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External Ca-independent release of norepinephrine by sympathomimetics and its role in negative feedback modulation

(norepinephrine release/negative feedback Ca-independent release/adrenoceptor/cytoplasmic release)

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ABSTRACT **The release of [^{3}H]norepinephrine from isolated mouse vas deferens has been measured. 1-Phenylephrine and 1-norepinephrine significantly enhanced the spontaneous release of radioactivity. As shown by a combination of HPLC and scintillation spectrometry, the release of total radioactivity in response to 1-phenylephrine or 1-norepinephrine consisted mainly of [^{3}H]norepinephrine. Evidence has been obtained that the release of endogenous norepinephrine by exogenous norepinephrine and 1-phenylephrine is independent of the external Ca^{2+} concentration. The released endogenous norepinephrine in turn inhibits the release of norepinephrine in response to electrical field stimulation. In the presence of yohimbine, the enhancement of spontaneous release due to 1-phenylephrine (or to 1-norepinephrine) was not affected, whereas there was a significant superimposed release of [^{3}H]norepinephrine in response to field stimulation, indicating that the inhibition of stimulation-induced norepinephrine release is an α_2-adrenoceptor-mediated process. An important consequence of these findings is to question previous interpretations that the effects of administration of 1-norepinephrine or 1-phenylephrine are due exclusively to their direct effects on the effector cells. The Ca-independent release of endogenous norepinephrine might partly initiate their pharmacological responses. It is concluded that this Ca-independent release is of functional importance, since norepinephrine may accumulate in a concentration sufficient to modulate the release of norepinephrine from varicosities in response to electrical stimulation.**

It is generally accepted (1) that 1-norepinephrine and 1-phenylephrine act directly on effector cells, rather than indirectly, via release of endogenously stored substances. However, in previous studies, evidence has been obtained that sympathomimetic amines can release norepinephrine from various tissues (2–10). In spite of this evidence, it has been concluded that the contribution of this indirectly mediated release to the pharmacological effects of, for example, 1-phenylephrine is negligible (5, 8).

In the present study, an attempt was made to determine whether endogenous norepinephrine released by the addition of 1-norepinephrine, or the α_1-adrenoceptor agonist, 1-phenylephrine, could be involved in the presynaptic modulation of further norepinephrine release evoked by axonal stimulation. Neurochemical evidence has been obtained that the endogenous norepinephrine released in a Ca-independent manner by 1-phenylephrine or 1-norepinephrine inhibits the release of norepinephrine in response to electrical stimulation via an α_2-adrenoceptor mediated mechanism.

EXPERIMENTAL PROCEDURES

Release of Radioactivity from Mouse Vas Deferens Preloaded with [^{3}H]norepinephrine. Two vasa deferentia were loaded with 1-[7, 8-^{3}H]norepinephrine (13 μCi/ml; 1 Ci = 37 GBq) for 45 min at 35°C in Krebs' solution (in mM: NaCl, 113; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11.5 mM) containing 0.3 mM ascorbic acid and 30 μM Na_2EDTA. The solution was aerated with 95%O_2/5%CO_2. After 45 min of loading, paired vasa were transferred to a 2-ml organ bath and superfused at a steady rate of 3 ml/min for 120 min with Krebs' solution containing 0.3 mM ascorbic acid 30 μM Na_2EDTA, and 2.7 μM prednisone at 35°C. For the first 120 min, effluent was discarded: subsequently, 3-min fractions were collected. The radioactivity released was measured as described by Vizi *et al.* (11). The amount (820,600 ± 25,200 Bq/g, n = 22) of radioactivity was also measured (11) at the end of the experiment.

Evoked release was defined as the tritium (Bq/g) in excess of that of resting outflow in samples during and following stimulation. To quantify the effects of drugs on stimulation-evoked release of tritium, the evoked release in the presence of the drug (S_2) was divided by the release evoked by stimulation prior to inclusion of the drug (S_1). Drugs were added to the superfusion medium 21 min before S_2. To quantify the effect of drug on spontaneous release, the fractional release in the 3 min before S_2 (Sp_2) was divided by the fractional release in the 3-min period before S_1 (Sp_1). The Sp_2/Sp_1 ratio reflects the prestimulation (spontaneous) efflux of radioactivity in the presence of drug (Sp_2) divided by the prestimulation efflux in the absence of the drug (Sp_1). Electrical field stimulation was applied (10 V/cm, 1 ms) at 1 Hz frequency as described by Paton and Vizi (12).

Reverse-Phase HPLC Combined with Electrochemical and Radiochemical Detection. The HPLC system (Biotronik Wissentschaftliche Gerate, Frankfurt am Main, F.R.G.) was used as described (11). [^{3}H]norepinephrine and metabolites released from the tissue were separated by reverse-phase HPLC and quantified by measuring the radioactivity. A 250-μl aliquot of diluted standard or sample was injected onto the column and the following retention times for 1-norepinephrine and its metabolites were recorded by electrochemical detection (in min): 1-norepinephrine, 7.8; MOPEG, 12.6; DOPEG, 18.0; VMA and NMA, 14.1; DOMA, 19.0 min. During the resting period, 14.6 ± 2.1% of the total radioactivity released was [^{3}H]norepinephrine (n = 6). This value is similar to those obtained by others (13, 14).

Drugs. The following drugs were used: 1-norepinephrine tartrate (Sigma), 1-phenylephrine hydrochloride (Koch-Light Laboratories, Bucks, England), yohimbine hydrochloride (Sigma), xylazine (Bayern), prednisone (Di-Adreson, Organon-Holland), pargyline hydrochloride (Sigma), 1-[7, 8-^{3}H]norepinephrine (36 Ci/mmol; Amersham), tetrodotoxin (Sigma), and reserpine (K & K; solution prepared with 10% ascorbic acid). When Ca-free solutions were used, $CaCl_2$ was omitted and 1 mM EGTA was added. Reserpine (5 mg/kg) was injected intraperitoneally into mice 48 and 24 hr prior to experiments. Pargyline (10 mg/kg) was injected subcutaneously 48 and 24 hr prior to experiments.

Statistics. Statistical analysis of data was carried out using analysis of variance or Student's t test. For calculating

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Table 1. Effect of 1-norepinephrine and 1-phenylephrine on release of radioactivity from mouse isolated vas deferentia preloaded with [^{3}H]norepinephrine in the presence and absence of calcium

Exp.	Drug	Conc., μM	Ca^{2+} in medium during second stimulation or resting period, mM	S_2/S_1 (as release of radioactivity)					
				Spontaneous			Stimulation evoked		
				Geometric mean	Arithmetic mean of log	SD of data	Geometric mean	Arithmetic mean of log	SD of data
1	Control	—	2.5	0.97 (6)	−0.033	0.050	0.98 (6)	−0.020	0.083
2	Control	—	0	0.96 (6)	−0.044	0.049	0.02 (6)	−3.739	0.774
3	1-Norepinephrine	0.1	2.5	—	—	—	0.55 (6)	−0.584	0.142
4	1-Norepinephrine	1	2.5	3.37 (6)	1.216	0.054	0.08 (6)	−2.534	0.546
5	1-Norepinephrine	1	0	3.16 (8)	1.150	0.029	—	—	—
6	Xylazine	0.1	2.5	0.97 (7)	−0.030	0.044	0.46 (6)	−0.765	0.159
7	Xylazine	10	2.5	0.95 (6)	−0.054	0.031	0.16 (6)	−1.825	0.246
8	1-Norepinephrine	1	2.5	3.42 (6)	−1.229	0.069	—	—	—
9	Yohimbine	1	2.5	0.98 (7)	−0.021	0.020	3.25 (6)	1.179	0.253
10	1-Phenylephrine	10	2.5	3.51 (6)	1.292	0.041	—	—	—
11	1-Phenylephrine	10	0	3.40 (6)	1.230	0.016	—	—	—

Vasa were stimulated twice (S_1 and S_2) for 3 min each at 1 Hz (180 shocks). Drugs were added 21 min before S_2 and 20 min after S_1. Effects of drugs on spontaneous release of radioactivity are expressed as changes in the ratio between the release (as % of tissue tritium) evoked by S_2 and S_1. When calcium was omitted, 1 mM EGTA was added to the medium 20 min before S_2. Numbers in parentheses represent numbers of experiments. Significance ($P > 0.05$): Spontaneous release [by one way analysis of variance and the Dunnett test ($F = 261.39$)], exp. 1 vs. exp. 4, exp. 1 vs. exp. 10, exp. 2 vs. exp. 5, exp. 7 vs. exp. 10, exp. 2 vs. exp. 11; stimulation [by one way analysis of variance and the Dunnett test ($F = 105.79$)], exp. 1 vs. exp. 2, exp. 1 vs. exp. 3, exp. 1 vs. exp. 4, exp. 1 vs. exp. 6, exp. 1 vs. exp. 7, exp. 1 vs. exp. 9.

fractional release, a computer program on a Hewlett-Packard 41 CV was used.

RESULTS

Release of Radioactivity Evoked by 1-Norepinephrine and 1-Phenylephrine. After 120 min of perfusion, the spontaneous efflux of radioactivity measured in 3-min samples from isolated mouse vasa deferentia loaded with [^{3}H]norepinephrine had become low and stable, representing 0.2–0.6% of the radioactivity remaining in the tissue. Electrical field stimulation with 1 Hz (180 shocks) increased the release (S_1) of radioactivity by 3082 ± 292 Bq/g ($n = 6$) above the spontaneous level. When the stimulation was repeated 41 min later (S_2), the amount of radioactivity (2996 ± 197 Bq/g, $n = 6$) did not differ significantly from that evoked by the first stimulation ($P > 0.05$, Student's t test). The ratio S_2/S_1 was 0.98 ± 0.11 ($n = 6$). That the release was subject to presynaptic modulation was demonstrated by finding that yohimbine, an α_2-adrenoceptor antagonist, enhanced the S_2/S_1 ratio to 3.25 ± 0.21 and xylazine, an α_2-agonist, reduced this ratio by >80% (Table 1). 1-Norepinephrine produced a nearly complete inhibition of the stimulation-induced release. The S_2/S_1 ratio in the presence of 1 μM 1-norepinephrine was 0.08 ± 0.02 ($n = 6$). In contrast, the spontaneous release was enhanced by 1-norepinephrine; there was a 3.38-fold increase in the release of radioactivity.

Table 1 shows that, although the stimulation-evoked release was Ca dependent, the effect of 1-norepinephrine on spontaneous release was Ca independent. The release evoked by 1-norepinephrine proved to be tetrodotoxin insensitive (Fig. 1). A similar observation was made with 1-phenylephrine (10 μM). The question arises as to whether or not the norepinephrine released from endogenous stores in a Ca-independent manner is of functional importance—i.e., whether it is involved in the presynaptic modulation of norepinephrine release.

Effect of norepinephrine. The effect of 1-norepinephrine on the spontaneous and evoked release of radioactivity is shown in Fig. 2*A*. While 1-norepinephrine significantly enhanced the fractional spontaneous release of radioactivity (from 2.2 ± 0.05 × 10^{-3} per 3 min to 8.41 ± 0.07 × 10^{-3} per 3 min), it completely inhibited the effect of electrical stimulation (Fig. 2*A*). However, in the presence of yohimbine and 1-norepinephrine together (Fig. 2*B*), electrical stimulation produced a significant superimposed spike, a release of radioactivity above the spontaneous level already enhanced by 1-norepinephrine. The ratio (S_2/S_1 = 2.30 ± 0.15, $n = 4$) between the

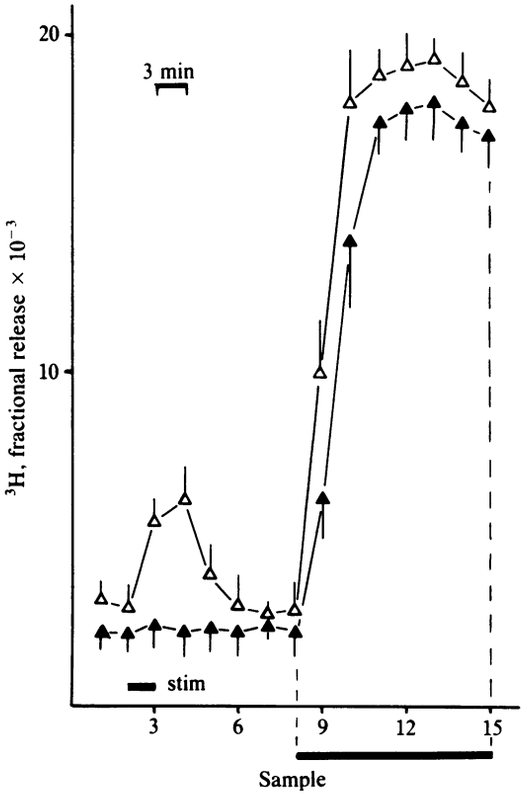

FIG. 1. Tetrodotoxin (TTX; 50 μM)-insensitive release of tritium by 1-norepinephrine (20 μM) from mouse vas deferens preloaded with [^{3}H]norepinephrine. Results shown represent mean ± SEM of four identical experiments. 3-min samples were collected; perfusion rate, 3 ml/min; stimulation (stim), 1 Hz and 180 shocks. Note that blockade of sodium channels by TTX inhibits the release of tritium evoked by stimulation but does not affect that evoked by exogenous 1-norepinephrine. ▲, with TTX; △, without TTX.

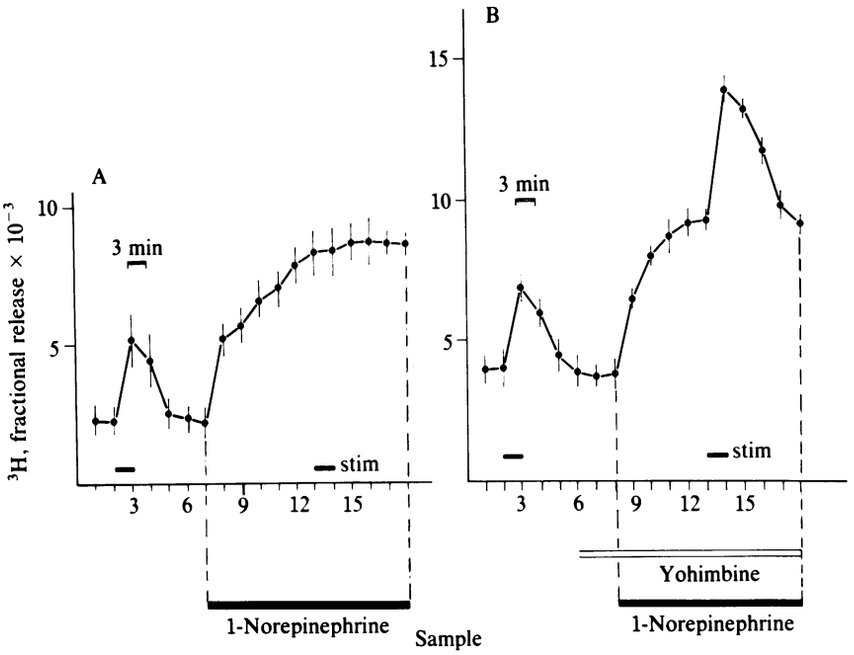

FIG. 2. Release of tritium by 1-norepinephrine. Inhibition of stimulation-evoked release of tritium by 1-norepinephrine (10 μM) (*A*) and its reversal by yohimbine (1 μM) (*B*). Mouse vas deferens were preloaded with [^{3}H]norepinephrine; 3-min samples were collected; perfusion rate, 3 ml/min; stimulation (stim), 1 Hz and 180 shocks. Drugs were added to the perfusion fluid as indicated. Note that in the presence of yohimbine plus 1-norepinephrine there is an increased response to stimulation, indicating that the release is inhibited by α_2-adrenoceptor stimulation.

radioactivity released by the superimposed outflow and that evoked by stimulation in the absence of 1-norepinephrine plus yohimbine was significantly higher ($P < 0.01$, Student's t test) than in controls (0.97 ± 0.11, Table 1). These data show that 1-norepinephrine (10 μM) causes an efflux of radioactivity that represents the release of stored norepinephrine and that it inhibits the effect of electrical stimulation via α_2-adrenoceptor stimulation, but they do not elucidate the role of norepinephrine-released endogenous norepinephrine in the presynaptic modulation of norepinephrine release. Clearly, it is not possible to distinguish between the effect of norepinephrine derived from endogenous stores and the effect of that added to the medium. It is generally accepted that 1-phenylephrine has no significant α_2-adrenoceptor stimulant activity (15–17). Therefore it seemed that 1-phenylephrine might be useful to clarify the role of endogenous norepinephrine released in a Ca-independent way of presynaptic modulation.

Effect of 1-phenylephrine. The effect of 1-phenylephrine on the release of radioactivity from mouse vas deferens preloaded with [^{3}H]norepinephrine is shown in Fig. 3*A*. 1-Phenylephrine (0.1 mM) promptly enhanced the fractional release of radioactivity (from 4.03 ± 0.45 × 10^{-3} per 3 min to 23.05 ± 3.56 × 10^{-3} per 3 min) and completely eliminated the release induced by electrical stimulation. Administration of yohimbine did not affect the action of 1-phenylephrine (Fig. 3*B*) on spontaneous release (18.38 ± 2.00 × 10^{-3} per 3 min) but, in its presence, electrical stimulation released a large amount of radioactivity. In the presence of yohimbine plus 1-phenylephrine the release of radioactivity (13600 ± 2500 Bq/g; n = 3) in response to electrical stimulation was significantly higher ($P < 0.01$, Student's t test) than that in the absence of drugs (3150 ± 405 Bq/g; Fig. 3*B*). In the presence of yohimbine, the response to stimulation was superimposed on the enhanced spontaneous efflux of radioactivity.

Evidence that 1-Norepinephrine and 1-Phenylephrine Release [^{3}H]norepinephrine: HPLC Study. Since the basal out-

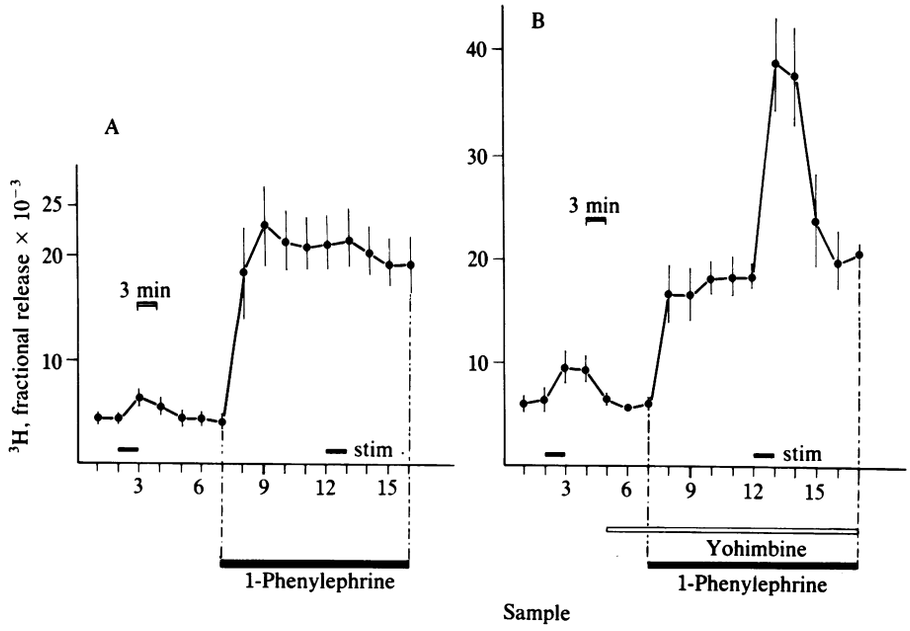

FIG. 3. Release of tritium by 1-phenylephrine. Inhibition of stimulation-evoked release of tritium by 1-phenylephrine (0.1 mM) (*A*) and its reversal by yohimbine (1 μM) (*B*). Three-minute samples were collected; perfusion rate, 3 ml/min; stimulation (stim), 1 Hz and 180 shocks. Drugs were added as indicated. Note that in the presence of yohimbine plus 1-phenylephrine there is a superimposed response to stimulation, indicating that the release is inhibited by α_2-adrenoceptor stimulation.

flow of total radioactivity from noradrenergically innervated tissues preloaded with [^{3}H]norepinephrine consists mostly of ^{3}H-labeled metabolites (18, 19) and the evoked release consists largely of [^{3}H]norepinephrine, reverse-phase HPLC combined with scintillation spectrometry was used to measure [^{3}H]norepinephrine release. The outflow of [^{3}H]norepinephrine at rest, in response to stimulation, and during exposure to l-norepinephrine and l-phenylephrine is shown in Table 2. At rest, 23.1 ± 1.9% of the total radioactivity was [^{3}H]norepinephrine. The amount of [^{3}H]norepinephrine released in response to l-norepinephrine administration was found to be much higher than the amount released during rest; l-norepinephrine induced a release of mainly norepinephrine, which represented 73.0 ± 9.2% (n = 3) of the total radioactivity released. If we compare the release of [^{3}H]norepinephrine measured during rest and that obtained during rest in the presence of l-norepinephrine or l-phenylephrine, [^{3}H]norepinephrine output was increased by factors of 30.1 ± 5.8 and 20.9 ± 8.7, respectively. A similar increase was seen when 5 Hz (900 shocks) of stimulation was applied; the release of [^{3}H]norepinephrine was 13.2 ± 5.0 times that during rest. While the release of [^{3}H]norepinephrine in response to electrical stimulation is Ca dependent, the release evoked by l-phenylephrine proved to be Ca_0 independent (data not shown).

Effect of Reserpine and Monoamine Oxidase Inhibition. Since the carrier-mediated, cocaine-sensitive release of axoplasmic norepinephrine may well be involved in the indirect sympathomimetic effects of amines (20), an attempt was made to study the effects of l-norepinephrine and l-phenylephrine on [^{3}H]norepinephrine release in tissue that had been pretreated with reserpine and pargyline (to inhibit monoamine oxidase)—i.e., tissue in which the [^{3}H]norepinephrine is exclusively located in the cytoplasm (21). Under these conditions the resting release was significantly higher ($16.3 \pm 0.89 \times 10^{-3}$ per 3 min; $n = 3$; $P < 0.01$), and l-norepinephrine (10 μM) and l-phenylephrine (10 μM) enhanced the fractional release of radioactivity (from $16.1 \pm 0.9 \times 10^{-3}$ to $26.6 \pm 0.64 \times 10^{-3}$ and from $15.31 \pm 0.88 \times 10^{-3}$ to $25.30 \pm 0.31 \times 10^{-3}$ per 3 min, respectively; for both cases, $P < 0.01$; $n = 3$). Electrical stimulation (1 Hz, 180 shocks) did not enhance the release. Although favorable conditions for filling the axoplasmic space with [^{3}H]norepinephrine prevailed (inhibition of vesicular uptake and blockade of monoamine oxidase activity), the release evoked by l-norepinephrine or l-phenylephrine in the tissue was less than the release evoked by l-norepinephrine or l-phenylephrine from vas deferens obtained from nontreated mouse.

DISCUSSION

Although previous studies have shown that l-phenylephrine can release norepinephrine (4, 5, 8, 9), little attention has been paid to this phenomenon. l-Phenylephrine has been considered as a direct acting α_1-adrenoceptor agonist (16). In addition, evidence has been obtained that radioactivity can be released by other sympathomimetic amines from heart that has been loaded with [^{3}H]norepinephrine (2–4, 6, 10, 22, 23). However, no evidence has been found that the released radioactivity is due mainly to [^{3}H]norepinephrine. In this study, we have found that l-norepinephrine and l-phenylephrine are able to release radioactivity from isolated mouse vas deferens. A combination of reverse-phase HPLC and scincillation spectrometry showed that the released radioactivity induced by l-norepinephrine or l-phenylephrine is due to the release of [^{3}H]norepinephrine and not to ^{3}H-labeled metabolites. Evidence has been obtained that l-norepinephrine (10 μM) produces an ≈30-fold increase in endogenous norepinephrine release, which does not depend on the presence of Ca in the bathing fluid. Similarly, the release by tyramine (24) is also Ca independent. Although the stimulation-evoked release of [^{3}H]norepinephrine requires external Ca, the release evoked by l-norepinephrine or l-phenylephrine is independent of the external Ca^{2+} concentration. In addition, evidence has been obtained that the mechanism of release by l-norepinephrine (l-phenylephrine) is different from that induced by electrical stimulation. In reserpine-treated tissue containing a monoamine oxidase inhibitor, in which [^{3}H]norepinephrine is located in the cytoplasm (21), l-norepinephrine and l-phenylephrine released radioactivity but electrical stimulation did not. Therefore, it seems likely that l-norepinephrine- and l-phenylephrine-evoked release is of cytoplasmic origin. A similar mechanism has been suggested by Trendelenburg (25) for the indirectly acting sympathomimetics.

Considering the large increase in spontaneous [^{3}H]norepinephrine efflux caused by l-norepinephrine or l-phenylephrine, one could argue that what appears to be an inhibition of stimulation-evoked release may, in reality, be due to failure to detect increased efflux against an already high background release. However experiments with yohimbine, a relatively pure α_2-adrenoceptor antagonist, indicate that this is not likely. Yohimbine completely reversed the inhibitory effects of l-norepinephrine and l-phenylephrine on stimulation-evoked [^{3}H]norepinephrine release (producing values even higher than control) without changing their effects on spontaneous release. This suggests that the spontaneous release and that evoked by axonal stimulation are mediated by different mechanisms and can therefore be studied simultaneously.

The data obtained with l-norepinephrine did not prove that the release of endogenous norepinephrine contributes to the modulation induced by norepinephrine added to the tissue since one cannot differentiate the actions of norepinephrine of different origins (endogenous or exogenous). The findings obtained with l-phenylephrine, a drug that has been generally

Table 2. Release of [^{3}H]norepinephrine from isolated vas deferens: Effects of l-phenylephrine and l-norepinephrine

	Tritium, Bq/g	[^{3}H]norepinephrine released in 3 min, Bq/g	[^{3}H]norepinephrine release per 3 min, % total radioactivity released	[^{3}H]norepinephrine released, % tissue content	Factor of [^{3}H]norepinephrine release*
Resting (6)	4,551.5 ± 186.6	1,055.2 ± 537.8	23.1 ± 1.9	0.16 ± 0.09	1
Stimulation (1 Hz, 180 shocks) (3)	6,993.8 ± 432.6	2,146.3 ± 285.6	35.8 ± 3.7	0.30 ± 0.09	2.03 ± 0.17
1-Phenylephrine (3)	37,658.3 ± 12,432.7	22,125.3 ± 9,435.6	58.7 ± 10.8	2.64 ± 1.32	20.9 ± 8.7
1-Norepinephrine (3)	43,546.7 ± 9,840.6	31,793.7 ± 6,721.9	73.0 ± 9.2	2.97 ± 0.65	30.1 ± 5.8

Results represent mean ± SEM. Numbers in parentheses represent numbers of experiments. Each value represents release per 3 min calculated from the two highest consecutive-release fractions following stimulation or administration of drugs. Exposure time to l-phenylephrine (0.1 mM) or to l norepinephrine (20 μM) was 30 min.

*Factor of [^{3}H]norepinephrine release, ratio between drug (stimulation)-evoked release and resting release (resting release = 1).

regarded as a pure direct-acting α_1-adrenoceptor agonist, provides convincing evidence that endogenous norepinephrine released in a manner that is independent of the external Ca concentration may be involved in presynaptic modulation. Thus, the presynaptic modulatory effect of l-norepinephrine (cf. refs. 17, 19, 26, 27) and l-phenylephrine (5, 9) cannot simply be attributed to their direct effects on α_2-adrenoceptors present on noradrenergic varicosities; an indirect component must also be involved in these actions.

The release of l-phenylephrine is not subject to presynaptic modulation; yohimbine, an α_2-adrenoceptor antagonist, failed to influence it. In addition, it is interesting to note that the tyramine-induced release is also not affected by α_2-adrenoceptor blocking agents (28).

In the present experiments, we found that endogenous norepinephrine released by l-norepinephrine and l-phenylephrine reached a concentration in the vicinity of the axon terminals (where presynaptic modulation of norepinephrine release evoked by axon stimulation takes place) high enough to inhibit further stimulation-induced release via presynaptic α_2-adrenoceptors. This is substantiated by the findings that l-norepinephrine and l-phenylephrine enhance the spontaneous release of radioactivity but actually inhibit the stimulation-evoked release of [^{3}H]norepinephrine. Several unexpected effects of l-phenylephrine have been reported (9, 29–34)—for example, β-adrenoceptor (35, 36)-and α-adrenoceptor (5)-mediated positive inotropic effects. These could be explained by our finding that l-phenylephrine releases endogenous norepinephrine, which may contribute to the response of the effector cells. The concentration of endogenous norepinephrine released in a Ca-independent manner into the vicinity of release sites may be high enough to inhibit the release of norepinephrine in response to axonal stimulation (Ca-dependent release). It is suggested, therefore, that this indirect effect of l-phenylephrine (or l-norepinephrine) may be partly or fully responsible for the pharmacological effects of added l-norepinephrine or l-phenylephrine or for the physiological effect of locally released or circulating norepinephrine and adrenaline. If, under physiological conditions, the norepinephrine released in a Ca-dependent fashion releases additional norepinephrine in a Ca-independent manner (Fig. 4), the target cells will not be able to distinguish between norepinephrine of different origins. Therefore, this release mechanism seems to be of both physiological and pharmacological significance.

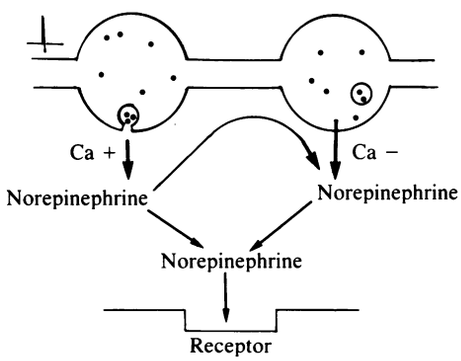

FIG. 4. Possible mode of action of l-norepinephrine. Varicosities are invaded by depolarization with the result that they release norepinephrine in a Ca-dependent manner (Ca^+). If the norepinephrine concentration is sufficiently high, it may produce a further release of norepinephrine from the varicosities but in a Ca-independent way (Ca−). The Ca-independent release of norepinephrine is of cytoplasmic origin. The released norepinephrine diffuses locally and influences a large number of varicosity branches that are equipped with α_2-adrenoceptors. Those boutons located distally from varicosities containing α_2-adrenoceptors will not be invaded and therefore norepinephrine will not be released.

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