J. Physiol. (1972), **221**, *pp.* 601–615
With 5 text-figures
Printed in Great Britain

MODIFICATION OF THE EVOKED RELEASE OF NORADRENALINE FROM THE PERFUSED CAT SPLEEN BY VARIOUS IONS AND AGENTS

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(*Received 9 August 1971*)

SUMMARY

1. Cat spleens were perfused with Krebs-bicarbonate solution at a rate of about 7 ml./min at 33–35° C. Noradrenaline release after splenic nerve stimulation at 10 or 30 Hz was measured. Effects of various ions and drugs on noradrenaline release were determined.

2. Perfusion of phenoxybenzamine- and [^{3}H]noradrenaline-treated spleens with 1, 2·5 and 5 mM cobalt or nickel–2 Krebs solution markedly reduced the release of noradrenaline by nerve stimulation. Lanthanum was the most potent inhibitor of noradrenaline release. Increasing the calcium concentration or adding tetraethylammonium chloride (TEA) partially counteracted the inhibitory effects of cobalt on release. Cobalt did not inhibit release induced by tyramine.

3. Calcium did not cause spontaneous release of noradrenaline either when high concentrations were injected directly into the spleen or after first perfusing the spleen with calcium-free medium.

4. Carbachol, protoveratrine and high potassium inhibit, whereas TEA, barium and rubidium enhance, the evoked release of noradrenaline.

5. The relation of noradrenaline release to influx of calcium ions and its modification by various agents has been discussed.

INTRODUCTION

Studies on different sympathetically innervated organs have established the critical role of calcium in the release of noradrenaline from adrenergic nerves. Huković & Muscholl (1962) first showed that the release of noradrenaline by stimulation of cardiac accelerator nerves was considerably diminished in low calcium solutions. More detailed studies on the role of cations on the release of adrenergic transmitter were carried out by

Kirpekar & Misu (1967), Kirpekar & Wakade (1968), on the cat spleen perfused *in situ*, and Boullin (1967) on the cat colon perfused *in vitro*. These studies showed that removal of calcium from the external medium almost abolished release, and that noradrenaline output varied directly with the external calcium concentration up to 7·5 mM. Magnesium antagonized release, and barium and strontium were able to replace calcium in sustaining the release process.

The present investigation was undertaken to probe further into the mechanism of action of calcium. Experiments were carried out to determine the effects of certain transition elements – which specifically block the late phase of calcium entry in giant axons (Baker, Meves & Ridgway, 1971) and block transmitter release in stellate ganglia of the squid (Katz & Miledi, 1969) – on noradrenaline release by sympathetic nerve stimulation. In the present study, the perfused cat spleen was used as the test preparation, since it had been previously shown that easily measurable and reproducible quantities of noradrenaline are released following splenic nerve stimulation (Brown & Gillespie, 1957).

Our results show that transition elements and lanthanum decrease the evoked release of noradrenaline, probably by blocking calcium permeability into the sympathetic nerves. It is also suggested that carbachol, protoveratrine and high potassium which inhibit, and TEA, barium and rubidium which enhance, release do so by modulating the calcium entry into the sympathetic nerve endings. A preliminary report of some of these findings has been published (Kirpekar, Wakade, Puig & Prat, 1971; Puig, Wakade & Kirpekar, 1971).

METHODS

The technique of perfusing the cat's spleen with Krebs-bicarbonate solution has been previously described (Kirpekar & Misu, 1967). Briefly, cats were anaesthetized with ether, followed by chloralose (60 mg/kg I.V.). The abdomen was opened by a mid line incision and the animal eviscerated from stomach to colon. Both adrenals were also removed. Spleens were perfused with Krebs-bicarbonate solution equilibrated with 95 % O_2–5 % CO_2, or 100 % O_2, at 35° C. Venous samples were collected by placing a cannula in the superior mesenteric vein. In some experiments the endogenous pool of noradrenaline was labelled by infusing [^{3}H]noradrenaline (specific activity 5·7 c/m-mole) into the spleen at a rate of 20 ng/min for 20 min. After the infusion of [^{3}H]noradrenaline, spleens were perfused with Krebs-bicarbonate solution containing phenoxybenzamine (10 μg/ml.) for 20 min, followed by normal Krebs-bicarbonate solution for a similar period. The spleens were also pre-treated with phenoxybenzamine without prior infusion of [^{3}H]noradrenaline. This procedure was followed since phenoxybenzamine markedly increases the transmitter output (Brown & Gillespie, 1957), and allows one, in addition, to study release in isolation from the uptake process. Spleens were then perfused with experimental solutions of different ionic compositions, and finally with Krebs-bicarbonate solution. In untreated spleens the nerves were stimulated at 30 Hz for 7 sec, and in phenoxybenzamine-treated spleens they were stimulated at 10 Hz for 10 sec. Control samples

without nerve stimulation were taken 1 min before stimulation, and each venous sample was collected over a period of 1 min. In some experiments noradrenaline was released by perfusing the spleen for 6 min with Krebs-bicarbonate solution containing tyramine (20 μg/ml.).

KH_2PO_4 and $NaHCO_3$ were not added to Krebs-bicarbonate solutions containing cobalt ($CoCl_2$), nickel ($NiCl_2$) and lanthanum ($LaCl_3$), and these solutions contained 1 mM-Tris buffer. The pH of the final solutions was about 7·2. In experiments with barium ($BaCl_2$) the $MgSO_4$ of the Krebs-bicarbonate solution was replaced by $MgCl_2$, to avoid precipitation. High potassium solutions were made by adding solid K_2SO_4 to the Krebs-bicarbonate solution.

Noradrenaline content of the venous samples was determined by a chemical assay procedure of Anton & Sayre (1962), using alumina. This procedure separates methylated metabolites from [^{3}H]noradrenaline. Measurements of total radioactivity and [^{3}H]noradrenaline content of the venous samples were done in a Packard Tri-carb liquid scintillation counter by adding 0·5 ml. of venous sample or extracted sample directly to 10 ml. of Bray's scintillation fluid; quenching was corrected by the use of automatic external standard.

RESULTS

Effect of cobalt and nickel on the evoked release of noradrenaline

Perfusion of the spleen with cobalt-Krebs solution markedly reduced the evoked release of noradrenaline. Treatment of the spleen with 1 mM and 2·5 mM cobalt solution reduced the noradrenaline output by about 60 and 90%, respectively. Increasing the concentration of cobalt to 5 mM eliminated release completely. Recovery of noradrenaline output in normal Krebs solution after perfusion with 2·5 mM cobalt-Krebs solution was 77 ± 4% of the initial output. Recovery of noradrenaline output after perfusion with 5 mM cobaltous-Krebs solution was less than 50% of the initial output. Similarly, nickel (2·5 mM) caused a marked reduction in noradrenaline output (Table 1).

Effects of high calcium on the inhibitory action of cobalt on noradrenaline release

In this and the following two sections, noradrenaline release is expressed in terms of 'net increase in radioactivity' after nerve stimulation, since such increase in radioactivity represents the release of endogenous noradrenaline (Hertting & Axelrod, 1961; Gillespie & Kirpekar, 1966). Another reason for expressing the release in this manner was that venous samples containing high calcium and cobalt interfered with the chemical analysis of noradrenaline.

In three control experiments it was found that noradrenaline output induced by nerve stimulation was depressed by about 30% in the presence of 20 mM calcium-Krebs solution. Fig. 1 shows that during perfusion with 20 mM calcium and 2·5 mM cobalt-Krebs solution the noradrenaline output was nearly 3–4 times greater than a subsequent output in 2·5 mM cobalt-

Krebs solution alone. In four experiments the mean net increase in radioactivity in 20 mM calcium and 2·5 mM cobalt-Krebs solution was 43 ± 12 % of the control output, which was significantly ($P < 0{\cdot}01$) greater than the output in 2·5 mM cobalt-Krebs solution alone (12 ± 2·6 %). On reperfusing the spleens with the normal Krebs solution, the mean output was restored to 87 ± 6·4 % of the control value. The lower panel (Fig. 1*b*) shows that if the inhibitory effect of cobalt was first established and then the spleens were perfused with 20 mM calcium and 2·5 mM cobalt-Krebs solution, a

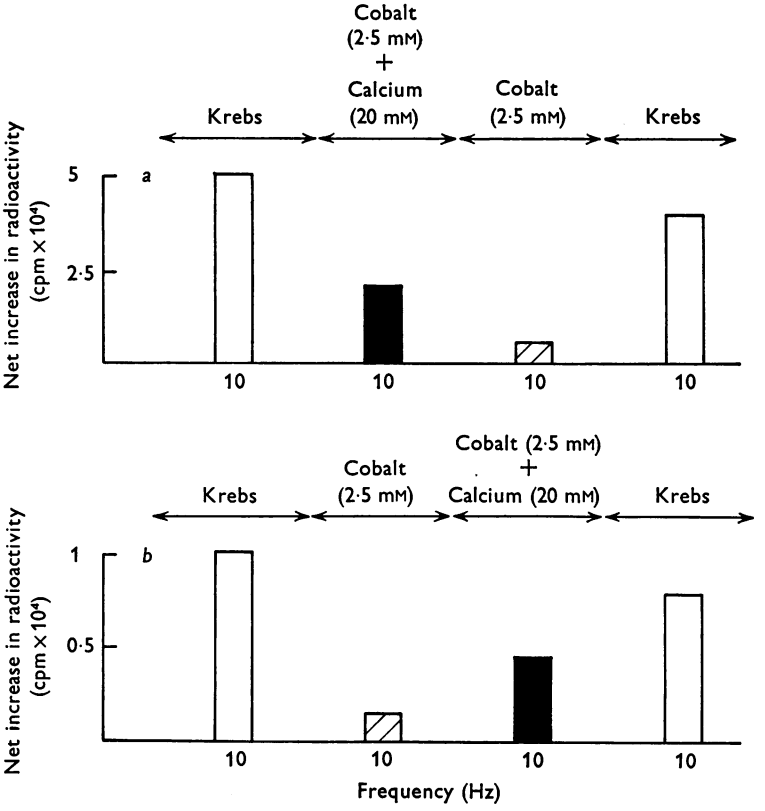

Fig. 1. Effect of high calcium concentration on the inhibitory effect of cobalt on noradrenaline release. [^{3}H]noradrenaline (20 ng/min) was infused into the spleen for 30 min. After the end of [^{3}H]noradrenaline infusion, the spleen was first perfused with phenoxybenzamine (10 μg/ml.) for about 20 min, and then with normal Krebs-bicarbonate solution for a similar period. Both panels show net increases in radioactivity due to nerve stimulation. In the experiment shown in the upper panel, the spleen was perfused with cobalt (2·5 mM) + calcium (20 mM) Krebs solution before the effect of cobalt was established. The lower panel shows the effect of high calcium solution after initial perfusion of the spleen with cobalt-Krebs solution for 20 min.

partial restoration of noradrenaline release was obtained in the presence of high calcium. In four experiments the mean noradrenaline output was reduced to $9{\cdot}3 \pm 2{\cdot}3\%$ of the control value during perfusion with 2·5 mM cobalt-Krebs solution, which was increased to $31 \pm 7{\cdot}5\%$ during perfusion with 2·5 mM cobalt and 20 mM calcium-Krebs solution ($P < 0{\cdot}01$). After perfusing with normal Krebs solution, the noradrenaline outputs recovered only partially, to $57 \pm 11{\cdot}7\%$ of the initial control value. If the inhibitory effect of high calcium (20 mM) on release is taken into account, the protection afforded by high calcium against cobalt becomes even more significant. Calcium at 10 mM did not antagonize the cobalt effect (two experiments).

Effect of TEA on the inhibitory action of cobalt on noradrenaline release

Perfusion of TEA (10 mM), either before or after establishing the effects of cobalt, partially compensated for the inhibitory effect of cobalt on noradrenaline release. In each of the two experiments the net increase in radioactivity in 2·5 mM cobalt and 10 mM-TEA-Krebs solution was 67% of the control output, which was considerably greater than the 10% output obtained in 2·5 mM cobalt-Krebs solution alone. Similarly, in three experiments the mean noradrenaline output, which was reduced to $17 \pm 3\%$ of the control output during perfusion with 2·5 mM cobalt-Krebs solution, was increased to $66 \pm 1{\cdot}5\%$ during perfusion with 2·5 mM cobalt and 10 mM-TEA ($P < 0{\cdot}01$). After reperfusion with normal Krebs solution, the mean noradrenaline output was $70 \pm 7{\cdot}5\%$ of the initial control output. TEA (10 mM) alone nearly doubled ($188 \pm 11\%$) noradrenaline release by nerve stimulation, and even if this facilitatory effect of TEA on noradrenaline release is taken into consideration, the protection afforded by TEA against inhibition of release by cobalt is still apparent. Thoenen, Haefely & Staehelin (1967) have previously shown the potentiating effect of TEA on noradrenaline release.

Effect of cobalt on noradrenaline release by tyramine

Since noradrenaline release from adrenergic nerves by an indirectly acting sympathomimetic amine such as tyramine does not depend upon the presence of calcium in the external medium (Lindmar, Löffelholz & Muscholl, 1967), it was of interest to investigate the effect of cobalt on tyramine-induced release of noradrenaline. Noradrenaline release caused by an infusion of tyramine (20 μg/ml.) was not significantly affected during perfusion with 2·5 mM cobalt. In three experiments the mean output was $83 \pm 9{\cdot}2\%$ of the control value during exposure to cobalt-Krebs solution.

Effect of lanthanum on the evoked release of noradrenaline

Table 1 shows the inhibitory effect of lanthanum on noradrenaline release. Lanthanum in low concentration (0·01 mM) had no effect on release. However, 0·05 mM lanthanum reduced noradrenaline output to $14 \pm 8{\cdot}4\%$ of the control value. Further increase in the concentration of lanthanum to 0·1 mM caused almost complete block of release. Reperfusion of spleens with normal Krebs solution after high concentrations of lanthanum failed to restore release.

Effects of graded concentrations of calcium on the spontaneous release of noradrenaline

The evidence presented above, as well as the previous studies (Huković & Muscholl, 1962; Burn & Gibbons, 1965; Kirpekar & Misu, 1967; Boullin, 1967; Kirpekar & Wakade, 1968), show the importance of calcium in the evoked release of noradrenaline from sympathetic nerves. Experiments were therefore carried out to determine whether calcium itself can stimulate the release of noradrenaline without electrical excitation. Simply increasing the calcium concentration of the perfusing medium from the normal 2·5 to 20 mM did not release noradrenaline, as determined by the efflux of [^{3}H]noradrenaline. When the spleen was first perfused for some time with calcium-free Krebs solution, reperfusion either with normal Krebs solution or with Krebs solution containing higher concentrations of calcium, up to 20 mM, did not stimulate release. This is in contrast to the observations of Douglas & Rubin (1963), who showed the releasing effect of calcium on the medullary secretion of catecholamines by first perfusing the adrenal glands with calcium-free medium.

Effects of various agents on the evoked release of noradrenaline

Carbachol

Initial studies were carried out with carbachol, whose effect on noradrenaline release was examined in several experiments. As shown in Table 1, carbachol at a concentration of 10^{-5} g/ml. inhibited the release reversibly. In five experiments the mean noradrenaline release was reduced to $42 \pm 9\%$ of the control output. Carbachol at 10^{-6} g/ml. did not significantly affect release. The inhibitory effect of carbachol was readily reversed by atropine (10^{-6} g/ml.), and by TEA (1 and 5 mM). In three experiments carbachol reduced the mean control noradrenaline output to $35 \pm 4\%$, which was restored to $116 \pm 27\%$ and $145 \pm 13\%$ by a simultaneous perfusion with Krebs solution containing carbachol and 1 or 5 mM-TEA, respectively. Previously, Löffelholz & Muscholl (1969) demonstrated a muscarinic

TABLE 1. Effects of various agents on noradrenaline output from the perfused spleen

Agent	No. of observations	Stimulation frequency (Hz)	Spontaneous release (ng/min)	Evoked release (ng/stim period) (percentage of control)		
				Before agent (control)	In presence of agent	After agent
Cobalt (1 mM)	4	10*	0	581 ± 115	203 ± 58 (39)	438 ± 123 (77)
Cobalt (2·5 mM)	12	10*	0	378 ± 61	32 ± 5 (9·8)	262 ± 47 (77)
Cobalt (5·0 mM)	4	10*	0	438 ± 123	37 ± 2 (0·5)	329 ± 89 (47)
Nickel (2·5 mM)	2	10*	0	218	16 (8)	189 (90)
Lanthanum (0·01 mM)	1	30	0	146	155	138
Lanthanum (0·05 mM)	3	30	0	119 ± 18	18 ± 12·5 (14)	95 ± 27 (76)
Lanthanum (0·1 mM)	2	30	0	261	0 (0)	81 (31)
Carbachol (10^{-6} g/ml.)	5	30	0	147 ± 30	126 ± 46 (87)	—
Carbachol (10^{-5} g/ml.)	5	30	0	147 ± 30	54 ± 15 (42)	181 ± 34 (131)
Protoveratrine (10^{-6} g/ml.)	3	30	0	196 ± 38	95 ± 19 (48)	148 ± 20 (83)
Potassium (10 mM)	5	30	0	148 ± 27	97 ± 16 (68)	—
Potassium (15 mM)	5	30	0	—	52 ± 8 (38)	—
Potassium (20 mM)	5	30	6, 9†	—	24 ± 4 (18)	104 ± 19 (72)
Rubidium (5·9 mM)	4	30	0	161 ± 68	281 ± 15 (170)	217 ± 101 (162)

* Pre-treated with phenoxybenzamine; 10 Hz for 10 sec.

† Only two out of five experiments showed spontaneous release.

inhibition of the noradrenaline release from perfused rabbit heart by stimulation of the cardiac accelerator nerves.

Protoveratrine

Protoveratrine (10^{-6} g/ml.) blocked the release of noradrenaline by about 50 % (Table 1), which was restored during a simultaneous perfusion with 1 and 5 mM-TEA and protoveratrine (Fig. 2). Mean reduction in three

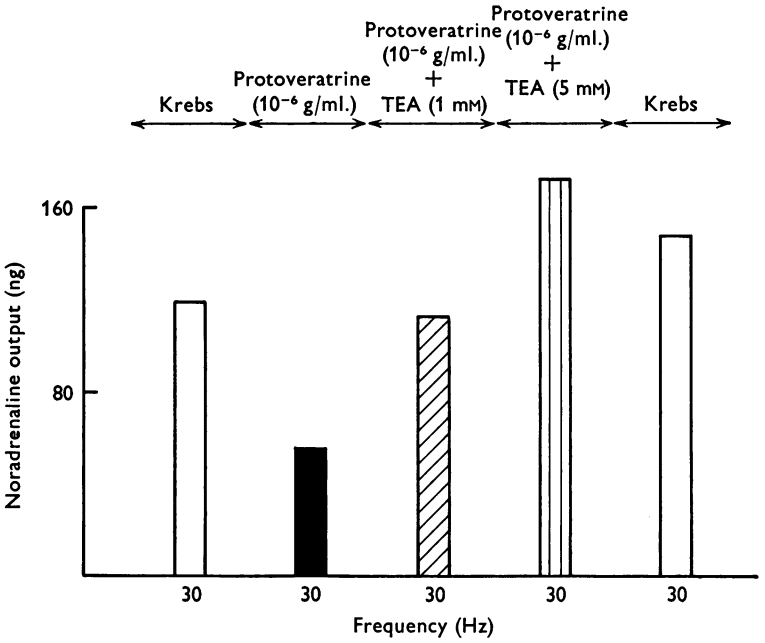

Fig. 2. The effect of protoveratrine on noradrenaline release. Inhibitory effect of protoveratrine on noradrenaline release is largely antagonized by a simultaneous perfusion of TEA and protoveratrine.

experiments was 48 ± 1 %. In three experiments, neither protoveratrine, 10^{-7} g/ml., nor carbachol, 10^{-6} g/ml., affected the evoked release, but the combination of the two drugs in their respective concentrations reduced the mean release by 49 ± 16 %.

High potassium

Increasing the potassium concentration of the bathing medium from the normal 5·9 to 20 mM progressively blocked release. In five experiments the mean noradrenaline output was reduced to 68 ± 10 %, 38 ± 7 % and $18 \pm 3{\cdot}5$ in 10, 15 and 20 mM potassium-Krebs solution, respectively (Table 1). Following reperfusion with normal Krebs solution, the mean

noradrenaline output was restored to $72 \pm 9\%$ of the control output. The inhibitory effect of high potassium on release probably is not due to block of nervous conduction, since Furness (1970) showed that the excitatory junction potentials in the mouse vas deferens evoked by stimulation of the hypogastric nerve or intramural nerves were observed in high potassium, up to 30 mM.

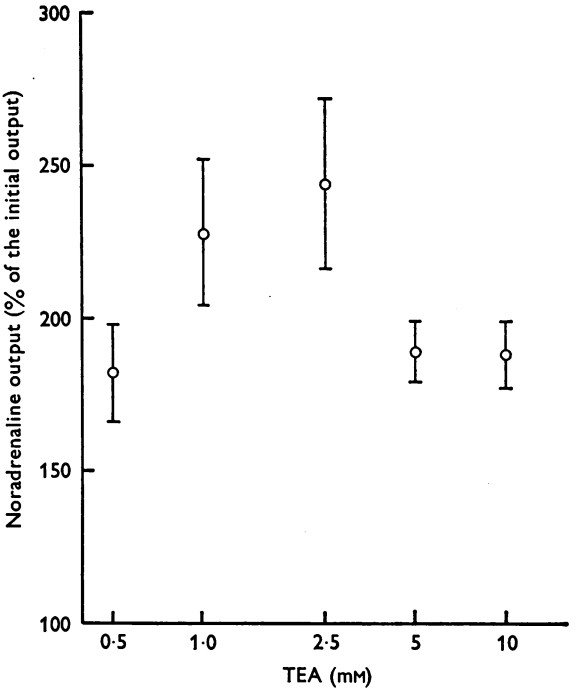

Fig. 3. The relationship between the logarithm of the TEA concentration in Krebs-bicarbonate solution and the noradrenaline release, expressed as a percent increase over that released during a stimulation period without TEA. Results from three spleens, pretreated with phenoxybenzamine, have been used to calculate the values.

Rubidium

The effect of rubidium on potassium was studied by Adrian (1964), who showed that rubidium induces potassium inactivation in frog muscle. Baker, Hodgkin & Shaw (1962) showed that the action potential of the squid axon was very much prolonged when the axon was perfused with high rubidium solution. In the present experiments, potassium in the Krebs solution was replaced by rubidium. Table 1 shows that rubidium (5·9 mM) increased noradrenaline release by about 70%. In four experiments the mean increase in output was $170 \pm 21{\cdot}1\%$ over the control value.

TEA

TEA specifically blocks the potassium current, and Katz & Miledi (1969) have explained the actions of TEA on transmitter release on the basis of this property alone. Fig. 3 shows the effect of different concentrations of TEA (0·5 to 10 mM) on noradrenaline release. Noradrenaline output is about doubled at any of the TEA concentrations used, the maximum output being obtained at 2·5 mM-TEA. In three experiments, TEA (2·5 mM) increased the control output of 233 ± 60 ng to 562 ± 136 ng.

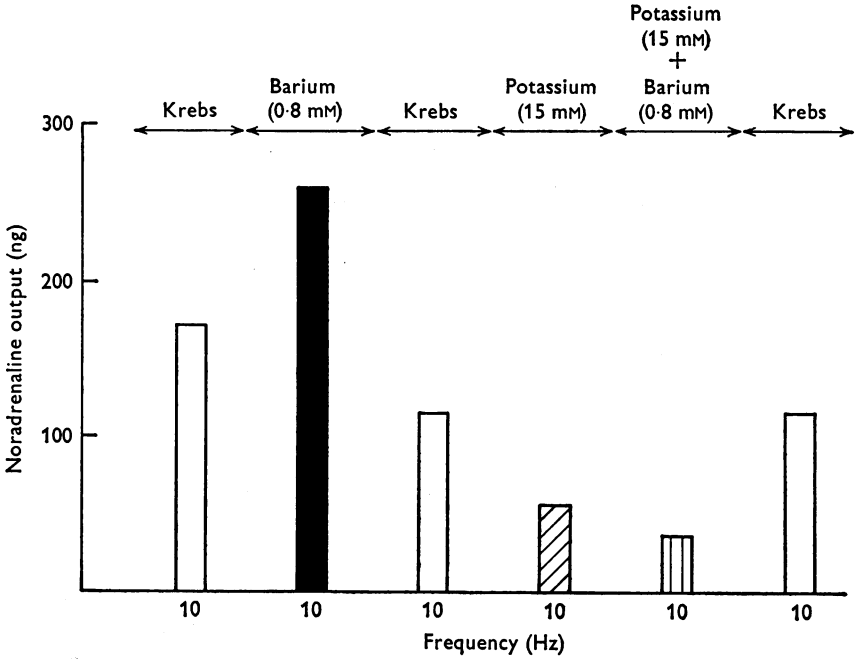

Fig. 4. The effect of barium on noradrenaline release following nerve stimulation in phenoxybenzamine-treated spleen. Barium increases the spontaneous release of noradrenaline. Noradrenaline outputs by nerve stimulation in barium-Krebs solution were therefore corrected for the background activity. Note that barium increased noradrenaline release by nerve stimulation. The same experiment also shows that high potassium antagonizes the potentiating effect of barium on noradrenaline release.

Barium

Release of noradrenaline by nerve stimulation was enhanced in the presence of barium (0·8 to 10 mM) in Krebs solution. Experiments with barium were, however, complicated due to the fact that exposure of the spleen to barium invariably reduced the noradrenaline output during a subsequent reperfusion with normal Krebs solution. Because of this

difficulty, the effect of different concentrations of barium could not be studied in the same spleen. However, it was clear from different experiments that higher concentrations of barium (7·5 and 10 mM) were more effective two to threefold than the lower concentrations (0·8 and 2·5 mM) in enhancing noradrenaline release induced by nerve stimulation. Barium (0·8 to 10 mM) always enhanced the spontaneous release of noradrenaline (range 18–64 ng/min). The facilitatory effect on release of barium was antagonized by increasing the concentration of potassium in the perfusing solution. Fig. 4 shows that barium (0·8 mM) enhances noradrenaline release induced by nerve stimulation by about 50%, and during a subsequent perfusion with normal Krebs solution the output was depressed by about 40%, which is considered as a control output for the rest of the experiment. During perfusion with 15 mM potassium Krebs solution, the output was depressed by about 50%, which was even further depressed during a perfusion with 15 mM potassium plus 0·8 mM barium solution. In three experiments the mean noradrenaline output in 15 mM potassium solution was depressed to 29 ± 15% of the control, which was not significantly altered during a simultaneous perfusion with 15 mM potassium and 0·8 mM barium. Following reperfusion with normal Krebs solution, the outputs were essentially restored.

DISCUSSION

Experiments reported in this paper provide additional support to the hypothesis that calcium is essential for the release of noradrenaline by sympathetic nerve stimulation. Earlier studies showed that manganese ions exert a marked inhibitory effect on the release of sympathetic transmitter (Kirpekar, Dixon & Prat, 1970). Our present findings with other transitional elements, such as cobalt and nickel, also demonstrated a powerful inhibitory action on the evoked release of noradrenaline from the perfused cat spleen. The inhibitory effects of transitional elements and lanthanum on release are not due to interference in conduction, since H^3-noradrenaline release from the vas deferens and seminal vesicle of the guinea-pig by excess potassium was also effectively suppressed by these elements (Wakade & Kirpekar, 1971). Moreover, additional evidence for the antagonistic action of transitional elements with calcium ions was obtained by showing that excess calcium in the perfusion medium was able partially to reverse the blockade of noradrenaline release by cobalt. Previously, several investigators have shown that manganese specifically suppresses the calcium permeability in various types of muscle cells (Hagiwara & Nakajima, 1966; Coraboeuf & Vassort, 1967; Lu & Brooks, 1969) and axons (Baker *et al.* 1971; Katz & Miledi, 1969), and the present

results also suggest a suppressive effect of these metals on calcium permeability in sympathetic nerves, causing an inhibition of noradrenaline release. A marked inhibitory action of lanthanum on transmitter release can also be attributed to the selective inhibitory property of this element on calcium entry in different tissues (Hagiwara & Takahashi, 1967; Miledi, 1971). In keeping with the concept of an interaction between calcium and manganese, cobalt, nickel or lanthanum is the finding that calcium-independent release of noradrenaline from adrenergic nerves by the indirectly acting sympathomimetic amine, tyramine, was not interfered with by the presence of cobalt in Krebs solution.

The changes observed in noradrenaline release by a number of agents cannot possibly be explained on the basis of a unitary hypothesis. It is conceivable that all these procedures, acting through different mechanisms, would ultimately increase or decrease the calcium entry to modify transmitter release. The calcium entry probably depends on (*a*) the resting membrane potential, (*b*) the presence of agents which block calcium channels, and (*c*) the duration of action potential in the nerve terminals. The recent work of Baker *et al.* (1971) has provided further information on the voltage-dependent changes in calcium permeability in squid axons. Sustained depolarization of the axon had a phasic effect on the tetrodotoxin-resistant calcium entry, i.e. an initial rapid activation of calcium channels, followed by their inactivation. If depolarization of the sympathetic nerve endings affects the calcium entry in a similar manner, then it seems likely that carbachol, protoveratrine and high potassium which might depolarize the nerve terminals would inactivate the calcium channels, thereby reducing the evoked release of noradrenaline. The inhibitory actions of transitional elements and lanthanum on release can be attributed to the blockade of calcium channels, since Baker *et al.* (1971) showed that manganese and cobalt ions specifically block the late phase of calcium entry. Finally, enhancement of transmitter release by TEA, barium and rubidium may be due to prolongation of the duration of the action potential, which would allow the calcium gates to remain open longer, and more calcium to enter.

The inhibitory and facilitatory actions of various agents on release can also be alternately explained on the basis of the scheme presented in Fig. 5*a*. According to this scheme, the inward movement of calcium ions required for noradrenaline release, and which is probably initiated by the depolarization of nerve membrane, is opposed by the outward movement of potassium. The outward movement of potassium, in effect, controls the release of noradrenaline by modulating the calcium entry into the sympathetic nerves. In support of such a view, agents such as carbachol and high potassium, which increase potassium efflux at rest from vagal C fibres

(Keynes & Ritchie, 1965), were found in the present study to reduce noradrenaline release (Fig. 5*b*). Although one cannot be certain whether these treatments would also enhance the efflux of potassium from adrenergic nerve endings during electrical stimulation, it is encouraging that these agents had an inhibitory action on release in response to nerve stimulation.

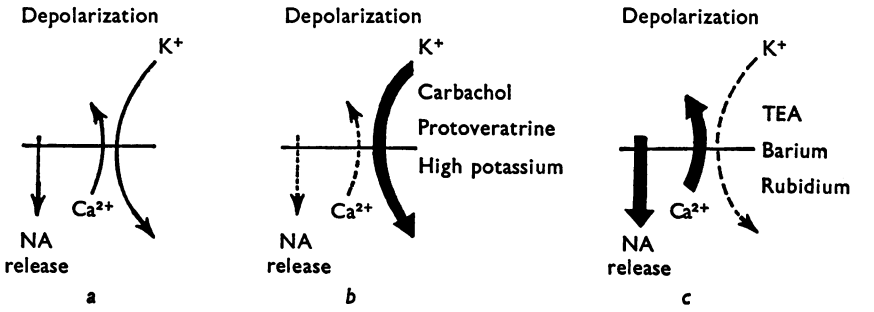

Fig. 5. A schematic representation of the hypothesis advanced to explain the release of noradrenaline on the basis of a possible interaction between calcium and potassium ions. Fig. 5*a* shows that the calcium-dependent noradrenaline (NA) release is related to the outward movement of potassium which controls the entry of calcium during the course of an action potential. Fig. 5*b* shows that agents such as carbachol, protoveratrine and high potassium, which are believed to increase the potassium efflux, decrease the transmitter release by opposing the calcium entry. Fig. 5*c* shows that agents such as TEA, barium and rubidium, which decrease the potassium efflux, enhance the transmitter release by increasing the calcium entry.

Keynes & Ritchie (1965) obtained a great increase in potassium efflux with veratrine during stimulation, and the present results also show an inhibitory effect of protoveratrine on noradrenaline release. Similarly, Weidmann (1956) showed that the duration of the cardiac action potential could be considerably shortened by an injection of potassium during the repolarization phase. It is also well known that acetylcholine or vagal stimulation (Burgen & Terroux, 1953; Hutter & Trautwein, 1956) shortens the cardiac action potential. These effects could be explained by a further increase in potassium permeability during repolarization by high potassium and acetylcholine. From evidence such as this, we have assumed that carbachol, protoveratrine and high potassium would probably enhance the efflux of potassium from the sympathetic nerves during stimulation.

Experiments with TEA and barium provide additional support to the above hypothesis, since their effects on potassium are better documented (Keynes & Ritchie, 1965; Armstrong & Binstock, 1965; Katz & Miledi, 1969). Both TEA and barium markedly reduce the potassium efflux during stimulation, and according to the above hypothesis (Fig. 5*c*) these

agents should increase the transmitter output by allowing greater entry of calcium into the nerves. Katz & Miledi (1969) have previously advanced a similar hypothesis to explain the action of TEA on the transmitter release in squid ganglia. It is interesting to note that the release of noradrenaline from mammalian post-ganglionic sympathetic C fibres can also be explained on this hypothesis.

This work was supported by a U.S. Public Health Service Grant, No. HE-05237.

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