

using animals that had been older (80-100 g) at the start of the thiouracil treatment. In this case, control rats with body weights of 242 g had hearts weighing 636 mg and hypothyroid rats weighing 176 g had heart weights of 446 mg. The MAO half-lives were 12.0 and 12.3 days respectively.

These results suggest that the half-life of cardiac MAO in the rat is related solely to the age of the animal, and cannot be affected by the artificial alteration of either the body weight or the weight of the heart by hormonal manipulation such as that used here although significant changes in enzyme activity may be produced. These results also suggest that thyroid hormones increase the rate of synthesis of rat heart MAO.

G.A.L. is a Medical Research Council Scholar.

References

- CALLINGHAM, B.A. & DELLA CORTE, L. (1972). The influence of growth and of adrenalectomy upon some rat heart enzymes. *Br. J. Pharmac.*, **46**, 530-531P.
- CALLINGHAM, B.A. & LAVERTY, R. (1973). Studies on the nature of the increased monoamine oxidase activity in the rat heart after adrenalectomy. *J. Pharm. Pharmac.*, **25**, 940-947.
- HORITA, A. (1967). Cardiac monoamine oxidase in rat. *Nature, Lond.*, **215**, 411-412.

A gas-liquid chromatographic method for the estimation of the acidic metabolites of dopamine in cerebrospinal fluid and brain tissues

J.D.M. PEARSON* & D.F. SHARMAN

Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

Fluorimetric methods have not proved sensitive enough for the estimation of the acidic metabolites of dopamine 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA) in discrete areas of the brains of small laboratory rodents without pooling tissues from several animals. The present method was developed to study the possible relation between the cerebral metabolism of dopamine in different brain regions and behaviour in individual rats and mice.

The formation of a trifluoroacetyl-hexafluoroisopropyl derivative of HVA and its measurement by gas-liquid chromatography (GLC) with electron capture detection (ECD) was reported by Dziedzic, Bertani, Clarke & Gitlow (1972). We have applied the formation of such derivatives to the simultaneous estimation of DOPAC, HVA and 3-hydroxy-4-methoxyphenylacetic acid (homovanillic acid; *iso*-HVA). The acids were extracted from acidic, KCl saturated, deproteinized tissue extracts into ethyl acetate. This organic phase was evaporated to dryness and reacted with trifluoroacetic anhydride and hexafluoroisopropanol in a closed reaction vial at 100°C for 60 min. The reaction mixture was

cooled and evaporated just to dryness under a stream of nitrogen at room temperature. The residue was dissolved in a measured volume of dry ethyl acetate containing a known concentration of pentafluorophenyl benzoate which serves as a reference standard for the quantification of the trifluoroacetyl hexafluoroisopropyl derivatives of DOPAC, HVA and *iso*-HVA. Crystalline samples of the three derivatives have been prepared to determine standard peak height ratios. Up to 3 µl of the final solution was used for gas chromatography using a Pye Model 104 gas chromatograph fitted with an electron capture detector, using a 9 ft column (glass) packed with 2% SE52 on Diatomite CQ. The carrier gas was Argon/5% methane at flow rates of 40-60 ml/minute. Recoveries of authentic DOPAC and HVA added to brain tissue carried through the procedure averaged $53 \pm 4\%$ (s.e. mean) ($n = 14$) for DOPAC and $64 \pm 8\%$ (s.e. mean) ($n = 14$) for HVA. The method has been successfully applied to the estimation of DOPAC and HVA in discrete regions of the rat and mouse brain, the pig and rabbit superior cervical ganglion, aqueous and vitreous humours, retinal tissue and to samples of cerebrospinal fluid from the pig and the sheep.

J.D.M.P. is supported by the Medical Research Council.

Reference

- DZIEDZIC, S.W., BERTANI, L.M., CLARKE, D.D. & GITLOW, S.E. (1972). A new derivative for the gas-liquid chromatographic determination of homovanillic acid. *Analyt. Biochem.*, **47**, 592-600.