Effects of amphetamine, tyramine, and protriptyline on reserpine-resistant amine-concentrating mechanisms of adrenergic nerves

SIR,—The ability of adrenergic nerves to take up and concentrate amines even after blockade of the amine-storing granules by reserpine has been demonstrated, and evidence supporting the existence of a reserpine-resistant uptake mechanism at the level of the neuronal cell membrane ("the membrane pump") has been presented (see Malmfors, 1965; Carlsson & Waldeck, 1965a). Whether the amines that accumulate in the adrenergic nerves under these conditions exist entirely free in the cytoplasm or are partly bound extragranularly, has not been elucidated. An observation of Malmfors (1965) may indicate that the latter alternative is true. He found that even the virtually complete blockade of the "membrane pump" by desipramine was relatively inefficient in releasing extragranular noradrenaline or α-methylnoradrenaline. Nevertheless, tyramine caused rapid release of these amines. The present data lend additional support to this view. Tyramine, and particularly amphetamine, have been found to release extragranular ^{3}H-noradrenaline under conditions where a large dose of an efficient "membrane pump"-blocking agent was found to be more or less inactive.

Mice were pretreated with reserpine and nialamide and then received ^{3}H-noradrenaline in certain experiments together with carrier noradrenaline. The drugs to be investigated were given 15 min after the ^{3}H-noradrenaline, and the animals were killed after another 15 min. For further experimental details, see Tables 1 and 2 and Carlsson & Waldeck (1963).

TABLE 1. RELEASE BY AMPHETAMINE OF ^{3}H-NORADRENALINE FROM THE HEARTS OF MICE PRETREATED WITH RESERPINE AND NIALAMIDE. Reserpine (10 mg/kg) and nialamide (10 mg/kg) were given intraperitoneally 6 and 2 hr, respectively, before the intravenous injection of ^{3}H-noradrenaline (1 μg/kg; approximately 6 c/mM). The amphetamine was given 15 min after this injection, and the animals killed after another 15 min. There were 6 animals/group.

Drug	Dose mg/kg i.v.	^{3}H-noradrenaline, ng/g		
		Mean	Range	No. of groups
Control	—	0·50	0·35–0·67	4
(±)-Amphetamine	0·15	0·15	0·07–0·23	2
,,	0·4	0·11		1
,,	1·5	0·08		1
,,	4	0·11	0·09–0·12	2
(+)-Amphetamine	0·1	0·15	0·09–0·21	2

TABLE 2. COMPARISON OF ^{3}H-NORADRENALINE RELEASING ACTIVITY OF TYRAMINE AND PROTRIPTYLINE FROM THE HEARTS OF MICE PRETREATED WITH RESERPINE AND NIALAMIDE. Reserpine (10 mg/kg) and nialamide (100 mg/kg) were given intraperitoneally 15–21 and 2 hr, respectively, before the intravenous injection of ^{3}H-noradrenaline (1 μg/kg; approximately 6 c/mM). The drugs were given 15 min after this injection, and the animals killed after another 15 min. There were 6 animals/group.

	Dose of drug mg/kg i.v.	Dose of carrier (−)-noradrenaline mg/kg	^{3}H-noradrenaline, ng/g		
			Mean	Range	No. of groups
Control	—	—	0·36	0·23–0·49	3
Tyramine HCl	10	—	0·17	0·16–0·18	3
Protriptyline HCl	10	—	0·36	0·23–0·49	2
Control	—	0·1–0·2	0·33	0·30–0·39	4
Tyramine HCl	10	0·1–0·2	0·10	0·09–0·12	3
Protriptyline HCl	10	0·1–0·2	0·26	0·24–0·31	3

Under the conditions described above, amphetamine in doses down to 0·10–0·15 mg/kg caused a marked release of ^{3}H-noradrenaline accumulated in heart (Table 1). In contrast, amphetamine given to non-reserpine-treated mice in a dose of 1·5 mg/kg causes little or no release of ^{3}H-noradrenaline previously taken up in heart (data not shown).

Under the present conditions tyramine also caused release of ^{3}H-noradrenaline, whereas protriptyline was inactive in a dose of 10 mg/kg (Table 2). We have discovered that this drug blocks ^{3}H-noradrenaline uptake in doses down to 1 mg/kg.

In the present experiments the animals had been pretreated with a large dose of reserpine, and thus the noradrenaline uptake by the amine-storing particles was blocked. The ^{3}H-noradrenaline taken up by the adrenergic nerves thus accumulated outside the granules and was protected from destruction by the monoamine oxidase inhibitor nialamide. Blockade of the "membrane pump" by a large dose of protriptyline did not result in detectable release of this ^{3}H-noradrenaline. This is in contrast to ^{3}H-metaraminol which is rapidly lost after blockade of the "membrane pump" (Carlsson & Waldeck, 1965b; Carlsson, 1965). The explanation of this difference may be that the more lipid soluble metaraminol leaks out through the cell membrane more rapidly and its intraneuronal retention is thus more dependent on the "membrane pump". The question then arises how tyramine, and particularly amphetamine in doses which do not block the "membrane pump" efficiently (Carlsson & Waldeck, 1965c), are capable of releasing extragranular ^{3}H-noradrenaline. The most likely explanation appears to be that the ^{3}H-noradrenaline under the present conditions is largely bound to extragranular sites and that these drugs are capable of displacing the ^{3}H-noradrenaline from these binding sites.

The question also arises whether the effects of tyramine and amphetamine described may be involved in their actions as indirect sympathomimetics. This question is difficult to answer, since the binding sites proposed above must be assumed to be occupied by noradrenaline to a much smaller degree normally than under the present conditions, where both monoamine oxidase and storage granules have been blocked. Further work is necessary to settle this point.

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