Distribution of amphetamine and its hydroxylated metabolites in various areas of the rat brain

Amphetamine is metabolized by the rat to *p*-hydroxyamphetamine (Axelrod, 1954) and *p*-hydroxynorephedrine (Goldstein & Anagnoste, 1965). These hydroxylated metabolites exert a depleting action on the brain catecholamine stores (Brodie, Cho & Gessa, 1970; Lewander, 1971) and have been implicated in some pharmacological effects of the parent compound. It has been suggested, for instance, that *p*-hydroxynorephedrine may be responsible for the persistent decrease of catecholamine concentrations after single doses of amphetamine (Groppetti & Costa, 1969) and for the tolerance which appears after repeated amphetamine treatment (Brodie & others, 1970). We have used a specific and sensitive gas-chromatographic method (Belvedere, Caccia & others, 1973) to measure the relative concentrations of amphetamine and its hydroxylated metabolites in various areas of the rat brain at different times after a single dose of amphetamine.

Charles River female rats (160 ± 10 g) were injected intraperitoneally with (+)-amphetamine sulphate (kindly supplied by Recordati S.p.A., Milan) at 15, 10 or 5 mg kg^{-1} and were decapitated 1, 2, 5, 8 and 12 h after the dose. Brains were immediately removed, dissected on dry ice and stored at −20° for a maximum period of three days. The gross anatomical areas dissected from brains were: cerebellum, brainstem (including medulla oblongata, pons, mesencephalon and diencephalon), striatum and the remaining hemispheres (telencephalon excluding striatum). Determinations were made on single or pooled areas. The samples were homogenized in acetone-formic acid mixture as previously reported (Belvedere & others, 1973). Amphetamine was determined according to Änggård, Gunne & Niklasson (1970)

Table 1. *Concentrations of amphetamine*, p-*hydroxyamphetamine and* p-*hydroxynorephedrine in various areas of the rat brain after amphetamine.*

Brain areas	Interval between treatment and death	Amphetamine	*p*-OH-Amphetamine	*p*-OH-Norephedrine
			ng g^{-1} ± s.e.	
Striatum	1	11615±874	92±6	<25
	2	6006±242	79±6	38±1·5
	5	1099±86	46±5	57±3
	8	266±30	31±1	44±5
	12	100±11	<25	31±1
Brainstem	1	14763±778	105±18	32±3
	2	5826±822	62±5	81±7
	5	852±87	37±6	168±15
	8	248±20	<25	87±9
	12	75±8	—	53±1
Cerebellum	1	9663±777	83±9	32±5
	2	4660±286	51±9	62±9
	5	596±56	<25	84±8
	8	221±22	—	57±3
	12	82±5	—	43±3
Hemispheres	1	13413±488	105±6	27±2
	2	5140±549	62±8	51±4
	5	628±44	<25	120±5
	8	233±58	—	63±5
	12	91±14	—	41±5

(+)-Amphetamine sulphate was given intraperitoneally at the dose of 15 mg kg^{-1}.
Each figure is the average of 4 determinations.

Table 2. *Concentrations of* p-*hydroxyamphetamine and* p-*hydroxynorephedrine in the brainstem after various doses of amphetamine.*

(+)-Amphetamine sulphate (mg kg^{-1}, i.p.)	*p*-OH-amphetamine	*p*-OH-norephedrine
	ng g^{-1} brainstem $\pm$ s.e.	
15	37 ± 6	168 ± 15
10	<25*	72 ± 8
5	—	31 ± 2

Determinations were made 5 h after the administration of (+)-amphetamine sulphate.
Each figure is the average of 4 determinations.
* = peaks visible but not quantitatively determinable.

and *p*-hydroxyamphetamine and *p*-hydroxynorephedrine according to Belvedere & others (1973).

The results reported in Table 1 show that localization of amphetamine and its metabolites in various parts of the brain differs. Amphetamine reached high levels in all the areas studied, but it seemed to be preferentially localized in the brainstem and hemispheres. The pattern of amphetamine localization in the brain has also been described by Placidi, Masuoka & Earle (1972) and by Morselli (personal communications), although differences in the animal species, in the methods used and in the time course of the experiments may be responsible for some discrepancies in respect to the present results.

As is well known, amphetamine disappears rapidly from the brain, so that 12 h after the administration the levels of the drug are less than 1/100th of those found in the same area 1 h after the injection. In this respect no remarkable differences seem to exist among the brain areas.

p-Hydroxyamphetamine, formed from the parent compound mostly by liver metabolism (Axelrod, 1955; Dingell & Bass, 1969), shows a distribution in the brain similar to that of amphetamine, although at a different range of concentrations. The rate of disappearance of this metabolite is, however, slower than that of the amphetamine in all the other brain areas examined.

Table 1 shows also that *p*-hydroxynorephedrine reaches maximum levels at 5 h after the dose of amphetamine and accumulates in decreasing order in brainstem, hemispheres, cerebellum and striatum. The brainstem level of this metabolite is proportional to the dose of the amphetamine administered (Table 2).

The low concentration of *p*-hydroxynorephedrine in the striatum observed also by Cattabeni, Racagni & Groppetti (1973) is justified by the presence in this area of a large number of dopamine nerve endings lacking dopamine 3-hydroxylase, the enzyme responsible for the hydroxylation of *p*-hydroxyamphetamine to *p*-hydroxynorephedrine (Goldstein & Contrera, 1962). In all the areas, the latter attains its maximum level slowly, but it is present for a relatively long time.

Young & Gordon (1962), Goldstein & Anagnoste (1965) and Placidi & others (1972) found in rats and mice that most of the radioactivity initially present after an injection of [^{14}C]amphetamine was associated with unchanged amphetamine. Our data do not disagree, but the sensitivity and the specificity of the gas chromatographic method used by us allows relatively low concentrations of the metabolites to be detected in the presence of high concentrations of the parent compound.

Finally, the persistence of large concentrations of *p*-hydroxynorephedrine in a noradrenergic area, such as the brainstem, is consistent with the hypothesis that this metabolite is retained in the noradrenaline nerve-endings, and is responsible for the amphetamine-induced long-lasting noradrenaline depletion.

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Red back spider venom and inhibitory transmission

The venom of the black widow spider, *Latrodectus mactans tredecimguttatus*, has been shown to cause release of transmitter from the aminergic systems of insects (d'Ajello, Magni & Bettini, 1971; Majori, Bettini & Casaglia, 1972; Cull-Candy, Neal & Usherwood, 1973; Griffiths & Smyth, 1973), from the crustacean neuromuscular system (Grasso & Paggi, 1967; Kawai, Mauro & Grundfest, 1972), and from the amphibian neuromuscular system (Clark, Hurlbut & Mauro, 1972). Similar effects of the venom have also been demonstrated in mammalian tissue supplied by adrenergic nerves (Frontali, Granata & Parisi, 1972) and cholinergic nerves (Longenecker, Hurlbut & others, 1970; Paggi & Rossi, 1971; Okamoto, Longenecker & others, 1971). It was previously reported that the venom of the Australian red back spider, *Latrodectus mactans hasselti*, causes the depletion of vesicles within the adrenergic nerve terminals of the mouse vas deferens, and also produces an increased frequency of spontaneous excitatory junction potentials in this tissue (Einhorn & Hamilton, 1973). As the action of the venom thus seems to be independent of the nature of the transmitter, it was of interest to study the effects of red back spider venom on the purinergic nervous system (Burnstock, 1972) of the guinea-pig ileum.

Segments of ileum isolated from adult guinea-pigs of either sex, were cut along the mesenteric border, and mounted with the serosal surface uppermost, in a 15 ml organ bath. Intracellular recordings were obtained with KCl-filled glass capillary microelectrodes of 60–100 Mohm resistance. The composition of the perfusion medium and details of circuitry have been described previously (Hashimoto & Holman, 1967). Transmural silver wire electrodes were used for electrical stimulation (pulses of 0·5 ms from a Grass S4 stimulator). 2×10^{-7} gml^{-1} atropine sulphate was added to the perfusion medium to prevent excitation due to stimulation of cholinergic neurons. Experiments were conducted at 25° to minimize spontaneous muscle movement. After each experiment the tissues were fixed in either glutaraldehyde in cacodylate buffer or glutaraldehyde in 110 mM magnesium solution, and examined with the electron microscope.