homogenates of hypothalamus were measured according to Horn & Snyder (1972).

Mazindol rivalled desipramine and (+)-amphetamine in blocking the *in vitro* uptake of [^{3}H]-NA and [^{3}H]-DA by hypothalamic and striatal synaptosomes respectively. Of the compounds studied for inhibition of [^{3}H]-5-HT uptake mazindol, desipramine and fenfluramine were of comparable potency and all three compounds were less potent than either chlorimipramine or imipramine.

Following 1 h pretreatment mazindol was approximately 2.5 times more potent than desipramine in blocking hypothalamic synaptosomal [^{3}H]-NA uptake. Mazindol, by contrast with tricyclic antidepressants, also inhibited striatal [^{3}H]-DA uptake. At 1 h after injection of desipramine, fenfluramine and chlorimipramine (each 20 mg/kg i.p.), [^{3}H]-5-HT uptake by hypothalamic synaptosomes was blocked 14.6 ± 2.5 ($P<0.05$), 36.9 ± 2.1 ($P<0.001$) and 50.9 ± 1.0% ($P<0.001$) respectively. By contrast, [^{3}H]-5-HT uptake was not significantly inhibited (4.6 ± 2.3%) following 1 h pretreatment with mazindol (30 mg/kg, i.p.).

Results from *ex vivo* experiments agree with data from *in vivo* studies. For example, mazindol is not only more potent than desipramine in blocking NA uptake but is also essentially devoid of effect on 5-HT uptake. In contrast to the *ex vivo* and *in vivo* findings mazindol blocks 5-HT uptake *in vitro* and has a potency comparable to that of desipramine and fenfluramine, drugs which significantly inhibit the *ex vivo* uptake of the monoamine. Why mazindol is active in blocking 5-HT uptake *in vitro* but not *ex vivo* and *in vivo* is not readily apparent. Furthermore, these findings suggest that it may be invalid to extrapolate an *in vitro* inhibition of monoamine uptake to the *in vivo* situation.

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Effect of acute α-methyldopa administration on catecholamine levels in anterior hypothalamic-preoptic and medullary nuclei in rat brain

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α-Methyldopa (α-MDOPA) is now considered to produce a major component of its hypotensive effect via the central actions of its metabolite, α-methylnoradrenaline (Henning, 1975). Injection of α-methylnoradrenaline into specific areas within the anterior hypothalamic-preoptic (AH/PO) region and into the nucleus (n.) tractus solitarius in the medulla oblongata produces a fall in blood pressure in anaesthetized rats (Struyker-Boudier, Smeets, Brouwer & Van Rossum, 1975; De Jong & Nijkamp, 1976). These authors have suggested that these areas may be directly involved in producing the hypotensive effect of α-MDOPA. However, no studies have yet looked at levels of the metabolites of α-MDOPA in these areas after α-MDOPA injection. We have therefore, examined the effects of acute administration of α-MDOPA on catecholamine levels in several AH/PO nuclei and in the n. tractus solitarius and n. tractus spinalis nervi trigemini in the medulla oblongata.

Nuclei were dissected from fresh 400 μm thick coronal sections of brain tissue from adult Sprague-Dawley rats. Nuclei from three animals were pooled and catecholamine levels were estimated by modifying the radiochemical-enzymatic assay procedure of Van Der Gugten, Palkovits, Wijnen & Versteeg (1976). After acute administration of α-MDOPA (200 mg/kg s.c.) levels of both noradrenaline and dopamine declined in all nuclei. Noradrenaline levels were lower 12 h after α-MDOPA than 4 h and ranged from 16 ± 6.7% (mean ± s.e. mean, $n = 5$) of control in the n. interstitialis striae terminalis (100 ± 6.7%, $P < 0.001$) to 23 ± 5.1% of control in the n. preopticus medialis (100 ± 12.7%, $P < 0.001$). At 12 h no noradrenaline was detected in the medullary nuclei. Endogenous dopamine levels were undetectable in the medullary nuclei. At 4 h after α-MDOPA dopamine could not be detected in the n. anterior (hypothalami), n. lateralis (hypothalami) and n. preopticus medialis while dopamine levels had been reduced to 19 ± 8.9% of control in the n. interstitialis striae terminalis (100 ± 29.3%, $P < 0.05$) and 13 ± 6.9% in the n. preopticus lateralis (100 ± 32.1%, $P < 0.05$). Dopamine levels were still depressed at 12 hours. α-Methyldopamine

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.

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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).

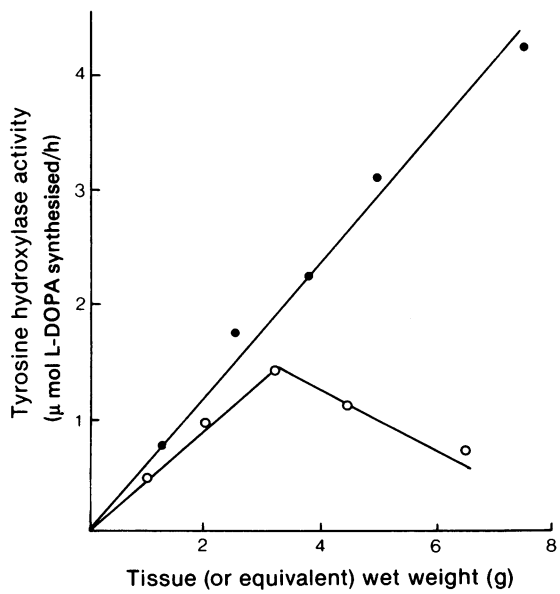

Figure 1 The production of L-DOPA with increasing amounts of guinea pig caudate nucleus homogenate supernatant (○) and acetone powder supernatant (●).