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Interaction between desipramine, tyramine, and amphetamine at adrenergic neurones

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1. Small doses of tyramine (10 μg/kg intravenously) are taken up by rat heart, which can concentrate the amine 7.3-fold over the plasma level.

2. Desipramine (DMI) blocks the uptake of small doses of tyramine by rat heart, but in doses of 20 mg/kg intraperitoneally, it does not affect the cardiac concentrations of tyramine following 20 mg/kg intramuscularly of the drug. The same dose of DMI prevents the depletion of noradrenaline (NA) elicited by intramuscular injection of tyramine 20 mg/kg.

3. DMI prevents the depletion of heart NA elicited by (+)-amphetamine 5 mg/kg given intraperitoneally to demedullated rats and enhances the heart concentration of amphetamine.

4. Liposoluble amphetamine enters sympathetic nerves by simple diffusion. It is suggested that DMI prevents the release of heart NA caused by this indirect sympathomimetic through an action within the adrenergic neurone.

The sympathomimetic effects of tyramine, like those of guanethidine, are commonly thought to be mediated through the release of noradrenaline (NA) from adrenergic neurones (Burn & Rand, 1958; Chang, Costa & Brodie, 1965). The sympathomimetic and NA-releasing actions of these drugs are antagonized by desipramine (desmethylimipramine) (DMI) and a number of other chemically related antidepressants (Matsumoto, Costa & Brodie, 1964; Stone, Porter, Stavorski, Ludden & Totaro, 1964), which block the accumulation of NA into adrenergic neurones (Cuenca, Salvá & Valdecasas, 1964; Gessa, Vargiu & Crabai, 1966).

The present paper describes the interaction of tyramine and DMI with NA storage sites in adrenergic neurones. DMI blocks the neuronal accumulation of small doses of tyramine, but it does not affect the cardiac concentration of tyramine when given in large doses. DMI, however, antagonizes the loss of cardiac NA elicited by these doses of tyramine. The release of cardiac NA by amphetamine,

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a liposoluble indirect sympathomimetic substance, is also blocked by DMI, so it is suggested that DMI prevents the release of NA elicited by these two indirect sympathomimetics through an action within the nerve terminal.

Methods

Experiments were carried out in male Sprague–Dawley rats, weighing about 200 g. Because DMI potentiates the central effects of amphetamine, the association of these two drugs might elicit a release of catecholamines from adrenal medulla. Hence the effects of DMI on the release of heart NA by amphetamine was studied with demedullated rats. Adrenal demedullated male Sprague–Dawley rats, weighing about 200 g, were purchased from Hormone Assay Laboratories (Chicago, Illinois). ^{3}H-tyramine HCl (specific activity 1.6 c/m-mole) and (±)-NA-7-^{3}H bitartrate (specific activity 5 c/m-mole) and ^{3}H(+) amphetamine sulphate (specific activity 4.23 c/m-mole) were purchased from New England Nuclear Corp. (Boston, Massachusetts). The generally labelled ^{3}H-tyramine and ^{3}H-amphetamine were diluted with various amounts of non-labelled amines. DMI was donated by Geigy Research Laboratories. Intravenous injections were made through the tail vein. At various times after the administration of tyramine the rats were killed by a blow on the head. The hearts were removed as rapidly as possible and stored at −20° C.

Chemical methods

Tyramine was assayed fluorimetrically by the method of Spector, Melman, Lovenberg & Sjoerdsma (1963), which extracts tyramine and only negligible amounts of octopamine from tissues. NA was assayed as previously described (Brodie, Comer, Costa & Dlabac, 1966). Tissue levels of ^{3}H-amphetamine were determined according to the method of Sulser, Owens & Dingell (1966).

Assay of ^{3}H-tyramine plus ^{3}H-octopamine (labelled tyramine bases)

The labelled tyramine bases were extracted from the heart by homogenization with 10 ml. of *n*-butanol (containing 0.85 ml. of concentrated HCl). Non-labelled tyramine and octopamine were added as carriers, the homogenate was centrifuged, and 5 ml. of the butanol phase was transferred to a 50 ml. glass-stoppered centrifuge tube containing 2 ml. water and 10 ml. *n*-heptane. The tube was shaken for 10 min and the heptane-butanol phase removed by aspiration. The aqueous phase was adjusted to pH 6.5 with 0.2 ml. 2M sodium acetate, the tyramine bases adsorbed on to a Dowex 50 (H+) column (200–250 mesh, 7 × 50 mm) and then eluted with 2 ml. of 3N-NH_4OH. An aliquot of the eluate was assayed for radioactivity by liquid scintillation counting as described by Beaven & Maickel (1964). The recovery of ^{3}H-tyramine added to heart homogenate averaged 70%.

To assay labelled octopamine, the tyramine bases in the column eluate were treated with acetic anhydride and the acetylated products extracted into methylene chloride, concentrated and separated by ascending paper chromatography as described by Goldstein, Friedhoff & Simmons (1959). Two radioactive spots corresponding to those of the authenticated acetylated derivatives of octopamine (R_F 0.10) and tyramine (R_F 0.49) were obtained.

Sucrose gradient studies

Rats injected with 1.2 μg of ^{3}H-NA (50 μc/rat intravenously) were divided into three groups. The first group was used as controls; the second group received tyramine (20 mg/kg intraperitoneally), 75 min after the injection of ^{3}H-NA; the third group received DMI (20 mg/kg intraperitoneally) 45 min after the injection of ^{3}H-NA and tyramine was injected 30 min later. All animals were killed 105 min after the intravenous injection of ^{3}H-NA. The hearts were homogenized and the subcellular distribution of ^{3}H-NA was carried out by a sucrose gradient technique as described by Michaelson, Richardson, Snyder & Titus (1964).

Results

Relationship of tyramine concentration to NA release

Within 3 min of the injection of tyramine (20 mg/kg intraperitoneally), the concentration of this amine in the heart reached a value of about 5 μg/g; after 5 min the concentration quickly declined to 0.9 μg/g, corresponding to a half-life of about 5 min (Table 1). The concentration of heart NA fell and within 30 min levelled off at about 60% of the initial concentration. As indicated in a previous report, the rapid disappearance of tyramine is a major factor in limiting the extent of cardiac NA depletion elicited by this sympathomimetic substance (Neff, Tozer, Hammer & Brodie, 1965).

Because tyramine localized in sympathetic nerve endings is converted to octopamine (Kopin, Fischer, Musacchio, Horst & Weise, 1965), the possibility was enter-

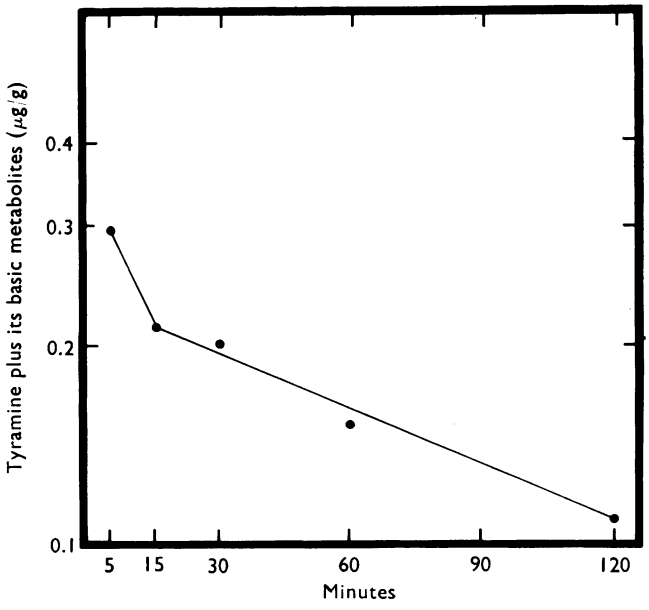

FIG. 1. Concentration of labelled tyramine bases in rat heart at various times after giving 1.25 mg/kg intravenously of ^{3}H-tyramine (specific activity 11 mc/m-mole). Each point is mean of six experiments.

tained that considerable amounts of this derivative accumulated in the heart. Fig. 1 shows that after the intravenous injection of ^{3}H-tyramine 1.25 mg/kg, the level of total labelled bases (octopamine plus tyramine) at first declined rapidly, but after 15 min the rate of decline was greatly reduced. Chromatographic analysis showed that

TABLE 1. *Concentrations of NA and tyramine in rat heart at various times after injection of tyramine (20 mg/kg intraperitoneally)*

Time (min)	NA concentration* (μg/g±S.D.)	Tyramine concentration* (μg/g±S.D.)
0	1·34±0·20	0
3	—	5·35±1·08
5	—	5·12±0·35
10	—	—
15	0·97±0·08	0·9±0·32
20	0·88±0·14	—
30	0·83±0·24	—

* Each value is mean of six experiments.

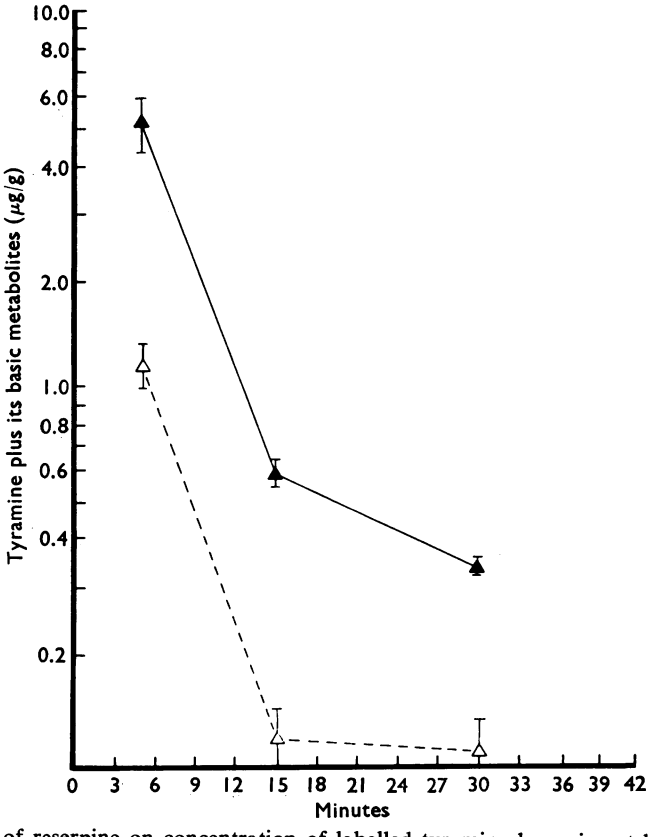

FIG. 2. Effects of reserpine on concentration of labelled tyramine bases in rat heart at various times after giving 10 mg/kg intravenously of ^{3}H-tyramine to control and reserpine-treated rats. Animals were treated with reserpine (2 mg/kg intravenously) (△---△) and 4 hr later received ^{3}H-tyramine (10 mg/kg intravenously, 6.8 mc/m-mole). Control animals received only ^{3}H-tyramine (▲——▲). The heart content of ^{3}H-tyramine bases of the two groups of animals at various times was significantly different ($P<0.01$). Each value is mean of six experiments. Bars represent S.D.

at this time almost all the total labelled bases consisted of octopamine, which disappeared with a half-life of about 110 min. This half-life is similar to that reported previously by Kopin *et al.* (1965). A diphasic decline of tyramine bases also occurred when ^{3}H-tyramine was given in doses large enough (10 mg/kg intravenously) to release a sizable proportion (about 25%) of heart NA (Fig. 2). In this

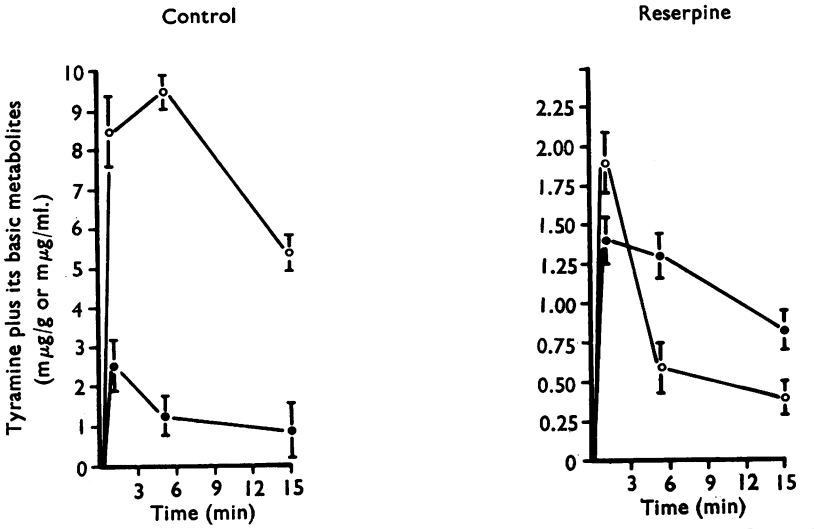

FIG. 3. Effects of reserpine on concentration of ^{3}H-tyramine bases in plasma (●——●) and hearts (○——○) of rats at various times after giving ^{3}H-tyramine (10 μg/kg intravenously, 1.56 c/m-mole) to control and reserpine-treated rats (2 mg/kg intravenously 4 hr before). Note that the ordinate of the two graphs are in different scale. Vertical bars indicate S.D. Each value is mean of six experiments. Heart content of basic metabolites of rats given reserpine is significantly lower than that of controls ($P<0.001$).

TABLE 2. *Effects of various doses of DMI on the uptake of tyramine by rat heart*

Dose of DMI (mg/kg i.p.)	Cardiac concentration of labelled bases (mμg/g±S.D.)
0	5·5±0·2
1·0	3·0±0·3
3·0	0·5±0·2
5·0	0·6±0·3

^{3}H-Tyramine, 10 μg/kg (1·56 c/m-mole intravenously), was given to control rats and to rats 30 min after treatment with DMI. Animals were killed 35 min later. Each value is mean of six animals.

TABLE 3. *Effects of large doses of DMI on the uptake of various doses of tyramine by rat heart*

Dose of tyramine (mg/kg i.v.)	Heart concentration of labelled bases: Controls (μg/g±S.D.)	Heart concentration of labelled bases: DMI-treated (μg/g±S.D.)
0·0055	0·009±0·002	0
0·25	0·064±0·001	0·02±0·002
1·25	0·22±0·04	0·14±0·06
10	0·62±0·07	0·50±0·09

^{3}H-Tyramine (50 μc diluted with appropriate amounts of non-labelled drug) was given to control rats and to rats 30 min after treatment with DMI (20 mg/kg intraperitoneally). Animals were killed 15 min later. Each value is mean of six animals.

experiment, 15 min after the injection of ^{3}H-tyramine, about 70% of the labelled bases consisted of octopamine (0.42 μg/g). Because of the slow rate at which octopamine disappeared, it may be presumed that this concentration is not too different from the maximum level of octopamine attained in these experiments. ^{3}H-tyramine given in small doses (10 μg/kg intravenously) was rapidly localized in heart against a concentration gradient. Figure 3 shows that 1 min after the injection of ^{3}H-tyramine, treatment with reserpine reduced by about 80% the concentration of amine in heart, while that in plasma was reduced by about 40%. Five minutes after the injection of ^{3}H-tyramine the concentration ratio of labelled bases for heart and plasma was 7.3 in control rats and 0.4 in reserpine-treated rats (Fig. 3). After 15 min this ratio was 6.1 in control and 0.5 in reserpine-treated rats (Fig. 3). The cardiac concentration of ^{3}H-tyramine given in doses of 10 mg/kg was also decreased by reserpine treatment (Fig. 2).

Effect of DMI on the uptake of tyramine

Table 2 shows the effects of various doses of DMI on the uptake and retention of tyramine bases 30 min after the administration (10 μg/kg) of ^{3}H-labelled amine. Treatment with DMI 1 mg/kg reduced the accumulation of tyramine by about 50%, whereas DMI 3 and 5 mg/kg blocked the accumulation almost completely.

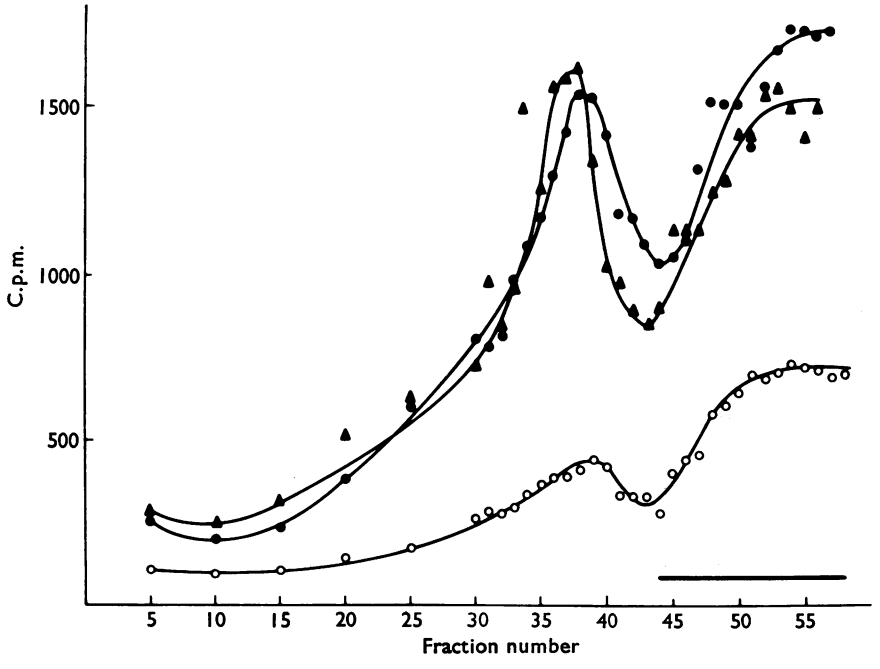

FIG. 4. Effects of DMI on tyramine-induced release of ^{3}H-NA from microsomal fraction of rat heart homogenates, isolated in sucrose gradient. One group of rats was given ^{3}H-NA (50 μc/rat intravenously); a second group was injected with ^{3}H-NA and then tyramine (20 mg/kg intraperitoneally) 75 min later; a third group was injected with ^{3}H-NA, 45 min later were given DMI (20 mg/kg intravenously) followed in 30 min by tyramine. Hearts were homogenized and the subcellular distribution of ^{3}H-NA carried out as described in **Methods.** For each experiment, three hearts were pooled. ●, Control; ○, tyramine treated; △, DMI and tyramine treated.

Table 3 shows the effect of a single large dose of DMI (20 mg/kg intraperitoneally) on the retention of labelled bases after the administration of ^{3}H-tyramine in various doses. The retention of tyramine bases was almost completely antagonized after tracer doses of the labelled amine, but the retention of tyramine was not appreciably affected when the sympathomimetic was given in large doses (1.25 mg/kg and 10 mg/kg).

Effects of DMI on NA release elicited by tyramine

Table 4 shows the effects of a large dose of DMI (20 mg/kg intraperitoneally) on the cardiac concentrations of NA and tyramine in rats given 20 mg/kg of the drug intramuscularly. DMI did not reduce the retention of tyramine by the heart. Although DMI failed to reduce cardiac concentrations of tyramine, it blocked almost completely the depletion of endogenous NA content.

Effects of DMI on the subcellular distribution of tyramine and NA

Figure 4 (typical of three experiments) illustrates the effects of pretreatment with DMI on the subcellular localization of NA in rat hearts after the injection of large doses of tyramine. The animals were given ^{3}H-NA intravenously (50 μc/rat) and the effects of tyramine were compared in hearts of control and DMI treated animals. Tyramine caused a considerable reduction in the content of ^{3}H-NA in the microsomes from control rats but not from those rats that had been treated with DMI.

TABLE 4. *Concentrations of NA and tyramine in heart at various times after injection of tyramine (20 mg/kg i.m.) in control rats and rats pretreated with DMI (20 mg/kg i.p.)*

Treatment	NA concentration (μg/g±S.D.)					Tyramine concentration (μg/g±S.D.)		
	0	5	15	30	45	5	15	30
Tyramine	1·31±0·04	1·11±0·04	1·04±0·06	0·75±0·05	0·75±0·06	2·99±0·80	5·16±0·73	0·37±0·18
DMI+ tyramine	1·21±0·06	—	1·12±0·08	1·12±0·07	1·26±0·03	2·91±0·82	5·61±0·78	0·70±0·20

Tyramine was given 30 min after treatment with DMI. Each value for heart NA is mean of eight animals; each value for heart tyramine is mean of four animals.

TABLE 5. *Concentrations of NA and amphetamine in heart at various times after the injection of (+)-amphetamine in control rats and rats pretreated with DMI*

Treatment	NA concentration (μg/g±S.D.)		Amphetamine concentration (μg/g±S.D.)	
	0 min	40 min	20 min	40 min
Amphetamine	1·45±0·14	1·04±0·18	1·45±0·46	1·06±0·30
DMI+ amphetamine	1·33±0·16	1·24±0·08	1·72±0·96	1·78±0·60

Statistical calculations of the variance (Snedecor, 1956) within the 24 NA determination included in this table show that the NA cardiac concentrations 40 min after amphetamine are significantly lower ($P<0·01$) than those given amphetamine 45 min after DMI.
(+)-^{3}H-Amphetamine sulphate (5 mg/kg i.p.; specific activity 2 mc/m-mole) was given 45 min after treatment with DMI (10 mg/kg i.p.). Each value is the mean of at least six determinations.

Effects of DMI on NA release elicited by (+)-amphetamine

Table 5 shows the effects of DMI (10 mg/kg intraperitoneally) on the depletion of NA by amphetamine (5 mg/kg intraperitoneally). In confirmation of reports by Sulser, Owens & Dingell (1966), the cardiac concentrations of amphetamine after pretreatment with DMI were higher than those in the controls. Despite the higher concentrations of amphetamine after pretreatment with DMI, the depletion of cardiac NA was almost completely prevented. These results indicate that DMI prevents the depletion of NA by amphetamine through an action within the neurone. In fact, the uptake of (+)-amphetamine into noradrenergic neurones does not depend on the function of the amine pump localized in the neuronal membrane (Roos & Renyi, 1966).

Discussion

The present studies support the view that tyramine, as well as octopamine, to which it is converted, are localized in sympathetic nerve endings of the rat heart. Tyramine in small doses (10 μg/kg intravenously) is taken up against a concentration gradient (Fig. 3), so it might be inferred that the drug is taken up from the bloodstream by the same specialized process of neuronal membranes which concentrates NA in nerve endings. A similar suggestion had been previously made by Carlsson & Waldeck (1963) and by Fischer, Musacchio, Kopin & Axelrod (1964), for the uptake of tyramine into the rat salivary gland. Because of the short half-life of tyramine, it is difficult to define the process responsible for the retention and uptake of this amine by nerve endings using conventional kinetic methods. Moreover, because the tyramine is a good substrate for monoamine oxidase (MAO) the difference in tyramine concentrations in hearts of normal and reserpine treated animals is not a precise measure of tyramine bindings to adrenergic neurones.

After the intravenous or intraperitoneal administration of ^{3}H-tyramine, almost all the parent drug disappears within 15 min (within 30 min after intramuscular administration). At this time the label in the heart consists almost entirely of octopamine, which is held more tenaciously than tyramine (half-life about 2 hr). A relatively large fraction of a small dose of tyramine is converted to octopamine; however, the data in Fig. 2 support the view that after a large dose of tyramine the proportion converted to octopamine is relatively small.

The profile of the distribution of tyramine is reminiscent of results obtained with guanethidine (Chang *et al.*, 1965). The two drugs seem to have the following characteristics in common: (1) their uptake into heart is maintained by a specialized membrane process; (2) a considerable fraction of the NA release by both drugs from the isolated heart reaches the effluent as unchanged NA (Nash, Costa & Brodie, 1964; Kopin & Gordon, 1962); (3) the drugs release NA from heart slices *in vitro* at the same maximum rate constant (Leitz & Bogdanski, 1967); (4) finally, DMI blocks the cardiac uptake of tyramine (Table 3) and guanethidine (Chang *et al.*, 1965) given in tracer doses, but does not block the uptake of large doses of tyramine (Table 3).

The demonstration that DMI can prevent the release of NA by large doses of tyramine suggests that DMI may act not only on the uptake mechanism at the neuronal membrane, but also within the neurone to prevent the release of NA by

tyramine and guanethidine (Stone, Porter, Stavorski, Ludden & Totaro, 1964). The uptake of tyramine by adrenergic nerves cannot be easily studied kinetically because of its high affinity for intraneuronal MAO, so we have carried out experiments with amphetamine, a substance which is not metabolized by monoamine oxidase and which crosses body membranes by simple diffusion. The experiments showing that DMI prevents amphetamine from releasing heart NA corroborate the view that DMI prevents the effects of indirect sympathomimetics by an action other than preventing their access to intraneuronal storage sites. Further indications of the possibility that DMI acts intraneuronally are studies which show that DMI decreases the spontaneous release of NA from nerve endings (Reid, Volicer & Brodie, 1967). In addition, these workers have investigated the possibility that DMI exerts an action on microsomes. They have shown that after the injection of large doses of NA into rats the catecholamine is taken up by adrenergic neurones in considerable amount and displaces about 80% of the endogenous NA (previously labelled with ^{3}H-NA). When animals are first given DMI and then given the large dose of NA, the exogenous catecholamine is taken up by adrenergic neurones to the same extent, but the displacement of endogenous NA is markedly reduced. These authors concluded that, despite the failure of DMI to block the cardiac uptake of large doses of NA, it prevents the exogenous amine from freely mixing with the endogenous NA stored in neuronal vesicles. This conclusion is corroborated by the data reported in Fig. 4 showing that DMI antagonizes the release of NA from synaptic vesicles elicited by tyramine.

This effect of DMI on NA stored in nerve endings *in vitro* is reminiscent of the effects of desipramine-like compounds on isolated synaptic vesicles studied *in vitro*. In both cases the uptake of NA can be blocked by amounts of drug that do not release the transmitter (Carlsson, 1966 ; Kirschner, 1965 ; Stjarne, 1964). Again, desipramine-like compounds reduce the spontaneous release of NA from adrenergic neurones (Reid *et al.*, 1967) and from nerve granules (Stjarne, 1964). Finally, high doses of desipramine-like substances cause the release of NA from adrenomedullary particles (Weil-Malherbe & Posner, 1963), from neurones in isolated and perfused heart (Titus, Matussek, Spiegel & Brodie, 1966) and from heart slices (Leitz & Bogdanski, 1967). These results invite the speculation that desipramine-like substances exert an action on the synaptic vesicles.

The present report shows the hazards of drawing conclusions about mechanisms of drug action based on studies using tracer doses of the drug. From the results with tracer doses of ^{3}H-tyramine (Fig. 1) it might be inferred that this amine is taken up by adrenergic neurones and converted almost entirely to octopamine, which in turn releases axonal NA. After large doses of tyramine which deplete cardiac NA, however, the extent of this conversion is quite small and the release of NA is probably mediated largely through the parent drug. Similarly, from studies showing that DMI blocks the uptake of tracer doses of tyramine (Table 3) or guanethidine (Brodie *et al.*, 1964), it might be assumed that DMI blocks the effects of these substances by an action at the neuronal membrane. In contrast, DMI blocks the NA depletion by amphetamine (Table 5) and by large doses of tyramine (Table 4) without reducing tissue levels of either drug. Because (+)-amphetamine accumulates in tissues by a process resistant to cocaine, DMI, reserpine, and ouabain (Roos and Renyi, 1966), the mechanism of DMI action cannot be linked to the sites of the cell membrane that transfer monoamines. The results shown in Table 5 are compatible

with the view that the effect of DMI is exerted intraneuronally, perhaps on the membrane of synaptic vesicles. Furthermore, this view is substantiated by the data of Fig. 4 showing that DMI prevents the release of NA from cardiac microsomal fraction which contains NA bound to synaptic vesicles (Michaelson *et al.*, 1964).

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