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Modification of potassium-evoked release of noradrenaline by various ions and agents

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1 Release of noradrenaline (NA) from isolated spleen slices of the cat by high K^+ and tetraethylammonium (TEA) was investigated. Studies were conducted with spleen slices whose tissue stores were prelabelled with [^{3}H]-noradrenaline ([^{3}H]-NA).

2 Release by high K^+ was related to the K^+ concentration of the incubation medium. Release of [^{3}H]-NA by 28.5 mM K^+ was only barely detectable over the background, while 70 mM K^+ enhanced release to more than 600% of the background output. Tetrodotoxin (TTX) did not block responses to 28.5 or 35 mM K^+.

3 Background release was not modified by 1 or 3 mM TEA, but 10 and 30 mM TEA enhanced the release of [^{3}H]-NA by about 50% and 150%, respectively, over the background level. Neither TTX nor hexamethonium (C_6) blocked the TEA response. Release by TEA was also not blocked in Ca^{2+}-free medium or in Ca^{2+}-free medium containing up to 3 mM EGTA. Release by TEA was blocked in Ca^{2+}-free medium containing 5 mM EGTA, and by La^{3+} or Mn^{2+}.

4 The response to 35 mM K^+ was not modified by 1 or 3 mM TEA; 10 mM TEA had an additive effect; and 30 mM TEA with 35 mM K^+ produced a response which was greater than the simple sum of responses to 35 mM K^+ and 30 mM TEA. At 45 mM K^+, 3 and 10 mM TEA potentiated the response, and at 30 mM K^+ only 1 mM TEA showed potentiation. TTX did not alter the response to high K^+ plus TEA.

5 When TEA (30 mM) was added during prolonged incubation with 140 mM K^+, the response was only slightly enhanced. This suggests that a large part of the secretory response to TEA is mediated through mobilization of Ca^{2+} activated by depolarization.

6 Phenoxybenzamine (3.3 μM) potentiated responses to 35 and 140 mM K^+ by about 50%, and TTX did not influence this potentiation. Acetylcholine (ACh) blocked responses to 28.5 and 35 mM K^+, and 1 mM TEA antagonized this ACh blockade.

7 In the perfused adrenal gland of the cat, the secretory response to TEA was related to its concentration. The response was not diminished by low Na^+, TTX, or C_6, but was markedly attenuated when TEA was applied 10 min after the start of perfusion with high K^+.

Introduction

Kirpekar & Wakade (1968) showed that high K^+ evokes secretion of noradrenaline (NA) from the sympathetic nerves of the cat spleen in a calcium-dependent manner. It has also been reported that agents which increase the duration of the action potential, such as tetraethylammonium (TEA) and 4-aminopyridine (4-AP), markedly potentiate the release evoked by nerve stimulation (Thoenen, Haefely & Staehelin, 1967; Gillespie & Tilmisany, 1976; Wakade, 1980) but are ineffective in enhancing release induced by high K^+ (Kirpekar, Wakade & Prat, 1976; Kirpekar, Kirpekar & Prat, 1977).

Recently, Wakade (1980) showed in rat vas deferens that tetrodotoxin (TTX) reduced K^+ (45 mM)-evoked release of [^{3}H]-noradrenaline ([^{3}H]-NA) by 30%. He also showed that TEA (10 mM) enhanced the K^+-evoked release by about 5 fold, and that TTX reduced the response by 65%. In the presence of TTX, the response to TEA and 45 mM K^+, even though reduced, still remained at more than twice the response to 45 mM K^+ plus TTX alone. Wakade & Wakade (1981a, 1982) further extended these observations in the guinea-pig heart, showing that TEA (20 mM) enhanced the response to 35 and 70 mM K^+

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by 5 fold and 1.5 fold, respectively, and that TTX blocked this potentiation. They suggested that a part of the release induced by high K^+ is due to regenerative activity, since the response was partially blocked by TTX and potentiated by TEA.

The primary purpose of this study was to reassess the secretory response to excess K^+ in the cat spleen. Our previous investigations of K^+-evoked NA release in the cat spleen focused on responses to very high K^+ concentrations (bolus injection of 3.7 M K^+ or prolonged application of 140 mM K^+) (Kirpekar & Wakade, 1968; Kirpekar *et al.*, 1976; Kirpekar *et al.*, 1977; Garcia, Kirpekar & Pascual, 1978). In the present study, we have used intermediate concentrations of K^+ to re-examine the role of regenerative depolarization in K^+-evoked secretion and the mechanisms of muscarinic inhibition and α-autoinhibition in the splenic nerve terminals.

Methods

Spleen slices

Cats were anaesthetized with ether. The abdomen was opened by a midline incision, then the spleen was quickly removed and cut into transverse sections (0.5 to 0.7 mm thick), using a tissue slicer. To label the NA stores of the splenic nerve, the slices were incubated for 30 min with 10 ml of Krebs solution containing 10 μCi of (±)-[^{3}H]-NA (specific activity 7.5 Ci/mmol), then washed 3 times in 20 ml of Krebs solution over a 30 min period. In each experiment, a number of slices (approximately 100 mg) were incubated in 10 ml of Krebs solution for 5 min to determine the background release, then transferred to 10 ml of a test solution for 5 min. The incubation temperature was 37°C.

Perfused adrenal gland

Left and right adrenal glands were prepared for retrograde perfusion at room temperature according to the procedure of Garcia, Hernandez, Horga & Sanchez-Garcia (1980). The perfusion rate was about 1 ml/min. Glands were perfused for about 30 min before the start of each experiment.

Perfusion and incubation solutions

Krebs bicarbonate buffer consisted of (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 10. When excess K^+ (as K_2SO_4) was added, an osmotic equivalent of NaCl was concomitantly removed. For studies with La^{3+}, Mn^{2+}, or 10 mM Na^+, a Tris-buffered solution was used, consisting of (mM): NaCl 143, KCl 6.7, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$, 2.4, Tris (hydroxymethyl)-aminomethane (Tris) 5.0, and glucose 10. When NaCl was decreased to 10 mM, 266 mM sucrose was added to the solution. Bicarbonate-buffered solutions were equilibrated with a mixture of 95% O_2 and 5% CO_2; Tris-buffered solutions were equilibrated with 100% O_2. The pH for all solutions was adjusted to 7.4.

Assays of noradrenaline and analysis of results

The background and evoked release of NA from spleen slices was estimated by measuring the ^{3}H content of incubating solutions and the ^{3}H extracted from tissue slices homogenized in 0.4 N perchloric acid. Radioactivity was determined in a Tri-Carb Liquid Scintillation Spectrometer (Packard Instrument Company, Inc., La Grange, IL) from a 0.5 ml aliquot of each sample added directly to 5 ml of Aquasol. Tritium release is expressed either as a percentage of the total tissue content of tritium, or as a percentage of the spontaneous release obtained immediately prior to the treatment. Catecholamine (CA) release from the perfused adrenal gland was determined according to the fluorometric assay of Anton & Sayre (1962) without the intermediate alumina adsorption procedure.

Each experiment was repeated at least 4 times. Results of experiments in spleen slices are expressed as means with standard errors. For experiments with perfused adrenal glands, typical results of single experiments are shown.

Material

The following drugs were used: acetylcholine chloride (ACh) (Aldrich, Milwaukee, WI), hexamethonium chloride (C_6) (Nutritional Biochemicals, Cleveland, OH), phenoxybenzamine (Pbz) (Smith, Kline and French, Philadelphia), tetraethylammonium chloride (TEA) (Eastman Kodak, Rochester, N.Y.), tetrodotoxin (TTX) (Calbiochem, La Jolla, CA). Aquasol and (±)-[^{3}H]-noradrenaline were obtained from New England Nuclear (Boston, MA).

Results

Release of noradrenaline by potassium in cat spleen slices

Cat spleen slices were incubated in the presence of 28.5, 35, 45, or 70 mM K^+ to study the effect of moderate depolarization on release of [^{3}H]-NA. Spontaneous release immediately prior to these trials measured 1 to 4% per 5 min of the total [^{3}H]-NA

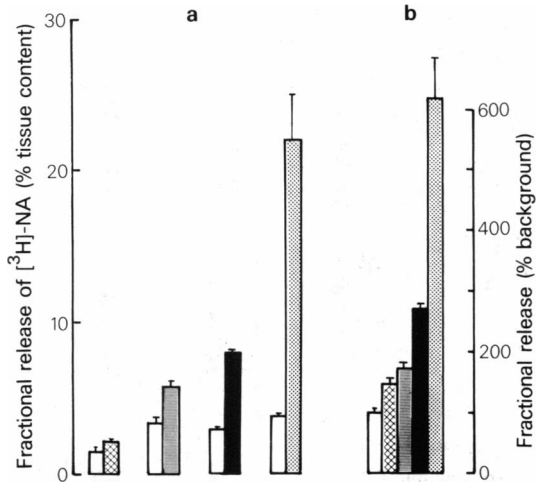

Figure 1 Effect of K^+ on tritium release from cat spleen slices. (a) Tritium release evoked by 28.5 (cross hatched column), 35 (lined column), 45 (solid column) and 70 mM (stippled column) K^+. Background (open columns) and evoked release of tritium is expressed as a percentage of the total tritium present in the tissue at the start of each incubation period. (b) Each response in section (a) re-expressed as a percentage of the background release measured immediately before treatment at each respective K^+ concentration. Results are expressed in this manner in subsequent figures.

content (Figure 1a). The net release of [^{3}H]-NA was less than 1% in the presence of 28.5 mM K^+ and approximately 2% in the presence of 35 mM K^+. With 45 and 70 mM K^+, the net response was further increased to about 5% and 18%, respectively, of the total tissue [^{3}H]-NA content. These results were normalized in Figure 1b with reference to the mean background [^{3}H]-NA output obtained in each set of trials. When [^{3}H]-NA secretion was re-expressed as a percentage of the background ^{3}H output, responses ranged from about 150% as background in the presence of 28.5 mM K^+ to more than 600% of background in the presence of 70 mM K^+. The output of $168 \pm 10\%$ ($n = 12$) at 35 mM K^+ was not significantly different from that of $146 \pm 10\%$ ($n = 16$) at 28.5 mM K^+. However, this may be attributed to the variability of responsiveness in spleens obtained from different cats. In 6 experiments, when these responses were compared in the same spleens, the output at 35 mM K^+ was 30% higher ($P < 0.05$) than that at 28.5 mM K^+. Thus, Figure 1 shows that [^{3}H]-NA overflow consistently increased as [K^+] was increased from 28.5 to 70 mM K^+.

Effect of tetraethylammonium on the background release of [^{3}H]-noradrenaline

Because one objective of the present series of experiments was to study the effect of TEA on the release of [^{3}H]-NA induced by low concentrations of excess K^+, the effect of TEA on spontaneous release of [^{3}H]-NA was examined. Figure 2a shows that 1 and 3 mM TEA had no effect on the background release, but that 10 mM TEA increased release to about 165% of the control and 30 mM TEA increased release to about 255% of the control. TTX (1 μM) failed to block the TEA response (Figure 2b). Hexamethonium (C_6) (0.1 mM) also had no effect on the response to 30 mM TEA, and even 1 to 10 mM C_6 did not diminish the TEA response (Figure 2c).

Removal of Ca^{2+} from the incubation medium did not diminish the secretory effect of TEA. The TEA response was not blocked by 1 or 3 mM EGTA, but was markedly reduced by 5 mM EGTA. The Ca^{2+} dependence of TEA-induced release was shown by the fact that La^{3+} (1 mM) or Mn^{2+} (2.5 mM), both potent blockers of the Ca^{2+} channel, abolished the secretory response to TEA.

Effect of tetraethylammonium on potassium-evoked release

Figure 3 shows the effect of 1, 3, 10, and 30 mM TEA

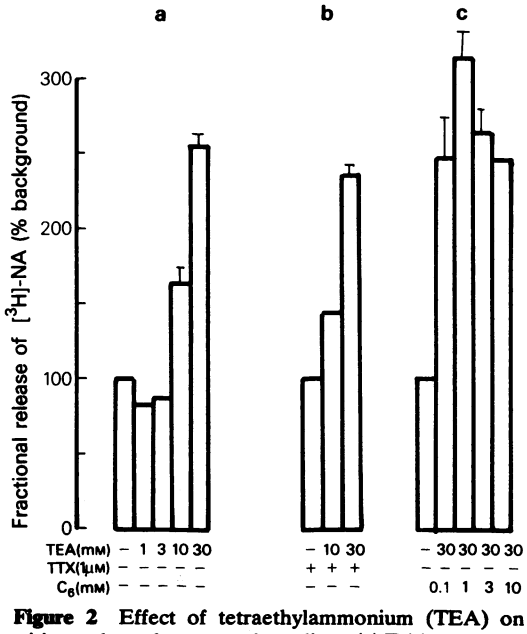

Figure 2 Effect of tetraethylammonium (TEA) on tritium release from cat spleen slices. (a) Tritium release evoked by 1, 3, 10, and 30 mM TEA expressed as a percentage of the background release measured immediately before treatment at each respective K^+ concentration. (b) Responses to 10 and 30 mM TEA re-evaluated in the presence of 1 μM TTX. (c) The response to 30 mM TEA re-evaluated in the presence of 0.1, 1, 3, and 10 mM C_6.

on the release of [^{3}H]-NA induced by 35, 45, or 70 mM K^+. In these experiments, because the secretory effect of TEA tends to decline during prolonged TEA treatment, control incubations were carried out in normal Krebs solution, rather than in solutions containing TEA.

TEA 1 and 3 mM, which did not modify the background release of [^{3}H]-NA, also had no effect on the response to 35 mM K^+; while 10 and 30 mM TEA, which enhanced the background release to 165% and 255%, respectively, enhanced the response to 35 mM K^+ by 90% and 270%, respectively, i.e., ^{3}H release was 185% of background in the presence of 35 mM K^+ alone, 350% of background in the presence of 35 mM K^+ plus 10 mM TEA, and 685% of background in the presence of 35 mM K^+ plus 30 mM TEA (Figure 3a). The response to 45 mM K^+ was potentiated 50% by 3 mM TEA, and about 160% by both 10 and 30 mM TEA (Figure 3b). The response to 70 mM K^+ was increased only 50% or less by 3, 10, or 30 mM TEA, but 1 mM TEA, which had no effect by itself or in combination with 35 or 45 mM K^+, also enhanced the response to 70 mM K^+ by about 40% (Figure 3c).

These data suggest that while the effect of TEA on K^+-evoked release of [^{3}H]-NA was diminished with increasing concentrations of K^+, the response tends to become more sensitive to enhancement by TEA. Thus, the lowest effective TEA concentration is 10 mM in the presence of 35 mM K^+, 3 mM in the presence of 45 mM K^+, and 1 mM in the presence of 70 mM K^+.

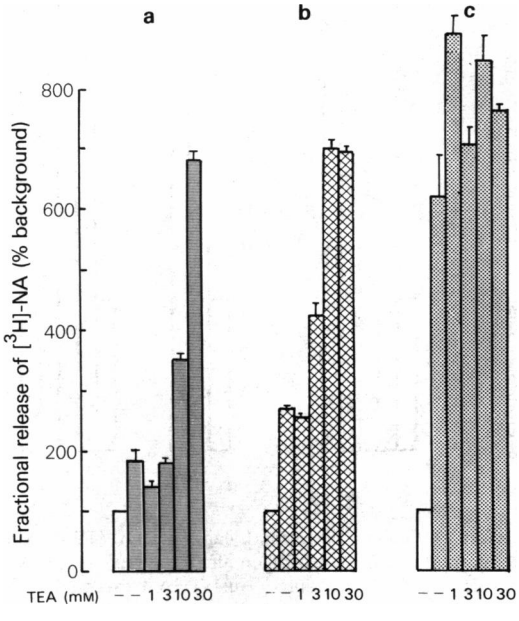

Figure 3 Effect of tetraethylammonium (TEA) (1, 3, 10, and 30 mM) on tritium release from cat spleen slices evoked by high K^+: (a) 35 mM K^+ (lined columns); (b) 45 mM K^+ (cross hatched columns); (c) 70 mM K^+ (stippled columns).

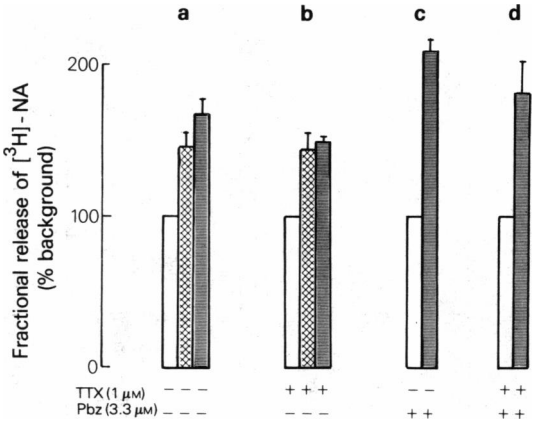

Figure 4 Effect of tetrodotoxin (TTX) and phenoxybenzamine (Pbz) on tritium release from cat spleen slices evoked by high K^+. (a) Control: release evoked by 28.5 (cross-hatched column) and 35 (lined column) mM K^+ in normal medium. (b) Release evoked by 28.5 (cross-hatched column) and 35 mM (lined column) K^+ in the presence of 1 μM TTX. (c) Release evoked by 35 mM K^+ (lined column) in the presence of 3.3 μM Pbz. (d) Release evoked by 35 mM K^+ (lined column) in the presence of both TTX and Pbz. Open columns represent background release in each case.

Effect of tetrodotoxin on potassium-evoked release

To determine whether a part of the release induced by low concentrations of K^+ in isolated spleen slices was due to generation of action potentials, secretory responses to 28.5 and 35 mM K^+ in the presence of 1 μM TTX were studied. Figure 4(a, b) shows that TTX did not block the secretory response to 28.5 or 35 mM K^+. Tetrodotoxin also failed to block the response to 35 mM K^+ in spleen slices treated with 3.3 μM Pbz (Figure 4c, d). The effect of TTX on the response to combined treatment with high K^+ and TEA was also studied. The responses to 35 and 70 mM K^+ with 1, 3, 10, or 30 mM TEA in the presence of TTX (1 μM) were comparable to those obtained in the absence of TTX. Thus, TTX did not reduce the response to high K^+ plus TEA.

Effect of tetraethylammonium during prolonged application of high potassium

To evaluate further the role of depolarization in the response to TEA, spleen slices were treated with

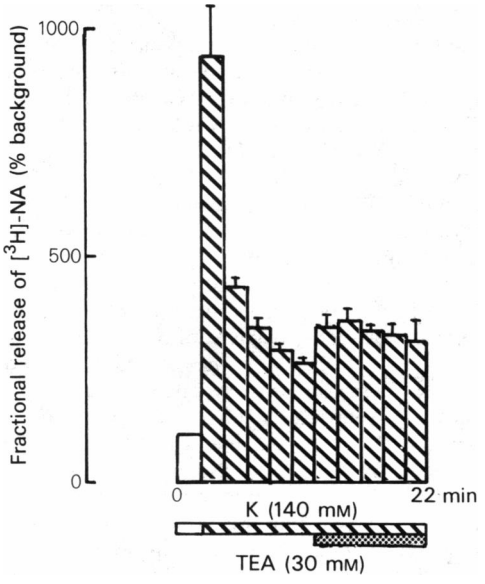

Figure 5 Effect of prolonged depolarization by high K^+ on tritium release from cat spleen slices induced by 30 mM tetraethylammonium (TEA). After obtaining the spontaneous release in normal Krebs solution, the slices were incubated in 140 mM K^+ solution for 20 min and samples were collected at 2 min intervals; TEA was added during the 10th and 20th min of high K^+ treatment.

30 mM TEA in the presence of 140 mM K^+, which should depolarize the nerve terminal to about 0 mV. Figure 5 shows that during prolonged incubation of spleen slices with 140 mM K^+ the release was more than 900% of the background in the first 2 min, and subsequently declined to about 250% of the background (about 20% of the maximal response) in 10 min. Tetraethylammonium (30 mM) only slightly enhanced the response to 140 mM K^+ when it was introduced at the 10th min of high K^+ treatment.

Effect of phenoxybenzamine and acetylcholine on potassium-evoked release

Secretory responses to 35 mM K^+ in cat spleen slices were evaluated in the presence of Pbz or ACh. Comparison of Figure 4a and c shows that 3.3 μM Pbz potentiated the response to 35 mM K^+ by only 50% of the background. Figure 6 shows that 3 to 10 μM ACh almost completely blocked the response to 35 mM K^+ in the presence or absence of TTX (1 μM). TEA (1 mM), which had no effect on the secretory response to 35 mM K^+ alone (Figure 3a), reversed the inhibitory effect of ACh on release evoked by 35 mM K^+.

Effect of tetraethylammonium on catecholamine release from the cat adrenal gland

Tetraethylammonium promoted catecholamine release from the adrenal medulla as well as from the spleen, and the effect appeared to be similarly dose-dependent. The spontaneous secretion was not affected by 1 or 3 mM TEA, but 10 mM TEA released 1.0 ± 0.1 μg catecholamine in the first 2 min, and 30 mM TEA initially released 4.5 ± 0.6 μg/2 min.

The response to TEA was markedly attenuated when TEA was applied late during perfusion with high K^+. Figure 7 shows that catecholamine secretion was vigorous at the start of perfusion with 140 mM K^+, but that it subsequently faded to about 10% of the initial response by the 8th to 10th min. TEA 30 mM produced only a feeble enhancement of the secretory response when it was added at the 10th min of high K^+ treatment, and the response to TEA

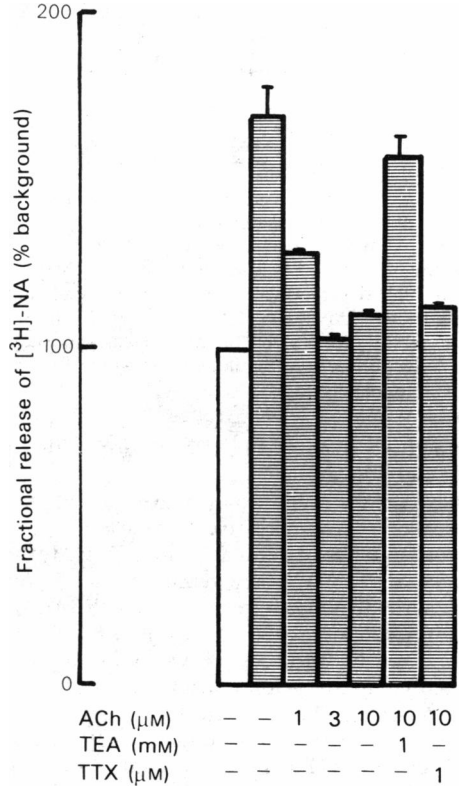

Figure 6 Effect of acetylcholine (ACh) on tritium release from cat spleen slices evoked by 35 mM K^+. The response to 35 mM K^+ in normal solution is compared with responses to 35 mM K^+ in the presence of 1, 3, and 10 μM ACh; 35 mM K^+ and 10 μM ACh were also added together in the presence of 1 mM TEA or 1 μM TTX. Open column: background; lined column: 35 mM K^+.

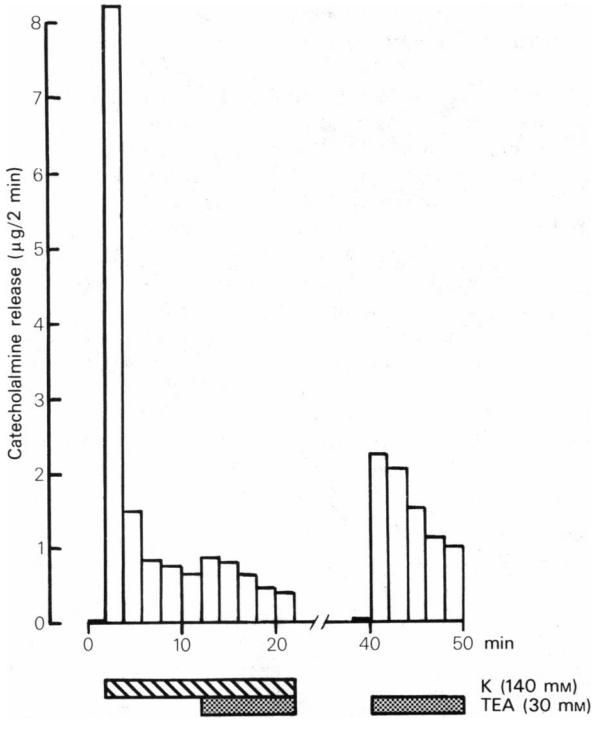

Figure 7 Loss of sensitivity to tetraethylammonium (TEA) during adrenal perfusion with high K^+. The adrenal gland was first treated with 140 mM K^+ for 20 min, and 30 mM TEA was added from the 10th and 20th min of high K^+ treatment; 20 min later, the gland was treated with 30 mM TEA alone for 10 min. Each column represents the total catecholamine released during a 2 min collection period. Similar results were obtained in three other experiments.

was restored on subsequent perfusion with normal Krebs solution.

These results suggest that in the adrenal gland, as in the spleen, TEA may stimulate secretion primarily as a consequence of depolarization. To study the role of regenerative Na^+ entry in the adrenal response to TEA, adrenal glands were perfused with 30 mM TEA in the presence of 1 μM TTX (Figure 8). The secretion evoked by 30 mM TEA was not diminished by TTX, as compared with bracketed control responses to TEA alone. The response to TEA was also well maintained when Na^+ was reduced to 10 mM.

The effect of TEA in the adrenal gland, as in the spleen, seemed to be unrelated to stimulation of the nicotinic receptor, inasmuch as C_6 (100 μM) failed to diminish TEA-induced secretion.

Discussion

The finding that transmitter release induced by 28.5 or 35 mM K^+ was not inhibited by TTX suggests that regenerative activity contributes little to the secretory response evoked by moderate concentrations of K^+. This observation contrasts with the report that TTX can partially block NA release from the rat vas deferens evoked by 45 mM K^+ (Wakade, 1980) or from the guinea-pig heart by 35 mM K^+ (Wakade & Wakade, 1982). Kirpekar and co-workers have shown that splenic NA release in response to nerve stimulation was 80% inhibited by 20 mM K^+ (Kirpekar, Prat, Puig, & Wakade, 1972; Kirpekar *et al.*, 1976). The response was completely blocked by 30 mM K^+, probably because of blockade of impulse conduction. If part of the release induced by 35 mM K^+ is mediated by regenerative activity (Na^+ potentials), then electrical stimulation should have promoted NA release even in the presence of 35 mM K^+. However, in the mouse vas deferens, 30 mM K^+ blocks the conduction of nerve impulses (Furness, 1970), and the conduction of impulses should be virtually impossible in high K^+ solution (Hodgkin, 1947). It is difficult, then, to reconcile the present results with those of Wakade (Wakade, 1980; Wakade & Wakade, 1982), except to note that different tissues were used in those studies.

TEA also releases catecholamines from both the spleen and the adrenal gland of the cat. The earlier failure to demonstrate release by 10 mM TEA (Kirpekar *et al.*, 1976) might be attributed to the relatively low sensitivity of the NA assay used and to the fact that NA release in the previous study was measured after 15 min perfusion with TEA, during which time the response would have faded. The failure of C_6 to diminish the secretory response to TEA suggests that TEA does not promote secretion by stimulating the nicotinic receptor. TTX did not diminish the secretory response to TEA. By its specific property of blocking K^+ conductance, TEA may cause depolarization, and this property alone may account for transmitter release, inasmuch as blockade of the TTX-sensitive Na^+ channel did not diminish the response to TEA. Alternatively, spontaneous Ca^{2+} action potentials may have contributed to the TEA response, but the fact that TEA-evoked secretion is graded, rather than all-or-none, suggests that TEA simply causes sustained depolarization of sympathetic nerves or adrenal chromaffin cells of the cat. If the TEA response was due to discharge of spontaneous action potentials, then the responses to 3 and 10 mM TEA should have been comparable. In the perfused cat spleen, 3 and 10 mM TEA comparably enhance NA output evoked by nerve stimulation (Kirpekar *et al.*, 1976). Similarly, in the anococcygeus muscle, 10 and 20 mM TEA were equally effective in potentiating the nerve-induced response to stimulation at 2 Hz (Gillespie & Tilmisany, 1976). The graded response to TEA in the cat spleen or adrenal gland, then, may

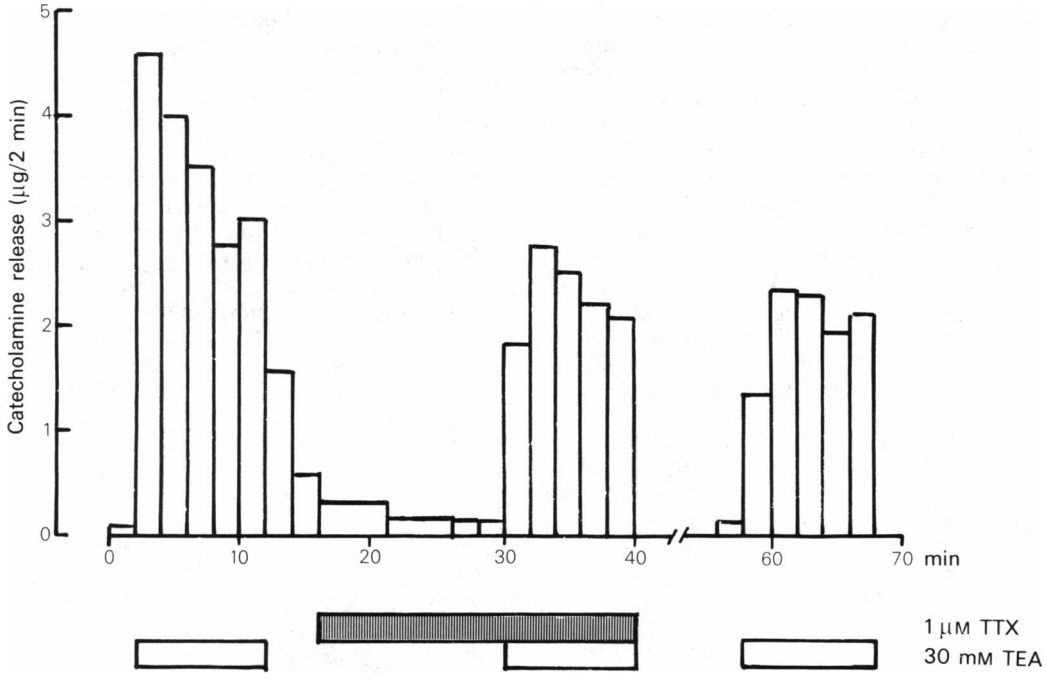

Figure 8 Effect of tetrodotoxin (TTX) on adrenal catecholamine release evoked by 30 mM tetraethylammonium (TEA). TTX (1 μM) was added during the second TEA treatment. Similar results were obtained in three other experiments.

be attributed simply to a greater depolarization by 30 than by 10 mM TEA, and is consistent with the proposal that TEA promotes catecholamine release by depolarizing the cell without increasing the discharge of spontaneous action potentials.

The secretory effect of TEA in cat spleen slices was not diminished in nominally Ca^{2+}-free medium, but a requirement for extracellular Ca^{2+} was demonstrated by the fact that the TEA response could be blocked by EGTA, La^{3+}, or Mn^{2+}. Wakade & Wakade (1981b) have reported that the TEA response in the isolated perfused heart of guinea-pig is Ca^{2+}-dependent, although they observed that TEA releases [^{3}H]-NA in 0.1 and 0.3 mM Ca^{2+}, but not in 2.5 mM Ca^+. They suggested that cardiac sympathetic nerves develop spontaneous action potentials in low-Ca^{2+} medium, and that TEA prolongs the duration of these spontaneous action potentials to enhance Ca^{2+} entry and thus triggers massive release of NA. It is not clear whether this suggestion would also account for the effect of TEA in the cat spleen inasmuch as the Krebs solution contained about 1.2 mM Mg^{2+}, which should stabilize the neuronal membrane even in a Ca^{2+}-free solution (Frankenhaeuser & Meves, 1956).

Enhancement of K^+-evoked secretion by TEA in the present study has been evaluated with reference to the enhancement of [^{3}H]-NA release by TEA alone. Only 30 mM TEA potentiated the response to 35 mM K^+ to an extent greater than its effect or spontaneous release; 3 and 10 mM TEA similarly potentiated the response to 45 mM K^+; only 1 mM TEA was more than additive in enhancing the response to 70 mM K^+. The lower TEA concentrations (1 to 3 mM) may fail to potentiate the release evoked by 35 mM K^+ because these concentrations may not appreciably enhance the depolarization caused by 35 mM K^+. On the other hand, because the cell membrane is considerably depolarized by 70 mM K^+, 1 mM TEA may be able to enhance secretion by its small increment of depolarization, while 30 mM TEA may cause no greater enhancement of either the depolarization or the secretion evoked by 70 mM K^+ plus 1 mM TEA. The observations that even the response to 70 mM K^+ was enhanced by TEA, and that TTX did not diminish the effect of TEA on K^+-induced release, both offer additional evidence that the secretory response to excess K^+ is due to sustained depolarization of the nerve terminals and that conducted action potentials do not contribute to the response.

It was previously reported that the secretory effect of 140 mM K^+ may be diminished by methacholine in the isolated heart of rabbit (Dubey, Muscholl, & Pfeiffer, 1975) or by ACh in cat spleen slices (Garcia *et al.*, 1978). The present experiments have shown

that ACh also inhibits the catecholamine release induced by moderate concentrations of K^+, and that this effect is reversed by TEA. When Kirpekar *et al.*, (1972) evaluated the inhibitory action of carbachol on electrically evoked NA release from splenic nerves and its reversal by TEA, they suggested that inasmuch as TEA specifically blocks K^+ efflux and enhances Ca^{2+} influx, muscarinic stimulation may inhibit Ca^{2+} entry. In order to account for the ACh inhibition of electrically evoked NA release from the guinea-pig heart, Wakade & Wakade (1981a, 1982) suggested that enhancement of K^+-conductance in cardiac sympathetic nerves by ACh would probably alter other electrical properties (e.g., conduction, resting membrane potential, and duration of nerve action potential). Increased K^+-conductance, however, cannot account for the ability of ACh to diminish the secretory response to moderate or high concentrations of K^+. Moreover, the reversal of ACh inhibition by TEA may be ascribed to the cholinoceptor antagonist properities of TEA. Gillespie & Tilmisany (1976) have reported that, in the anococcygeus muscle, the atropine-sensitive increase of tone evoked by carbachol is competitively blocked by TEA ($K_B \sim 2.5 \times 10^{-4}$ M). In the sympathetic nerve, ACh may inhibit secretion by directly interfering with Ca^{2+} entry. Consistent with this mechanism are the observations that ACh inhibition of electrically evoked NA release from the perfused cat spleen is more pronounced at low than at high stimulation frequencies (Kirpekar, Prat, & Wakade, 1975), and that the inhibitory effect of methacholine on K^+-evoked secretion in the rabbit isolated heart is potentiated by lowering the Ca^{2+} concentration (Dubey *et al.*, 1975). Thus, direct inhibition of Ca^{2+} entry could explain the inhibitory effect of ACh on the secretory response to electrical stimulation or excess K^+.

Pbz (3.3 μM) enhanced the secretory response to 28.5 mM K^+ by only 50%. Garcia *et al.* (1978) previously reported that the response to 140 mM K^+ in cat spleen slices was enhanced 50% by 10 μM Pbz. Because this concentration of Pbz blocks neuronal uptake of NA, and because 10 μM cocaine similarly enhanced NA release evoked by high K^+, Garcia *et al.* (1978) concluded that blockade of reuptake, rather than blockade of feedback inhibition, accounted for the enhancement of K^+-evoked NA release by Pbz. They suggested that prolonged K^+-depolarization may interfere with α-autoinhibition of NA release by desensitizing presynaptic α-receptors. However, Alberts, Bartfai, & Stjärne (1981) have noted that K^+-evoked secretion results from direct depolarization of the nerve terminal, whereas electrically evoked secretion results from indirect depolarization, i.e., recruitment of varicosities; they proposed that NA may selectively interfere with electrically evoked secretion chiefly by blocking conduction of nerve impulses between varicosities. This would account for both the ability of α-adrenoceptor antagonists to enhance secretion evoked by electrical stimulation and their failure to modify secretion evoked by high K^+ or veratridine.

An alternative explanation was proposed by Wakade & Wakade (1981c), who observed that the noradrenaline released from the isolated perfused heart following a single electrical pulse in the presence of TEA is distinctly facilitated by α-adrenoceptor antagonists. They concluded that the major target of presynaptic modulation in one-pulse experiments is at the level of Ca^{2+} availability. This conclusion, however, does not address the possibility that regeneratively conducted invasion of varicosities could account for much of the response to a single transmural field-stimulation pulse. Moreover, the conclusion that presynaptic α-inhibition directly reduces Ca^{2+} influx cannot account for the observations that secretory responses to treatments which directly depolarize the nerve terminals, such as high K^+ in isolated spleen slices (Garcia *et al.*, 1978; present results) or veratridine in isolated vas deferens (Stjärne, 1979), are not markedly enhanced by α-adrenoceptor antagonists.

In conclusion, high K^+ and TEA activate catecholamine release from the cat spleen and adrenal gland by maintained depolarization rather than regenerative depolarization of the nerve terminals or chromaffin cells. Acetylcholine may inhibit secretion by directly diminishing Ca^{2+} entry, whereas feedback inhibition by NA may primarily involve alteration of other membrane properties, such as conduction. It is also possible that feedback inhibition by NA does not contribute to the regulation of K^+-evoked secretion because prolonged depolarization may interfere with presynaptic α-inhibition, either by reducing the number of available α-receptors or by imposing a conformational change on the presynaptic α-receptor-effector complex which decreases receptor-agonist affinity or modifies the effector so that it fails to respond to activation of the receptor.

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