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Effect of Desmethylimipramine on the Release of [^{3}H]Norepinephrine Induced by Various Agents in Hypothalamic Synaptosomes

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SUMMARY

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In order to discriminate between carrier-mediated and non-carrier-mediated mechanisms of norepinephrine release, hypothalamic synaptosomes labeled with [^{3}H]norepinephrine were treated with desmethylimipramine, a blocker of the norepinephrine transport system, and then superfused under various conditions stimulating the release of the labeled amine. The stimulatory effect on release elicited by unlabeled norepinephrine, *p*-tyramine, and β-phenylethylamine was blocked in desmethylimipramine-treated synaptosomes. The effect of β-phenylethylamine, but not that of norepinephrine, was largely maintained in synaptosomes superfused with a sodium-free medium, in the absence of desmethylimipramine; thus β-phenylethylamine could enter synaptosomes without utilizing the norepinephrine carrier and displace the catecholamine, which would then be transported out of the particles through a desmethylimipramine-sensitive carrier. The release of [^{3}H]norepinephrine was increased upon superfusion with a sodium-free medium, and desmethylimipramine largely prevented this release. The release of [^{3}H]norepinephrine from reserpine-treated synaptosomes induced by lack of sodium was totally blocked by desmethylimipramine. Superfusion with a potassium-free medium stimulated [^{3}H]norepinephrine release, which was affected only slightly by prior treatment with desmethylimipramine. Depolarization of synaptosomes by 56 mM KCl, in the presence of calcium, or addition of the ionophore A23187 to the superfusion medium caused a desmethylimipramine-insensitive release of labeled amine, a finding compatible with exocytotic release. The release of ^{3}H-labeled deaminated metabolites of [^{3}H]norepinephrine induced by reserpine was not affected by desmethylimipramine.

INTRODUCTION

The mechanism by which norepinephrine is released from noradrenergic nerve terminals under various experimental conditions is not fully understood. It is generally agreed that the release, coupled to the depolarization of nerve terminals, occurs by a mechanism of exocytosis. Evidence favoring this mechanism has been obtained essentially in studies on periph-

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eral nerves (1–3), but recent results of investigations on nerve ending preparations obtained from the central nervous system may be compatible with the idea that exocytotic release also operates in central nerve terminals (4, 5). As "an alternative to exocytosis," Bogdanski (6) proposed that the amine released by depolarizing stimuli leaves the nerve terminal through a carrier-mediated process having characteristics similar to those of the uptake system. On the other hand, a carrier-mediated process seems to be involved in the release of norepinephrine elicited under some experimental conditions by sympathomimetic amines, as suggested, for example, by the studies of Paton (7) on atria from reserpine-treated rabbits. Finally, since the carrier-mediated uptake of norepinephrine is dependent on the Na^+ gradient across the nerve ending membrane (6, 8), a carrier-mediated efflux of norepinephrine might be expected under conditions tending to reverse the direction of the Na^+ gradient.

In attempting to discriminating between carrier-mediated and non-carrier-mediated release of norepinephrine from nerve terminals, we analyzed the release of the radioactive amine induced by several experimental conditions from hypothalamic synaptosomes treated with desmethylimipramine, a drug causing long-lasting inhibition of the carrier-mediated transport of norepinephrine.

METHODS

Crude synaptosomal fractions (P_2) prepared from adult male Wistar rat hypothalamus according to Gray and Whittaker (9) were washed once with 0.32 M sucrose and resuspended in 0.32 M glucose at a protein concentration of about 8 mg/ml. The suspension was diluted 1:10 with Krebs-Ringer medium (128 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5 mM Na_2HPO_4, and 10 mM Tris-HCl buffer at pH 7.35) containing (unless otherwise stated) ascorbic acid (1 mM) and nialamide (12.5 μM) and equilibrated for 10 min at 37° in a rotary water bath. Synaptosomes were then labeled for 10 min with 0.1 μM *l*-[7-^{3}H]norepinephrine (specific activity, 9.8 Ci/mmole; Radiochemical Centre). Aliquots of the suspension (1 ml) were transferred to as many as 18 parallel superfusion chambers (10) and washed with 15 ml of oxygenated, glucose-containing superfusion medium at 37°, under moderate vacuum. Superfusion was then started, at a rate of 0.5 ml/min, with standard medium or with a medium containing 10 μM DMI.[1] After 10 min the particles were washed with two 5-ml portions of standard medium and then superfused again for 7 min, after which the superfusion medium of some of the chambers was replaced with new medium whose composition was altered as indicated in the figures. The superfusion was stopped after 6–8 min. Fractions were collected at 1-min intervals from the various chambers and counted for radioactivity. The radioactivity remaining in the tissue was also measured at the end of the superfusion period. Since, under the superfusion conditions used, reuptake of the spontaneously released [^{3}H]norepinephrine was completely prevented (10–12), the increased release observed under the various experimental conditions tested could not be attributed to inhibition of reuptake of the released amine. In some experiments the amount of ^{3}H-labeled deaminated metabolites of [^{3}H]norepinephrine was measured in the superfusate by the method described by Baldessarini and Kopin (13).

RESULTS

Release by sympathomimetic amines. One method used for studying the mechanism of norepinephrine release by sympathomimetic amines has been pharmacological blockade of the norepinephrine carrier before or during administration of the releasing drug (7, 14–17). Reduction of the releasing activity subsequent to inhibition of the carrier has often been attributed to inhibition of the entry of the sympathomimetic amine into the nerve terminals through the norepinephrine transport system.

In recent studies we have shown that β-phenylethylamine, *p*-tyramine, and nor-

[1] The abbreviation used is: DMI, desmethylimipramine.

epinephrine itself stimulate the release of [^{3}H]norepinephrine from hypothalamic synaptosomes (18–20). Figure 1 shows that the release induced by any of the three amines at 10 μM was completely abolished when 1 μM DMI was added to the superfusion fluid together with the releasing amine, in order to block the norepinephrine carrier. DMI alone did not appreciably influence the spontaneous release of [^{3}H]norepinephrine. Very strong inhibition of [^{3}H]norepinephrine release by β-phenylethylamine and norepinephrine (10 μM) was also detected in synaptosomes previously treated with 10 μM DMI and then washed for several minutes before addition of the releasing agents (Fig. 2). The inhibition was somewhat less when 1 μM DMI was used (data not shown). The spontaneous release of [^{3}H]norepinephrine was minimally influenced only (difference not statistically significant) by the prior DMI treatment alone.

The inhibition of the releasing activity of norepinephrine and *p*-tyramine by DMI was probably due largely to prevention of their entry into synaptosomes consequent to the blockade of the norepinephrine carrier (21). However, it has been reported that some phenylethylamine derivatives, including β-phenylethylamine, are not good substrates for the norepinephrine transport system and that they could enter nerve terminals by passive diffusion (16, 22). If this were the case, β-phenylethylamine might also be expected to exhibit

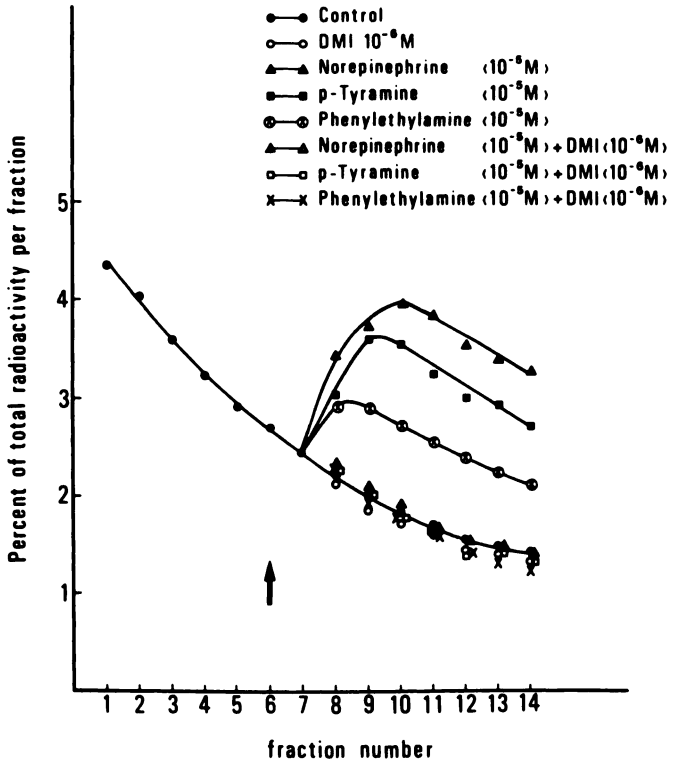

FIG. 1. *Inhibitory effect of desmethylimipramine on [^{3}H]norepinephrine release induced by sympathomimetic amines*

Crude synaptosomal fractions from rat hypothalamus were labeled with 0.1 μM [^{3}H]norepinephrine for 10 min and then superfused in 18 parallel superfusion chambers with standard medium. After 6 min (see arrow), the medium in two or three chambers was replaced with new medium containing either 1 μM DMI or 10 μM unlabeled amine, or both, as indicated. Radioactivity was measured in superfusate fractions collected at 1-min intervals and in tissue at the end of the superfusion period. The counts recovered in each fraction are expressed as a percentage of the total counts (fractions plus tissue). Each curve is the average of two duplicate or triplicate experiments performed on different days.

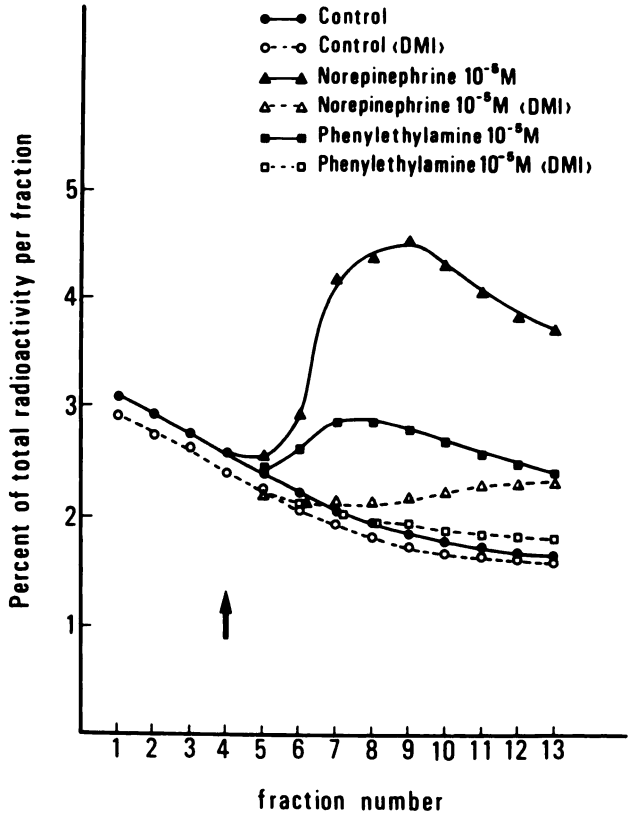

FIG. 2. *Effect of prior treatment with desmethylimipramine on release of [³H]norepinephrine induced by unlabeled β-phenylethylamine and norepinephrine*

Crude hypothalamic synaptosomal fractions were labeled with 0.1 μM [³H]norepinephrine for 10 min and then superfused for 10 min with standard medium or with medium containing 10 μM DMI. The fractions collected during this period were discarded. After washing with standard medium, the particles were superfused for 6 min with the same medium, which was then replaced (see arrow) with new medium containing 10 μM norepinephrine or β-phenylethylamine. Each curve is the average of two triplicate experiments performed on different days. The additional 10 min of superfusion to which synaptosomes were subjected in these experiments and in those presented in Figs. 5–8 accounts for the slower rates of [³H]norepinephrine release in the prestimulation part of the curves, compared with the rates presented in Figs. 1, 3, and 4.

releasing activity in the presence of DMI. The inhibition that was instead observed (Figs. 1 and 2) might suggest that DMI blocked the outward transport of the [³H]norepinephrine displaced from its storage sites by β-phenylethylamine. In order to test this possibility, we had to determine whether β-phenylethylamine would maintain its releasing capacity under conditions (lack of extacellular Na^+) preventing the influx (23) but not the efflux (see following paragraph) of norepinephrine through its carrier. Figure 3 shows that the release of [³H]norepinephrine induced by β-phenylethylamine was only moderately reduced in a Na^+-free medium; in contrast, that induced by unlabeled norepinephrine was very strongly inhibited (Fig. 4).

Release induced by lack of sodium or potassium. The release of [³H]norepinephrine from hypothalamic synaptosomes was increased when Na^+ was removed from the superfusion medium and replaced with sucrose (Fig. 5), confirming previous results of Bogdanski *et al.* (24) obtained in whole brain synaptosomes. Prior treatment of the particles with DMI largely prevented

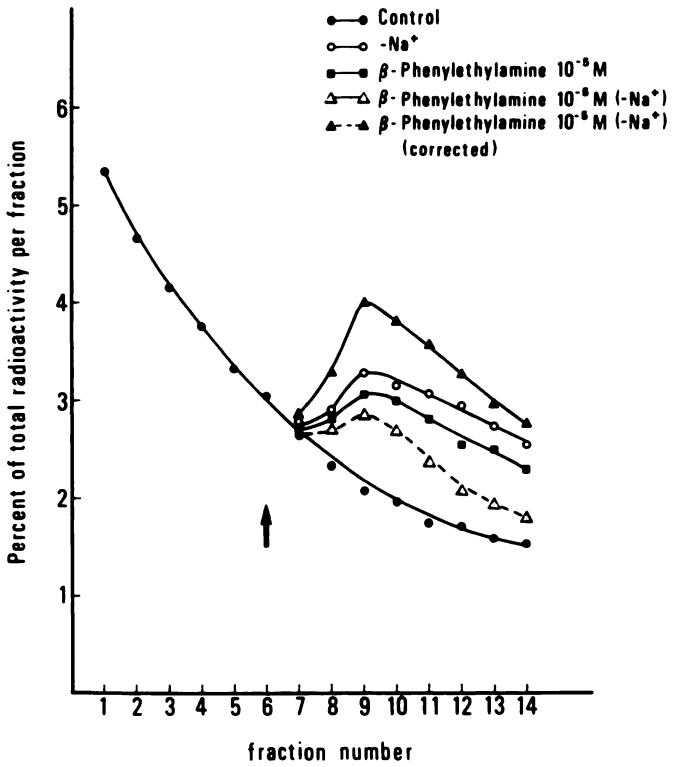

FIG. 3. *Release of [³H]norepinephrine induced by β-phenylethylamine in the absence of extracellular sodium*

Crude hypothalamic synaptosomal fractions labeled with 0.1 μM [³H]norepinephrine were superfused for 6 min with standard medium. The medium was then replaced with the following new media: Na^+-free medium (128 mM NaCl replaced by 256 mM sucrose), Na^+-free medium with 10 μM β-phenylethylamine, standard medium, or standard medium with 10 μM β-phenylethylamine. Each curve is the average of two experiments performed in triplicate on different days. The dashed curve was obtained by subtracting the release induced by the Na^+-free medium from that elicited by β-phenylethylamine in the absence of Na^+.

this increase, in agreement with similar observations made by Paton in atria from reserpine-treated rabbits (17).

In order to obtain information on the origin of the norepinephrine released by sodium deprivation, reserpine-treated synaptosomes were labeled with [³H]norepinephrine and then superfused with a sodium-free medium in the presence and absence of DMI. Figure 6 shows that DMI completely blocked the [³H]norepinephrine release due to sodium deprivation, and also that DMI caused a pronounced decrease in spontaneous [³H]norepinephrine release from reserpine-treated synaptosomes.

When synaptosomes were superfused with a medium lacking K^+, the release of [³H]norepinephrine was also increased, although to a lesser extent than in the absence of Na^+ (Fig. 7). However, the release elicited by lack of K^+ was diminished only slightly in DMI-treated synaptosomes. The release elicited by lack of either Na^+ or K^+ was totally accounted for by unmetabolized [³H]norepinephrine. ³H-Labeled deaminated metabolites, which represented about 25% of the total ³H spontaneously released in standard medium containing nialamide, were reduced to about 5–7% under conditions of Na^+ deprivation.[2]

Calcium-dependent release. In a previous study (4) we showed that depolarization of synaptosomes by 56 mM KCl, in the presence of Ca^{2+}, caused the release of unmetabolized [³H]norepinephrine. In order

[2] Unpublished observations.

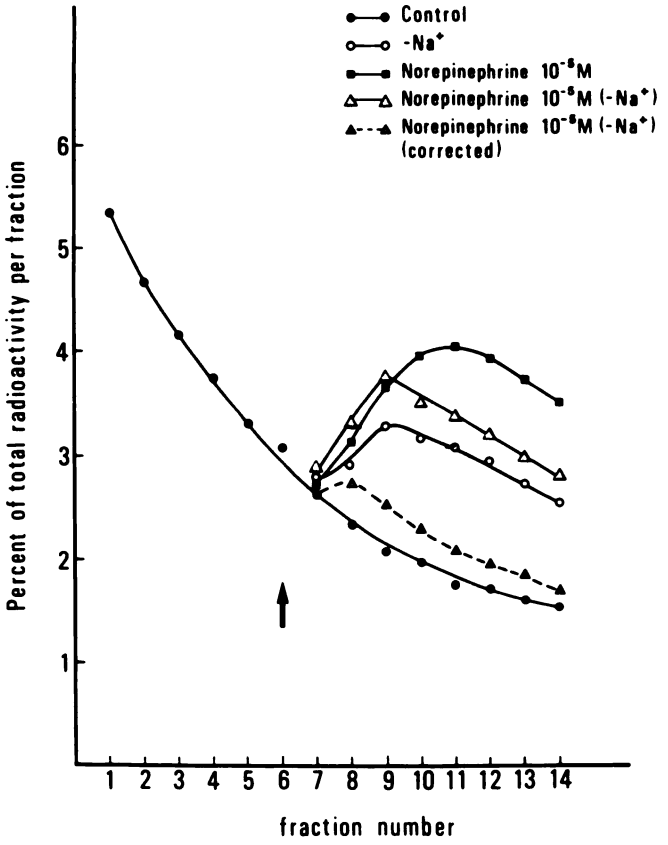

FIG. 4. *Release of [³H]norepinephrine induced by unlabeled norepinephrine in the absence of extracellular sodium*

Experimental details were the same as for Fig. 3, except for the unlabeled amine added to the superfusion medium. Each curve is the average of two experiments performed in triplicate on different days.

to clarify the mechanism of this stimulated release, synaptosomes were subjected to a depolarizing concentration of KCl after being treated with the norepinephrine carrier-blocking agent DMI. Figure 8 shows identical patterns of [³H]norepinephrine release in DMI-treated and control synaptosomes.

It has recently been shown that the calcium ionophore A23187 releases largely unmetabolized [³H]norepinephrine from peripheral and central nerve endings (5, 25). Figure 9 shows that also this stimulated release was not inhibited in DMI-treated synaptosomes.

Reserpine-induced release. Reserpine (1 μM) caused an increase in the release of total ³H from synaptosomes labeled with [³H]norepinephrine (Fig. 10). The reserpine-induced release was not altered in synaptosomes previously treated with DMI. In both control and DMI-treated nerve endings essentially all the ³H released by reserpine consisted of ³H-labeled deaminated metabolites. A pattern identical with that reported in Fig. 10 was obtained in experiments in which DMI was present in the superfusion medium at 10 μM during the reserpine-induced release.

DISCUSSION

Since norepinephrine, according to the available literature (1–7), can be released from nerve terminals by at least two mechanisms (carrier-mediated transport and exocytosis), it was thought that the utilization of an experimental procedure blocking one of these mechanisms would help to elucidate the modality of norepinephrine release under a variety of experimental

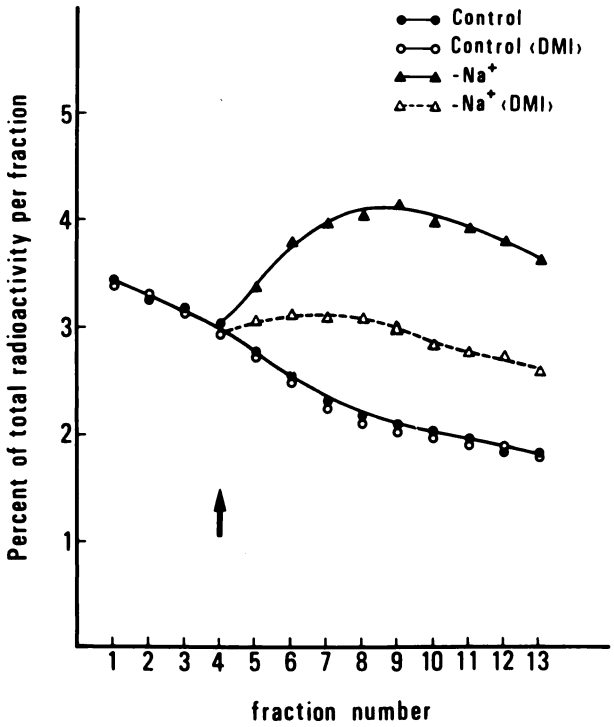

FIG. 5. *Effect of prior treatment with desmethylimipramine on release of [³H]norepinephrine induced by a sodium-free medium*

Experimental details were the same as for Fig. 2, except for the superfusion media added at the sixth minute. Each curve is the average of four experiments performed in duplicate or triplicate on different days.

conditions. In the present study we attempted to block synaptosomal carrier-mediated norepinephrine release with DMI (a drug amply described as an inhibitor of norepinephrine uptake in central and peripheral nervous tissues), on the assumption that the outward carrier-mediated release of [³H]norepinephrine is DMI-sensitive. The IC_{50} values reported for norepinephrine uptake inhibition by DMI are in the range of 10 nM (26), and in the present study synaptosomes were first treated with 10 μM DMI in order to achieve almost complete blockade of the norepinephrine pump.

Spontaneous release. About 75% of the ³H released spontaneously from superfused synaptosomes in the presence of nialamide consisted of unmetabolized [³H]-norepinephrine.[2] The finding that prior treatment with DMI had little if any inhibibitory effect on the spontaneous release suggests that most of the [³H]norepinephrine exits from synaptosomes without utilizing the carrier system blocked by the drug. Whether this release represents diffusion, possibly from damaged synaptosomes, or takes place by other mechanisms can not be established at present. It is noteworthy, however, that when the cytoplasmic concentration of [³H]norepinephrine was increased by treating the synaptosomes with reserpine before labeling them with [³H]norepinephrine in the presence of a monoamine oxidase inhibitor, the subsequent spontaneous release was substantially decreased by the addition of DMI to the superfusion fluid. A substantial decrease in the spontaneous release of [³H]norepinephrine could also be observed in synaptosomes prepared from rats treated with reserpine as described by Paton (17) and superfused in the presence of DMI.[2]

Release by sympathomimetic amines. Phenylethylamine derivatives having

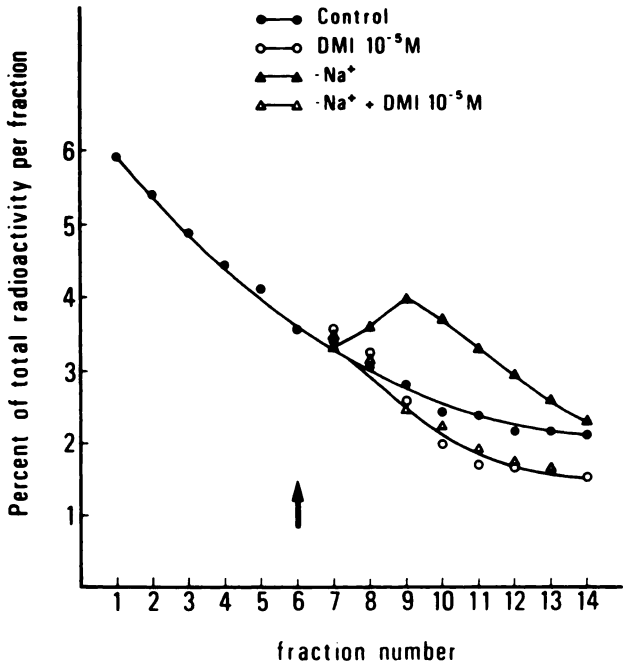

FIG. 6. *Effect of desmethylimipramine on release of [³H]norepinephrine induced by a sodium-free medium in reserpine-treated synaptosomes*

Crude hypothalamic synaptosomes which had been incubated for 10 min in the presence of 1 μM reserpine and labeled with 0.5 μM [³H]norepinephrine (in the presence of reserpine) were superfused for 6 min with standard medium. Then the medium was replaced with new media as indicated. Incubation and superfusion were performed in the presence of 0.5 mM pargyline. Each curve is the average of the data obtained in one experiment performed in triplicate. Another triplicate experiment, utilizing synaptosomes from reserpine-treated rats, gave qualitatively similar results.

phenolic hydroxyl groups are not only inhibitors of norepinephrine uptake but also substrates for the norepinephrine transport system (16, 22, 26). On the other hand, compounds lacking phenolic hydroxyl groups, such as β-phenylethylamine, are not considered good substrates for the norepinephrine carrier (16, 22). Therefore the inhibition by DMI of the [³H]norepinephrine-releasing effect of β-phenylethylamine, *p*-tyramine, and unlabeled norepinephrine (Figs. 1 and 2) can be attributed to inhibition of the entry of the releasing compound into synaptosomes only in the cases of *p*-tyramine and norepinephrine. β-Phenylethylamine largely retained its releasing effect in the absence of extracellular Na^+ (Fig. 3), a condition that prevents uptake through the norepinephrine transport system (23, 26) and that led to a large reduction in [³H]norepinephrine release by unlabeled norepinephrine (Fig. 4). Thus the inhibitory effect of DMI on β-phenylethylamine-induced release may have been due to inhibition of carrier-mediated outward transport of the [³H]norepinephrine displaced by β-phenylethylamine from intraterminal storage sites. A similar interpretation was given by Paton (27) to the inhibition by cocaine of the release of [³H]norepinephrine from reserpine-treated rabbit atria induced by phenylethylamines lacking phenolic hydroxyl groups.

Release induced by lack of sodium or potassium. According to the Na^+ gradient hypothesis (28), the establishment of an outward downhill Na^+ gradient across the synaptosomal membrane should facilitate outward norepinephrine carrier-mediated transport (6). The finding that the increased release observed upon superfusion with a Na^+-free medium consisted of unmetabolized [³H]norepinephrine, together

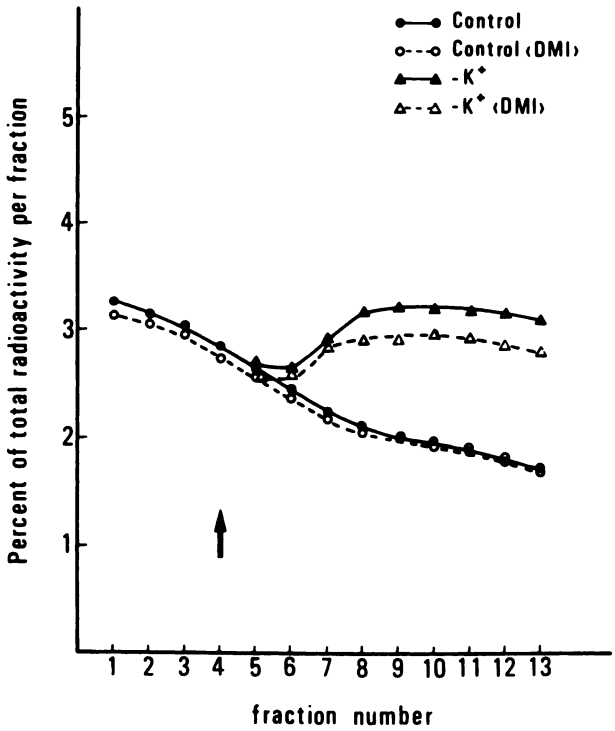

FIG. 7. *Effect of prior treatment with desmethylimipramine on release of [³H]norepinephrine induced by a potassium-free medium*

Experimental details were the same as for Fig. 2, except for the superfusion media added at the sixth minute. Each curve is the average of four experiments performed in duplicate or triplicate on different days.

with the observation that prior treatment of the synaptosomes with DMI largely prevented this increased release (Fig. 5), is in keeping with this expectation.

When present in the superfusion fluid, DMI also blocked the release (both spontaneous and stimulated by lack of sodium) of [³H]norepinephrine accumulated by reserpine-treated synaptosomes in the presence of a monoamine oxidase inhibitor (Fig. 6), or by synaptosomes from reserpine-treated animals (data not shown). This result supports the idea that cytoplasmic norepinephrine can exit from the nerve endings through a DMI-sensitive carrier located in the plasma membrane.

The effects of DMI on [³H]norepinephrine release from reserpine-treated synaptosomes could not be observed unless DMI was present in the superfusion medium, in contrast with the observations made with non-reserpine-treated synaptosomes, which had been treated beforehand with DMI. A possible explanation is that the two drugs compete for the plasma membrane lipids, for which reserpine would have an affinity higher than DMI. Reserpine might thus hinder stable binding of DMI to the membrane, or displace it in the case of synaptosomes previously treated with DMI; however, when present in the superfusion fluid at 10 μM, DMI was apparently able to interact with the norepinephrine transport sites, even in reserpine-treated synaptosomes.

The observation that the inhibition by DMI of [³H]norepinephrine release induced by lack of Na^+ was not complete (Fig. 5), a finding similar to that reported by Paton in atria from reserpine-treated rabbits (17), is not likely to have been due to a generalized increase in membrane permeability, as suggested by Paton, since under identical conditions of sodium deprivation the release of [³H]γ-aminobutyric acid, whose synaptosomal localization is

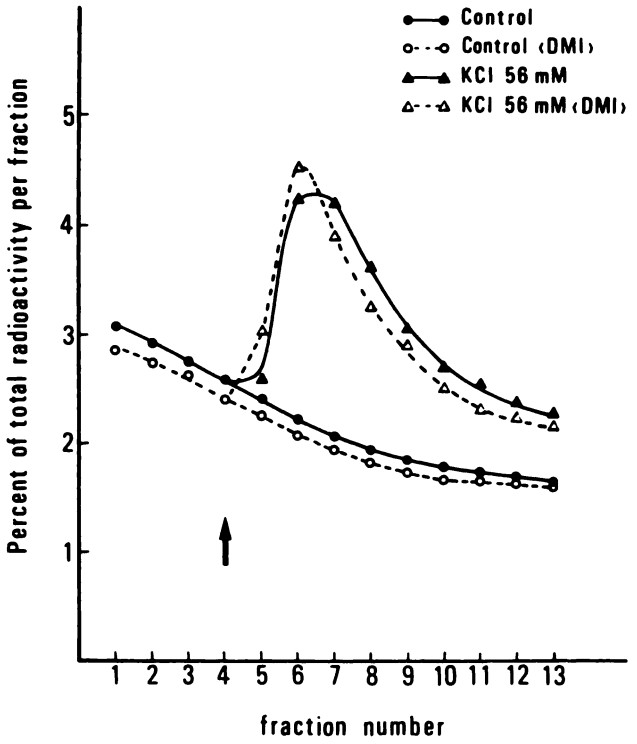

FIG. 8. *Effect of prior treatment with desmethylimipramine on release of [³H]norepinephrine induced by 56 mM KCl*

Experimental details were the same as for Fig. 2. In the medium containing 56 mM KCl, the excess KCl added replaced an equimolar concentration of NaCl. Each curve is the average of three experiments performed in triplicate on different days.

largely cytoplasmic, was unaltered (29, 30). Another possibility that should be considered is that part of the [³H]norepinephrine release observed in the absence of Na^+ is related to an increased influx of Ca^{2+}. In fact, Blaustein and Oborn (31) have shown that Ca^{2+} influx into synaptosomes increases as the extracellular concentration of Na^+ decreases. As shown in Fig. 8, the release of [³H]norepinephrine induced by high K^+, which is Ca^{2+}-dependent (4, 32), was not inhibited by DMI (see also following paragraph). It should be noted that in another system (heart slices) Ca^{2+}-dependent, DMI-sensitive release of [³H]norepinephrine under conditions of sodium deprivation was reported by Bogdanski (6).

It is known that ouabain or lack of K^+ inhibits the (Na^+ + K^+)-ATPase (8), with a consequent increase in the intracellular Na^+ concentration. Under these conditions, the less unfavorable Na^+ gradient should facilitate outward carrier-mediated norepinephrine transport (24). Data in keeping with this expectation were reported by Paton in reserpine-treated atria (17). Under our experimental conditions ouabain (0.1 mM) had a very modest releasing effect on synaptosomal [³H]norepinephrine (33), and was therefore not used in the present investigation. The releasing effect of the K^+-free medium was much greater than that of ouabain and was only moderately inhibited in synaptosomes previously treated with DMI (Fig. 7). Probably, therefore, the bulk of the release caused by the K^+-deficient medium is not mediated by the DMI-sensitive carrier. The data of Paton in reserpine-treated atria (17) are in marked contrast with those presented here, since under his conditions the release caused by ouabain was much greater than that induced by lack of

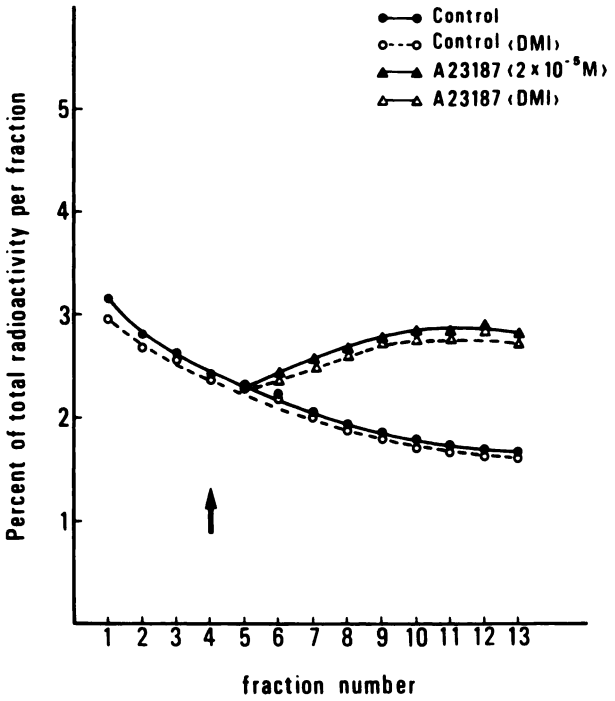

FIG. 9. *Effect of prior treatment with desmethylimipramine on release of [³H]norepinephrine induced by the ionophore A23187*

Experimental details were the same as for Fig. 2, except for the superfusion media added at the sixth minute. A23187 was added to the medium, just before use, as an ethanolic solution (7 μl/ml); control superfusion media contained the same amount of ethanol. Each curve is the average of four experiments performed in duplicate or triplicate on different days.

K^+, and both were strongly inhibited by DMI and cocaine. It is possible that following the combined effect of reserpine and monoamine oxidase inhibition (17), more free norepinephrine becomes available in the cytoplasm for the carrier-mediated exit favored by the increased Na^+ concentration. Under our conditions, the cytoplasmic norepinephrine concentration is likely to be negligible, and the creation of a less unfavorable Na^+ gradient may not be sufficient to promote large outward transport. The release of small amounts through the carrier, however, can be inferred from the fraction of [³H]norepinephrine release inhibited by DMI (Fig. 7). The mechanism by which the absence of extracellular K^+ causes substantial release of unmetabolized norepinephrine by a process which is largely DMI-insensitive is still unclear. The possibility cannot be excluded that part of this release is related to increased availability of free intracellular Ca^{2+}, which may follow the increased intracellular Na^+ concentration (31).

Calcium-dependent release. We have previously shown that depolarization of synaptosomes, in the presence of Ca^{2+}, causes the release of unmetabolized [³H]-norepinephrine (4). The calcium ionophore A23187 was also shown to release largely unmetabolized [³H]norepinephrine from synaptosomes (5). In the present study we have shown that both stimuli (which are generally considered to mimic the physiological stimulation of nerve endings) caused release of [³H]norepinephrine that could not be inhibited by DMI. Altogether these findings are consistent with an exocytotic release of norepinephrine triggered by the entry of Ca^{2+} into nerve endings. On the other hand, the release of norepinephrine induced by depolarization has been considered by Bogdanski (6) to be compatible with carrier-mediated transport. Bogdanski attributed the lack of in-

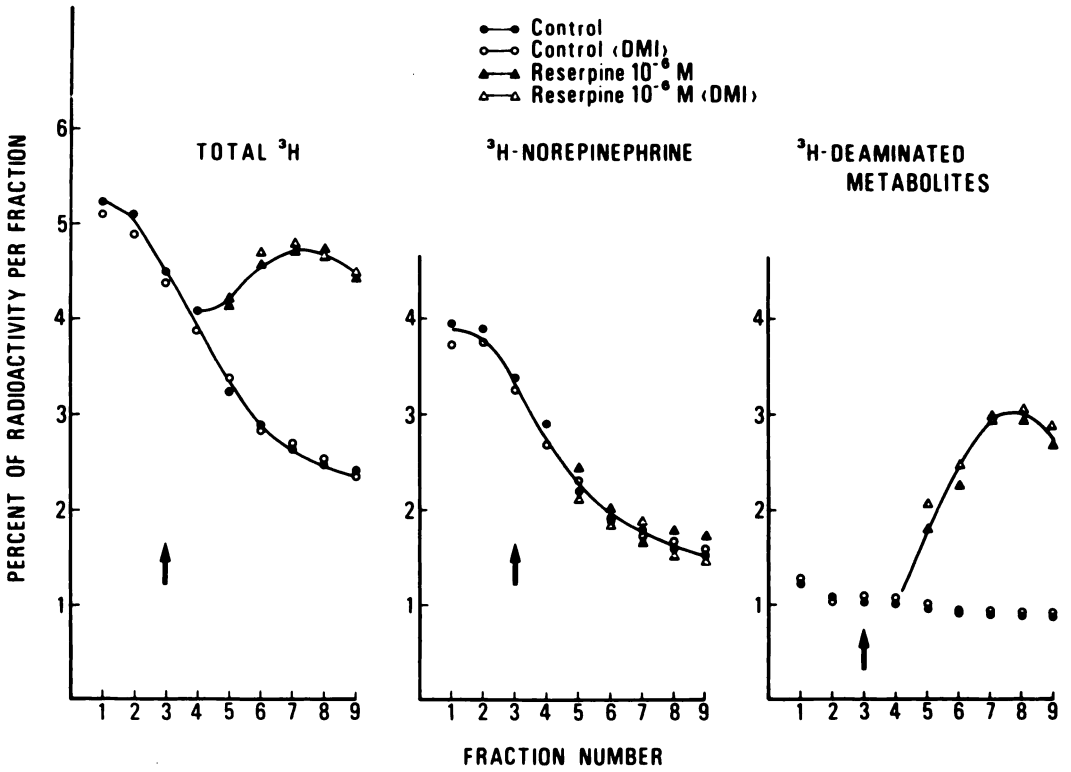

FIG. 10. *Effect of prior treatment with desmethylimipramine on release of [³H]norepinephrine induced by reserpine*

Experimental details were the same as for Fig. 2, except that incubation and superfusion media did not contain monoamine oxidase inhibitors, and fractions were collected every 2 min and aliquots were used for the determination of deaminated metabolites. The release of [³H]norepinephrine (center) was calculated by subtracting the radioactivity accounted for by deaminated metabolites (right) from the total radioactivity released (left). Each curve is the average of two experiments performed in triplicate on different days.

hibitory effect of DMI on [³H]norepinephrine release from rat heart slices elicited by high K^+ to a possible K^+-induced conformational change of the norepinephrine carrier, which would hinder the binding of DMI. Our data do not support this interpretation. In fact, prior treatment with DMI was without effect not only on the release of [³H]norepinephrine induced by 56 mM KCl (Fig. 8), but also on that provoked by the ionophore A23187 in the absence of high K^+ (Fig. 9). Since both high K^+ and A23187 are believed to cause the release of [³H]norepinephrine triggered by the entry of Ca^{2+} into synaptosomes (4, 5), it would appear that the Ca^{2+}-dependent norepinephrine release is not mediated by the DMI-sensitive carrier system. In this respect, it is pertinent that tricyclic antidepressants like DMI generally do not inhibit adrenergic transmission (21, 26).

In conclusion, DMI, known as a norepinephrine uptake inhibitor without appreciable effect on spontaneous release of the amine, has been shown in the present investigation to inhibit selectively some types of stimulated [³H]norepinephrine release mediated by the membrane norepinephrine carrier system. It therefore seems important, when experimenting with a new neuroactive drug, also to evaluate its possible effects on neurotransmitter release stimulated under various conditions.

Interaction with the carrier-mediated release of norepinephrine represents a target of drug action that has not previously been considered. It should be emphasized

that an action at this level may have physiological implications. For example, endogenous phenylethylamines, which have been proposed to modulate monoaminergic transmission, might also do so by causing the release of amines (34), which, according to the present study, is carrier-mediated and drug-inhibitable.

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