capture properties conferred by trifluoroacetylation of hydroxyl groups.[16]

MHPE-trifluoroacetate was separated from other interfering compounds derived from CSF on a 6 ft × 4 mm (i.d.) column of 2% XF-1105–3% OV-17 (3:1, w/w) coated on gas chrom Q 80–100 mesh at 125° and at a nitrogen flow rate of 35 cc/min. Addition of authentic MHPE (15–500 ng) to parallel CSF samples and carried through the procedure yielded peak areas proportional to the amount of added MHPE.

No MHPE was detected in any of the CSF specimens analyzed. With 2 ml CSF the limit of detectability was 7·5 ng/ml. A 10-ml specimen was then analyzed in a similar fashion and MHPE was not detectable. Under the latter conditions, the limit of detectability was 1·5 ng/ml. Since HVA levels of 23–40 ng/ml have been reported,[17,18] it appears likely that the reductive pathway for dopamine metabolism in the human brain is a minor one, which is in accord with animal studies and underlines the differences in the metabolism of dopamine and norepinephrine in brain.[2–4,19]

The method described may be of value in the assay of MHPE in cerebrospinal fluid after treatment with L-DOPA, aldehyde dehydrogenase inhibitors, ethanol and other drugs that might elevate MHPE.

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* This investigation was supported by a Public Health Service Research Career Development Award (No. 1K4-GM-40, 793-01).

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Biochemical Pharmacology, Vol. 20, pp. 2096–2099. Pergamon Press, 1971. Printed in Great Britain

Microsomal metabolism of morphine in a hyperbaric helium environment*

(*Received* 6 *August* 1970; *accepted* 16 *October* 1970)

AS MAN begins to explore the continental shelf at depths of 600 ft or greater, it is inevitable that his confrontation with the environment will result in conditions demanding immediate treatment with drugs. Treatment will be necessarily administered at depth since safe decompression may take a considerable time period. Because of nitrogen's narcotic effect at depths approaching 250 ft, an atmosphere in which nitrogen is at least partially replaced by helium must be used in deeper saturation diving. A helium–oxygen atmosphere has been shown to support life in man at pressures equivalent to 1500 ft,[1] and in animals to 4000 ft[2] without apparent ill effects. Due to the change in gaseous environment and the possible effects of pressure *per se*, it is conceivable that a drug's interaction with man may be qualitatively and/or quantitatively different at depth than on the surface. The effect of an environment of 20·8 atmospheres absolute (at.a.) of helium with 0·2 at.a. of oxygen on the ability of rat liver microsomes to metabolize morphine was undertaken as an *in vitro* approach to this problem. From this investigation it was concluded that hyperbaric helium does not affect the *in vitro* metabolism of morphine.

Male Sprague–Dawley rats (180–200 g) were decapitated, and their livers were removed and placed in an ice-cold solution of 1·15% KCl–0·02 M tris buffer (pH = 7·4). The livers were blotted, weighed,

* From the Bureau of Medicine and Surgery, Naval Department, Research Task No. MR005.22-0001A. The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. The experiments were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care".