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An effect of thyroid hormones upon monoamine oxidase activity

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It is known that the activity of the monoamine oxidase (MAO) in the rat heart increases with age (Horita, 1967). It may be that this increase is related to other consequences of ageing, such as the increases in heart and body weights. Previous studies of MAO recovery rates after pargyline treatment suggest that the age-related increase in enzyme activity is due to a decrease in the rate of enzyme degradation (Callingham & Della Corte, 1972).

Male Wistar rats were made hyperthyroid by daily injections of (−)-thyroxine (660 μg/kg, s.c. for at least 16 days), or hypothyroid by the addition of 2-thiouracil (0.2%) to their diet for at least six weeks. At the beginning of each experiment the rats weighed between 50 and 70 g. MAO activity in tissue homogenates was measured by a radiochemical method and expressed in terms of mg of protein (Callingham & Laverty, 1973). For the determination of MAO half-lives a single dose of pargyline was administered (25 mg/kg, s.c.) while continuing thyroid or drug treatment.

Treatment with (−)-thyroxine caused an increase in the activity of the cardiac MAO, particularly in younger animals, together with hypertrophy of the tissue. In control rats weighing 133 g the mean weight of their hearts was 445 mg and the half-life of their cardiac MAO was 3.7 days. Hyperthyroid rats weighed about the same (114 g) but the weight of their hearts was 592 mg with a half-life of 4.2 days for their MAO. However, older control rats with hearts weighing about the same as those of hyperthyroid animals had a half-life of about 9 days for their cardiac MAO.

In hypothyroid rats the cardiac MAO activity was significantly reduced to 71% of that in the controls ($P < 0.02$). But after pargyline there was no difference in the duration of the half-lives of the MAO from the two groups being between 8.5 and 9.0 days for both, although both body and heart weights were significantly less in the hypothyroid animals compared with the controls (body weights: 96 and 208 g, and heart weights: 313 and 580 mg respectively). This result could be repeated

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.

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

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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-hexafluoro-*iso*propyl 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 (homo-*iso*vanillic 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 hexafluoro*iso*propyl 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.

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