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EFFECTS OF TETRAETHYLAMMONIUM CHLORIDE ON THE MEMBRANE ACTIVITY OF GUINEA-PIG STOMACH SMOOTH MUSCLE

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SUMMARY

1. The effects of tetraethylammonium (TEA) on the membrane activity of the antral circular muscle of the guinea-pig stomach were investigated with micro-electrode and double sucrose gap methods.

2. In a concentration of $1–1{\cdot}5 \times 10^{-3}$ g/ml. (3–5 mM), the membrane potential was not influenced; the membrane resistance measured by inward current pulses remained the same but the rectifying property of the membrane was suppressed.

3. TEA ($1–1{\cdot}5 \times 10^{-3}$ g/ml.) enhanced the spike amplitude markedly even from fibres which generated graded responses.

4. TEA ($1–1{\cdot}5 \times 10^{-3}$ g/ml.) did not increase the maximum rate of rise of the spike but decreased the maximum rate of fall of the spike markedly.

5. In Na-free (Tris or sucrose) solution, in K-deficient and excess-K solutions, TEA ($1–1{\cdot}5 \times 10^{-3}$ g/ml.) suppressed the rectifying property of the membrane and enhanced the spike amplitude.

6. Atropine (10^{-6} g/ml.) had no effect on the enhancement of the spike amplitude produced by TEA.

7. The minimum concentration of Ca ions required for the effect of TEA on the spike amplitude was one fifth of the normal concentration. TEA also enhanced the spike amplitude in Sr–Krebs.

8. The possible role of TEA on the membrane activity is considered to be due to suppression of the K conductance when the membrane is depolarized. Alternative possible roles of TEA on the spike amplitude are also discussed.

INTRODUCTION

Choline and tetraethyl ammonium (TEA) are often used as a substitute for the Na ion to investigate the membrane property of the many excitable tissues.

In the squid giant axon, the injection of TEA increased Na conductance, and after depolarization Na conductance did not decline completely. This incomplete reversal of Na conductance with the delayed elevation of K conductance produced a post-spike plateau (Tasaki & Hagiwara, 1957). Recently, Nakajima (1966) reported that in the supramedullary cells of puffer fish, TEA reduced the K conductance in a manner which is independent of the membrane potential and unaccompanied by a shift of the inactivation curve of K conductance along the voltage axis, i.e. TEA acted on the slow phase of K activation rather than on the rapid phase of the K activation. Thus the effect of TEA on the K conductance of the supramedullary cells was similar to the action of alcohol or local anaesthetics on the squid giant axon.

In crustacean muscle, Fatt & Katz (1953) observed that Na-free TEA solution enlarged the spike amplitude and prolonged the spike duration. In skeletal muscle, Hagiwara & Watanabe (1955) observed that TEA prevented a rise in K conductance which normally hastens the end of the spike. They also observed that the spike amplitude recorded in the TEA solution was greater than that recorded in choline solution.

In the circular muscle layer of the guinea-pig stomach, some of the fibres generated spikes during the slow potential changes (slow wave or basic electrical rhythm); however the amplitude of these spikes was low, and it was difficult to record the all-or-none type of spike with full amplitude.

Treatment with low concentrations of TEA (2–5 mM) in Krebs solution enlarged the spike amplitude, and the all-or-none type of the spike could then be recorded spontaneously during the slow potential changes (T. Osa, personal communication).

The present experiment was carried out to investigate the effect of TEA on the spike generating mechanism of the guinea-pig stomach circular muscle. Micro-electrode and double sucrose gap methods were used to measure the absolute and relative changes of the membrane properties.

METHODS

Guinea-pigs weighing 250–300 g were stunned and bled. The stomach was dissected from the abdomen, and the connective tissue was removed carefully in Krebs solution at room temperature. The muscle layers were separated from the mucous membrane. The double sucrose gap and micro-electrode methods were employed to measure the effects of tetraethyl ammonium chloride (TEA–Cl) on the membrane activity of the antral smooth muscle. The double sucrose gap method employed was the same as that described by Kuriyama & Tomita (1970), i.e. stripes of the circular muscle of the antrum were dissected about 1 mm wide. A small portion (less than 2 mm) in the central part of the tissue was exposed to the test solution and the remaining parts on both sides were perfused by isotonic sucrose solution. Stimulating current pulses were applied to one side immersed in sucrose

solution through a resistor of 50 MΩ. The changes in the membrane potential in the central part were recorded across the sucrose gap on the other side of the tissue. The changes of the isometric tension in the central part were recorded with a strain gauge attached to the end of the tissue strip on the stimulating slide of the gap.

The micro-electrode method was also applied to measure the properties of the resting and active membrane. The arrangements of stimulating and recording electrodes were the same as those described by Tomita (1966), Abe & Tomita (1968) and Kuriyama, Osa & Tasaki (1970). Electrical stimulation to the tissue was applied extracellularly using stimulating partitions and the current intensity was measured by the recording electrodes inserted into the stimulating chamber as described by Abe & Tomita (1968).

A modified Krebs solution of the following composition was used (mM); Na^+ 137·4; K^+ 5·9; Mg^{2+} 1·2; Ca^{2+} 2·5; Cl^- 134·0; HCO_3^- 15·5; $H_2PO_4^-$ 1·2 and glucose 11·5; equilibrated with 97 % O_2 and 3 % CO_2.

When a low concentration of TEA was added in Krebs solution, the concentration of TEA was indicated as g/ml. and when Na ion was replaced by TEA, the concentration of TEA was indicated as mM.

The pHs of the Na-free sucrose and Na-free TEA Krebs solutions were adjusted to 7·3 with Tris (hydroxymethylaminoethane) buffer.

In the text, NaCl-free Krebs solution means that NaCl is replaced with other substances but that the Na ion concentration is still 15·5 mM in the form of $NaHCO_3$. Na-free solution means all the Na ion was replaced.

RESULTS

Effects of tetraethylammonium chloride (TEA–Cl) on the membrane activity

The resting membrane potential of the antral circular muscle of the guinea-pig stomach was −58·4 mV (S.D. = ±2·5, n = 30) in normal Krebs. A twice osmolar solution (95 g sucrose added to 1000 ml. Krebs) hyperpolarized the membrane to −64·3 mV (S.D. = ±2·1, n = 30). The contraction of the tissue elicited by the spike was abolished in this solution, and insertion of the electrode into the cell was facilitated (Tomita 1966; Kuriyama *et al.* 1970).

Extracellularly applied inward current pulse produced an electrotonic potential. The space constant measured from the amplitude of the electrotonic potentials at the various distances from the stimulating electrode was 1·8 mm (n = 3), and the time constant of the electrotonic potential (the time required to reach 85 % of the steady measurements) of the membrane predicted from the cable equations was 192 msec (n = 5). These results confirmed the observations made on the same tissue by Kuriyama *et al.* (1970).

An outward current pulse applied to the cell also evoked an electrotonic potential with a lower amplitude than that recorded by the inward current, i.e. a rectifying property of the membrane could be observed. A spike could be elicited by application of a strong outward current pulse

but not in all fibres. However, its amplitude was small and irregular. After treatment with TEA ($1{\cdot}1 \times 10^{-3}$ g/ml.), the spike amplitude was enlarged and an overshoot potential could be recorded. The amplitude of the spike in Krebs solution was, in many fibres, not more than 30 mV, but when TEA was added in Krebs, the spike amplitude exceeded 60 mV.

Fig. 1 shows the relationships between current intensities and amplitudes of the electrotonic potential before and after treatment with TEA

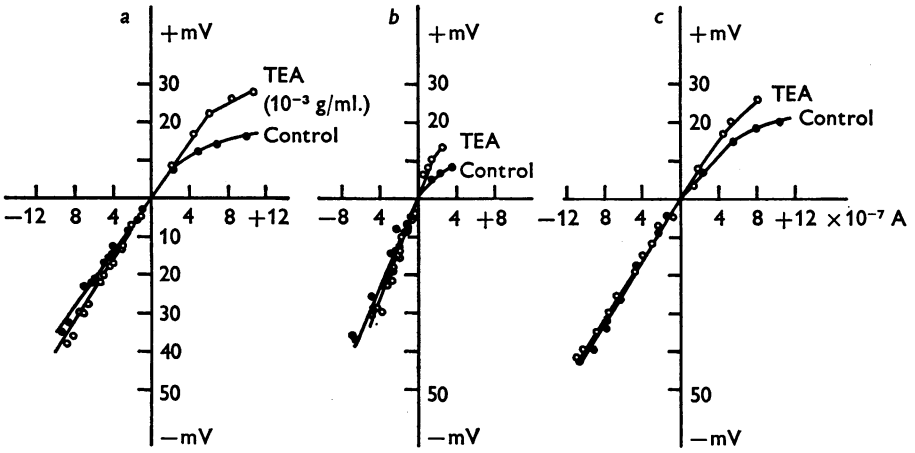

Fig. 1. Current–voltage relationship observed before and after treatment with TEA (10^{-3} g/ml.); three different experiments are illustrated. The potential changes are recorded at 0·5 mm distance from the stimulating electrode in *a*, 0·3 mm in *b* and 0·7 mm in *c*. The micro-electrode was inserted into the same muscle fibre throughout the experiment. The effects of TEA are observed after 10 min of perfusion.

(10^{-3} g/ml.) from the three different fibres. The electrotonic potentials were recorded at three different distances from the stimulating partition (0·5 mm in *a*, 0·3 mm in *b* and 0·7 mm in *c*). The micro-electrode was inserted into the same fibre throughout the experiments. The membrane resistances measured by the applications of the inward current pulses showed nearly the same value before and after treatment with TEA. However, the suppression of the rectifying property of the membrane was observed after treatment with TEA. In the fibre of *a*, the abortive spike was elicited, in *b* the full size of the spike, and in *c*, the spike was not recorded in Krebs solution. However, after treatment with TEA (10^{-3} g/ml., the full size of the spike (more than 50 mV) could be recorded. Threshold to trigger the spike became high after treatment with TEA.

Fig. 2 shows the effects of TEA ($1{\cdot}1 \times 10^{-3}$ g/ml.) on the membrane activity of the antral smooth muscle. The records were taken from the same muscle cell throughout the experiment. The membrane resistance

measured from the amplitude of the electrotonic potentials produced by extracellularly applied inward current showed nearly the same value before and after treatment with TEA. A remarkable change was, however, observed on the spike amplitude.

Fig. 3 also shows the effects of TEA (10^{-3} g/ml.) on the membrane activity recorded by micro-electrode technique measured after 20 min of perfusion. The micro-electrode was inserted into the same muscle fibre

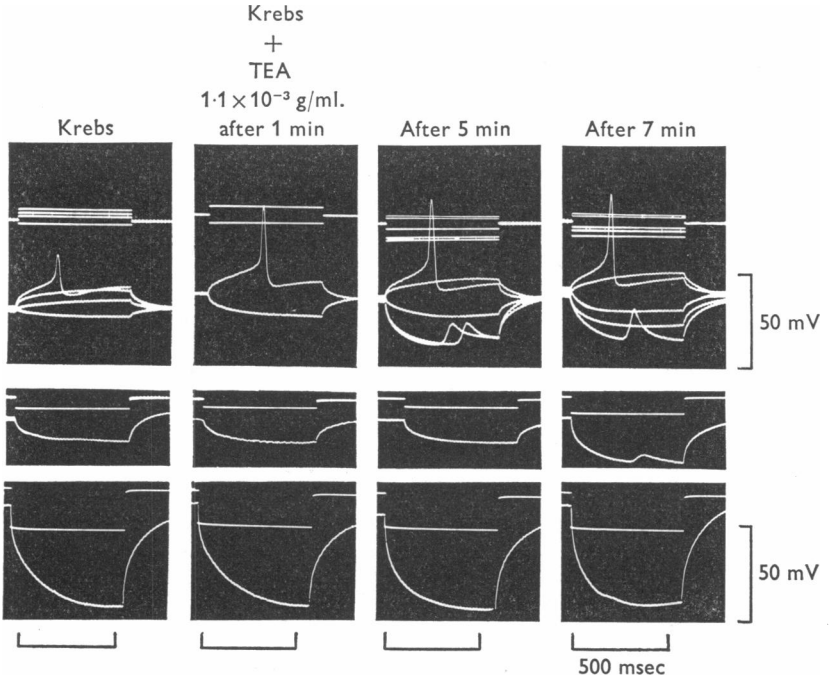

Fig. 2. Effects of TEA ($1{\cdot}1 \times 10^{-3}$ g/ml.) on the membrane activity of the antral smooth muscle. The records are taken from the same muscle cell throughout the experiment. The recording electrode is placed at 0·2 mm distance from the stimulating plate. Note that the small depolarizing potentials of the membrane could be recorded by applications of the middle grade of the inward current pulses to the tissue. Presumably the spike elicited by the distant side of the stimulating electrode may be picked up by the recording electrode electrotonically.

throughout the experiment. The electrical threshold to elicit a spike, the spike amplitude and the maximum rate of rise of the spike recorded by a differential integration method are illustrated simultaneously. The applied current intensity was fixed at a level just above the threshold for spike production on the break of the depolarizing pulse. TEA enhanced the spike amplitude but the maximum rate of rise of the spike was not changed. On the other hand, the maximum rate of fall of the spike was lowered

markedly. The mean overshoot potential was $8 \pm 1{\cdot}4$ mV ($n = 20$), and the mean values of the maximum rate of rise of the spike measured before the treatment was 3·3 V/sec and after the treatment was 3·4 V/sec ($n = 20$). In spite of no significant change in the rate of rise of the spike, the rate of fall was lowered markedly from 3·2 V/sec to 1·5 V/sec ($n = 20$). The duration of the spike at 50 % peak height was $32 \pm 3{\cdot}1$ msec ($n = 20$) and it was prolonged by TEA ($67 \pm 4{\cdot}6$ msec, $n = 20$).

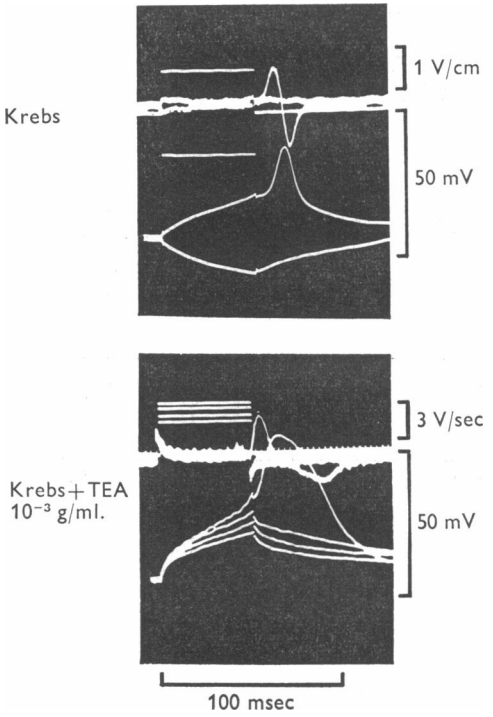

Fig. 3. Effects of TEA (10^{-3} g/ml.) on the membrane activity of the antral smooth muscle. The micro-electrode was inserted into the same muscle fibre throughout the experiments. The applied current, differential integration of the rates of rise and fall of the spike and the potential changes of the membrane were recorded in the photographs. The recording electrode was placed at 0·3 mm distance from the stimulating plate. The effect of TEA was observed after 20 min of perfusion.

A similar experiment was carried out using the double sucrose gap method. The membrane resistance measured from the electrotonic potential produced by inward current pulses was not influenced by treatment with TEA (10^{-3} g/ml.). However, the rectifying property of the membrane was slightly reduced, and the amplitude of the spike increased. Fig. 4 shows the effects of TEA (10^{-3} g/ml.) on the electrical and mechanical

threshold, and on the membrane activity in the antral smooth muscle recorded by the double sucrose gap method.

By this method, it was difficult to analyse the factors which caused the augmentation of spike amplitude, since the spike recorded by the gap method was a compound one. Therefore, differentiation between an increased number of the cells contributing to the generation of the observed

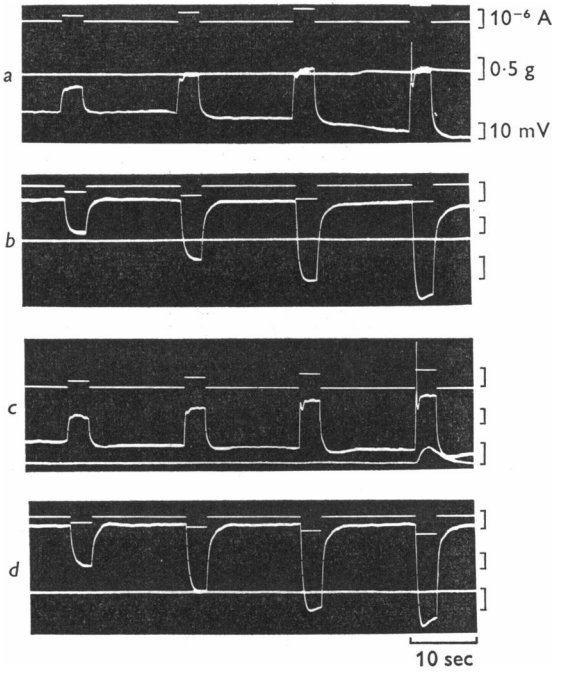

Fig. 4. Effects of TEA (10^{-3} g/ml.) on the membrane activity of the antral smooth muscle recorded by the double sucrose gap method. *a* and *b*: control; *c* and *d*: after 15–20 min of perfusion in TEA–Krebs. Pulse duration is 3 sec. Current intensities are varied from 2×10^{-7} to 10^{-6} A. Note: beams of tension recording were artificially shifted and therefore the levels did not indicate the absolute value of the resting tension.

spike and the augmentation of the spike amplitude of the individual muscle cells cannot be made. However, the result obtained by the microelectrode method might indicate that the large amplitude of the spike is mainly due to augmentation of the individual spike amplitudes.

The amplitude of the phasic contraction was increased in proportion to the increase in the amplitude of the spike; as a consequence the amplitude of the development tension was much larger with TEA than that recorded in Krebs solution alone.

Effects of TEA on the membrane activity of smooth muscle in Na-free or Na-deficient solution

The effects of TEA on NaCl-free solution (substituted by sucrose or $Tris^+$) on the electrical and mechanical activity of the stomach smooth muscle have been studied after 30 min of perfusion. When NaCl was replaced with sucrose, the membrane resistance was increased; on the other hand, it was reduced with Tris–Cl substitution. Both solutions hyperpolarized the membrane (Y. Sakamoto & H. Kuriyama, to be published).

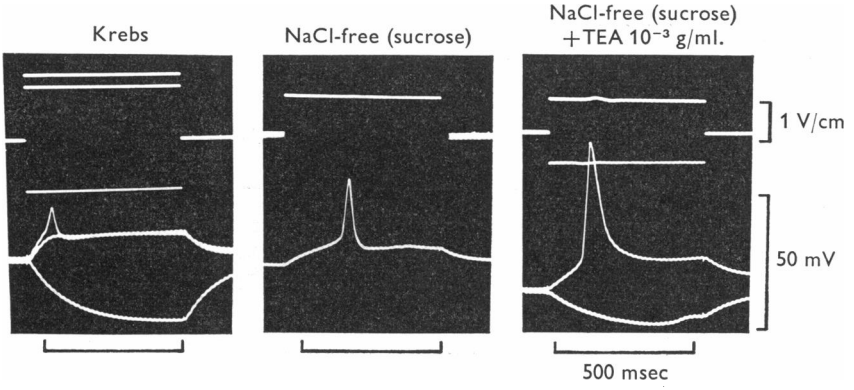

Fig. 5. Effects of TEA (10^{-3} g/ml.) in NaCl-free Krebs solution on the membrane activity of the antral smooth muscle recorded by the microelectrode method. NaCl was replaced by sucrose. The recording microelectrode was placed at 0·3 mm distance from the stimulating plate. The records were taken after 30 min of perfusion.

Fig. 5 shows the effect of NaCl-free (substituted by sucrose) solution on the membrane activity of the stomach muscle. In the Na-deficient solution (NaCl was substituted by sucrose but the concentration of Na ions was kept at 15·5 mM, as $NaHCO_3$) the membrane was slightly hyperpolarized ($-61{\cdot}8 \pm 2{\cdot}4$ mV, $n = 10$). The spike amplitude was increased by treatment with TEA ($1{\cdot}4 \times 10^{-3}$ g/ml.). When spikes which were more than 50 mV in amplitude in Krebs solution were chosen, the spike amplitude increased from $54{\cdot}1 \pm 3{\cdot}1$ mV ($n = 6$) to $63{\cdot}8 \pm 2{\cdot}8$ mV ($n = 6$), and the maximum rate of rise of the spike was slightly enhanced from $3{\cdot}2 \pm 0{\cdot}4$ V/sec ($n = 6$) to $3{\cdot}9 \pm 0{\cdot}3$ V/sec ($n = 6$). However, when spikes were chosen which were less than 30 mV in amplitude, the mean amplitude of the spike without TEA was $26{\cdot}0 \pm 3{\cdot}4$ mV ($n = 20$), and it was enhanced to $42{\cdot}8 \pm 2{\cdot}6$ mV with TEA ($n = 20$). The rate of rise of the spike in the Na-deficient solution alone was $2{\cdot}5 \pm 0{\cdot}4$ V/sec ($n = 20$), and it was enhanced to $3{\cdot}5 \pm 0{\cdot}3$ V/sec ($n = 20$) by treatment with TEA ($1{\cdot}4 \times 10^{-3}$ g/ml.).

The spike amplitude was enhanced in the Na-free solution; with addition of TEA (10^{-3} g/ml.), the spike amplitude was further enlarged. In three preparations, the membrane potential increased from $-54{\cdot}2 \pm 4{\cdot}2$ mV ($n = 20$) to $-59{\cdot}1 \pm 3{\cdot}4$ mV ($n = 20$) in the Na-free solution. Treatment with TEA, the spike amplitude increased from $23 \pm 4{\cdot}5$ mV ($n = 10$) to $58 \pm 3{\cdot}1$ mV ($n = 10$). The maximum rates of rise and fall of the spike were changed from $2{\cdot}8 \pm 0{\cdot}8$ V/sec and $2{\cdot}0 \pm 0{\cdot}7$ V/sec to $3{\cdot}2 \pm 0{\cdot}9$ V/sec and $1{\cdot}2 \pm 0{\cdot}6$ V/sec, $n = 10$, respectively.

Effects of high concentrations of TEA on the membrane activity of the smooth muscle

The Na ion in Krebs solution was replaced by TEA–Cl in steps, and the pH was adjusted to 7·3 with Tris. Fig. 6 shows the effects of various concentrations of TEA substituted isotonically for the Na ion on the membrane activity measured by double sucrose gap method (5, 62 and 122 mM respectively). At a concentration of 5 mM, spike amplitude was increased. When TEA concentration was increased up to 62 and 122 mM respectively, the spike amplitude was further increased.

Fig. 7 shows the relationship between the spike amplitudes and the external concentrations of TEA, $[TEA]_o$, in the Na-free (sucrose) solution. The solutions were prepared as isotonic solutions. The maximum increase in spike amplitude produced by a tenfold change in the external concentration of TEA by sucrose gap method was 26 mV ($n = 6$) calculated from the slope of the graph extrapolated at the concentrations between 2·2 and 8 mM-TEA, and was 14 mV ($n = 6$) calculated from the concentrations between 8 and 62 mM-TEA.

To investigate the effects of TEA as a cholinergic substance on the membrane activity of the antral smooth muscle, atropine (5×10^{-6} g/ml.) was added in the Na-free TEA Krebs solution. Spikes were elicited by outward current pulses and the amplitude often exceeded 60 mV as observed in the Na-free TEA solution. Therefore, the effects of Na-free TEA on the membrane activity were similar with or without atropine. The above results indicate that the enhancement of the spike amplitude in TEA solution was not due to acetylcholine-like action of TEA on the smooth muscle membrane.

Effects of TEA on the membrane activity of the stomach smooth muscle in relation to divalent cations

These experiments were carried out with the double sucrose gap method. When the external concentration of Ca ions was reduced to zero or treated with EDTA (2 mM) after the Na ion had been completely replaced with TEA, the membrane resistance was reduced and no spike and phasic

contraction could be elicited. After readministration of the Ca ion, the membrane resistance was again slightly increased and spikes with an enlarged amplitude of more than 70 mV were elicited. This effect of the Ca ion could not be reproduced by the Mg ion.

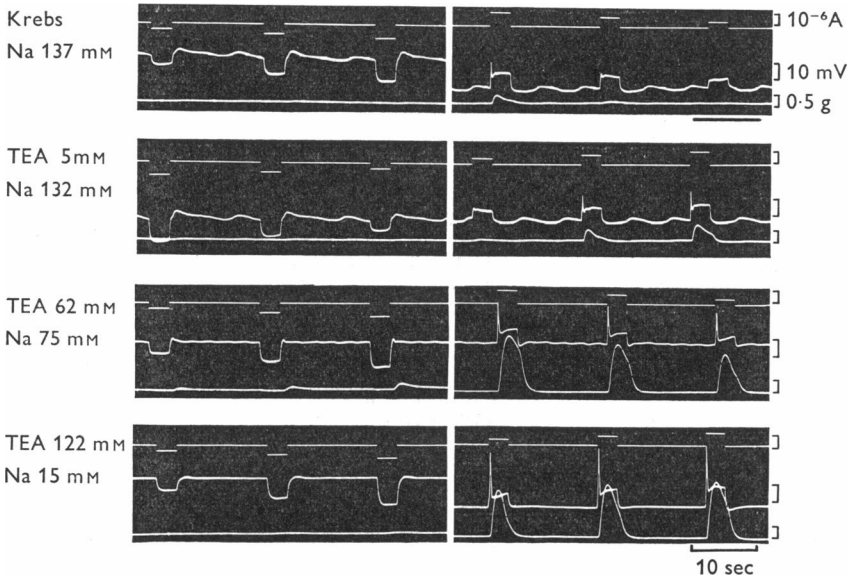

Fig. 6. Effects of various concentrations of TEA on the membrane activity observed by the double sucrose gap method. Na ion was replaced by TEA in various steps as illustrated in the Figure. Records were taken after 15–20 min of perfusion.

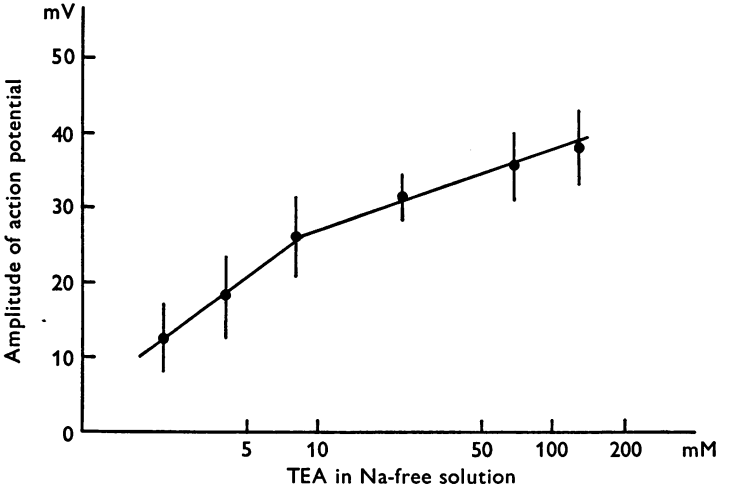

Fig. 7. Relationship between the spike amplitudes and $[TEA]_o$ in the Na-free (sucrose) solution. Horizontal bars indicate twice the S.D. ($n = 10 \sim 25$).

When the Ca ion was completely removed and a similar concentration of Mg ions was added to the solution (the total concentration of Mg ions was 4 mM), the membrane resistance was slightly reduced and spike could not be evoked in the Ca-free Mg–TEA solution. When one fifth of the Ca ion concentration in Krebs was added to the solution (0·6 mM), a spike could be elicited.

The Sr ion could be substituted for the Ca ion to elicit spikes in TEA solution. Fig. 8 shows the effects of Sr–TEA Krebs on the membrane activity of smooth muscle recorded by the gap method. When Krebs

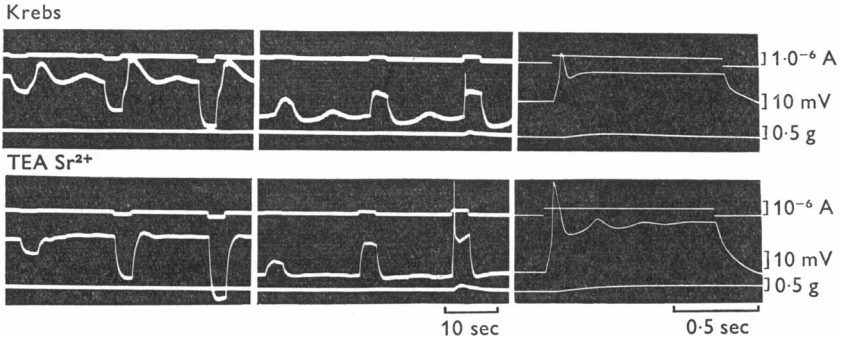

Fig. 8. Effects of the Sr ion in NaCl-free TEA Krebs on the membrane activity of the antral smooth muscle recorded by the double sucrose gap method. A solution of Sr ions (2·8 mM) was added instead of 2·8 mM-Ca. Records were taken after 20 min of perfusion in the test solution.

solution was replaced with Sr–TEA Krebs solution, the slow potential changes disappeared and the membrane resistance increased. The spike amplitude enlarged remarkably and contraction was also enhanced compared to that in the Sr–Krebs.

In the four different preparations, the membrane resistance in the Sr–TEA Krebs was increased to 1·4 times the control value and the spike amplitude was enhanced to 2·3 times the control value.

Effects of TEA on the membrane activity of the stomach muscle with various K concentrations

Table 1 shows the changes of the membrane potential and relative membrane resistance at various concentrations of K ions before and after treatment with TEA (10^{-3} g/ml.).

Low concentration of K ions. The effects of low concentrations of K ions on the membrane activity of stomach circular muscle was observed with the micro-electrode method. A fifth of the normal concentration of K ions slightly hyperpolarized from $-58{\cdot}4 \pm 3{\cdot}2$ to $-63{\cdot}1 \pm 2{\cdot}4$ mV the membrane and increased the membrane resistance (1·1 times the control value), and

evoked spike activity was largely suppressed. However, after treatment with TEA (10^{-3} g/ml.), the membrane potential and the membrane resistance showed nearly the same values with those measured before the treatment ($-61{\cdot}1 \pm 3{\cdot}0$ mV and 1·1 times the control). The rectifying property of the membrane had, however, almost disappeared. The membrane activity was restored by TEA, and spikes were produced with an amplitude of more than 50 mV. Fig. 9 shows the effect of TEA ($1{\cdot}4 \times 10^{-3}$ g/ml.) in the K-deficient (1·2 mM) solution. The records illustrated in the Figure were

TABLE 1. Changes of the membrane potential and relative membrane resistance (control = 1) at various concentrations of K ion before and after treatment with TEA ($1{\cdot}1 \times 10^{-3}$ g/ml.). The membrane potential was measured by the micro-electrode, and the relative change of the membrane resistance was measured by the sucrose gap method

	Control		TEA 10^{-3} g/ml.	
Concentration of K ion (mM)	Membrane potential (mV)	Relative membrane resistance	Membrane potential (mV)	Relative membrane resistance
0·58	$64{\cdot}5 \pm 3{\cdot}5$	1·3	$62{\cdot}5 \pm 1{\cdot}8$	1·4
	$n = 10$	$n = 3$	$n = 20$	$n = 3$
1·2	$63{\cdot}1 \pm 2{\cdot}4$	1·1	$61{\cdot}1 \pm 3{\cdot}0$	1·1
	$n = 10$	$n = 3$	$n = 10$	$n = 3$
5·8	$56{\cdot}6 \pm 0{\cdot}5$	1·0	$55{\cdot}8 \pm 1{\cdot}3$	1·0
	$n = 50$		$n = 20$	$n = 8$
11·6	$50{\cdot}4 \pm 1{\cdot}6$	0·74	$51{\cdot}2 \pm 1{\cdot}9$	0·88
	$n = 30$	$n = 3$	$n = 20$	$n = 3$
29	$44{\cdot}8 \pm 1{\cdot}2$	0·48	$48{\cdot}2 \pm 2{\cdot}4$	0·62
	$n = 30$	$n = 5$	$n = 10$	$n = 3$

recorded in sequence from the same muscle fibre. TEA restored the membrane activity, and the spike amplitude was much larger than that recorded in Krebs solution. Overshoot potentials and a uniform amplitude of spike were produced. In a tenth of the K concentration (0·56 mM), the membrane was hyperpolarized from $-56{\cdot}4 \pm 2{\cdot}6$ to $-64{\cdot}4 \pm 3{\cdot}5$ mV and abortive spike could be evoked by electrical stimulation. Treatment with TEA (10^{-3} g/ml.), large evoked spikes could be recorded ($52 \pm 2{\cdot}4$ mV ($n = 10$)). The rectifying property of the membrane was nearly suppressed.

High concentration of K ions. Fig. 10 shows the effect of TEA (10^{-3} g/ml.) in excess-K solution on the membrane activity of the stomach circular muscle recorded using micro-electrode technique. At a concentration of 11·6 mM, K ions depolarized the membrane from $-56{\cdot}1 \pm 2{\cdot}8$ to $-50{\cdot}4 \pm 1{\cdot}6$ mV, and decreased the membrane resistance (0·74 times the control value). The amplitude of the spike was also decreased. Treatment with TEA (10^{-3} g/ml.) increased the membrane resistance slightly than that in the excess-K solution (0·88 times the control), and enlarged the spike

amplitude even in excess-K ion solution. As shown in Fig. 9, the amplitude of the spike was increased from 21 to 56 mV. Increased concentration of K ions (five times the normal concentration; 29 mM) depolarized the membrane further ($-44{\cdot}0 \pm 1{\cdot}2$ mV) and also decreased the membrane resistance (0·48 times the control value). TEA (10^{-3} g/ml.) repolarized the membrane ($-48{\cdot}2 \pm 2{\cdot}4$ mV) and increased the membrane resistance

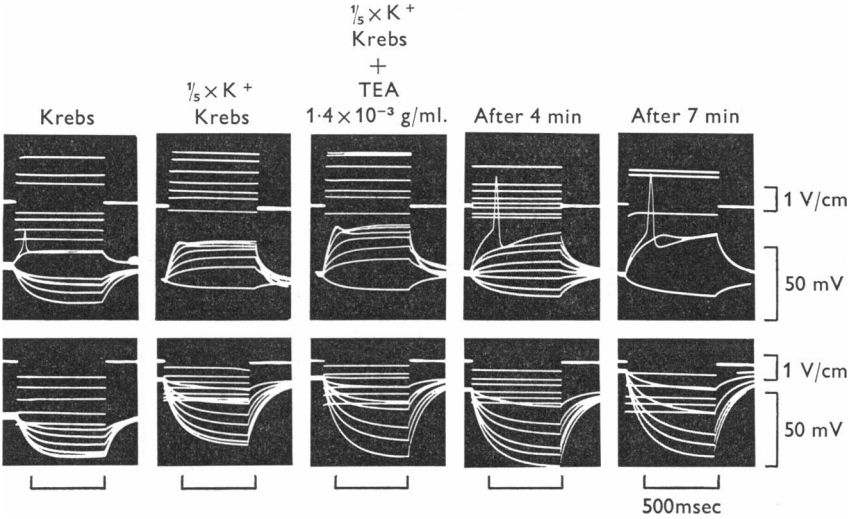

Fig. 9. Effects of TEA ($1{\cdot}4 \times 10^{-3}$ g/ml.) in K-deficient Krebs solution (1·2 mM) on the membrane activity of the antral smooth muscle recorded by the micro-electrode method. Throughout the experiment, the electrode was inserted into the same cell. The recording micro-electrode was placed at 0·3 mm distance from the stimulating electrode.

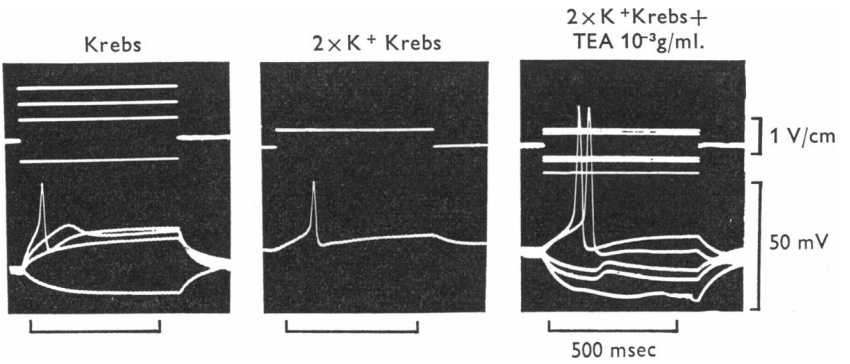

Fig. 10. Effects of TEA (10^{-3} g/ml.) in excess K-Krebs solution (11·6 mM) on the membrane activity of the antral smooth muscle recorded with the micro-electrode method. The records were taken after 10 min of perfusion in 2 × K-Krebs and after 5 min of perfusion in 2 × K-Krebs with TEA.

(0·62 times the control value). The rectifying property could not be measured, since the outward current pulse easily elicited the spike.

DISCUSSION

Effects of TEA on the membrane activity on the excitable tissue have been studied by many investigators (Fatt & Katz, 1953; Hagiwara & Watanabe, 1955; Tasaki & Hagiwara, 1957; Koketsu, 1958; Hagiwara & Saito, 1959; Washizu, 1959; Armstrong & Binstock, 1965; Nakajima, 1966; Schmidt & Stampfli, 1966; Koppenhöfer, 1967; Hille, 1967; Kusano, Livengood & Werman, 1967*a*, *b*; Bergmann, Nonner & Stampfli, 1968; Katz & Miledi, 1969; see also the review of Shanes, 1958). Many of the above investigators led to the general conclusion that TEA was a depressant of the K-activation mechanism, i.e. the Na conductance increased almost as before, but that subsequently it did not decline completely to zero during the plateau, and that an increase in K conductance did not occur during the plateau. On the other hand, Armstrong & Binstock (1965) reported that the K channel showed an anomalous rectifying property after the internal application of TEA in the squid giant axon.

In the guinea-pig taenia coli, Suzuki, Nishiyama & Inomata (1961) reported the prolongation of the spike duration on treatment with TEA.

Recently, Liu, Prosser & Job (1969) reported that concentrations below 1 mM-TEA had no effect on intestinal activity of the cat, and that when high concentrations of TEA (20 mM) were used in conjunction with 1 mM atropine, the rates of rise and fall of the slow waves were reduced by only 70–80 % and the rates of both rise and fall of the spikes were reduced by 30–40 %. He concluded that spikes and slow waves generated from the cat intestine were less resistant to TEA than taenia coli of the guinea-pig.

On treatment with TEA, the most striking change in the membrane activity in the stomach muscle of the guinea-pig was in the spike amplitude. Even in the electrically low excitable muscle fibres, a spike with overshoot could be elicited by 5 mM-TEA ($1{\cdot}1 \times 10^{-3}$ g/ml.), and increased several times above that observed in normal conditions using the double sucrose gap method. This enlargement of the spike amplitude did not require the presence of Na ions, and neither did one tenth or five times the normal K ion concentration prevent the enlargement of the spike.

The rectifying property of the membrane observed from the voltage–intensity relation curve might indicate increased K conductance by application of the outward current pulse. Low concentration of TEA suppressed the rectifying property of the membrane but almost no change in the membrane resistance was observed before and after treatment with TEA ($1{\cdot}1 \times 10^{-3}$ g/ml.) measured by application of the inward current pulses.

According to the voltage clamp experiment on the stomach smooth

muscle with the double sucrose gap method a suppression of the delayed outward going current was detected by treatment with TEA (Y. Sakamoto & H. Kuriyama, unpublished observations).

Results obtained from the present experiment might indicate that the enhancement of the spike amplitude in the low concentration of TEA is due mainly to the suppression of the K conductance when the membrane was depolarized. However, it is not yet clear whether this effect of TEA is due to the delayed onset of the K conductance together with suppression of the K conductance, or to suppression of the K conductance alone. In a high concentration of TEA, the slope of the graph of the spike amplitude against $[TEA]_o$ was nearly the same as that against $[Tris]_o$ of 16 mV Y. Sakamoto & H. Kuriyama (to be published).

The spike in the circular muscle of the stomach was thought to be evoked by inward movement of the Ca ion, since the spike was generated in the Na-free solution, and in the presence of tetrodotoxin but was blocked by treatment with $MnCl_2$. The Sr ion could also evoke the spike in Na- and Ca-free solution. The role of the Na ion during the active and resting states of the membrane was not clear. Although a lowering of the membrane potential by prevention of K permeability and a competitive action with Ca ions to the binding site in the membrane have been postulated (Bülbring & Tomita, 1969; Kuriyama & Tomita, 1970). In the presence of either Ca ion or Sr ion, TEA enhanced the amplitude of the spike. Beaulieu & Frank (1967) found that TEA facilitated the movement of Ca ions through the membrane in skeletal muscle of frog. TEA also might possibly facilitate the inward movement of Ca ions in the smooth muscle. However, in the stomach muscle, TEA enhanced the amplitude of the spike without any change of the maximum rate of rise of the spike.

Another possibility is that the Ca ion and the TEA ion may interact with the lipoprotein layer of the muscle membrane, because TEA has been shown to produce a large phase boundary potential in the excitable cell membrane (Davies & Rideal, 1955). Therefore, the electrical changes recorded in the presence of TEA might show a substantially larger potential change than the value expected from the ionic distribution and the membrane conductances in the smooth muscle.

REFERENCES

ABE, Y. & TOMITA, T. (1968). Cable properties of smooth muscle. *J. Physiol.* **196**, 87–100.

ARMSTRONG, C. M. & BINSTOCK, L. (1965). Anomalous rectification in the squid axon injected with tetraethylammonium chloride. *J. gen. Physiol.* **47**, 859–872.

BEAULIEU, G. & FRANK, G. B. (1967). Tetraethylammonium-induced contractions of frog's skeletal muscle. III. Mechanism of action by calcium release. *Can. J. Physiol. Pharmac.* **45**, 845–855.

BERGMANN, C., NONNER, W. & STAMPFLI, R. (1968). Sustained spontaneous activity of Ranvier nodes induced by the combined actions of TEA and lack of calcium. *Pflügers Arch. ges. Physiol.* **302**, 24–37.

BÜLBRING, E. & TOMITA, T. (1969). Effect of calcium, barium and manganese on the action of adrenaline in the smooth muscle of the guinea-pig taenia coli. *Proc. R. Soc.* B **172**, 121–136.

DAVIES, J. T. & RIDEAL, E. (1955). Interfacial potentials. *Can. J. Chem.* **33**, 947–960.

FATT, P. & KATZ, B. (1953). The electrical properties of crustacean fibres. *J. Physiol.* **120**, 171–204.

HAGIWARA, S. & SAITO, N. (1959). Voltage–current relation in nerve cell membrane of *Onchidium verruculatum*. *J. Physiol.* **148**, 161–178.

HAGIWARA, S. & WATANABE, A. (1955). The effect of tetraethylammonium chloride on the muscle membrane examined with an intracellular electrode. *J. Physiol.* **129**, 513–527.

HILLE, B. (1967). The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. gen. Physiol.* **50**, 1287–1302.

KATZ, B. & MILEDI, R. (1969). Spontaneous and evoked activity of motor nerve endings in calcium Ringer. *J. Physiol.* **203**, 689–706.

KOKETSU, K. (1958). Action of tetraethylammonium chloride on neuromuscular transmission in frogs. *Am. J. Physiol.* **193**, 213–218.

KOPPENHÖFER, E. (1967). Die Wirkung von Tetraäthylammoniumchlorid auf die Membranströme Ranvierscher Schnürringe von *Xenopus laevis*. *Pflügers Arch. ges. Physiol.* **293**, 34–38.

KURIYAMA, H., OSA, T. & TASAKI, H. (1970). Electrophysiological studies of the antrum muscle fibres of the guinea-pig stomach. *J. gen. Physiol.* **55**, 48–62.

KURIYAMA, H. & TOMITA, T. (1970). Action potential in taenia coli and ureter of guinea-pig studied by double sucrose gap method. *J. gen. Physiol.* **55**, 147–162.

KUSANO, K., LIVENGOOD, D. R. & WERMAN, R. (1967*a*). Tetraethylammonium ions; effect of presynaptic injection on synaptic transmission. *Science, N.Y.* **155**, 1257.

KUSANO, K., LIVENGOOD, D. R. & WERMAN, R. (1967*b*). Correlation of transmitter release with membrane properties of the presynaptic fibre of the squid giant synapse. *J. gen. Physiol.* **50**, 2579–2601.

LIU, J., PROSSER, C. L. & JOB, D. D. (1969). Ionic dependence of slow waves and spikes in intestinal muscle. *Am. J. Physiol.* **218**, 1542–1547.

NAKAJIMA, S. (1966). Analysis of K-inactivation and TEA action in the supramedullary cells of puffer. *J. gen. Physiol.* **49**, 629–640.

SCHMIDT, H. & STAMPFLI, R. (1966). Die Wirkung von Tetraäthylammoniumchlorid auf den einzelnen Ranvierschen Schnürring. *Pflügers Arch. ges. Physiol.* **278**, 311–325.

SHANES, A. M. (1958). Electrochemical aspects of physiological and pharmacological action in excitable cells. *Pharmac. Rev.* **10**, 59–273.

SUZUKI, T., NISHIYAMA, A. & INOMATA, H. (1961). Effect of TEA ion on the electrical activity of smooth muscle cell. *Nature, Lond.* **197**, 908.

TASAKI, I. & HAGIWARA, S. (1957). Capacity of muscle fiber membrane. *Am. J. Physiol.* **188**, 423–429.

TOMITA, T. (1966). Electrical responses of smooth muscle to external stimulation in hypertonic solution. *J. Physiol.* **183**, 450–468.

WASHIZU, Y. (1959). The effect of TEA on the electrical activities of spinal motoneurones. *Jap. J. Physiol.* **9**, 311–321.