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The effect of (+)-amphetamine on various central and peripheral catecholamine-containing neurones

SIR,—In previous experiments with rat brain (Carlsson, Lindqvist, Dahlström, Fuxe & Masuoka, 1965), support was obtained for the view that substances of the amphetamine group are capable of causing the release of extragranular catecholamines, that is, of catecholamines located intraneuronally outside the storage granules. Furthermore, it was found that in large doses these drugs may also cause the release of catecholamines from the granules. In the present work the effect of (+)-amphetamine has been further examined for its effect on extragranular amines. Special attention has been paid to the sensitivity of different catecholamine neurone systems to this drug.

Adult, male Sprague-Dawley rats, 200–300 g, were used. Since the extragranular amine fraction normally seems to be very small, the experiments were made on animals whose amine stores had been emptied by reserpine. Loading of the extragranular space was then brought about by means of the monoamine oxidase inhibitor nialamide, followed by the catecholamine precursor L-3,4-dihydroxyphenylalanine (L-dopa).

In vivo *experiments.* The animals were treated with reserpine, 10 mg/kg, i.p., 20–22 hr before being killed, nialamide, 100 mg/kg, i.p., 4 hr before death, and dopa, 25–50 mg/kg, s.c., 30 min before death. (+)-Amphetamine was administered in various doses (calculated as the base) 15 min before the dopa.

Dopamine, noradrenaline and 3-methoxytyramine were measured fluorimetrically (Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962; Carlsson & Lindqvist, 1963; Carlsson & Waldeck, 1964).

Fifteen animals were taken for the cellular localization of monoamines in the brain, heart and vas deferens (Falck, Hillarp, Thieme & Torp, 1962; Falck, 1962; see review by Hillarp, Fuxe & Dahlström, 1965), one control group (4 animals) and two groups receiving (+)-amphetamine (0·75 mg/kg, 5 animals, and 0·4 mg/kg, 6 animals).

In vitro *experiments.* Brain slices of the neostriatum, hypothalamus, neocortex and the vas deferens of reserpine-treated rats (10 mg/kg, 12–18 hr before killing) were incubated for 30 min (Hamberger & Masuoka, 1965) with α-methylnoradrenaline, 1 or 0·03 μg/ml. In the test experiments the slices were preincubated for 15 min with (+)-amphetamine (0·0075–0·75 μg/ml), whereupon the α-methylnoradrenaline was added to the medium.

Results in vivo. Table 1 shows the effects of (+)-amphetamine on the dopa-induced noradrenaline and dopamine accumulation in different parts of the brain. Doses of (+)-amphetamine down to 0·1 mg/kg partially blocked the noradrenaline accumulation in the brain compared with the controls, the hemispheres being more sensitive to the drug than the brain stem. The dopamine levels in brain were not affected by the lower doses of (+)-amphetamine. In the higher dose range a decrease in the dopamine : methoxytyramine ratio is evident (Table 2), suggesting increased release of dopamine into the extra-neuronal space. In the heart a decrease in noradrenaline was seen after the higher doses of (+)-amphetamine; no change in dopamine was detected.

TABLE 1. EFFECT OF VARIOUS DOSES OF (+)-AMPHETAMINE ON L-DOPA-INDUCED CATECHOLAMINE ACCUMULATION IN DIFFERENT PARTS OF RAT BRAIN

(+)-Amphetamine mg/kg i.p.	Noradrenaline			Dopamine			Number of experiments
	Stem	Hemispheres	Striatum	Stem	Hemispheres	Striatum	
0·50	73 ±6·9	25 ±3·5	26 ±7·5	128 ±12·8	106 ±17·0	124 ±17·2	3
0·15	88 ±6·1	60 ±2·9	53 ±8·6	115 ±8·2	108 ±18·0	104 ±16·6	3
0·10	76 ±1·6	55 ±1·3	85 ±15·0	100 ±7·1	101 ±20·7	86 ±12·0	2
0	0·29 ±0·02	0·12 ±0·01	0·09 ±0·01	1·12 ±0·08	0·95 ±0·08	1·73 ±0·14	9

(+)-Amphetamine was given 45 min, dopa (25 mg/kg s.c.) 30 min before killing. The rats were pre-treated with reserpine (10 mg/kg i.p.) and nialamide (100 mg/kg i.p.) 22 and 4 hr before killing, respectively. Controls in which no (+)-amphetamine was given were run in parallel.

The values are means ± s.e. of the means. The single values were expressed as % of parallel controls. The bottom row gives control values in μg/g tissue. Each experiment was on pooled tissue parts of 3 rats.

TABLE 2. EFFECT OF VARIOUS DOSES OF (+)-AMPHETAMINE ON L-DOPA-INDUCED AMINE ACCUMULATION IN RAT BRAIN AND HEART

(+)-Amphetamine mg/kg i.p.	Heart		Brain		
	Noradrenaline	Dopamine	Noradrenaline	Dopamine	3-Methoxy-tyramine
2·5	34 (2) ±6·5	77 (2) ±7·4	31 (2) ±2·4	70 (2) ±10·8	167 (2) ±8·1
0·5	57 (6) ±9·9	91 (5) ±15·8	40 (3) ±7·7	108 (3) ±23·6	140 (3) ±18·7
0·25	56 (2) ±18·8	88 (2) ±24·9	54 (2) ±3·6	84 (2) ±4·1	136 (2) ±45·6
0·15	71 (3) ±21·6	137 (3) ±1·7	—	—	—
0·10	93 (3) ±11·2	112 (3) ±19·8	75 (1)	93 (1)	83 (1)
0	0·10 (16) ±0·01	1·86 (15) ±0·13	0·13 (8) ±0·01	1·29 (8) ±0·10	0·87 (8) ±0·09

The details are as described in Table 1.

With the histochemical fluorescence technique, effects were seen only after the larger dose (0·75 mg/kg) of (+)-amphetamine and changes were then only observed in the noradrenaline terminals of the neocortex, compared with the controls, whereas the noradrenaline terminals of, for example, the hypothalamus, the preoptic, septal and vagus areas as well as peripheral organs appeared to be unaffected. The fluorescence intensity of areas rich in dopamine terminals was also unaffected. This preferential sensitivity of the noradrenaline terminals

of the neopallium is in good agreement with the biochemical results. The decrease in noradrenaline obtained with the smaller dose was probably too low to be detected by the histochemical fluorescence technique. The catecholamine nerve cell bodies were not affected by the (+)-amphetamine treatment.

Results in vitro. (+)-Amphetamine in concentrations down to 0·2 μg/ml inhibited almost completely the accumulation of α-methylnoradrenaline in the noradrenaline nerve terminals of the hypothalamus, the neocortex and the vas deferens as well as the dopamine nerve terminals of the neostriatum. In a concentration of 0·075 μg/ml, the drug still had a clearcut effect on the neocortex but no definite effect on the other tissues, which is in general agreement with the *in vivo* experiments.

The present results support the view (Carlsson & others, 1965) that (+)-amphetamine may act by releasing extragranular catecholamines. It should be noted that effects are obtained with doses as low as 0·1 mg/kg *in vivo*, which appear to be threshold doses with respect to gross behaviour, and with low concentrations (0·075 μg/ml) *in vitro*. The particularly high sensitivity of the noradrenaline neocortical systems suggests that these terminal systems are of importance for the psychomotor stimulation induced by amphetamine. However, these suggestions should be considered tentative until the effect of (+)-amphetamine described above has been demonstrated under less artificial conditions.

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