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Ion dependence of the release of noradrenaline by tetraethylammonium and 4-aminopyridine from cat splenic slices

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1 Cat splenic slices prelabelled with [^{3}H]-noradrenaline were incubated in oxygenated Krebs-bicarbonate solution at 37°C, and the spontaneous total ^{3}H release into different incubation media monitored. In normal Krebs bicarbonate solution, the spontaneous tritium fractional release amounted to 3.7% of the tissue radiactivity content per 5 min collection period.

2 Tetraethylammonium (TEA) increased spontaneous transmitter release in a concentration-dependent manner; the release was maximal at 30 mM and was 3.5 times the basal release.

3 4-Aminopyridine (4-AP) also enhanced the spontaneous release of tritium. The response increased linearly with 4-AP concentration (1–10 mM). With 10 mM 4-AP, the release was as much as 6 times the basal transmitter release. Guanidine was much less potent than either TEA or 4-AP.

4 The secretory response to TEA or 4-AP was little affected by changes in external Ca^{2+}, Mg^{2+}, Na^+, Cl^-, $H_2PO_4^-$ or by tetrodotoxin.

5 However, transmitter release evoked by TEA or 4-AP strongly depended upon the concentration of HCO_3^- of the incubation solution; in fact, the secretory response varied almost linearly between 1 and 25 mM HCO_3^-.

6 The mechanisms underlying these effects are probably related to the well-known ability of TEA and 4-AP to block K^+ conductance that would cause depolarization of the splenic sympathetic nerve terminals. The HCO_3^- requirements for the secretory response are probably related to the ability of CO_2/HCO_3^- solutions to mobilize and release Ca^{2+} from intracellular organelles.

Introduction

Tetraethylammonium (TEA) and 4-aminopyridine (4-AP) increase the amounts of noradrenaline released upon electrical stimulation of sympathetic nerves (Thoenen *et al.*, 1967; Kirpekar *et al.*, 1972; 1977; Wakade, 1980). The TEA ion and 4-AP are known to block selectively the delayed outward K^+ current responsible for repolarization, thereby prolonging the duration of the action potential in several neuronal types (Thesleff, 1980); this situation allows Ca^{2+} channels to open longer and more Ca^{2+} to enter the nerve terminal to evoke an increased transmitter release.

In addition to this well-known effect, TEA and 4-AP have also been shown to enhance the spontaneous release of noradrenaline from perfused cat spleen (Kirpekar *et al.*, 1977), perfused guinea-pig heart (Wakade & Wakade, 1981), cat splenic slices and adrenal medulla (Kirpekar *et al.*, 1983).

As part of a study of the specificity of the effects of these drugs on evoked and spontaneous catecholamine release, we undertook this work to determine: first, how TEA and 4-AP increased spontaneous noradrenaline release from cat splenic slices; and second, the ionic requirements for the releasing effects of TEA and 4-AP.

Methods

Preparation of splenic slices

Cats were anaesthetized with ether and the abdomen was opened by a midline incision. The spleen was removed quickly and cut into transverse sections (0.5–0.7 mm thick) with a tissue slicer.

[1] Correspondence.

Experimental designs

To label the noradrenaline stores in splenic nerve terminals, about 3 g of slices were thoroughly washed in fresh Krebs solution, and then incubated for 30 min at 37°C in 10 ml Krebs-bicarbonate solution equilibrated with 95% O_2:5% CO_2, containing 10 μCi of (±)-[^{3}H]-noradrenaline (specific activity 25 Ci m mol^{-1}); then the slices were washed 3 times in 20 ml of fresh 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 solutions containing different concentrations of TEA, 4-AP or guanidine for 5 min and finally twice for 5 min each in drug-free solution. In every experiment, the secretory responses to each drug were tested in control (Krebs-bicarbonate) or modified solutions.

Incubation and perfusion solutions

The composition of normal Krebs-bicarbonate solution, as well as the modifications introduced in its concentrations of cations and anions, are summarized in Table 1. Solutions lacking HCO^-_3 (HEPES and Tris solutions) were oxygenated with pure O_2; all other solutions were equilibrated with 95% O_2:5% CO_2. The pH of all solutions was carefully adjusted to 7.4 after vigorous bubbling with the appropriate gas for 30 min. The solutions were not bubbled further either during the subsequent incubation of the slices or during the periods of collection of media after exposing the tissues to TEA or 4-AP. To avoid repetition, each solution will be named in the text in the abbreviated form given in Table 1.

Measurements of noradrenaline release

The spontaneous and evoked release of noradrenaline from cat splenic slices prelabelled with [^{3}H]-noradrenaline was estimated indirectly by measuring the tritium content of incubating solutions and the tritium extracted in supernatants of 0.4 N perchloric acid tissue homogenates. Radioactivity was determined in a Tri-Carb liquid scintillation spectrometer (Packard Instruments Company, Inc., La Grange, IL) from a 0.5 ml aliquot of incubation and tissue extract samples added directly to 5 ml Aquasol.

Tritium released was expressed as a percentage of the total tritium tissue content at the beginning of the experiment (fractional release). To estimate the net amount of release evoked by every drug concentration, the spontaneous release (first collected sample) was subtracted from the amounts of radioactivity found in the sample containing the drug and the two subsequent drug-free samples collected (see Figure 1). In several experimental conditions including the use of

Table 1 Composition of solutions used to study how different cations and anions affected the release of tritium evoked by tetraethylammonium and 4-aminopyridine from cat splenic slices prelabelled with [^{3}H]-noradrenaline (concentrations are in mM)

Solution	*NaCl*	*KCl*	*K_2SO_4*	*$CaCl_2$*	*$MgSO_4$*	*Mg_2Cl*	*KH_2PO_4*	*$NaHCO_3$*	*$KHCO_3$*	*Vanadate*	*Glucose*	*HEPES*	*Tris*	*Sucrose*	*Sodium pyruvate*
Krebs	119	4.7	—	2.5	1.2	—	1.2	25	—	—	11	—	—	—	—
O CO^{2+} (a)	119	4.7	—	—	1.2	—	1.2	25	—	—	11	—	—	—	—
70 K^+	53.9	4.7	32	2.5	1.2	—	1.2	25	—	—	11	—	—	—	—
12 Mg^{2+} (a)	119	4.7	—	2.5	1.2	—	1.2	25	—	—	11	—	—	—	—
HEPES	144	4.7	—	2.5	1.2	—	1.2	—	—	—	11	15	—	—	—
Tris	144	5.9	—	2.5	—	1.2	—	—	—	—	11	—	5	—	—
1,3,10,25 HCO_3^- (a)	119	4.7	—	2.5	1.2	—	1.2	1,3,10,25	—	—	11	—	—	—	—
30,80,130 Cl^- (b)	0,50,100	—	—	—	1.2	—	5.9	25	—	—	11	—	—	—	119,69,19
0,50,100 Na^+	0,50,100	4.7	—	—	1.2	—	1.2	—	25	—	11	—	—	238,138,38	—
6 Phosphate	119	—	—	2.5	1.2	—	6	—	—	—	11	—	—	—	—
25 Vanadate	119	5.9	—	2.5	—	1.2	—	—	—	25	11	—	—	—	—

(a) No osmotic adjustments were made
(b) During the 5 min incubation in TEA chloride, each solution contained the 30 mM Cl^- of the TEA salt; Cl^- concentrations in low NaCl solutions were therefore 30,80 and 130 mM.

TEA and 4-AP, it had been shown previously that total tritium monitoring reflected adequately the behavior of intact [^{3}H]-noradrenaline tissue content and release (Kirpekar *et al.*, 1976; 1980; Kirpekar & Prat, 1978; Wakade & Wakade, 1981).

Materials

The following drugs were used: tetraethylammonium chloride (Eastman Kodak, Rochester, N.Y.); 4-aminopyridine and sodium vanadate ($NaVO_3$; Sigma, St. Louis, MO), guanidine hydrochloride (Aldrich, Milwaukee, WI), tetrodotoxin (TTX, Calbiochem, La Jolla, CA), Aquasol and (±)-(3H)-noradrenaline (New England Nuclear, Boston, MA).

Results

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

After labelling cat splenic slices with [^{3}H]-noradrenaline, the washout period with radioactivity-free Krebs solution ensured that a stable spontaneous fractional release of tritium of about 3–4% per 5 min collection period occurred; this rate was unchanged in the presence of 1–3 mM TEA. However, 10 mM TEA clearly enhanced the spontaneous transmitter release (Figure 1); at 30mM a peak secretory effect was observed. At higher concentrations (up to 100 mM), the enhanced release was seen not only during the 5 min of TEA application but also in the subsequent 5 min collection period. Since TEA chloride was added to the Krebs solution without osmotic adjustment, the effects of increased osmotic pressure and chloride content of these solutions on tritium release were examined; excess amounts of NaCl, equivalent to the concentrations of TEA used, did not modify the spontaneous transmitter release.

Figure 2 plots the net releases of tritium evoked by increasing concentrations of TEA in experiments similar to those of Figure 1. Again, it seems that 30 mM maximally enhanced transmitter release.

Effects of 4-aminopyridine and guanidine on the spontaneous release of tritium from cat splenic slices

Like TEA, 4-AP also inhibits the ionic current flowing through the voltage-sensitive K^+ channel; it was

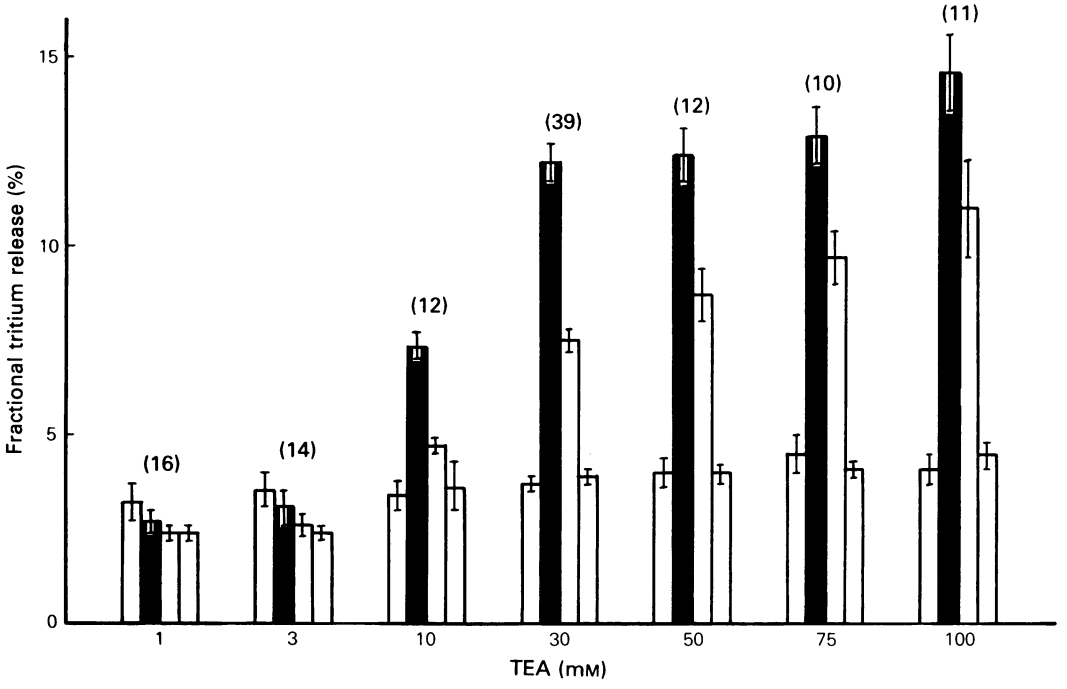

Figure 1 Effect of tetraethylammonium (TEA) on the release of tritium from cat spleen slices prelabelled with [^{3}H]-noradrenaline. Each group of columns represents the release of tritium in 5 min periods in the absence (open columns) and the presence (solid columns) of TEA at the concentrations shown on the abscissa scale. The ordinate scale shows the amounts of tritium released during each collection period of 5 min, expressed as fractional release (% of the total tissue content of [^{3}H]-noradrenaline at the beginning of every collection period). Data are means of the number of experiments shown in parentheses; vertical lines show s.e.means.

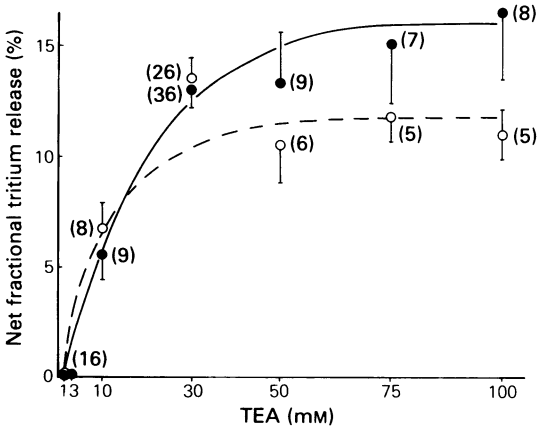

Figure 2 Release of tritium evoked by increasing concentrations of tetraethylammonium (TEA, abscissa scale) from cat splenic slices prelabelled with [^{3}H]-noradrenaline (●). Experimental design as in Figure 1; net fractional releases were calculated by substracting the spontaneous release obtained during the first 5 min sample collected from the following 3 samples (see Figure 1). A similar protocol was followed for slices exposed to 0 Ca^{2+} solution 20 min before and during TEA application (○). Data are means of the number of experiments shown in parentheses; vertical lines show s.e.means.

therefore interesting to test whether this drug was capable also of increasing the spontaneous release of tritium from cat spleen slices prelabelled with [^{3}H]-noradrenaline. Figure 3 shows clearly that 4-AP enhanced the spontaneous release of tritium; the secretory response was almost linear between 1 and 10 mM concentrations of the drug.

Guanidine was much less potent than either TEA or 4-AP; at 30 and 100 mM concentrations it released only 1.32 ± 0.26 and 4.53 ± 0.94% of the total ^{3}H-noradrenaline tissue content, respectively ($n = 4$).

Effects of divalent cations on the secretory responses to tetraethylammonium and 4-aminopyridine

At all TEA concentrations tested, the release of tritium obtained in the absence of Ca^{2+} was similar to that seen in the presence of 2.5 mM Ca^{2+} (Figure 2). This is very apparent at concentrations of TEA lower than 30 mM; in many of these experiments the secretory responses were almost identical (Figure 2).

In 0 Ca^{2+} solution, the release of tritium evoked by 4-AP was in fact higher than in the presence of this cation (Figure 3). The net fractional release obtained with 10 mM 4-AP amounted to 22 and 28% in 2.5 and 0 Ca^{2+}, respectively ($P = 0.05$).

Figure 4 shows that the secretory responses to 30 mM TEA or 10 mM 4-AP were not appreciably influenced in 12 Mg^{2+} solutions, either in the presence (2.5 mM) or the absence of Ca^{2+}. These data are in sharp contrast with the practical abolition by high Mg^{2+} of the release of tritium evoked by high K^+ solutions (Figure 4) or by nerve stimulation-evoked release of noradrenaline (Kirpekar & Misu, 1967; Kirpekar & Wakade, 1968).

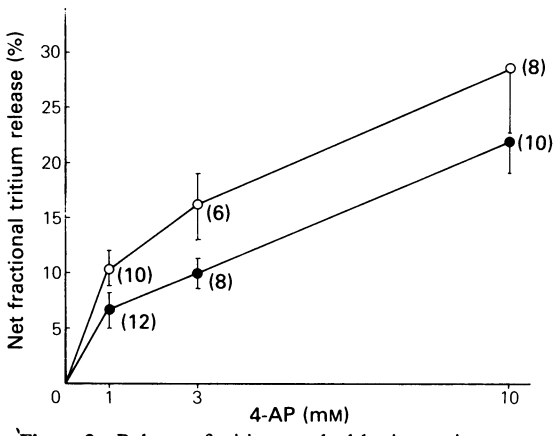

Figure 3 Release of tritium evoked by increasing concentrations of 4-aminopyridine (4-AP, abscissa scale) from cat splenic slices prelabelled with [^{3}H]-noradrenaline. Experimental design as in Figure 2. (●) Experiment carried out in normal Krebs solution; (○) experiment in 0 Ca^{2+} solution. Data are means of the number of experiments shown in parentheses; vertical lines show s.e.means.

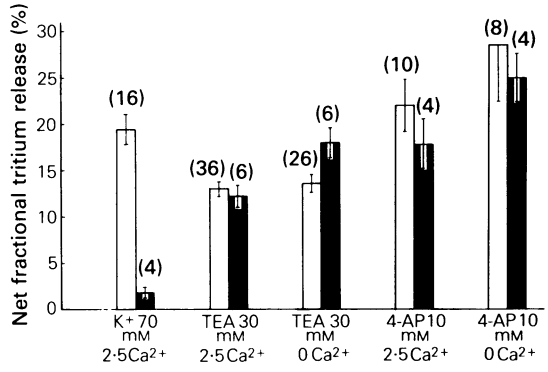

Figure 4 Effect of 12 Mg^{2+} solution (Krebs containing 10 × the normal concentration of Mg^{2+}) on the net release of tritium evoked by high K^+, tetraethylammonium (TEA, 30 mM) or 4-aminopyridine (4-AP, 10 mM) in the presence of 2.5 mM Ca^{2+} (2.5 Ca^{2+}) or 0 Ca^{2+} solution: open columns 1.2 Mg^{2+}; solid columns 12 Mg^{2+}. Slices were bathed and repeatedly washed in each solution 20 min before, during and after application of TEA or 4-AP. Data are mean net releases of the number of experiments shown in parentheses; vertical line shows s.e.means.

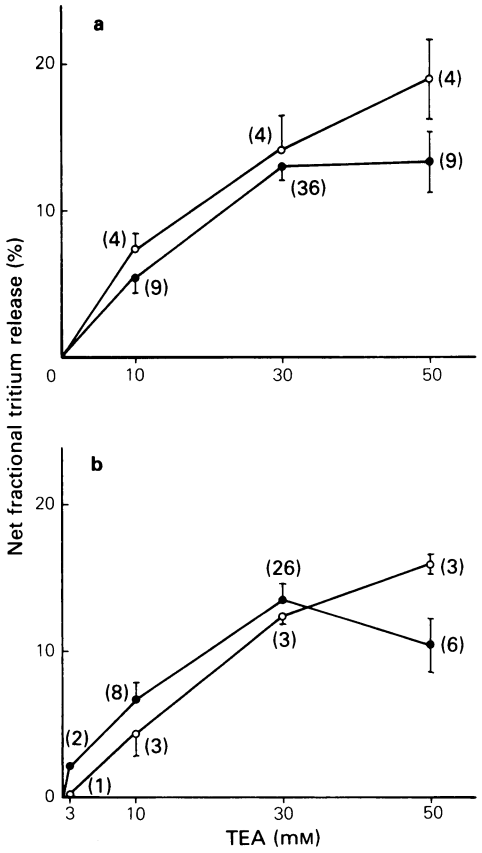

Figure 5 Effect of tetrodotoxin (TTX) on the net release of tritium evoked by tetraethylammonium (TEA) from cat splenic slices prelabelled with [^{3}H]-noradrenaline. Slices were incubated in Krebs (a) or 0 CA^{2+} (b) solutions for 15 min before adding TTX (1 μM); then, 5 min later TEA was applied for 5 min at the concentrations shown on the abscissa scale. 0 Ca solution was maintained for the rest of the experiment (similar protocol to that of Figure 1). (●) Control; (○) TTX 1 μM in both (a) and (b). Net fractional releases of tritium (ordinates) represent means of the number of experiments shown in parentheses; vertical lines show s.e.means.

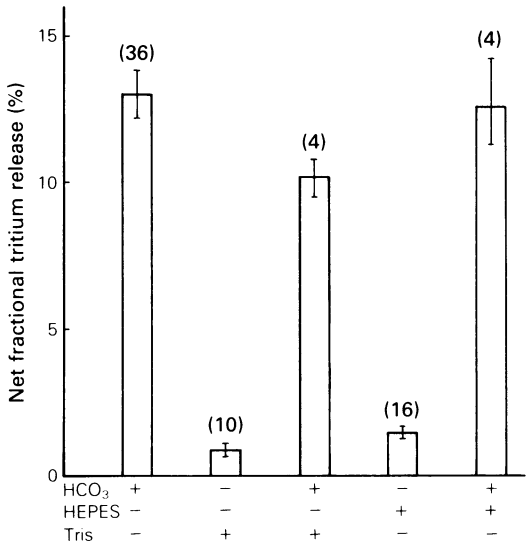

Figure 6 Effect of the HCO_3^- anion on the release of tritium evoked by tetraethylammonium (TEA, 30 mM for 5 min), from cat splenic slices prelabelled with [^{3}H]-noradrenaline. Before adding TEA, slices were preincubated for 15 min in modified Krebs solutions buffered with HCO_3^- (25 mM), HEPES (15 mM) or Tris (5 mM) as shown below each column; after incubation in TEA, there were two additional 5 min collecting periods in the presence of the corresponding buffer. Net fractional releases of tritium (ordinates) represent means of the number of experiments shown in parentheses; vertical lines show s.e.means.

Effect of tetrodotoxin on tetraethylammonium-induced tritium release

The effects of TTX on the release of tritium from cat splenic slices exposed to increasing concentrations of TEA both in Krebs or 0 Ca^{2+} solutions are shown in Figure 5. the secretory responses to TEA were similar in the presence or the absence of TTX (1 μM), both in 2.5 or 0 Ca^{2+} solutions, at all concentrations of TEA tested.

Effects of the HCO_3^- anion on the release of tritium evoked by tetraethylammonium and 4-aminopyridine

The effects of TEA in solutions lacking bicarbonate and phosphate were studied in a series of experiments. The results in Figure 6 suggest that TEA did increase noradrenaline release in the absence of HCO_3^-, though to a much smaller extent than in its presence. The s.e. bars suggest that the increase shown is unlikely to have occurred by chance (especially in the HEPES-buffered solution). The addition of HEPES (15 mM) or Tris (5 mM) to Krebs containing 25 mM HCO_3^- did not affect the secretory response obtained in Krebs solution, suggesting that these two buffers *per se* do not have an inhibitory effect.

It became clear from these experiments that the secretory response to TEA depended upon the presence of bicarbonate in the Krebs solution bathing splenic slices; therefore, we studied the TEA response in solutions containing different bicarbonate concentrations. Figure 7a shows that the response to 30 mM TEA was strictly dependent on the bicarbonate con-

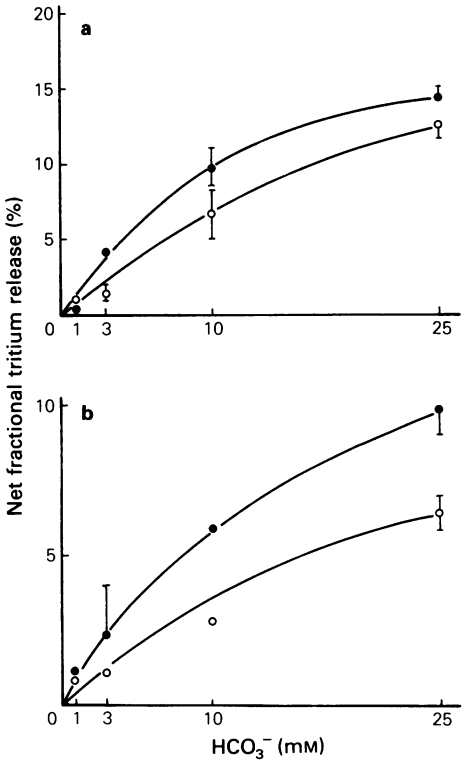

Figure 7 Effects of increasing concentrations of HCO_3^- (abscissa scale) on the release of tritium evoked by (a) tetraethylammonium (TEA, 30 mM for 5 min) or (b) 4-aminopyridine (10 mM for 5 min) from cat splenic slices prelabelled with [^{3}H]-noradrenaline. The experiments were repeated in the presence (○) or the abscence (●) of Ca^{2+}. Net fractional releases of tritium (ordinates) represents means of 3 experiments; vertical lines show s.e.means.

centration and it progressively increased from 1 to 25 mM; removal of Ca^{2+} had little effect on the secretory response to TEA. In Figure 7b it can be seen that the secretory response to 4-AP was also dependent on bicarbonate and that the absence of Ca^{2+}, if anything, enhanced the response.

Since the solutions used in the experiments described above were bubbled with 5% CO_2 in O_2 during pH adjustment, the final concentration of HCO_3^- might change as H_2CO_3 formed from the CO_2 bubbled into the solution dissociates into additional HCO_3^-. The fact that the secretory response is proportional to the concentration of HCO_3^- added as $NaHCO_3$ is against this explanation, though the concomitant change in pH may have been a complicating factor, which was not examined further. However, additional

Table 2 Effects of several anions and cations on the net release of tritium (increases over spontaneous release) evoked by tetraethylammonium (TEA, 30 mM for 5 min) from splenic slices prelabelled with [^{3}H]-noradrenaline

Incubation solution	n	*Net ^{3}H* fractional release (%)
Krebs	36	13 ± 0.8
6 Phosphate	4	2.4 ± 0.4
25 Vanadate	10	2.4 ± 0.5
30 Cl^-	6	5.6 ± 1.3
80 Cl^-	6	5.2 ± 1.1
130 Cl^-	6	5.1 ± 1.1
O Na^+	6	15.6 ± 1.4
50 Na^+	6	12.6 ± 2.1
100 Na^+	6	10.6 ± 0.8

Experimental protocol as in Figure 6. The composition of each solution appears in Table 1. Data are means ± s.e. of the number of experiments shown in *n*. The spontaneous fractional release obtained by incubating the slices in Krebs solution for 5 min was 3.7 ± 0.2% (n = 39).

similar experiments were performed using pure O_2 (instead of CO_2 + O_2) to bubble the incubation media. In this situation, the concentration of HCO_3^-, added as $NaHCO_3$, will surely remain constant. In the absence of HCO_3^-, fractional tritium release from splenic slices prelabelled with [^{3}H]-noradrenaline evoked by exposing them to 30 mM TEA for 5 min was 4.5 ± 0.41% (n = 5); when 25 mM HCO_3^- was added, the release was 15.9 ± 1.24% (n = 3).

Effect of anions and cations on the release of tritium evoked by tetraethylammonium

The ability of several anions to substitute for HCO_3^- and maintain the secretory response to TEA was also tested. Table 2 summarizes these data; the spontaneous release of ^{3}H was 3.7% in 5 min, and the net releases over this mean value obtained with different anions are shown.

In 6 mM phosphate solution, TEA increased the release of tritium over background levels by only 66%, compared with 350% obtained when the anion was HCO_3^-. Similar results were obtained when vanadate substituted for the anions phosphate and bicarbonate; in the presence of 25 mM $NaVO_3$, TEA enhanced the spontaneous release of ^{3}H by only 65%. Lowering the Cl^- concentrations of the Krebs solution did not produce a variation of the TEA-evoked secretory response proportional to such concentrations; although the release was lower than that obtained in normal Krebs solution, it was similar in the presence of

30,80 or 100 mM Cl^- since we tested only the chloride salt of TEA. To maintain constant the concentration of Na^+, sodium pyruvate was added in equiosmotic amounts to the reduced NaCl; this ionic substitution might contribute also to the poor secretory response.

Finally, the effects of several concentrations of NaCl at constant HCO_3^- on the release of ^3H, were tested. $NaHCO_3$ was substituted by $KHCO_3$; to avoid the high K^+ secretory effects, $CaCl_2$ was removed from these solutions. In 0,50 or 100 Na^+ solutions the release of ^3H evoked by TEA was similar to the release obtained in Krebs solution (Table 2). From all these experiments, it appears that phosphate, Cl^- or Na^+ ions contribute very little to the TEA secretory response and that the HCO_3^- effect in sustaining release is quite specific.

Discussion

We have shown in this paper that TEA or 4-AP increase markedly the spontaneous release of tritium from cat splenic slices prelabelled with [^3H]-noradrenaline in a concentration-dependent manner. The secretory response was relatively insensitive to Ca^{2+}, Mg^{2+}, Na^{2+}, Cl^-, phosphate or TTX but, surprisingly, depended upon the concentration of the anion HCO_3^- in the incubation medium.

Enhanced transmitter release has a common underlying mechanism both for tetraethylammonium and 4-aminopyridine

TEA had been shown previously to enhance the spontaneous release of catecholamines from perfused guinea-pig heart (Wakade & Wakade, 1981), cat splenic slices and perfused adrenal gland (Kirpekar *et al.*, 1983). Here, we have demonstrated a similar effect for 4-AP, guanidine being a much weaker secretagogue. Both TEA and 4-AP block selectively the delayed outward K^+ current responsible for repolarization, thereby prolonging the duration of the action potential in several neuronal types (Thesleff, 1980); on the other hand, the secretory response to both drugs depended upon the concentration of HCO_3^-. These two facts strongly suggest that these secretory responses have a common underlying mechanism, probably related to the ability of both drugs to block the outward K^+ current.

This idea is corroborated by the observation that guanidine behaved as a much weaker secretagogue. Like TEA and 4-AP, guanidine has been shown to enhance excitability of motor nerve fibres leading to multiple firing (Mathews & Wickelgren, 1977) and to potentiation of electrically-induced noradrenaline release (Hirsh *et al.*, 1980); however, unlike TEA and 4-AP, guanidine does not block K^+ conductance.

Propagated action potentials are not involved in the secretory effects of tetraethylammonium and 4-aminopyridine

Sodium-dependent action potentials could be responsible for the enhanced spontaneous transmitter release evoked by TEA and 4-AP. By blocking a large outward K^+ current (Thesleff, 1980; Bowman & Savage, 1981), TEA and 4-AP may cause sufficient localized depolarization of sympathetic nerve terminals to generate propagated Na^+-dependent action potentials primarily responsible for the release of noradrenaline. The insensitivity of such a secretory response to Na^+ deprivation or TTX suggests that there is no participation of Na^+-dependent action potentials in this response.

However, Ca^{2+}-dependent action potentials could be involved. At the giant synapse of the squid stellate ganglion pretreated with TTX and TEA, Katz & Miledi (1969) showed that a local regenerative response confined to the presynaptic terminal was obtained on depolarization, provided that the external Ca^{2+} concentration was sufficiently high. Under similar conditions, depolarization of sympathetic nerve terminals initiates a regenerative inward Ca^{2+} current which leads to an explosive release of noradrenaline (Kirpekar & Prat, 1978). Again, it is unlikely that our present results can be explained by this mechanism, essentially because the secretory responses to TEA and 4-AP, if anything, were enhanced in 0 Ca^{2+} solutions and high Mg^{2+} did not affect them.

This interpretation of our results contrasts with that given by Wakade & Wakade (1981) who observed that TEA increased spontaneous [^3H]-noradrenaline release from perfused guinea-pig hearts in low Ca^{2+} (0, 0.1 and 0.3 mM) Krebs-bicarbonate solution but the release was not enhanced at 1 or 2.5 mM Ca^{2+}. Since the release evoked by low Ca^{2+} and TEA was depressed by TTX, Mg^{2+} or La^{3+} these authors suggested that 'cardiac sympathetic nerves develop spontaneous action potentials in low Ca^{2+} and the duration of such action potentials is greatly enhanced by TEA, allowing more Ca^{2+} to enter and evoke release of [^3H]-noradrenaline'. This interpretation is unlikely since the low Ca^{2+} Krebs solution used by Wakade & Wakade (1981), like our solution, contained 1.2 mM Mg^{2+}, which is known to maintain (although less potently than Ca^{2+}; Orchardson, 1978) the normal permeability of cell membranes, even in the absence of Ca^{2+}, in nerve (Frankenhaeuser & Hodgkin, 1957; Frankenhaeuser & Meves, 1958) and chromaffin cells (Douglas & Rubin, 1963; García *et al.*, 1980; Montiel *et al.*, 1984). Only technical differences ([^3H]-noradrenaline versus total tritium monitoring) or the preparations used (guinea-pig heart versus cat splenic slices) remain to explain such conflicting results.

Depolarization is likely to be involved in the secretory response

Because TEA and 4-AP, by specifically blocking K^+ conductance, cause depolarization in a variety of cells (Bowman & Savage, 1981), it is reasonable to assume that such a depolarizing effect might be implicated in the secretory effects of the drugs (Kirpekar *et al.*, 1983). On the other hand, if depolarization of sympathetic nerve terminals were the unique mechanism involved, the releasing effects of TEA and 4-AP should mimic the secretory response obtained by other depolarizing stimuli such as high K^+ or veratridine. This is not the case since high K^+-evoked noradrenaline release is known to be Ca^{2+}-dependent and is antagonized by Mg^{2+} (Kirpekar & Wakade, 1968; Figure 4 of this paper) and the response to veratridine is inhibited by TTX, Na^+ deprivation or Ca^{2+} removal (Esquerro *et al.*, 1980). Therefore, it is clear that depolarization is not the only requirement for the secretory effects of TEA and 4-AP.

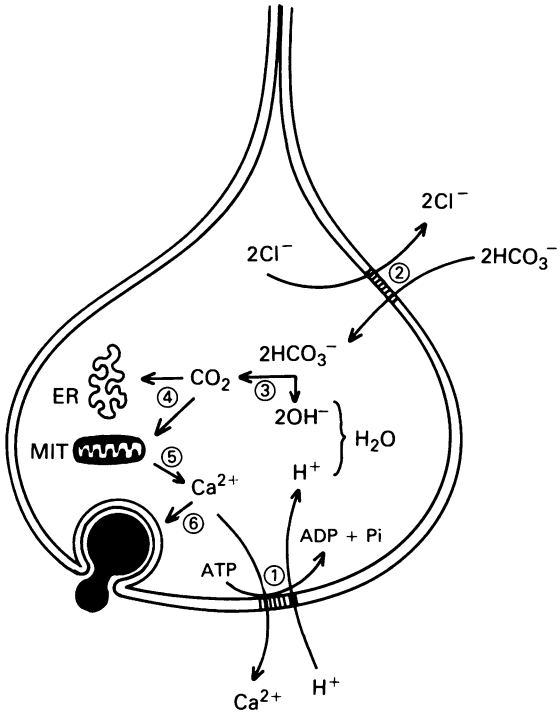

Figure 8 Scheme showing the sequence of events that might explain the permissive effect of HCO_3^- anions on the release of noradrenaline evoked by tetraethylammonium or 4-aminopyridine from sympathetic nerve terminals. (1) Ca^{2+} pump; (2) anion exchange system; (3) dissociation of HCO_3^- into H_2O and CO_2; (4) release of Ca^{2+} by CO_2 from intracellular organelles; (4) ER endoplasmic reticulum; (5) MIT mitochondria and (6) noradrenaline storage vesicles. The increase of intracellular ionized Ca^{2+} will cause the exocytotic release of noradrenaline.

The anion bicarbonate is involved in the secretory effects of tetraethylammonium and 4-aminopyridine

The observation that HCO_3^- could be involved in the increase of transmitter release caused by TEA and 4-AP was a casual finding. In a recent paper (Kirpekar *et al.*, 1983) we observed that La^{3+} and Mn^+ inhibited the release of tritium evoked by TEA; however, in the present paper high Mg^{2+} did not affect the secretory responses to TEA or 4-AP (Figure 4). This was a paradoxical finding since it is well known that Mg^{2+}, La^{3+} and Mn^{2+} behave as inorganic Ca^{2+} antagonists that inhibit equally noradrenaline release evoked by depolarizing stimuli (Kirpekar & Misu, 1967; Kirpekar & Wakade, 1968; Kirpekar *et al.*, 1972). Because La^{3+} and Mn^{2+} precipitate out in HCO_3^--containing solutions, we always used Tris (5 mM) to buffer solutions containing those cations and eliminated from them the anions HCO_3^- and $H_2PO_4^-$. Therefore, we decided to study the effects of TEA in solutions lacking these two anions; surprisingly, it turned out that TEA and 4-AP failed to increase the spontaneous tritium release to Tris or HEPES solutions and that the secretory response was proportional to the concentration of HCO_3^- present in the medium. This is a rather specific effect since Cl^-, vanadate or $H_2PO_4^-$ anions were not capable of replacing HCO_3^-.

Because depolarizing concentrations of K^+ release noradrenaline in the absence of HCO_3^-, it is clear that depolarization as such, is not a sufficient stimulus to trigger the enhanced transmitter release evoked by TEA and 4-AP. We suggest that HCO_3^- anions in the presence of depolarizing concentrations of TEA or 4-AP release Ca^{2+} from an intracellular source to cause the secretion of noradrenaline from sympathetic nerve terminals.

HCO_3^- is known to activate an ATPase and proton secretion in the gastric mucosa (Kasbeker & Durbin, 1965) and may be actively transported (Schulz, 1980; Steinmetz *et al.*, 1980) or exchanged for another anion such as Cl^- (Cabantehik & Rothstein, 1972). Niggli *et al.* (1982) have proposed that the Ca^{2+} pump of human erythrocyte membranes operates as an electroneutral $Ca^{2+}-H^+$ antiporter. This model predicts the formation of a pH gradient which is equilibrated mainly via an anion exchange system which involves $Cl^- - HCO_3^-$ exchange. If we extrapolate this model to the sympathetic nerve terminal, the sequence of events taking place in normal conditions might be those shown in Figure 8.

HCO_3^- exchanges with Cl^- and dissociates inside the nerve terminal to OH^- and CO_2. The proton gradient formed with the activation of the Ca^{2+} pump

is dissipated by the combination of H^+ and OH^- to form H_2O. The CO_2 formed might then act to favour the release of Ca^{2+} from intracellular sequestering organelles such as endoplasmic reticulum, mitochondria or noradrenergic vesicles. In fact, Lea & Ashley (1981) have shown that CO_2/HCO_3^- solutions can release Ca^{2+} from the sarcoplasmic reticulum of crustacean myofibrillar bundles. How TEA and 4-AP intervene in combination with HCO_3^- to trigger the secretory response remains to be elucidated, although it could be suggested that they somehow facilitate the transport of HCO_3^- inside the nerve terminal.

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