VMA IN SPINAL FLUID: EVALUATION OF THE PATHWAYS OF CEREBRAL CATECHOLAMINE METABOLISM IN MAN

SHERWIN WILK and ERIC WATSON
Department of Pharmacology, Mount Sinai School of Medicine of The City University of New York, Fifth Avenue and 100th Street, New York, N.Y. 10029, U.S.A.

THE PATHWAYS of cerebral catecholamine metabolism have been evaluated in animals by application of radioisotopic techniques. These studies involved incubation of brain slices with labelled norepinephrine (NE), dopamine (DA) and their precursors or intraventricular or intracisternal administration of these compounds. These studies have indicated that dopamine is preferentially metabolised to the acids, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC), whereas norepinephrine is preferentially metabolised to the alcohols, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) and dihydroxyphenylglycol (MANNARINO *et al.*, 1963; RUTLEDGE and JONASON, 1967; BREESE *et al.*, 1969; JONASON, 1969); Because of the inaccessibility of the human brain to direct study, similar information for man is lacking. Evaluation of cerebral catecholamine metabolism in man is best achieved by study of the metabolites of the catecholamines in cerebrospinal fluid (CSF) (MOIR *et al.*, 1970). The occurrence of HVA and MHPG in CSF has been documented and these metabolites can be accurately quantitated (ANDEN *et al.*, 1963; WILK *et al.*, 1971). In man the major NE metabolite excreted in the urine is vanillylmandelic acid (VMA) (ARMSTRONG *et al.*, 1957). The presence of this compound in brain and CSF of man or other species has not been documented (SHARMAN, 1971). To evaluate the metabolic significance of VMA in human brain, a highly sensitive method was developed which is of general use for the quantitation of acidic metabolites of biogenic amines in brain and CSF (WILK and WATSON, 1973). This procedure utilises gas–liquid chromatography and electron capture detection (GLC-ECD).

The esterification of acids with trichloroethanol using trifluoroacetic anhydride as catalyst was reported in 1971 (SMITH and TSAI, 1971). Utilising the principles described in this publication, we developed a new reagent, 20% pentafluoropropanol in pentafluoropropionic anhydride, for the preparation of derivatives of phenolic acids which possess excellent electron capture properties. In this reaction, the anhydride catalyses esterification of the carboxyl group with pentafluoropropanol and itself reacts with the hydroxyl groups. Derivatives of VMA, HVA, DOPAC, 5-hydroxy indoleacetic acid (5-HIAA), and *p*-hydroxy phenylacetic acid were prepared. Excellent separation of these compounds was obtained on a 3% OV-17 column. The derivative of 5-HIAA was chromatographed at 145° whereas the other acid derivatives were chromatographed at 115°.

Although as little as 15 pg of these derivatives can be detected, the true sensitivity of this method is dependent upon the fraction of sample that can be injected onto the column. This in turn is governed by the sample background. The anticipated low levels of VMA in CSF led to the consideration of a system that could offer the highest degree of sample purification. This was achieved by thin layer chromatography (TLC).

The procedure developed for the quantitation of VMA and HVA in CSF involves acidification of the sample and extraction of the acids into ethyl acetate. After evaporation of the solvent, the residue is applied to a cellulose coated TLC plate and chromatographed in a benzene–acetic acid–water (100–50–2) system. Zones corresponding to the acids are eluted, the eluate acidified and the acids reextracted into ethyl acetate. The solvent is removed under nitrogen and the residue treated with the anhydride–alcohol mixture for 15 min at 75°. The derivatives are chromatographed on the 3% OV-17 column. Recoveries have averaged 60 per cent. The purity of these samples permits one-tenth of the total sample to be applied to the column.

All spinal fluid samples examined had very low levels of VMA (< 2ng/ml). Accurate quantitation of a 10 ml CSF sample gave a value of 0·50 ng VMA/ml (Fig. 1). Because of these low levels the identity of the peak attributed to VMA could not be confirmed by auxillary techniques such as mass spectrometry. However, these studies indicate that VMA in CSF is present at levels less than 2 ng/ml. In contrast, HVA can be easily quantitated. HVA levels in CSF have averaged 30 ng/ml.

The alcohol metabolites of NE and DA can be measured by GLC-ECD after formation of trifluoroacetyl derivatives. Levels of MHPG in CSF average 16 ng/ml (WILK *et al.*, 1971). The alcohol metabolite of dopamine 3-methoxy-4-hydroxyphenyl ethanol, was not detected in CSF (WILK, 1971). If present levels of this compound are less than 1·5 ng/ml.

The MHPG/VMA ratio in CSF (~16) is in sharp contrast to this ratio in urine (~ 0·5) (WILK *et al.*, 1965; WILK *et al.*, 1967). To eliminate the possibility that VMA is rapidly transported out of CSF, preliminary studies were carried out on patients who were treated with large doses of probenecid (100 mg/kg) (GOODWIN *et al.*, 1973). Measurement of CSF probenecid levels in such studies is of importance since it was shown

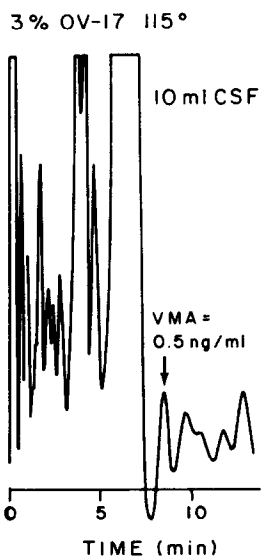

FIG. 1.—The demonstration of VMA in CSF. 10 ml of CSF was purified by thin layer chromatography where R_f values were: VMA = 0·25, 5-HIAA = 0·25, DOPAC = 0·25, *p*-hydroxyphenylacetic acid = 0·70, HVA = 0·80. Retention time of derivatives on 3% OV-17 at 115° relative to VMA: *p*-hydroxyphenylacetic acid = 0·58, DOPAC = 0·82, HVA = 1·90. The 5-HIAA derivative is chromatographed at 145°.

that the accumulation of acidic metabolites in CSF is linearly related to the CSF probenecid concentration (KORF and VAN PRAAG, 1971; SJOSTROM, 1972). The pentafluoropropanol–pentafluoropropionic anyhdride reagent can derivatise probenecid and measurement of CSF probenecid levels can be achieved using as little as 50 microliters of CSF. In these studies HVA accumulated to several hundred ng/ml whereas after probenecid the level of VMA in CSF averaged 2 ng/ml. Therefore rapid transport of VMA out of CSF cannot account for the finding of low levels of this metabolite.

The metabolism of catecholamines in human brain has been evaluated by measurement of the endogenous levels of metabolites in CSF. This approach avoids the difficulties inherent in the use of radioisotopes in which one assumes mixing of endogenous and exogenous material. The assumption made in the studies reported here is that metabolites in the CSF accurately reflect metabolism in brain. While there is good evidence that HVA in CSF has its origin in brain (PAPESCHI *et al.*, 1971; CURZON *et al.*, 1971) the relative contribution of spinal cord NE to the levels of the NE metabolites in CSF is still unknown.

On the basis of these gas chromatographic determinations one may conclude that within the central nervous system of man NE is preferentially metabolised to the alcohol, MHPG, whereas DA is preferentially metabolised to the acid HVA. These results are in agreement with a growing body of literature from animal studies and provide the first direct evidence that within the central nervous system of man the predominant pathway of NE metabolism is reductive to MHPG.

Acknowledgements—Supported by a grant from the NIMH: MH-21638-02 and a Research Career Development Award to S. W. #1K4-GM-40 793-01.

REFERENCES

ANDEN N.-E., ROOS B.-E. and WERDINIUS B. (1963) *Life Sci.* **2**, 448–458.
ARMSTRONG M. D., MCMILLAN A. and SHAW K. N. F. (1957) *Biochim. Biophys. Acta* **25**, 422–423.
BREESE G. R., CHASE T. N. and KOPIN I. J. (1969) *J. Pharmacol. Exp. Ther.* **165**, 9–13.
CURZON G., GUMPERT E. J. W. and SHARPE D. M. (1971) *Nature, New Biol.* **231**, 189–191.
GOODWIN F. K., POST R. M., DUNNER D. L. and GORDON E. K. (1973) *Am. J. Psychiat.* **130**, 73–79.
JONASON J. (1969) *Acta Physiol. Scand. Suppl.* **320**, 1–50.
KORF J. and VAN PRAAG H. M. (1971) *Brain Res.* **35**, 221–230.
MANNARINO E., KIRSHNER N. and NASHOLD B. (1963) *J. Neurochem.* **10**, 373–379.
MOIR A. T. B., ASHCROFT G. W., CRAWFORD T. B. B., ECCLESTON D. and GULDBERG H. C. (1970) *Brain* **93**, 357–368.
PAPESCHI R., SOURKES T. L., POIRIER L. J. and BOUCHER R. (1971) *Brain Res.* **28**, 527–533.
RUTLEDGE C. O. and JONASON J. (1967) *J. Pharmacol. Exp. Ther.* **157**, 493–502.
SHARMAN D. F. (1971) in *Methods of Neurochemistry* (FRIED R., ed.) Vol. 1, pp. 83–127. Marcel Dekker New York.
SJOSTROM R. (1972) *Psychopharmacologia (Berl.)* **25**, 96–100.
SMITH R. V. and TSAI S. L. (1971) *J. Chromatogr.* **61**, 29–34.
WILK S. (1971) *Biochem. Pharmacol.* **20**, 2095–2096.
WILK S., DAVIS K. L. and THACKER S. B. (1971) *Anal. Biochem.* **39**, 498–504.
WILK S., GITLOW S. E., CLARKE D. D. and PALEY D. H. (1967) *Clin. Chim. Acta* **16**, 403–408.
WILK S., GITLOW S. E., MENDLOWITZ M., FRANKLIN M. J., CARR H. E. and CLARKE D. D. (1965) *Anal. Biochem.* **13**, 544–551.
WILK S. and WATSON E. (1973) *Fedn. Proc.* **32**, 798.