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THE ACTION OF TETRAETHYLAMMONIUM CHLORIDE ON THE RESPONSE OF THE RAT ANOCOCCYGEUS MUSCLE TO MOTOR AND INHIBITORY NERVE STIMULATION AND TO SOME DRUGS

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1 Tetraethylammonium chloride (TEA) 0.125 mM to 20 mM potentiates the response of the anococcygeus muscle to field stimulation of the motor adrenergic nerves without affecting the response to noradrenaline suggesting a pre-synaptic origin of potentiation. The potentiation is greatest at low, submaximal, frequencies (2 Hz) of stimulation and only slight at the higher frequency of 20 Hz. This difference is due to the restraint imposed on the demonstration of potentiation by maximal or near maximal motor responses since reduction of the mechanical response at 20 Hz by either phentolamine (post-synaptic block) or guanethidine (pre-synaptic block) resulted in a great increase in potentiation of the response at this frequency.

2 TEA in concentrations up to 1 mM similarly potentites the response to inhibitory nerve stimulation and again the greatest effect is at low frequencies. Higher concentrations (5–20 mM) progressively depress the inhibitory response. It is suggested that TEA may specifically antagonize the post-synaptic action of the inhibitory transmitter and that at higher concentrations of TEA this effect dominates the pre-synaptic action in increasing transmitter release.

3 TEA has no effect on the motor response to tyramine.

4 TEA (5–20 mM) causes a maintained rise in muscle tone. Part of this is abolished by phentolamine but part is resistant. A similar muscle stimulant action of TEA is observed in muscles from rats previously treated with 6-hydroxydopamine in which indirect sympathomimetic drugs and field stimulation could no longer produce a motor response. These results suggest that part of the motor effect of TEA is due to an increased spontaneous release of noradrenaline and part to a direct action on the muscle.

5 TEA 0.125 mM to 20 mM antagonize the stimulant action of carbachol. Dose-response curves show a parallel shift to the right with no change in the maximum response suggesting a competitive atropine-like action. Such an effect has previously been reported in amphibian tissue but not so far as we can determine in mammalian preparations.

6 The possible mode of action of TEA is discussed.

Introduction

Tetraethylammonium chloride (TEA) possesses a variety of actions which result from its interference with cholinergic transmission. In addition to the effects at acetylcholine receptors, TEA has important effects on the ion permeability of the membranes of many excitable cells. One particular effect is to prolong the action potential in muscle and nerve by preventing the delayed rise in potassium permeability which contributes to the rapid restoration of the resting membrane potential (Fatt & Katz, 1953; Tasaki & Hagiwara, 1957; Armstrong & Binstock, 1965). This prolongation of the action potential increases the liberation of transmitter from both cholinergic (Kensler, 1950; Koketsu, 1958; Douglas & Lywood, 1961; Collier & Exley, 1963) and adrenergic nerves (Thoenen, Haefely & Staehelin, 1967; Kirpekar, Prat, Puig & Wakade, 1972; Stjarne, 1973).

The experiments described in this paper were done to determine whether TEA would increase the release of transmitter from the nerves to the anococcygeus muscle and, in particular, whether it would potentiate the response to stimulation of the inhibitory nerves, a non-cholinergic non-adrenergic neurone whose transmitter is at present unknown (Gillespie, 1972). A preliminary account of some of these results has been published (Gillespie & Tilmisany, 1974).

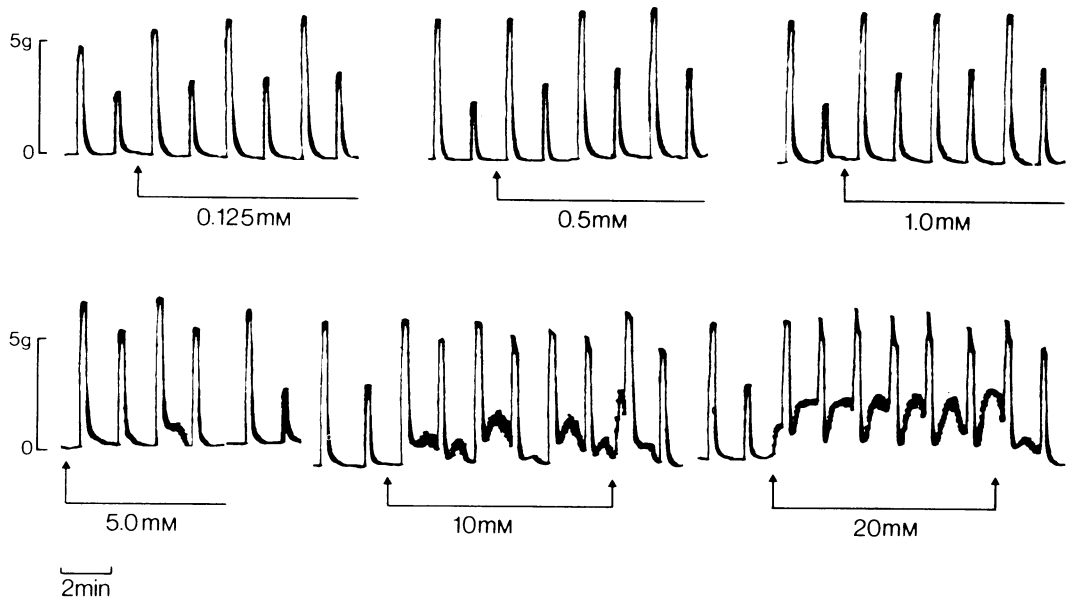

Figure 1 The effect of increasing concentrations of tetraethylammonium (TEA) on the motor response of the rat anococcygeus muscle to field stimulation. The preparation was stimulated alternatively at 20 and 2 Hz; TEA in the concentration shown below each trace was added at the arrows. TEA potentiated the motor response at both 20 and 2 Hz but the effect was greater at 2 Hz so that at a concentration of 20 mM TEA the responses at both frequencies became equal. TEA 10 and 20 mM also caused a sustained rise in muscle tone. In this and all subsequent figures the amplitude of the motor response was measured from the level of tone existing before the addition of TEA.

Method

Rat anococcygeus muscles were isolated and suspended in 20 ml baths of Krebs saline at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2 as previously described (Gillespie, 1972). Only male animals were used since in this species the muscles are thicker and stronger in the male and variability is reduced by using only one sex. The muscles were passed through platinum ring electrodes for field stimulation. Stimulation was by 1 ms pulses at supramaximal voltage and at the frequencies shown in the text. The tissue was stimulated for fixed periods of 10–20 s repeated at intervals varying in different experiments from 100 s to 3 minutes. The interval between periods of stimulation and the duration of stimulation were preset by appropriate timing devices. Drugs were added to the bath dissolved in 0.9% w/v NaCl solution (Saline) in volumes of 0.1 to 0.3 ml.

Drugs used were tetraethylammonium bromide (Koch-Light), carbachol (Sigma), guanethidine sulphate (Ciba), (−)-noradrenaline bitartrate (Koch-Light), hexamethonium bromide (Koch-Light), phentolamine mesylate (Ciba), atropine sulphate (BDH), (+)-tubocurarine (Burroughs Wellcome), 6-hydroxydopamine (Calbiochem) and tyramine (Sigma). Doses are given as moles of the base.

Results

The effect of TEA on the motor response to field stimulation

TEA in concentrations of 0.125 mM and higher potentiated the motor response to field stimulation (Figure 1) with little or no potentiation of the response to noradrenaline (Figure 4). The degree of potentiation of the response to field stimulation varied with frequency and with the concentration of TEA. Potentiation was most obvious at low frequencies and was relatively slight at the optimum frequency of stimulation of 20 Hz (Figure 1, Table 1). As a result, the effect of the drug was to make the response at 2 Hz equal to that at 20 Hz. Increasing the concentration of TEA increased the degree of potentiation up to at least a concentration of 20 mM (Table 1). Estimation of the effect of TEA concentrations greater than 5 mM was complicated by the maintained rise in

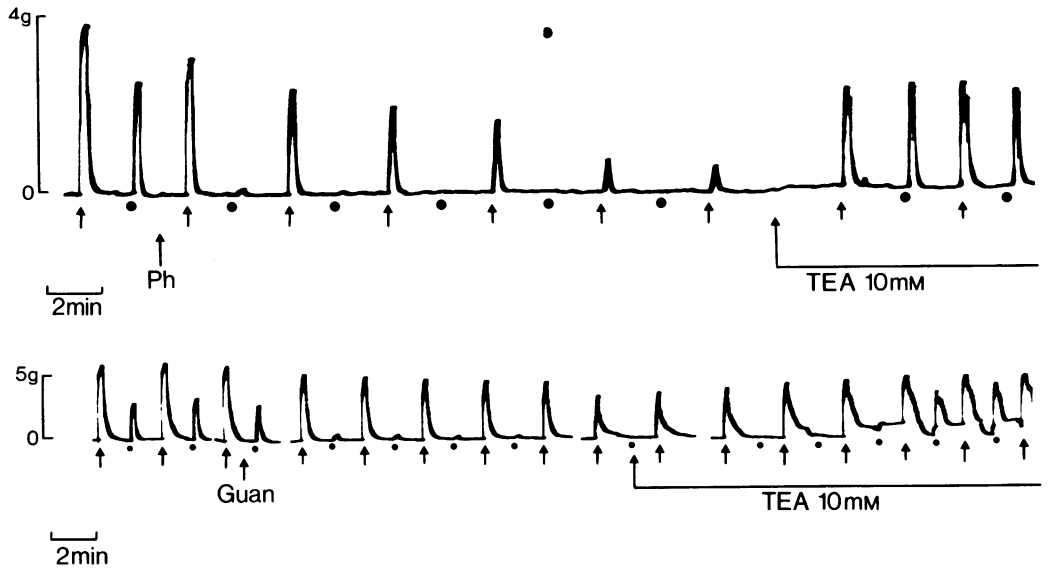

Figure 2 The effect of tetraethylammonium (TEA) 10 mM on the motor response of the rat anococcygeus to field stimulation in the presence of either phentolamine (Ph 20 μM) or guanethidine (Guan, 10 μM). The records are from two different muscles. In both the blocking agent reduces the motor response at 20 Hz (▲) and completely abolishes that at 2 Hz (●); TEA restores the response at both frequencies.

muscle tone which these concentrations induced (Figure 1) so that concentrations above 20 mM were not investigated.

The smallness of the mean potentiation at 20 Hz (see Table 1) could have been due to a failure of TEA to increase transmitter output at this frequency or to the fact that the muscle response was already near maximal and little or no increase in tension was possible whatever the effect on transmitter output. These possibilities were distinguished by the use of blocking drugs to impair transmission and produce a submaximal response at 20 Hz suitable for the detection of potentiation. Two blocking agents were separately used, phentolamine 2 μM to produce post-synaptic α-adrenoceptor blockade and guanethidine 1 μM to produce pre-synaptic blockade. The results are shown in Figure 2. TEA reversed both pre- and post-synaptic block.

Table 1 The mean potentiation or inhibition of the motor response to field stimulation produced by tetraethylammonium (TEA) at various concentrations

Concentration of TEA mM	*Potentiation or inhibition as a % of the maximum response*		
	50 Hz	*20 Hz*	*2 Hz*
0.125	2.1 ± 1.8	6.4 ± 2.5*	8.7 ± 2.7**
0.25	3.7 ± 1.9	8.3 ± 3.6*	9.6 ± 6.7***
0.5	−2.4 ± 2.9	7.7 ± 2.7*	16.7 ± 2.7***
1.0	−4.6 ± 5.5	6.2 ± 2.2**	22.3 ± 3.6***
5.0	−7.7 ± 4.3	5.2 ± 2.4*	40.8 ± 4.9***
10.0	−3.1 ± 5.9	9.2 ± 2.4***	47.6 ± 4.4***
20.0	−10.2 ± 5.0	14.1 ± 2.4***	54.4 ± 4.7***

In each experiment potentiation or inhibition was measured as the increase or decrease in the response compared with the control at that frequency and expressed as a percentage of the maximum response the tissue could produce. The amplitude of the motor response was measured from the level of tone existing before the addition of TEA. The values in the table are the means ± s.e. mean and the number of muscles tested varied from 6 to 39.

The significance of the results was assessed by Student's *t* test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

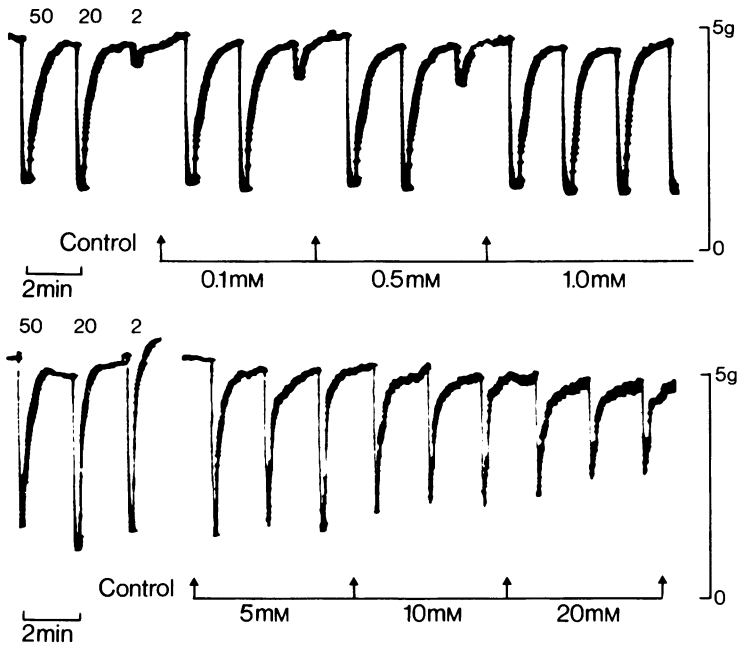

Figure 3 The effect of increasing concentrations of tetraethylammonium (TEA) on the inhibitory response of the rat anococcygeus muscle to field stimulation at 50, 20 or 2 Hz. The upper and lower records are from two different muscles; the concentrations of TEA are shown below the records. Guanethidine (30 μM) was present to block the motor response and raise tone. Concentration of TEA up to 1 mM potentiated the inhibitory response especially at 2 Hz; higher concentrations of 10 and 20 mM reduced both amplitude and duration of the response.

The effect of TEA on the inhibitory response to field stimulation

In these experiments guanethidine 30 μM was added to the bath to block the motor adrenergic nerves and simultaneously, by an indirect sympathomimetic action, to raise tone. It is on the background of this raised tone that the presence of an inhibitory innervation can be demonstrated. TEA in concentrations from 0.1 to 1 mM potentiated the inhibitory response to field stimulation and once again the effect was most marked with the lower frequencies of stimulation (Figure 3) so that the response to 2 Hz was increased so as to equal the maximum response. Higher concentrations of TEA reduced the inhibitory response both in amplitude and duration (Figure 3).

The effect of TEA on the response to tyramine

Transmitter may be liberated from an adrenergic nerve either spontaneously, in response to the arrival of an action potential, or by displacement from storage sites by indirect sympathomimetic drugs. The first two mechanisms require calcium, the last does not and it was therefore of interest to see whether transmitter liberation by an indirect sympathomimetic agent would be affected by TEA. As Figure 4 shows, TEA in concentrations which potentiate the response to nerve stimulation had no effect on the response to tyramine.

Direct effects of TEA

Concentrations of TEA greater than 5 mM caused a maintained rise in tone in the preparation (Figure 5). The possibility that this was due to an increased spontaneous release of noradrenaline was investigated by studying the effect of phentolamine. Part, but not all, of the rise in tone was abolished by phentolamine 10 μM confirming that part of the effect was due to the release of noradrenline but that there was in addition a direct action on the smooth muscle. This was further investigated by treating four rats with 6-hydroxydopamine (6-OHDA) to destroy the adrenergic nerve terminals and then examining the effect of TEA. Examination of these muscles with the Falck fluorescence technique showed no fluorescent adrenergic nerves and the muscles, though responding

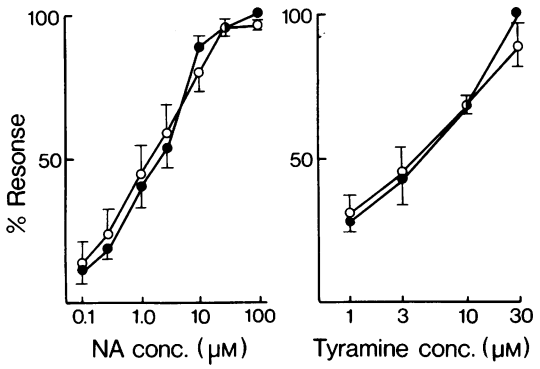

Figure 4 Dose-response curves of the rat anococcygeus muscle to noradrenaline (NA) and tyramine in the presence (O) and absence (●) of tetraethylammonium (TEA) 1 mM. In each experiment responses are expressed as a percentage of the maximum response in that experiment. Each point is the mean of observations and the vertical lines represent s.e. mean. TEA had no significant effect on the response to either agonist.

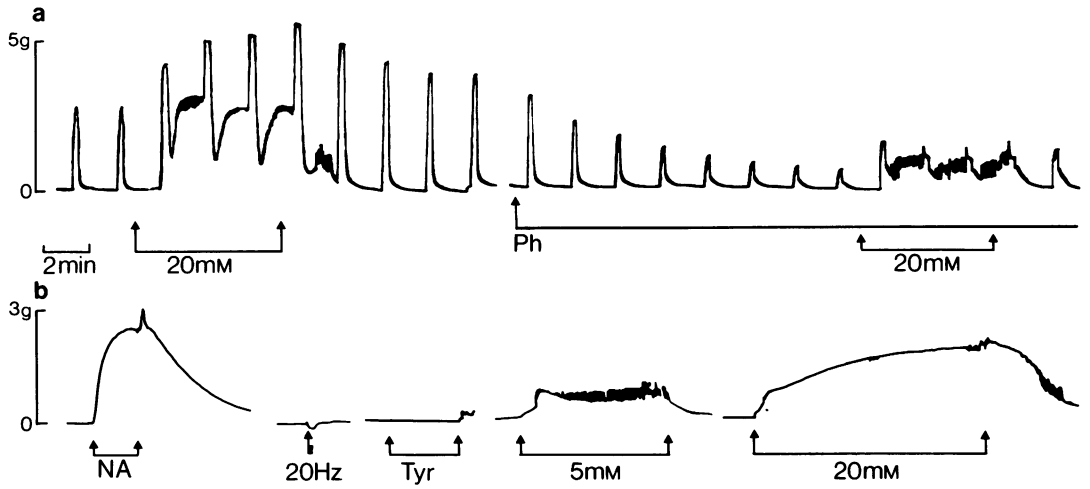

Figure 5 (a) The effects of tetraethylammonium (TEA) 20 mM in potentiating the motor response to field stimulation at 20 Hz and also causing a considerable rise in tone in a rat anococcygeus muscle. Phentolamine (Ph 10 μM) almost blocked the response to field stimulation and greatly reduced but did not abolish the rise in tone produced by TEA. (b) Records from a muscle from a rat previously treated with 6-hydroxydopamine to destroy the adrenergic nerves. The muscle was still sensitive to noradrenaline (NA, 3 μM) but neither field stimulation nor tyramine (Tyr, 10 μM) caused contraction. TEA 5 mM and 20 mM still produced a rise in tone.

to noradrenaline, gave no motor response to either field stimulation or the indirect sympathomimetic agent tyramine; nonetheless, TEA 5 and 20 mM produced a graded contraction (Figure 5).

Atropine-like action of TEA

In the experiments described in the previous paragraph the continuing presence of an intact inhibitory innervation in tissues from 6-OHDA-treated animals was checked by raising tone with carbachol since guanethidine was ineffective in these circumstances. It was found that TEA abolished this carbachol-induced tone. This was further investigated in muscles from normal animals and, as Figure 6 shows, TEA was able to abolish the effects of carbachol in a dose-related fashion. Dose-response curves of rise in tone for carbachol in the presence of

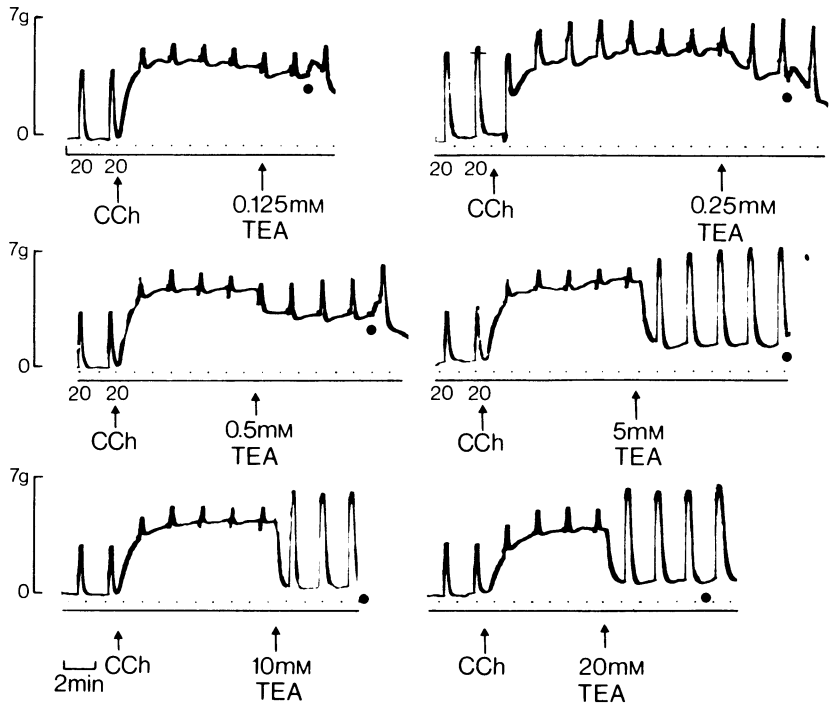

Figure 6 The effect on the rat anococcygeus muscle of increasing concentrations of tetraethylammonium (TEA) on the increase in muscle tone induced by carbachol (CCh, 5 μM). Field stimulation at 20 Hz was applied at 2 min intervals. The lowest concentration of TEA, 0.125 M, slightly lowered tone, an effect which increased with concentration up to a maximum at 10 mM. The response to field stimulation was also potentiated. At the black dot the bath was washed.

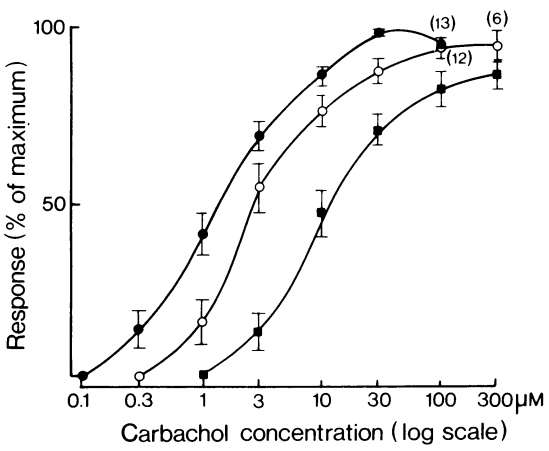

Figure 7 Mean dose-response curves of rise in tone to carbachol alone (●), and in the presence of tetraethylammonium (TEA) 0.25 mM (○) and 1.0 mM (■). Responses are expressed as a percentage of the maximum response to carbachol in the control. Number of muscles was 15 for all concentrations unless otherwise indicated. The vertical lines represent s.e. mean.

0.25 and 1.0 mM TEA are illustrated in Figure 7 and shows a parallel shift of the dose response curves with no change in the maximum response, consistent with a competitive blocking effect.

The mode of action of TEA

Adrenergic nerves are known to possess a variety of pre-synaptic receptors capable of feedback inhibition of transmitter release. Prominent among these are nicotinic and muscarinic acetylcholine receptors and the α-receptor for noradrenaline itself. We, therefore, investigated whether block of nicotinic receptors by hexamethonium or curare, of muscarinic receptors by atropine or of α-receptors by phentolamine would abolish the ability of TEA to cause potentiation. The results are shown in Figure 8. None of these blocking agents prevented either the potentiation of the response to motor nerve stimulation or the effect of TEA in raising muscle tone.

Discussion

TEA produces a variety of effects in the anococcygeus muscle. The potentiation of the response to both

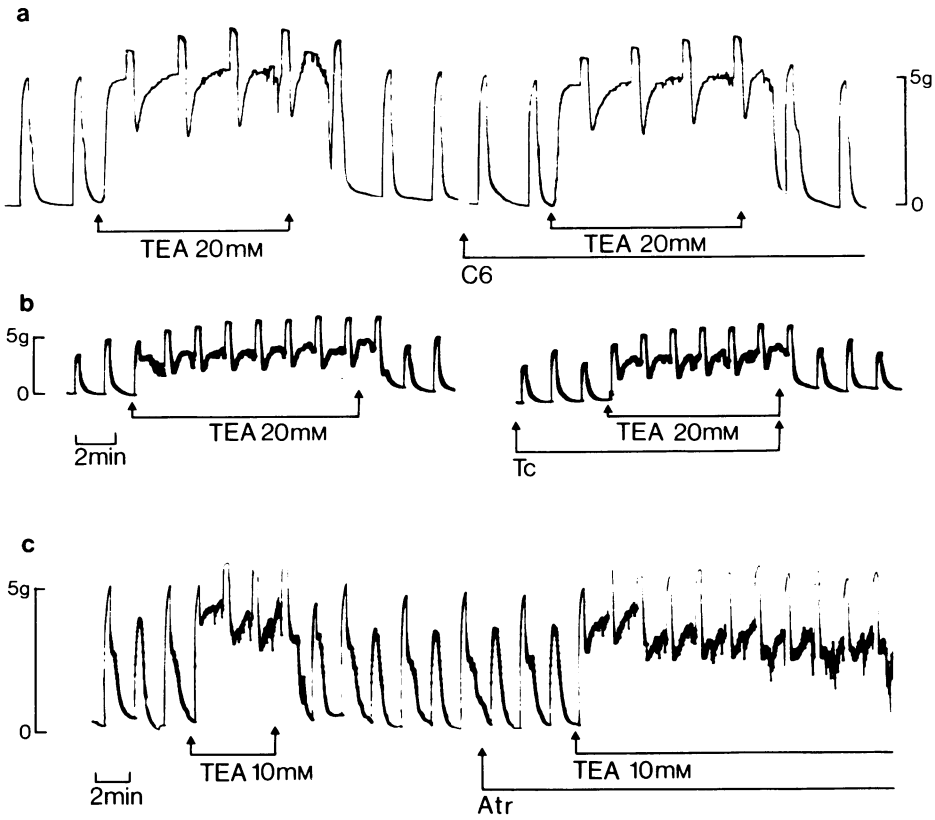

Figure 8 The responses of three different anococcygeus muscles to field stimulation and to tetraethylammonium (TEA) 20 mM and the effect on these of (a) hexamethonium (C6, 10 μM). (b) (+)-tubocurarine (Tc, 3 μM) and (c) atropine (Atr, 10 μM). The muscle in (a) was stimulated intermittently at 20 Hz. The muscles in (b) and (c) were stimulated at 2 and 20 Hz alternately. Neither the potentiation of the response to field stimulation nor the rise in muscle tone was affected by these blocking drugs.

motor and inhibitor nerve stimulation is almost certainly due to an increased liberation of their respective transmitters as a result of prolongation and augmentation of the nerve action potential and a consequent increased entry of calcium. Similar concentrations of TEA have been shown to prolong the action potential in muscle (Fatt, & Katz, 1953; Hagiwara & Watanabe, 1955) and nerve (Schmidt & Stämpfli, 1966; Taski & Hagiwara, 1957) and to increase transmitter liberation from cholinergic and adrenergic nerves (see earlier references). The lack of potentiation of the response to tyramine is in agreement with this explanation since calcium is not involved in the release of transmitter by this drug.

The phentolamine-sensitive rise in tone with concentrations of TEA above 5 mM is probably due to a direct liberation of noradrenaline by TEA. Such concentrations are likely to cause depolarization and instability of the terminal nerve membrane and this instability alone would increase the spontaneous liberation of transmitter. This instability might be sufficient to induce spontaneous action discharge but previous work suggests that higher concentrations of TEA are normally necessary for this (Beaulieu & Frank, 1967a). The phentolamine-resistant component of the stimulant action of TEA suggests an additional, direct, action on the smooth muscle. TEA has been reported to stimulate other smooth muscles; the guinea-pig ileum (Collins, 1948; Beleslin & Rakič, 1969, the guinea-pig stomach (Ito, Kuriyama & Sakamoto, 1970) and the bovine trachea (Kirkpatrick, 1975. The probable mechanism suggested by Kirkpatrick (1975) and Ito *et al.* (1970) is that TEA abolishes the rectifying properties of the muscle membrane by reducing the normally high potassium permeability. The result is some depolarization but more dramatically an instability of the membrane so that, for example, muscles previously incapable of generating spike potentials can now do so. Consistent with such an explanation is the finding that the

anococcygeus normally has a stable membrane potential, rarely generates spike potentials and these never show overshoot. In the presence of TEA the muscle is depolarized and generates large spike potentials with several millivolts of overshoot (Creed, Gillespie & Muir, 1975). It is only to be expected that in such circumstances the muscle would develop spontaneous tone.

We found little or no potentiation of the response to noradrenaline. In the cat spleen (Thoenen *et al.*, 1967) and the rabbit pulmonary artery (Bevan, 1963), TEA in addition to increasing transmitter release caused a two- to six-fold increase in sensitivity to noradrenaline. The lack of potentiation in the experiments described here is all the more surprising in view of the direct depolarizing effect of TEA on the muscle and the appearance of spikes with overshoot; effects which would be expected to be synergistic with any other depolarizing agonist. However, other workers have reported a failure of TEA to potentiate some agonists while markedly augmenting others on the same tissue (Collins, 1948).

TEA reversed the blocking action of both phentolamine and guanethidine; a similar reversal of guanethidine block by TEA has been reported in the guinea-pig vas deferens (Maanen & Werty, 1966). An increased release of transmitter with more effective competition with phentolamine would be a sufficient explanation of the reversal of post-synaptic block by TEA. The mechanism of guanethidine reversal is less obvious. It may be the augmented nerve action potential allows entry of calcium over a longer period and the resulting increase in neurosecretory coupling efficiency together with the post-synaptic hypersensitivity characteristic of guanethidine is sufficient explanation. A more interesting alternative is that the antagonism is more specifically related to the mode of action of guanethidine and involves an increase in the mobility of calcium within the membrane as suggested by Beaulieu & Frank (1967b). A similar hypothesis might account for the reduction by higher concentrations of TEA of the inhibitory response to field stimulation. Beaulieu & Frank (1967b) have called attention to the paradoxical effects of TEA which produces membrane instability in muscle and nerve in a way reminiscent of low calcium, yet appears able to potentiate the effects of calcium in, for example, the release of neurotransmitter or the restoration of potassium contractures previously abolished by low calcium. Beaulieu & Frank (1976b) suggest both phenomena are the result of a single action of TEA, the displacement of calcium from binding sites in the membrane. This causes membrane instability but at the same time the displaced calcium is made available within the cytoplasm for excitation-contraction or excitation-secretion coupling. If this explanation is correct then the mode of action of the inhibitory transmitter may be to increase calcium binding to the membrane, and TEA may be a relatively specific antagonist.

One other action of TEA was to inhibit the response to carbachol in a competitive fashion suggesting an atropine-like action on muscarinic receptors. Among the earliest work on TEA are reports of just such an effect (Schüller, 1920; Külz, 1922). However, these experiments were on amphibian tissue (frog rectum, frog heart) and a similar atropine-like action on the blood pressure and pupil of the cat could not be confirmed (Hunt & Renshaw, 1925). We have been unable to find convincing reference in the literature of an atropine-like action in the mammal. It may be that pre-existing parasympathetic tone potentiated by TEA has obscured the post-synaptic blocking effect just as at the skeletal neuromuscular junction the pre-synaptic, anti-curare action can obscure the curare-like block by TEA.

Finally, the site of action of TEA should be considered. TEA penetrates cells only with difficulty and when introduced by iontophoresis its effects are slow to develop and difficult to reverse (Kleinhaus & Prichard, 1975). All of the effects reported in this paper were rapid in onset and easily and completely reversed by washing, suggesting the effects were exerted on the outside of the various cell membranes.

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