was present in all nuclei at 4 h and varied from 145 ± 13.5% of control dopamine levels in the n. interstitialis striae terminalis (n.s.) to 1560 ± 97.1% in the n. anterior (hypothalami) (100 ± 39.5%, $P < 0.001$). At 12 h the levels of α-methyldopamine had dropped markedly in all nuclei. By comparison, noradrenaline levels were higher at 12 h than at 4 h in all AH/PO nuclei. At 12 h the combined levels of noradrenaline and α-methylnoradrenaline ranged from 137 ± 33.5% of control noradrenaline levels in n. interestitialis striae terminalis (n.s.) to 175 ± 12.0% of control noradrenaline in the n. lateralis (hypothalami) (100 ± 18.1%, $P<0.01$). In the medullary nuclei noradrenaline levels were lower at 12 h than at 4 h, however in the n. tractus solitarius levels of noradrenaline plus α-methylnoradrenaline at 4 h were 290 ± 21.6% of control noradrenaline levels (100 ± 17.8%, $P<0.001$).

These results indicate that, after acute α-MDOPA administration, α-methylnoradrenaline is formed in particular areas in the brain where it may produce a fall in blood pressure.

This research was supported by a grant from the National Health and Medical Research Council of Australia.

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Measurement of tyrosine hydroxylase in the guinea pig brain

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The hydroxylation of tyrosine *in vitro* is the first step in the synthesis of catecholamines. A method for the measurement of tyrosine hydroxylase activity *in vitro* has been described (Nagatsu, Levitt & Udenfriend, 1964). Later Nagatsu, Sudo & Nagatsu (1971) investigating tyrosine hydroxylase in the bovine caudate nucleus were unable to produce a linear increase in L-DOPA production with increasing amounts of tissue, unless the enzyme was purified. It has been shown that centrifuging homogenates of striatal tissue and assaying the enzyme activity in the supernatant gives a linear response when increasing amounts of supernatant are used (Coyle, 1972).

Using an adaptation of the Nagatsu method the caudate nucleaus of the guinea pig was homogenized in water (100 mg/ml) and the homogenate was incubated in a medium containing dimethyl glutarate buffer pH 6.0 (40 mM), $FeSO_4$ (0.2 M), dithiothreitol (1 mM), 6-MpH_4 (2 mM) and tyrosine (0.5 mM). Plots of enzyme activity against the amount of homogenate used showed, in many cases, a decrease in L-DOPA production as the amount of homogenate was increased. Assaying the supernatant after centrifugation (20,000 *g* for 30 min) produced a plot which showed an initial increase in L-DOPA production with increasing amounts of supernatant, but there was a subsequent decrease in activity as the higher concentrations of caudate nucleus extract were reached (Figure 1).

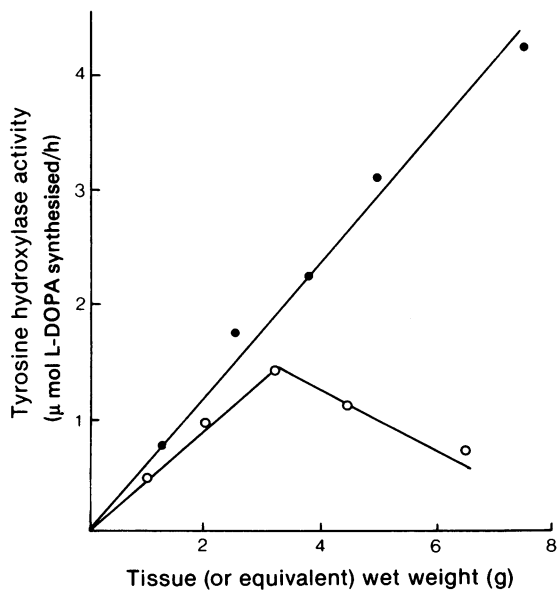

Figure 1 The production of L-DOPA with increasing amounts of guinea pig caudate nucleus homogenate supernatant (○) and acetone powder supernatant (●).

These observations suggest that an endogenous inhibitor is present in the caudate nucleus extract. In an attempt to remove this inhibitor, acetone-dried powders of the caudate nucleus were prepared. The activity was present in the supernatant after the powder had been resuspended in phosphate buffer pH 6.0 (0.1 M). Increasing concentration of this supernatant gave a linear increase in L-DOPA production (Figure 1). The acetone used for the preparation was evaporated and the residue taken up in buffer; this solution significantly inhibited the enzyme. The inhibitory factor in this solution has not yet been identified.

The tyrosine hydroxylase was very unstable both in tissue homogenates and in the supernatant of the acetone powder, losing more than 50% of its activity in 24 h at −18°C. The enzyme in the acetone dried powder was, however, reasonably stable at −70°C. The most satisfactory results were obtained when the enzyme activity in the powder was assayed immediately after the powder was prepared. The tyrosine hydroxylase activity was estimated to be 0.62 ± 0.044 μmol L-DOPA formed/g guinea pig caudate nucleus per hour.

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The effect of fasting on the formation of 4-hydroxy-3-methoxyphenyl-acetic acid (homovanillic acid) and 5-hydroxyindolylacetic acid in the brain of the mouse.

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Fasted rats have an increased concentration of brain 5-hydroxyindolylacetic acid (5-HIAA) which has been attributed to an increase in the synthesis and turnover of 5-hydroxytryptamine (5-HT) (Tagliamonte, Biggio, Vargiu & Gessa, 1973). The possibility that fasting also increases the concentration of homovanillic acid (HVA), one of the acidic metabolites of dopamine, was investigated.

The HVA content of the caudate nuclei and the 5-HIAA content of the forebrain were determined in male albino mice kept in reversed daylight and killed at least two h after the beginning of darkness. The HVA and 5-HIAA were estimated fluorimetrically by the methods of Murphy, Robinson & Sharman (1969) and Ahtee, Sharman & Vogt (1970).

After 20 h fasting, mice showed a higher content of HVA in the caudate nucleus and of 5-HIAA in the forebrain (2.1 ± 0.1 and 0.35 ± 0.01 μg/g respectively) than fed mice (1.3 ± 0.09 and 0.29 ± 0.01 μg/g respectively). Yet, the administration of probenecid produced a smaller increase in the HVA concentration in fasted than in fed mice (33.2% vs 108%). This result could be due to a blockade of the active transport system which removes this metabolite from the brain. If this explanation were correct, the administration of probenecid should affect the content of 5-HIAA in fasted and fed mice in the same way as it affected HVA because both metabolites use a similar transport system. However, after probenecid the increase of 5-HIAA in fasted mice was larger than in fed mice (the opposite result to that found with HVA), confirming that fasted animals have an increase in 5-HT turnover and suggesting a functionally unaltered transport system for HVA and 5-HIAA during fasting.

These results could be explained by the existence of a pool of HVA at a site within the caudate nucleus where the probenecid-sensitive transport is not effective. This hypothetical pool would be increased by fasting and might also be identical with the probenecid-resistant pool suggested by Sharman (1967) to explain the effect of some drugs on the striatal HVA content. Finally, some experiments were carried out to test whether fasting affected the concentration of dihydroxyphenylacetic acid (DOPAC) in the mouse caudate nucleus. No change was found, so the possibility should be considered that there is a subpopulation of dopaminergic neurons, the activity of which leads to the formation of HVA but not DOPAC.

Supported by a grant of the Consejo de Desarrollo Cientifico y Humanistico, Universidad Central de Venezuela, Caracas.