Central dopamine and noradrenaline receptor activity of the amines formed from *m*-tyrosine, α-methyl-*m*-tyrosine and α-methyldopa

Like the dopamine and noradrenaline precursor, L-3,4-dihydroxyphenylalanine, the amino-acids *m*-tyrosine, α-methyl-*m*-tryrosine and α-methyldopa are decarboxylated *in vivo* to amines, which are partly β-hydroxylated in the noradrenaline neurons (Carlsson & Lindqvist, 1962; 1967). Treatment with α-methyldopa but not with α-methyl-*m*-tyrosine produces hypotension in rats (Henning, 1967). The hypotensive effect of α-methyldopa appears to be due to formation of central amines since it is not observed after inhibition of the central decarboxylase activity (Davis, Drain & others, 1963) but is present after selective inhibition of the peripheral decarboxylase activity (Henning, 1969). Other pharmacological effects may also be induced by the amines formed in the central nervous system. Very little is known, however, about the central receptor activity of these dopamine and noradrenaline analogues. Therefore we have studied a possible stimulation of the dopamine receptors in the corpus striatum and of the noradrenaline receptors in the spinal cord after treatment with these amino-acids. To exclude an indirect effect of the amines formed, the animals were always pretreated with a large dose of reserpine (10 mg/kg i.p. 10–18 h before the amino-acids) and often also with the tyrosine hydroxylase inhibitor DL-α-methyltyrosine methylester HCl (H 44/68; 250 mg/kg i.p., 1–2 h before). The amines formed from *m*-tyrosine are, in contrast to the α-methylated amines, to a large extent oxidatively deaminated. Therefore, the monoamine oxidase inhibitor nialamide (50 mg/kg, i.p.) was given 1 h before injection of *m*-tyrosine but not before the other amino-acids.

The functional effect of the amine metabolites on the dopamine receptors in the corpus striatum was examined after unilateral removal of the corpus striatum of adult, male Sprague-Dawley rats (Andén, Dahlström & others, 1966). Stimulation of the noradrenaline receptors by the amine metabolites was tested in acutely spinalized rats by pinching the hindlegs and evaluating the strength of the flexor reflex activity (Andén, Corrodi & others, 1967). The locomotor activity of rats was recorded in Animex boxes (Svensson & Thieme, 1969).

The amines were purified and separated by cation exchange chromatography and determined spectrofluorimetrically after condensation with *o*-phthalaldehyde (*m*-tyramine, *m*-octopamine, α-methyl-*m*-tyramine, metaraminol; Shore & Alpers, 1964) or

Table 1. *Concentrations (μg/g) of dopamine and noradrenaline analogues in the rat brain and spinal cord at various times after injection of their precursor amino-acids. The rats were pretreated with reserpine* (10 mg/kg, i.p., 10–18 h *before*). *Means ± s.e. of* 3 *experiments*

Precursor	Amine	Mean recovery %	Brain (B) and spinal cord (S) 0 h B	0 h S	½ h B	½ h S	1½ h B	1½ h S	6 h B	6 h S
DL-*m*-Tyrosine (150 mg/kg, i.p., 1 h after nialamide 50 mg/kg, i.p.)	*m*-Tyramine	65	0·04 ±0·01	0·07 ±0·00	13·30 ±1·70	13·22 ±1·21	3·51 ±0·69	3·06 ±0·40	0·11 ±0·01	0·19 ±0·05
	m-Octopamine	81	0·01 ±0·00	0·01 ±0·00	0·15 ±0·01	0·15 ±0·01	0·17 ±0·02	0·15 ±0·09	0·07 ±0·01	0·08 ±0·05
DL-α-Methyl-*m*-tyrosine (800 mg/kg, i.p.)	α-Methyl-*m*-tyramine	91	0·03 ±0·00	0·04 ±0·01	—		0·55 ±0·11	0·21 ±0·03	0·37 ±0·04	0·25 ±0·01
	Metaraminol	80	0·03 ±0·00	0·02 ±0·00	—		0·19 ±0·03	0·15 ±0·02	0·33 ±0·06	0·26 ±0·04
L-α-Methyldopa (400 mg/kg, i.p.)	α-Methyl-dopamine	91	0·00 ±0·00	0·00 ±0·00	0·20 ±0·00	0·13 ±0·02	0·27 ±0·06	0·07 ±0·02	0·38 ±0·05	0·12 ±0·01
	α-Methyl-noradrenaline	87	0·02 ±0·01	0·02 ±0·00	0·06 ±0·00	0·04 ±0·01	0·14 ±0·01	0·08 ±0·02	0·21 ±0·02	0·12 ±0·04

after oxidation (α-methyldopamine; Carlsson & Lindqvist, 1962) or after boiling and oxidation (α-methylnoradrenaline; Waldeck, 1968). In each experiment, the pooled brains and the pooled spinal cords of 4 rats were analysed. The brain extract was divided in two aliquots and a known amount of amines was added to one of them. All values were corrected for the mean recovery (see Table 1).

Dopamine receptor activity. Injection of DL-*m*-tyrosine (150 mg/kg, i.p.) to unilaterally striatotomized rats pretreated with reserpine and nialamide caused the rats to turn the head and tail from the unoperated to the operated side. This effect was noted after approximately 15 min, was maximal after 30–60 min and had almost disappeared after 2 h. At the peak of action, the rats were active and rotated to the operated side. Injection of L-α-methyldopa (400 mg/kg, i.p.) usually produced a decrease in the reserpine-induced turning but never caused a deviation to the operated side. No change in the reserpine-induced turning was observed after injection of DL-α-methyl-*m*-tyrosine (800 mg/kg, i.p.). The effects mentioned were also seen after pretreatment with reserpine plus H 44/68, thus excluding an amphetamine-like action (see Andén, Carlsson & Häggendal, 1969).

Noradrenaline receptor stimulation. Injection of DL-*m*-tyrosine in doses up to 800 mg/kg, i.p., did not cause any marked increase in the flexor reflex activity of spinal rats pretreated with reserpine and nialamide. Treatment with DL-α-methyl-*m*-tyrosine (800 mg/kg, i.p.) was also ineffective. On the other hand, there was a pronounced increase in flexor reflex activity after administration of reserpine plus L-α-methyldopa (400 mg/kg, i.p.). This effect began after about $1\frac{1}{2}$ h, was maximal after 4–8 h and was still observed, though weaker, the following day. Pretreatment with H 44/68 had no effect on this increased flexor reflex activity. However, administration of the adrenergic α-receptor blocking agent phenoxybenzamine (20 mg/kg, i.p., 4 h after α-methyldopa) or the dopamine-β-hydroxylase inhibitor bis(4-methyl-1-homopiperazinylthiocarbonyl)disulphide (FLA-63 25 mg/kg, i.p. 30 min before α-methyldopa) did block this effect. The hypotensive effect of α-methyldopa is abolished by FLA-63 (Henning & Rubenson, unpublished observations).

Behaviour. The injection of DL-*m*-tyrosine (75–150 mg/kg, i.p.) to reserpine- and nialamide-treated rats induced a marked increase in motor activity to even above normal values in agreement with the findings in mice described by Blaschko & Chruściel (1960). The onset and duration of these effects corresponded to the dopamine receptor stimulation described above. Particularly after more than 30 min, the hyperkinesia was accompanied by stereotypies such as sniffing, chewing of woodshavings and backward walking. The effects were observed also after pretreatment with H 44/68. Injection of L-α-methyldopa (400 mg/kg, i.p.) somewhat increased the motor activity of the reserpine–H 44/68-treated rats although not as markedly as described by Uretsky & Seiden (1969). No behavioural changes were seen after administration of DL-α-methyl-*m*-tyrosine (800 mg/kg, i.p.).

Chemistry. The concentrations of the amine metabolites formed from the various amino-acids in the brain and the spinal cord are presented in Table 1. After injection of DL-*m*-tyrosine there was a rapid and marked accumulation of *m*-tyramine and a smaller accumulation of its β-hydroxylated derivative metaoctopamine. Both amines had almost disappeared after 6 h. After injection of DL-α-methyl-*m*-tyrosine or L-α-methyldopa, the peak concentrations of the corresponding amines were reached later and were much lower for the non-β-hydroxylated amines (α-methyl-*m*-tyramine, α-methyldopamine) than after *m*-tyrosine. The amine levels were of about the same magnitude after the two α-methylated amino-acids. No significant concentrations of dopamine, noradrenaline and the amines described above were observed after treatment with reserpine alone (0 h in Table 1). Treatment with FLA-63 (25 mg/kg,

i.p.) 30 min before L-α-methyldopa (400 mg/kg, i.p., 6 h, n = 3) did not significantly change the level of α-methyldopamine (0·54 ± 0·086 μg/g in the brain, 0·11 ± 0·024 μg/g in the spinal cord) but markedly reduced that of α-methylnoradrenaline (0·06 ± 0·090 μg/g in the brain, 0·01 ± 0·001 μg/g in the spinal cord).

From this functional and chemical evidence it appears that there is a correlation in time of the functional effects of *m*-tyrosine and α-methyldopa and the peak accumulation of *m*-tyramine and α-methylnoradrenaline, respectively. There was a much greater and faster accumulation of *m*-tyramine than of the other amines which may be of importance for the dopamine receptor stimulation. All the β-hydroxylated amines reached about the same peak concentrations but stimulation of the noradrenaline receptors was only seen after injection of α-methyldopa.

In conclusion, treatment with *m*-tyrosine and α-methyldopa caused a stimulation of central dopamine and noradrenaline receptors, respectively, whereas no effect on these receptors was observed after treatment with α-methyl-*m*-tyrosine.

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Effects of chronic morphine administration on the catecholamine depletion induced by reserpine

Morphine interferes with the depletion of brain noradrenaline seen after reserpine in acute (Freedman, Fram & Giarman, 1961) and chronic experiments (Gunne, 1963). After chronic administration of morphine there was only a 38% reduction of brain noradrenaline 20 h after an injection of reserpine compared with a 93% reduction in control animals without morphine.

To establish the cellular localization of these effects, especially those of the various