Uptake of tritiated tyramine and (+)-amphetamine by mouse heart slices

SIR,—The sympathomimetic action of tyramine and amphetamine is of the "indirect" type, by releasing noradrenaline from a minor, labile store (Trendelenburg, 1963). Cocaine antagonises the action of these amines (Tainter & Chang 1927; Burn & Rand, 1958). It has been proposed that cocaine counteracts the action of these amines by inhibiting their uptake into the noradrenergic neurones in the same way as it inhibits the uptake of noradrenaline at the neurone membrane transfer sites (cf. Muscholl, 1966). In a previous investigation we could demonstrate that tritiated tyramine and (+)-amphetamine were accumulated into mouse brain cortex slices (Ross & Renyi, 1966). Only the uptake of the former amine was, however, decreased by cocaine, desipramine and ouabain. Amphetamine seems therefore to be accumulated into the tissue mainly owing to its lipid solubility while tyramine in part is actively taken up by the tissue. Since it is of interest to know whether cocaine inhibits the uptake of amphetamine into tissues in which this amine affects the effector organs by the "indirect" way we have now examined the uptake of tritiated tyramine and (+)-amphetamine by mouse heart slices and the influence of cocaine upon this uptake.

Heart ventricle slices (100 mg) were incubated at 37° with 0·1 nmole/ml of the tritiated amine (tyramine hydrochloride, generally labelled, 1·73 c/mmole; (+)-amphetamine sulphate, generally labelled, 1·82 c/mmole, New England Nuclear Corp.) in 2 ml of Krebs-Henseleits solution, pH 7·4. The amines were extracted from the tissues in the same way as described for noradrenaline (Ross & Renyi, 1964). The radioactivity in the slices was regarded as the amount of the amine being taken up and was expressed as nmole/g. Each value is the mean of four determinations.

The results obtained are presented in Table 1. While both amines were accumulated into the tissue only that of tyramine was decreased by cocaine. Thus the heart tissue resembles the brain cortex tissue regarding the uptake mechanisms for tyramine and amphetamine.

TABLE 1. EFFECT OF COCAINE ON THE ACCUMULATION OF TRITIATED TYRAMINE AND (+)-AMPHETAMINE INTO MOUSE HEART SLICES

Amine	Incubation time, min	Amine uptake nmole/g ± s.e.	
		Control	Cocaine HCl 10 μg/ml
Tyramine-^{3}H	5	0·125 ± 0·003	0·083 ± 0·006‡
	30	0·160 ± 0·005	0·130 ± 0·003†
(+)-Amphetamine-^{3}H	5	0·162 ± 0·005	0·163 ± 0·007
	30	0·195 ± 0·010	0·199 ± 0·009

† $0{\cdot}01 > P > 0{\cdot}001$ ‡ $P < 0{\cdot}001$

The lack of effect of cocaine on the amphetamine uptake seems to contradict the possibility that cocaine antagonises the "indirect" action of amphetamine by inhibiting its uptake. The possibility remains that cocaine counteracts the amphetamine induced release of noradrenaline from an intra- or extra-neuronal site. If from an intra-neuronal site it has to be assumed that cocaine acts at different sites when inhibiting the noradrenaline uptake or when antagonising the action of the "indirectly" acting amphetamine. If an extra-neuronal site of release of noradrenaline is postulated the possibility exists that cocaine acts directed at the same site, especially if it is assumed that the "indirectly" acting

amine releases noradrenaline bound to the transfer (uptake) sites of the neurone membrane (Ross & Renyi, 1966).

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Reserpine and the neuromuscular junction

Sir,—The ineffectiveness of reserpine on the neuromuscular transmission (Bein, 1956) was questioned by Liebmann & Matthies (1964) who claimed that the drug displayed a powerful anticurare activity by increasing the acetylcholine release from the motor nerve endings. Their results, however, were not confirmed by Ledda & Baldi (1965).

We now summarise investigations made to establish whether reserpine affects both the amount of acetylcholine stores in the motor nerve, and the pattern of the end-plate potentials.

The experiments were made on the isolated phrenic nerve-diaphragms of the guinea-pig and rat; the methods for detecting acetylcholine and for intracellular recordings were those previously described (Beani, Bianchi & Ledda, 1966).

The right and left hemidiaphragms of five guinea-pigs were separately incubated for 2 hr in oxygenated Tyrode solution with dyflos 500 μg/ml, at 38° After washing out the dyflos, the preparations were indirectly stimulated at 50/sec for 10 min. They were then kept at rest for 1 hr and one hemidiaphragm of each pair was maintained in the presence of reserpine 1×10^{-5}M. Immediately after a second period of stimulation at 50/sec (10 min) the tissue acetylcholine was extracted both in the control and treated preparations.

TABLE 1. TOTAL TISSUE ACETYLCHOLINE (NG/HEMIDIAPHRAGM ± S.D.) AT THE END OF THE SECOND PERIOD OF STIMULATION AT 50/SEC IN HEMIDIAPHRAGMS KEPT IN TYRODE SOLUTION, AT 38°, WITH OR WITHOUT RESERPINE 1×10^{-5}M. PREINCUBATION WITH DYFLOS 500 μG/ML FOR 2 HR (each value is the mean of five experiments).

Treatment	Guinea-pig weight g ± s.d.	Hemidiaphragm weight mg ± s.d.	Acetylcholine ng/hemidiaphragm ± s.d.
Controls	330 ± 23	223 ± 33	81·5 ± 11·3
Reserpine	330 ± 23	250 ± 49	76·0 ± 8·0

As shown in Table 1, reserpine does not change the transmitter stores; in similar experimental conditions, the drug does not modify the acetylcholine release (Ledda & Baldi, 1965). The end-plate potentials were recorded from curarised rat diaphragms, kept in oxygenated Tyrode solution at 33°. The phrenic nerve was stimulated at 1, 10 and 100/sec for 10 sec.