MOLECULAR PHARMACOLOGY, 11, 10-18

On the Mechanism of Release of Norepinephrine from Sympathetic Nerves Induced by Depolarizing Agents and Sympathomimetic Drugs

NGUYEN B. THOA, G. FREDERICK WOOTEN, JULIUS AXELROD, AND IRWIN J. KOPIN

Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20014

(Received October 8, 1974)

SUMMARY

THOA, NGUYEN B., WOOTEN, G. FREDERICK, AXELROD, JULIUS & KOPIN, IRWIN J. (1975) On the mechanism of release of norepinephrine from sympathetic nerves induced by depolarizing agents and sympathomimetic drugs. *Mol. Pharmacol.*, **11**, 10-18.

Depolarization of isolated guinea pig vasa deferentia *in vitro* by either hypertonic KCl or veratridine resulted in a dose-dependent, proportional release of norepinephrine and dopamine β-hydroxylase. The ratio of norepinephrine to dopamine β-hydroxylase activity released by depolarizing agents was similar to that obtained by electrical stimulation of the hypogastric nerve. Thus exocytosis from sympathetic nerve terminals may be elicited by depolarizing drugs as well as by electrical stimulation. Incubation of vasa deferentia *in vitro* in the presence of reserpine or the sympathomimetic amines tyramine, *d*-amphetamine, or metaraminol resulted in the dose-dependent release of norepinephrine but not of dopamine β-hydroxylase. When calcium was omitted from the incubation medium, exocytotic release of norepinephrine and dopamine β-hydroxylase induced by depolarizing agents was blocked whereas norepinephrine release induced by sympathomimetic agents was not affected. Tetrodotoxin blocked veratridine but not tyramine-induced release. Colchicine, but not hexamethonium or atropine, blocked exocytotic release. None of these drugs affected tyramine-induced release of norepinephrine. In vasa deferentia from untreated animals, the ratio of supernatant (S_2) to particulate (P_2) norepinephrine concentration after centrifugation at 100,000 × *g* for 1 hr was 0.7. In animals treated with reserpine and a monoamine oxidase inhibitor this ratio increased to 1.3, suggesting a release of norepinephrine from vesicular into intraneuronal cytoplasmic stores. The release of norepinephrine by tyramine was enhanced in vasa deferentia from reserpine- and monoamine oxidase inhibitor-treated animals and resulted in a fall in the norepinephrine content of the S_2 fraction. This suggests that tyramine may act by displacing norepinephrine from cytoplasmic stores into the synaptic space. After treatment of guinea pigs with reserpine alone or in combination with a monoamine oxidase inhibitor, electrical stimulation of the hypogastric nerve to the guinea pig vas deferens resulted in release of dopamine β-hydroxylase but not of norepinephrine. This suggests that release by exocytosis may occur in the presence of a depleted amine store and that the norepinephrine released by exocytosis is derived predominantly from vesicular reserpine-sensitive stores.

INTRODUCTION

Norepinephrine and dopamine β-hydroxylase, the enzyme that catalyzes the conversion of dopamine to norepinephrine (1), are highly localized in vesicular structures at noradrenergic nerve terminals (2). Electrical stimulation of the sympathetic nerves to the isolated, perfused spleen resulted in the release of both norepinephrine and dopamine β-hydroxylase into the perfusion medium (3–5). More recent studies have demonstrated a proportional release of these substances from the isolated guinea pig vas deferens on electrical stimulation of the hypogastric nerve (6). The proportionality between released norepinephrine and dopamine β-hydroxylase, coupled with similar norepinephrine to dopamine-β-hydroxylase ratios found in the soluble fraction of vas deferens homogenates and the bath medium following stimulation, suggest that the transmitter and the enzyme are released by exocytosis. Furthermore, the proportional release of norepinephrine and dopamine β-hydroxylase from the vas deferens induced by electrical stimulation is dependent upon the presence of extracellular calcium (7, 8) and is blocked by colchicine and vinblastine, drugs that disaggregate microtubules (9). In contrast to electrical stimulation, the sympathomimetic amine tyramine releases norepinephrine independently of calcium or colchicine (10–12).

In the following study we report that depolarizing agents such as hypertonic KCl and veratridine likewise elicit release of norepinephrine and dopamine β-hydroxylase from noradrenergic nerve terminals by exocytosis. The sympathomimetic amines tyramine, metaraminol, and *d*-amphetamine, as well as reserpine, release the transmitter but not the enzyme. Some properties of both release mechanisms are also described.

METHODS

Preparation of organs. Male albino guinea pigs (500–700 g) were killed by a blow on the head and exsanguinated through their carotid arteries. The abdomen was opened, and both vasa deferentia were excised and placed in cold normal or calcium-free medium. The normal medium had the following composition, in grams per liter: NaCl, 8.06; KCl, 0.35; $CaCl_2 \cdot 2H_2O$, 0.30; $MgSO_4 \cdot 7H_2O$, 0.294; KH_2PO_4, 0.162; and dextrose, 2.0. The calcium-free medium was composed of normal medium from which $CaCl_2 \cdot 2H_2O$ was omitted. The media were adjusted to pH 7.2–7.4 before each experiment. Following excision, the vasa deferentia were washed five times with cold medium, then incubated for 15 min in medium containing 0.25% bovine serum albumin (Sigma Chemical Company). When used, tetrodotoxin, hexamethonium, atropine, or colchicine was present in the 15-min preliminary incubation medium as well as in the subsequent incubation medium. Following preliminary incubation the organs were transferred to 20-ml beakers to which had been added 5 ml of medium containing bovine serum albumin (0.25%), 50 μM phenoxybenzamine HCl to block reuptake of released norepinephrine, and the drug to be studied. The incubation continued for 30 min at 37° in a Dubnoff incubator with slight shaking under an atmosphere of 95% O_2 and 5% CO_2. During the incubation the pH was maintained at 7.2–7.4. Following the 30-min incubation, norepinephrine concentration and dopamine β-hydroxylase activity were determined in the medium. In experiments involving electrical stimulation of the hypogastric nerve, following excision, the vasa deferentia with attached hypogastric nerves were placed in 10-ml organ baths containing medium at 37° and aerated with 95% O_2 and 5% CO_2. After five changes, new medium containing 0.25% bovine serum albumin was introduced and kept for 10 min, then exchanged for 6 ml of normal medium containing 0.25% bovine serum albumin and phenoxybenzamine. Five minutes later the hypogastric nerve was stimulated electrically (5–7 V, 25 Hz, 5-msec duration) for 30 sec of each minute for 60 min. At the conclusion of the stimulation period, the incubation media were removed for assay of norepinephrine and dopamine β-hydroxylase.

Assay of norepinephrine and dopamine

β-hydroxylase in incubation media. Portions (200 μl) of each incubation medium were used to assay for dopamine β-hydroxylase by the method of Molinoff *et al.* (6, 13). Immediately before the assay, control incubation media were routinely added with each of the drugs used as releasing agents. Optimal enzyme activity in the bath medium was obtained in the presence of 20 μM copper sulfate to inactive endogenous inhibitors. Dopamine β-hydroxylase activity was expressed as nanomoles of octopamine formed per gram of tissue incubated in the bath per hour. The remaining incubation medium was acidified with 1 ml of 10 N perchloric acid and centrifuged for 10 min at 27,000 × *g* in a Sorvall refrigerated centrifuge. Sodium metabisulfite (100 mg), alumina (400 mg), and 10 ml of 2% EDTA were added to each supernatant, and the pH was adjusted to 8.6 with NaOH. The norepinephrine was adsorbed onto 400-mg alumina columns by the method of Anton and Sayre (14). The columns were washed once with 10 ml of sodium acetate, 0.2 N (pH 8.6), and then twice with 10 ml glass-distilled water. Norepinephrine was eluted with 6 ml of 0.2 N acetic acid. One-milliliter portions of each eluate were used for determination of norepinephrine by the trihydroxyindole method (15).

Assay of norepinephrine in tissue. Vasa deferentia were blotted dry, weighed, homogenized in 10 ml of 0.4 N perchloric acid, and assayed for norepinephrine as described above. In studies of the subcellular distribution of norepinephrine the P_2 and S_2 fractions were prepared according to Iversen *et al.* (16): vasa deferentia were homogenized in 10 ml of ice-cold 0.25 M sucrose in an all-glass homogenizer. The homogenates were centrifuged at 12,000 × *g* for 10 min. The resulting supernatants fractions were centrifuged at 100,000 × *g* for 1 hr. The supernatant (S_2) and particulate (P_2) fractions obtained were resuspended in perchloric acid to a final concentration of 0.4 N and assayed for norepinephrine as described above.

Drugs. Phenoxybenzamine HCl and *d*-amphetamine sulfate were obtained from Smith Kline & French Laboratories; tyramine HCl, colchicine, and tetrodotoxin, from Calbiochem; metaraminol bitartrate, and atropine sulfate, from Merck, Sharp & Dohme; reserpine (Serpasil), from Ciba Pharmaceuticals; iproniazid sulfate, from Sigma Chemical Company; and hexamethonium HCl, from K & K Laboratories.

RESULTS

Effects of drugs on release of dopamine β-hydroxylase and norepinephrine. Both hypertonic KCl and veratridine elicited a dose-dependent release of norepinephrine and dopamine β-hydroxylase activity into the bath medium (Fig. 1). The release of enzyme was proportional to that of norepinephrine whether release was elicited by hypertonic KCl or veratridine (Fig. 2). The correlation coefficient (0.75) between released dopamine β-hydroxylase and norepinephrine was significant at the $p < 0.001$ level. The ratios of transmitter to enzyme

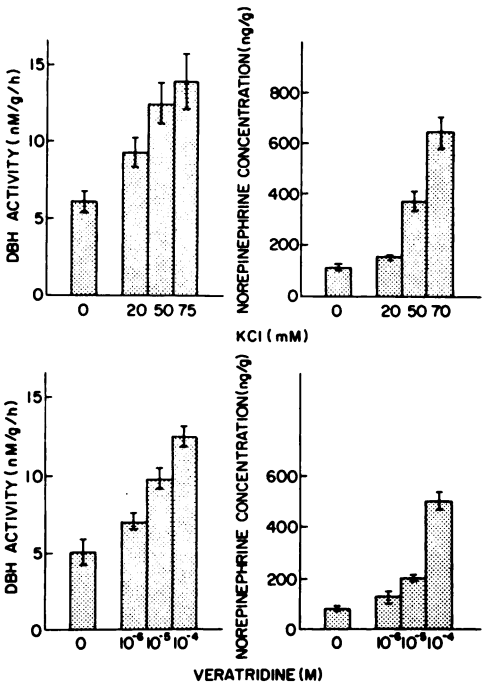

FIG. 1. *Release of dopamine β-hydroxylase (DBH) and norepinephrine into bath medium at various concentrations of KCl and veratridine*

Each bar represents mean ± standard error for at least eight experiments.

activity in the bath medium were similar for both hypertonic KCl and veratridine (Fig. 2). Furthermore, the release ratio in the bath medium produced by hypertonic KCl and veratridine was similar to that obtained by electrical stimulation as well as that found in the soluble fraction of vasa deferentia homogenates (Fig. 2 and ref. 6). Tyramine, *d*-amphetamine, metaraminol, and reserpine all caused a dose-dependent release of norepinephrine (Fig. 3). Neither tyramine nor amphetamine caused release of dopamine β-hydroxylase (Fig. 2). Also, release of norepinephrine by reserpine or metaraminol was not accompanied by release of dopamine β-hydroxylase.

Properties of hypertonic KCl-, veratridine-, and tyramine-induced release of dopamine β-hydroxylase and norepinephrine. When calcium was deleted from the bath medium, neither hypertonic KCl nor veratridine elicited an increase in norepinephrine and dopamine β-hydroxylase activity in the bath (Fig. 4). In contrast, the norepinephrine-releasing effect of tyramine, *d*-amphetamine, metaraminol, and reserpine remained unchanged. Tetrodotoxin blocked the release of both norepinephrine and dopamine β-hydroxylase by veratridine in the presence of normal calcium concentration (Table 1). Colchicine reduced the quantities of norepinephrine and enzyme released by veratridine and

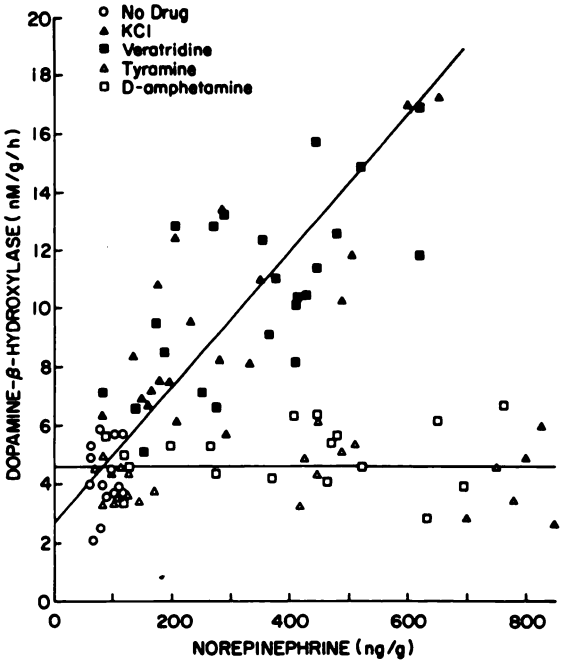

FIG. 2. *Release of dopamine β-hydroxylase and norepinephrine from vas deferens induced by depolarizing agents and sympathomimetic amines*

The dopamine-β-hydroxylase activity present in the incubation medium is plotted against the concentration of norepinephrine. Each preparation was incubated in the presence of one of several concentrations of veratridine, KCl, tyramine, or *d*-amphetamine. The correlation coefficient for dopamine-β-hydroxylase activity and norepinephrine concentration in preparations treated with veratridine or hypertonic KCl was 0.75 ($p < 0.001$; $N = 68$). The linear regression line for this correlation was $y = 2.7 + 0.014x$. In contrast, the correlation between norepinephrine and dopamine β-hydroxylase released by tyramine and *d*-amphetamine was not statistically significant. KCl (50–75 mM), veratridine (100 μM), tyramine (100 μM), and *d*-amphetamine (100 μM) released norepinephrine and dopamine β-hydroxylase in the following ratios: KCl, 34 ± 5; veratridine, 39 ± 2; tyramine, 95 ± 8; *d*-amphetamine, 87 ± 9. The ratio of norepinephrine to dopamine-β-hydroxylase in the incubation medium following hypogastric nerve stimulation was 49 ± 3 (6); the corresponding ratio in the soluble fraction of vasa deferentia homogenates was 28 ± 3 (6).

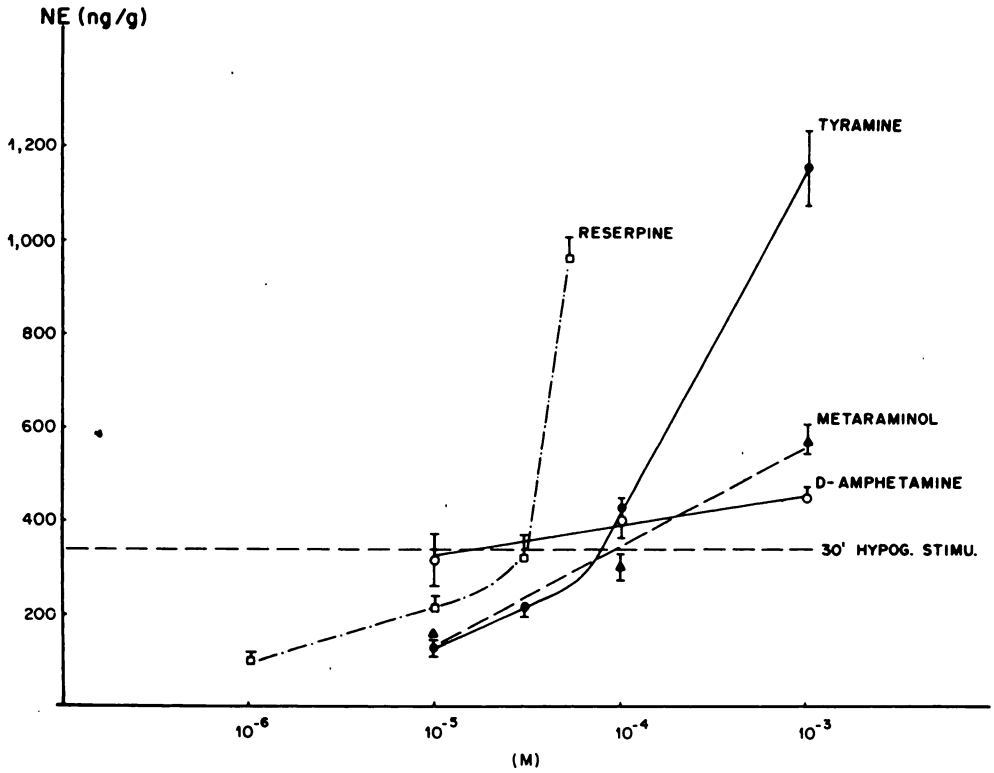

FIG. 3. *Release of norepinephrine (NE) into bath medium at various concentrations of reserpine, tyramine, metaraminol, and d-amphetamine and during 30-min electrical stimulation of hypogastric nerve*

Each point represents mean ± standard error for at least eight experiments. The dose-response values were obtained in the presence of phenoxybenzamine, 50 μM.

hypertonic KCl (Table 1). Neither blocking agent affected the norepinephrine-releasing effect of tyramine (Table 1). Neither hexamethonium (20 μg/ml) nor atropine (5 μg/ml) had any effect on the release of the amine and enzyme by veratridine, or of norepinephrine by tyramine.

Norepinephrine-releasing effect of tyramine after treatment with iproniazid and reserpine. Prior treatment with the monoamine oxidase inhibitor iproniazid increased the level of norepinephrine in vasa deferentia, but treatment with both iproniazid and reserpine decreased it. Tyramine (100 μM) released more norepinephrine from both iproniazid-treated groups than from the control group (Table 2). In both the untreated and the inhibitor-treated group, the proportions of norepinephrine in the S_2 and P_2 fractions were similar. In animals treated with both the monoamine oxidase inhibitor and reserpine the norepinephrine content decreased in both S_2 and P_2 fractions, but the decrease was less in the former than in the latter. Tyramine (100 μM) did not affect the norepinephrine content of the P_2 fraction but decreased its level in the S_2 fraction (Table 3).

Effect of iproniazid and reserpine treatment on norepinephrine and dopamine β-hydroxylase release induced by hypogastric stimulation. In the control group, electrical stimulation decreased the tissue content of norepinephrine and increased both the norepinephrine and dopamine β-hydroxylase released into the bath medium. Reserpine treatment reduced the tissue norepinephrine content to less than 1%, and treatment with both iproniazid and reserpine reduced it to 27%. In both reserpine-treated groups, electrical stimulation failed to affect the norepinephrine

level of the tissue; no increase of the amine was detected in the bath medium following electrical stimulation, although dopamine β-hydroxylase release was normal (Table 4).

DISCUSSION

Hypertonic KCl causes depolarization of nerve cell membranes by reducing the concentration gradient of potassium between the intracellular and extracellular space; veratridine elicits depolarization by increasing the permeability of the cell membrane to a variety of ions, including sodium (17). Depolarization of noradrenergic neurons in the guinea pig vas deferens by either compound results in a proportional release of norepinephrine and dopamine β-hydroxylase similar to that produced by electrical stimulation of the hypogastric nerve (6). The similarity of the ratio of norepinephrine to enzyme released by electrical stimulation or by depolarizing compounds such as hypertonic KCl or veratridine to the corresponding ratio in the soluble fraction of the vesicles in sympathetic nerves of the vas deferens (6) provides additional evidence that norepinephrine is liberated at depolarized sympathetic nerve terminals by exocytosis.

In contrast to hypertonic KCl and veratridine, reserpine and the sympathomimetic amines cause release only of the amine. Tyramine displaces norepinephrine from adrenergic vesicles (18, 19) and may release it from sympathetic nerves by this mechanism. In vasa deferentia obtained from animals treated with the monoamine oxidase inhibitor iproniazid alone or together with reserpine, the release of norepinephrine by tyramine is enhanced. In the vasa from iproniazid- and reserpine-treated animals, tyramine specifically lowers the supernatant (S_2) content of norepinephrine. The enhanced release of the amine by tyramine after monoamine oxidase inhibition may be the result of protection of the tyramine from destruction by

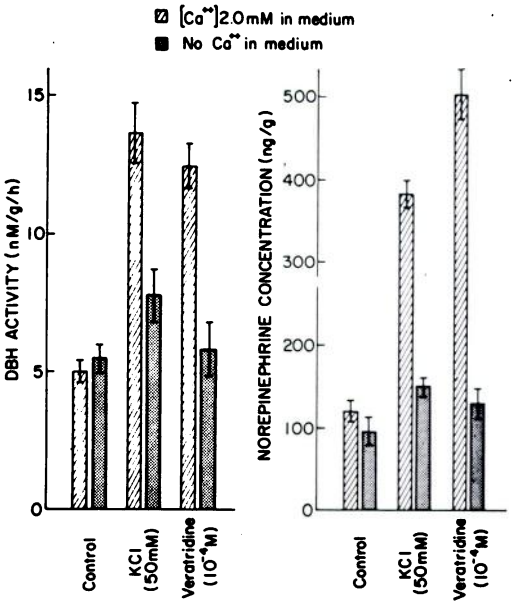

FIG. 4. *Effect of hypertonic KCl (50 mM) and veratridine (0.1 mM) on dopamine β-hydroxylase (DBH) and norepinephrine release from guinea pig vasa deferentia in calcium-free incubation medium*

Each bar represents the mean ± standard error for at least eight preparations. $p < 0.01$; norepinephrine and enzyme released by both veratridine and KCl in normal calcium-medium vs. release by veratridine and KCl in calcium-free medium.

TABLE 1

Effects of tetrodotoxin and colchicine on norepinephrine and dopamine β-hydroxylase release induced by depolarizing agents and tyramine

Values represent means ± standard errors of norepinephrine concentration and dopamine β-hydroxylase activity in the bath medium for at least six preparations.

Treatment	Norepinephrine	Dopamine β-hydroxylase
	ng/g	*nmoles/g/hr*
Control	83 ± 5	8.4 ± 0.6
Veratridine (100 μM)	829 ± 41	18.8 ± 0.8
Veratridine + tetrodotoxin (5 μM)	104 ± 6[a]	9.1 ± 0.8[a]
Veratridine + colchicine (1 mM)	189 ± 17[a]	10.4 ± 0.8[a]
KCl (75 mM)	620 ± 65	14.1 ± 0.8
KCl + colchicine	351 ± 21[a]	10.0 ± 0.7[a]
Tyramine (100 μM)	435 ± 15	
Tyramine + tetrodotoxin	367 ± 30	
Tyramine + colchicine	368 ± 37	

[a] $p < 0.05$ or less; releasing drug and blocking agent vs. releasing drug alone.

In contrast to hypertonic KCl and veratridine, reserpine and the sympathomimetic amines cause release only of the amine. Tyramine displaces norepinephrine from adrenergic vesicles (18, 19) and may release it from sympathetic nerves by this mechanism. In vasa deferentia obtained from animals treated with the monoamine oxidase inhibitor iproniazid alone or together with reserpine, the release of norepinephrine by tyramine is enhanced. In the vasa from iproniazid- and reserpine-treated animals, tyramine specifically lowers the supernatant (S_2) content of norepinephrine. The enhanced release of the amine by tyramine after monoamine oxidase inhibition may be the result of protection of the tyramine from destruction by

the enzyme. The selective lowering of the soluble norepinephrine, however, suggests that tyramine displaces it from the cytoplasm into the synaptic space.

Stimulation of exocytotic release of norepinephrine can be elicited by electrical stimulation of adrenergic nerves (4–9), by scorpion venom (20), by the calcium ionophore A-23187 (Eli Lilly) (21), by depolarizing drugs such as veratridine, and by hypertonic KCl. This mode of norepinephrine release is dependent upon extracellular calcium (4–9, 20, 21). In the absence of this cation an additional store of intracellular calcium seems to be available and can be mobilized by dibutyryl cyclic 3′,5′-AMP or theophylline (8). Liberation of norepinephrine by tyramine (10, 11), reserpine, *d*-amphetamine, or metaraminol does not require the presence of calcium.

TABLE 2

Effect of monoamine oxidase inhibitor and reserpine on norepinephrine-releasing effect of tyramine

Values represent means ± standard errors of norepinephrine concentration in the bath medium and in vasa deferentia for at least eight preparations.

Group	Norepinephrine concentration	
	Bath medium	Tissue
	ng/g	*ng/g*
Untreated	112 ± 12	15,800 ± 570
Untreated + tyramine (100 μM)	400 ± 42[a]	14,200 ± 800
Monoamine oxidase inhibitor[b]	181 ± 24[a]	17,800 ± 680[a]
Monoamine oxidase inhibitor[b] + tyramine	1,140 ± 40[c]	17,000 ± 800
Monoamine oxidase inhibitor, reserpine[d]	96 ± 16	4,300 ± 700[a]
Monoamine oxidase inhibitor, reserpine[d] + tyramine	968 ± 170[c]	4,200 ± 900[a]

[a] $p < 0.05$ or less; treated vs. untreated.

[b] Guinea pigs received the monoamine oxidase inhibitor iproniazid sulfate, 150 mg/kg intraperitoneally, on days 1 and 2 and were killed on day 3.

[c] $p < 0.05$ or less; treated vs. untreated plus tyramine.

[d] Guinea pigs were treated with monoamine oxidase inhibitor as above but also received reserpine, 5 mg/kg intraperitoneally, on day 2 immediately after iproniazid.

TABLE 3

Effect of monoamine oxidase inhibitor and reserpine on subcellular distribution of norepinephrine in control and tyramine-stimulated vasa deferentia

Values represent means ± standard errors for at least eight preparations.

Group	Norepinephrine concentration		
	P_2[a]	S_2[a]	S_2:P_2
	ng/g	*ng/g*	
Untreated	5680 ± 540	4030 ± 420	0.7 ± 0.1
Untreated + tyramine (100 μM)	5760 ± 1020	4280 ± 390	0.7 ± 0.1
Monoamine oxidase inhibitor[b]	6400 ± 780	5000 ± 230	0.8 ± 0.1
Monoamine oxidase inhibitor, reserpine[c]	2240 ± 410[d]	2930 ± 250[d]	1.3 ± 0.2[d]
Monoamine oxidase inhibitor, reserpine[c] + tyramine	2300 ± 200[d]	2070 ± 250[e]	0.9 ± 0.1

[a] Particulate and supernatant fractions obtained by centrifugation at 100,000 × *g* for 1 hr. (See methods)

[b] Guinea pigs were treated with iproniazid sulfate as described in Table 2.

[c] Guinea pigs were treated with iproniazid sulfate and reserpine as described in Table 2.

[d] $p < 0.05$ or less; treated vs. untreated.

[e] $p < 0.05$; monoamine oxidase inhibitor, reserpine plus tyramine group vs. monoamine oxidase inhibitor, reserpine group.

TABLE 4

Effect of monoamine oxidase inhibitor and reserpine treatment on norepinephrine and dopamine β-hydroxylase release induced by hypogastric nerve stimulation

Values represent means ± standard errors for at least eight preparations.

Group	Tissue norepinephrine	Bath medium norepinephrine	Bath medium dopamine β-hydroxylase
	ng/g	*ng/g*	*nmoles/g/hr*
Unstimulated control	15,800 ± 570	130 ± 10	11.6 ± 0.8
Stimulated control	9,370 ± 930[a]	1,040 ± 190[a]	27.9 ± 1.3[a]
Reserpine,[b] unstimulated	120 ± 30[c]	70 ± 20[c]	13.1 ± 0.9
Reserpine,[b] stimulated	150 ± 20	80 ± 10	27.6 ± 2.3[a]
Monoamine oxidase inhibitor, reserpine,[d] unstimulated	4,300 ± 330[c]	80 ± 10[c]	9.2 ± 1.9
Monoamine oxidase inhibitor, reserpine,[d] stimulated	3,800 ± 330	120 ± 30	30.2 ± 2.7[a]

[a] $p < 0.05$ or less- stimulated vs. corresponding unstimulated group.

[b] Guinea pigs received reserpine, 2.5 mg/kg intraperitoneally, on days 1 and 3 and were killed on day 4.

[c] $p < 0.05$ or less; drug-treated unstimulated group vs. control unstimulated group.

[d] Guinea pigs were treated with the monoamine oxidase inhibitor iproniazid sulfate, 150 mg/kg intraperitoneally, on days 1 and 2 and received reserpine, 5 mg/kg intraperitoneally, on day 2 immediately after iproniazid. They were killed on day 3.

Tetrodotoxin selectively blocks the mechanism whereby stimulation induces an increase in sodium permeability of the nerve membrane (22). This blocking effect occurs only when tetrodotoxin is applied to the outer surface of the nerve membrane (23). Since tetrodotoxin blocks exocytosis produced by veratridine, the action of veratridine may involve enhancement of sodium permeability at a site on the exterior of the cell membrane. Release of norepinephrine by tyramine does not seem to involve the sodium conductance, since it is unaffected by tetrodotoxin.

Colchicine, a drug which disaggregates microtubules (24), blocks exocytosis at noradrenergic nerve terminals elicited by electrical stimulation (9) or depolarizing drugs. It has been suggested (25) that the mechanism whereby colchicine blocks exocytosis in the adrenal medulla is not due to the disaggregation of microtubules but rather to an atropine-like effect. However, neither atropine (5 μg/ml) nor hexamethonium (20 μg/ml) affected the exocytosis caused by veratridine, while colchicine (1 mM) reduced the release of norepinephrine and dopamine β-hydroxylase by either veratridine or hypertonic KCl. At the concentrations used in this experiment, hexamethonium is an effective ganglionic blocking agent in the vas deferens (26), and atropine completely blocks the contractile response of the stripped vas deferens (devoid of ganglionic cells) to acetylcholine.[1] While colchicine may affect exocytosis by causing nonspecific damage to adrenergic nerve terminals, this also seems improbable because colchicine did not affect the norepinephrine-releasing action of tyramine. The results obtained with colchicine suggest that structural integrity of microtubules or other colchicine-sensitive structures may be necessary for the over-all process of exocytosis to occur at sympathetic nerve terminals. In view of the known inhibitory effect of colchicine on the axonal transport of norepinephrine vesicles (27), it is possible that the effects observed with this drug resulted partly from blockade of axonal transport and partly from direct inhibition of exocytosis.

Reserpine blocks the vesicular binding of norepinephrine (28, 29), leaving it unprotected from monoamine oxidase (30) and thereby causing depletion of the amine store. In vasa from the reserpine-treated animals, electrical stimulation did not

[1] Unpublished observations.

elicit release of norepinephrine although there was normal release of dopamine β-hydroxylase. Thus noradrenergic vesicles retain the ability to release their contents by exocytosis when the level of neurotransmitter is below normal. Stimulation-induced amine release appears to be predominantly a consequence of exocytosis, since after preliminary treatment with a monoamine oxidase inhibitor, which partly prevents the amine-depleting effect of reserpine by allowing preservation of soluble cytoplasmic norepinephrine, electrical stimulation still fails to elicit significant norepinephrine release.

ACKNOWLEDGMENT

We thank Mrs. P. Cover for her competent technical assistance with the experiments.

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