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THE EFFECT OF SODIUM AND CALCIUM ON THE ACTION POTENTIAL OF THE SMOOTH MUSCLE OF THE GUINEA-PIG TAENIA COLI

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SUMMARY

1. Spontaneous spike activity and action potentials evoked by external field stimulation were recorded, intracellularly and with the double sucrose gap method, from the smooth muscle of guinea-pig taenia coli.

2. Replacement of external NaCl with sucrose (leaving 10 mM-Na in the buffer) caused hyperpolarization and stopped spontaneous activity within 10 min. Spikes could, however, be evoked for 2–3 hr. The amplitude, the overshoot and rate of rise of the spike were increased.

3. In 10 mM-$[Na]_o$ the intracellular Na concentration was reduced from 35 to 24 mM, shifting the Na-equilibrium potential from +34 to −22 mV.

4. Excess Ca (12·5 mM) caused hyperpolarization and increased membrane conductance. The amplitude and the rate of rise of the spike were increased, the threshold was raised and the latency of the spike evoked by threshold stimulation became shorter.

5. The effect of reducing the external Ca concentration depended on the Na concentration present, being greater with higher external $[Na]_o$. When the membrane was depolarized and spikes deteriorated in low Ca (0·2–0·5 mM) reduction of Na to 10 mM caused repolarization and recovery of the action potential.

6. Mn (0·5–1·0 mM) blocked spontaneous spike discharge after 20 min. Higher concentrations (more than 2·0 mM) were required to block the evoked action potential.

7. The results indicate that the smooth muscle spike in taenia is due to Ca-entry and that Na influences spike activity indirectly by competing with Ca in controlling the membrane potential.

INTRODUCTION

The amplitude of the action potential in the smooth muscle of guinea-pig taenia coli is relatively insensitive to the external Na concentration. However, a contribution of Na has been suggested by the findings that

the rate of rise of the action potential is reduced by reducing the external Na concentration, and when it is less than 5 mM activity is lost (Holman, 1958; Bülbring & Kuriyama, 1963). Since the observations have been made on the spontaneously occurring spike, it is possible that the reduction of the rate of rise is due to an impairment of spike propagation and that the loss of the activity is due to the abolition of the pace-maker potential in low-Na-solution.

Bülbring & Kuriyama (1963) postulated that Ca ions may replace Na and carry the charge to generate the action potential in the absence of Na. The possibility of a 'Ca spike', even in the normal solution, has been considered based on the facts that tetrodotoxin is ineffective in blocking the spike (Nonomura, Hotta & Ohashi, 1966; Kuriyama, Osa & Toida, 1966; Bülbring & Tomita, 1967), that Ba is capable of substituting Ca for the spike and that Mn suppresses the spike (Nonomura *et al.* 1966; Hotta & Tsukui, 1968; Bülbring & Tomita, 1968*b*, 1969*a*), because these findings are similar to those on the crustacean muscle whose spike is due to the entry of Ca (Fatt & Ginsborg, 1958; Hagiwara & Naka, 1964; Hagiwara & Nakajima, 1966).

In the present experiments the spike mechanism was further investigated mainly by intracellular recording of action potentials evoked by external field stimulation (Tomita, 1966; Abe & Tomita, 1968) in low external Na (NaCl substituted with sucrose). The intracellular Na concentration was determined in the same condition. Some experiments were carried out with the double sucrose-gap method (Bülbring & Tomita, 1969*a*).

The results have led to the conclusion that the spike in taenia coli is mainly due to Ca entry and that Na influences the spike indirectly through competition with Ca in controlllng the membrane potential.

Some of the results have been communicated to the Physiological Society (Brading & Tomita, 1968).

METHODS

Intracellular and external recording was used for electrophysiological investigations. The arrangement for intracellular recording and external stimulation has been described by Abe & Tomita (1968). In this method the absolute intensity of the applied current could not be measured. In the records, therefore, only relative values of intensity are shown in the upper tracing. The double sucrose-gap method was used for external recording, as described by Bülbring & Tomita (1969*a*). All experiments were carried out at 37° C.

Ion contents were estimated by the methods described by Casteels & Kuriyama (1965). The fresh weights of the tissues were determined immediately after dissection, and the wet weights after equilibration with the bathing solution. The ratio of Na to K in the standard solution for flame photometry was matched to that in the tissue in order to avoid errors due to interaction of these ions in the flame.

The ionic composition of the solutions used is given in Table 1. The normal solution in

most experiments was Krebs solution (A); sometimes Locke solution (B) was used. When the NaCl was reduced it was replaced with sucrose (154 mM-NaCl is isosmotic with 292 mM-sucrose). The buffer was sometimes kept constant, as in solution (C), or a part of $NaHCO_3$ was replaced with 5·9 mM-$KHCO_3$, omitting KCl and NaH_2PO_4, and leaving about 10 mM-Na in the solution (D). In Locke solution containing zero Na, $NaHCO_3$ was replaced with $KHCO_3$ (E). Further changes in the ionic composition, introduced in the course of an experiment, are mentioned in the description of results.

For the studies by intracellular recording of the effect of excess Ca on the electrotonic potential, hypertonic solution (F) was used in order to suppress spontaneous activity (Tomita, 1966). The Krebs solution was made twice hyperosmotic by adding 292 mM-sucrose. NaH_2PO_4 was omitted to prevent Ca precipitation; this had no effect in the presence

TABLE 1. Ionic composition (mM)

Solution	Na	K	Ca	Mg	Cl	HCO_3	H_2PO_4	Glucose	O_2/CO_2 (%)
(A) Krebs	136·9	5·9	2·5	1·2	133·6	15·4	1·2	11·5	97/3
(B) Locke	155·8	5·6	2·2	—	161·8	1·8	—	5·6	100/0
(C) Low Na Krebs	25	5·9	2·5	1·2	21·7	15·4	1·2	11·5	97/3
(D) Low Na Krebs	9·6	5·9	2·5	1·2	7·4	15·4	—	11·5	97/3
(E) Zero Na Locke	—	5·6	2·2	—	8·2	1·8	—	5·6	100/0
(F) Hypertonic Krebs	135·7	5·9	2·5	1·2	133·6	15·4	—	11·5	97/3
(G) Tris-buffered Krebs	120·3	5·9	2·5	1·2	150·2	—	—	11·5	100/0

of a normal Ca concentration. When Mn was applied in some experiments Krebs solution with tris (-hydroxymethyl aminomethane) buffer (16·7 mM) instead of $NaHCO_3$ and NaH_2PO_4 was used (G) in order to prevent precipitation of Mn, in other experiments Locke solution was used. There was no essential difference between the Mn effects in tris-buffered Krebs and Locke solution.

RESULTS

Effects of low Na

Action potential. Since the taenia is normally spontaneously active, the shape of the evoked action potential recorded in normal solution varies with the interval from the preceding spontaneous spike. For this reason the spontaneous spikes in normal Krebs solution A (Fig. 1*a*, *d*) were compared with evoked spikes in low sodium solution D (Fig. 1*b*, *c*, *e*). When the NaCl was replaced with sucrose, leaving 10 mM-Na contained in the buffer, the membrane was hyperpolarized by about 10 mV and spontaneous spike activity stopped after about 10 min. However, spikes were easily evoked by a depolarizing current pulse, even after 2 or 3 hr exposure to a low Na solution.

In low Na, the spike amplitude was larger and the rate of rise was faster than in the normal solution. The maximum rate of rise was increased from 7 to 13 V/sec. The overshoot was usually increased by more than 10 mV and the amplitude, measured from the resting potential, often exceeded

80 mV. This was not simply due to the hyperpolarization of the membrane, because conditioning depolarization scarcely affected the overshoot of the spike as shown in Figs. 1 and 2.

The records shown in Fig. 2 were taken in 10 mM-Na (solution D) with (*b*, *d*) and without (*a*, *c*) conditioning depolarization. Neither the rate of

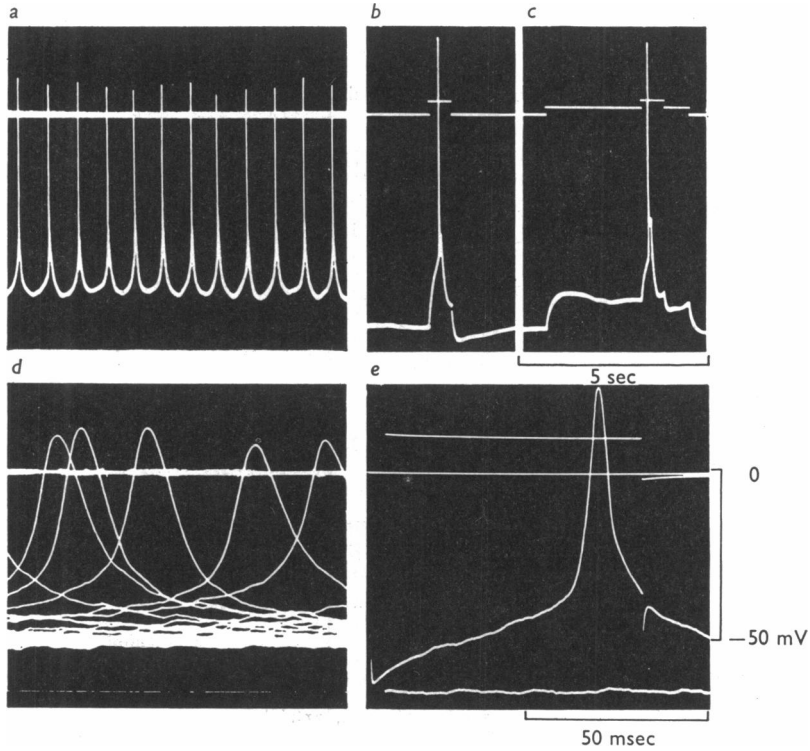

Fig. 1. Effects of a low external Na concentration on the action potential. Spontaneous spikes recorded intracellularly in normal Krebs solution, A, with (*a*) slow sweep and (*d*) fast sweep. After 1 hr in 10 mM Na solution, D (sucrose substitution), the spike was evoked by externally applied current in (*b*) by a 600 msec pulse taken at slow sweep speed, in (*e*) evoked by a 75 msec pulse and taken by fast sweep. (*c*), Spike evoked during conditioning depolarization. Upper trace shows zero potential level and relative current intensity (in *b*, *c* and *e*). Note hyperpolarization of the membrane, suppression of spontaneous activity, increase in amplitude and rate of the spike in low Na. Also note little change in overshoot by conditioning depolarization.

rise nor the overshoot of the spike was affected by conditioning depolarization.

The spikes in low Na solution shown in Figs. 1 and 2 were evoked by long current pulses so that stimulating current was flowing during the spike. However, the spikes evoked by short current pulses (5–10 msec) had the

same shape and height. When the intensity of long current pulses was increased, the peak of spike remained more or less at the same level, as seen in the top records of Fig. 3. A constant spike amplitude, independent of stimulating current intensity, was also observed in normal Na solution (Tomita, 1966) and may indicate that the membrane resistance is very much reduced during the spike.

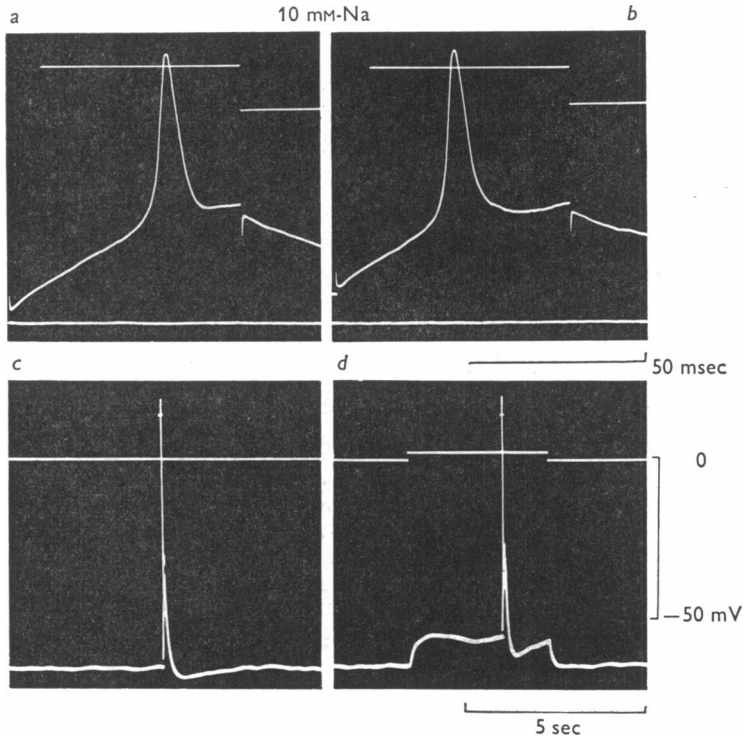

Fig. 2. Effects of conditioning depolarization on the evoked spike in low Na (10 mM, solution D). (*a*) and (*c*): spike evoked by a short current pulse (65 msec), (*b*) and (*d*): with preceding conditioning depolarization (300 msec). Upper trace shows zero potential level and relative intensity of applied current. Note little effect of conditioning depolarization on amplitude and rate of rise of the spike.

Ion content. The intracellular ionic composition was determined after 1 hr incubation in Krebs solution and in 10 mM-Na Krebs solution. The results are summarized in Table 2. In this table it is assumed that all ions and water are free in the cell. When the external Na concentration was reduced from 126 to 10 mM, the intracellular K concentration was increased from 184 to 234 mM because of a loss of cell water. The intracellular Na concentration fell from 35 to 24 mM. The equilibrium potential for Na was, therefore, shifted from +34 mV to −22 mV.

TABLE 2

Solution	Wet wt. / Fresh wt.	Wet wt. / Fresh wt. as % of normal	Extracellular space (% of fresh wt.)	Intracellular space (% of fresh wt.)	Cell water (% of fresh wt.)
Krebs (A)	89·7	100·00	37·1	52·6	35·8
Low Na (D)	72·8	81·2	36·3	36·1	19·4

Solution	Extracellular concentration (mM)			Intracellular concentration (m-mole/l. cell water)			Equilibrium potential (mV)		
	K	Na	Cl	K	Na	Cl	K	Na	Cl
Krebs (A)	5·9	126·2	133·6	184·1	35·0	73·9	−91·9	+34·3	−15·8
Low Na (D)	5·9	10·4	17·0	234·4	23·7	25·5	−98·3	−22·0	+10·8

Spontaneous activity. When the NaCl was reduced to less than 25 mM by replacing it with sucrose, the spontaneous spike activity usually stopped within 10–15 min. In most experiments, spontaneous activity remained absent and only occasionally spontaneous spikes reappeared at a very slow frequency (e.g. 1 in 10 sec). However, repetitive spikes could be evoked by applying a sustained depolarizing current as shown in Fig. 3 (top records). The spike amplitude remained more or less the same even during strong depolarization which produced spikes at a frequency higher than that of the spontaneous spikes in normal solution.

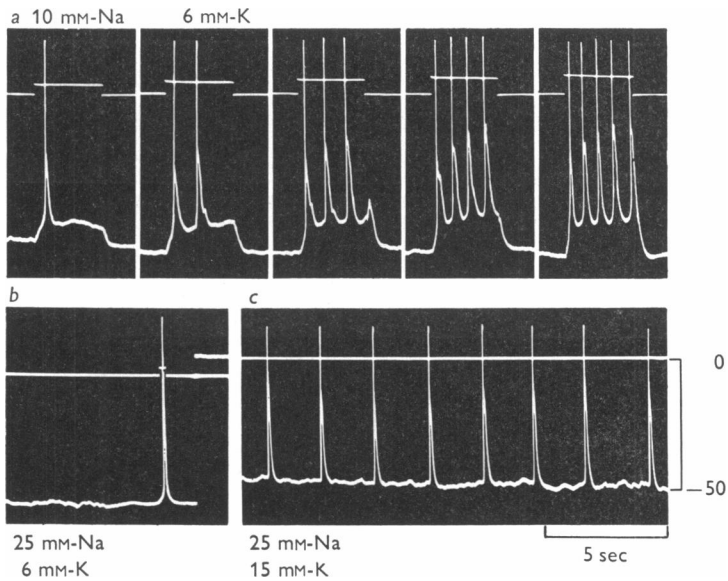

Fig. 3. Repetitive spike activity recorded intracellularly in Krebs solution containing low Na. (*a*): responses to depolarizing current pulses (3 sec duration) of increasing intensity (monitored in upper trace) in 10 mM-Na and normal K (6 mM, solution D); (*b*): evoked spike in 25 mM-Na and normal K (6 mM, solution C, no spontaneous activity). After the evoked spike, electrode came out of the cell; (*c*): spontaneous spikes in low Na (25 mM) produced by excess K (15 mM). For further description, see text.

Spontaneous spikes were also produced in low Na solution when the membrane was slightly depolarized by increasing the external K concentration (Fig. 3, bottom records). In this condition the spike amplitude was slightly reduced.

Effect of excess Ca

In experiments with the double sucrose-gap method it has been found that excess Ca reduces the membrane resistance (Bülbring & Tomita,

1968*a*, 1969*b*). In the present experiments this effect was further investigated by intracellular recording in Krebs solution made twice hypertonic with sucrose.

The records shown in Fig. 4 were obtained at 0·3 mm distance from the nearest stimulating electrode. When the external Ca concentration was

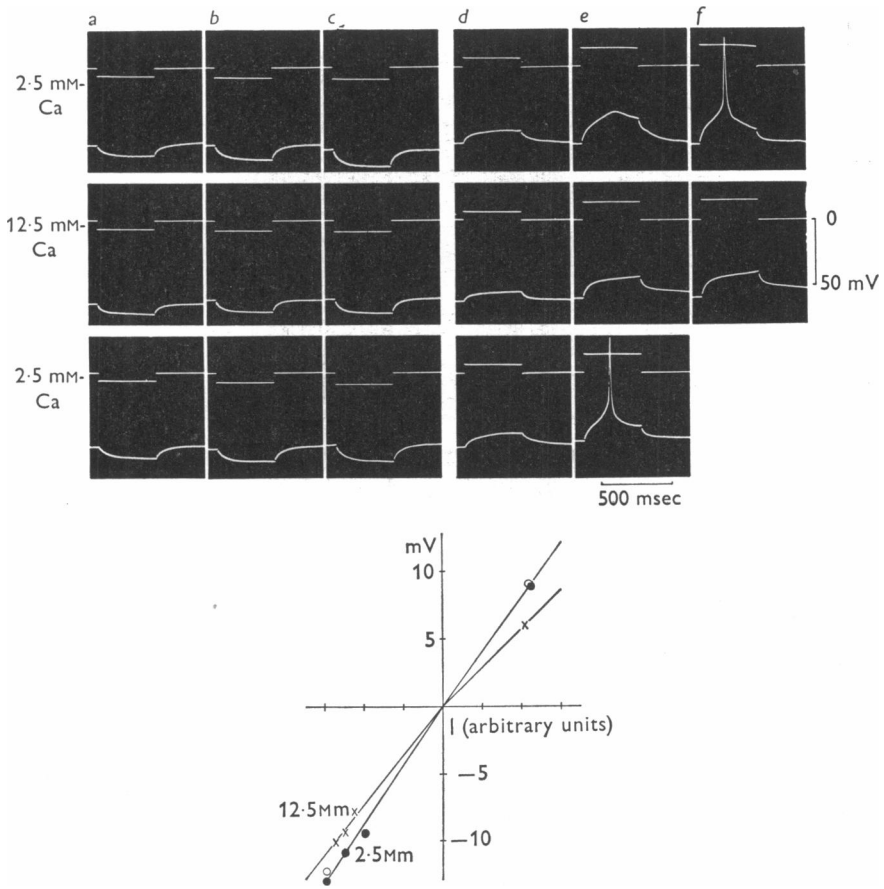

Fig. 4. Effect of excess Ca (12·5 mM) in Krebs solution of twice normal osmolarity by addition of sucrose (292 mM). Intracellular records of responses to current pulses of increasing intensity. All records were taken from the same cell at 0·3 mm distance from the nearest current supplying electrode. (*a–c*): records of hyperpolarization and (*d–f*): depolarization. Top: in normal Ca (2·5 mM), solution F. The strongest depolarizing current produced a spike. Middle: in 12·5 mM-Ca. No spike was evoked due to a higher threshold. Bottom: recovery in normal Ca. Note reduction of amplitude and shortening of time course of the electrotonic potentials by excess Ca. The graph shows current–voltage relations in the presence of 2·5 mM and 12·5 mM-Ca. Ordinates: mV; abscissae: current intensity in arbitrary units (see Tomita, 1966; Abe & Tomita, 1968). Filled and open circles: in 2·5 mM-Ca; crosses: in 12·5 mM-Ca.

increased from 2·5 to 12·5 mM the membrane was hyperpolarized by about 5 mV. The size of the electrotonic potential was decreased by about 20 % (Fig. 4), indicating a reduction of the membrane resistance, since excess Ca had no measurable effect on the longitudinal tissue impedance (T. Tomita, unpublished observation). A decrease in the membrane resistance was also suggested by a faster time course of the electrotonic potential in

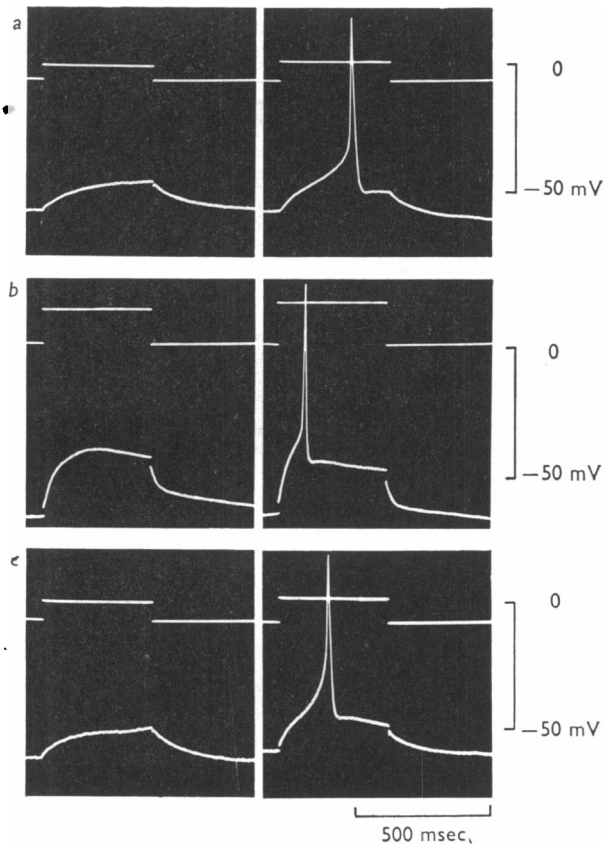

Fig. 5. Effect of excess Ca (12·5 mM) on subthreshold and threshold responses, obtained in twice hyperosmotic solution. Top and bottom: in normal Ca solution F; middle: in excess Ca. Note the shape of the subthreshold response, the increase in threshold and amplitude of the spike, and the short latency in excess Ca.

a solution containing excess Ca. The time to reach 84 % of the steady level was shortened by about 30 %.

Excess Ca increased the amplitude and the rate of rise of the spike as observed on the spontaneous spikes in the normal Krebs solution (Holman, 1958; Bülbring & Kuriyama, 1963). Excess Ca also increased the threshold, partly as a result of the membrane hyperpolarization and partly due to a

shift of the firing level towards a more positive inside potential (Fig. 5). In the presence of a normal Ca concentration the latency could easily be more than 200 msec at threshold stimulation, while in the presence of excess Ca it was relatively short (less than 100 msec).

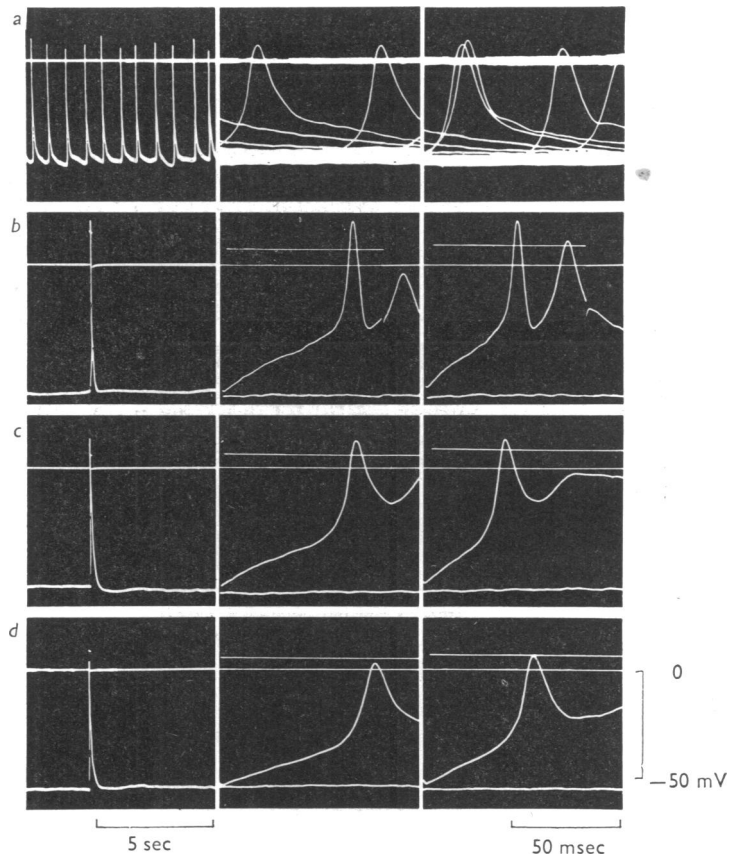

Fig. 6. Effects of low Ca in the presence of a low external Na concentration (10 mM). (*a*): spontaneous spikes recorded intracellularly in normal Krebs solution, A, at two different sweep speeds; (*b*): evoked spikes after 30 min in 10 mM-Na solution, D; (*c*): evoked spikes after 10 min and (*d*): after 40 min in 0·2 mM-Ca and 10 mM-Na. Note increase in rate of rise and amplitude of the spike in low Na and decrease after reducing Ca.

Effect of low Ca and low Na

Fig. 6 shows the effect of reducing Ca in a solution containing low Na (10 mM). Spontaneous spikes were recorded in normal Krebs solution, A (*a*). Low Na, solution D, increased the amplitude and the rate of rise of the spike (*b*). When the Ca concentration was then reduced from 2·5 to 0·2 mM the amplitude of the spike was gradually decreased from 77 to

62 mV with only a small depolarization (less than 5 mV) of the membrane (*c*, *d*). The rate of rise was also reduced from 13 to 6 V/sec, and the spike duration became longer. Conditioning hyperpolarization (up to 10 mV and 3 sec) of the membrane only slightly improved the spike in low Ca solution.

Figure 7 shows the effect of reducing Na in a solution containing low Ca. Spontaneous spikes were recorded in normal Krebs solution, A (*a*, *e*). When

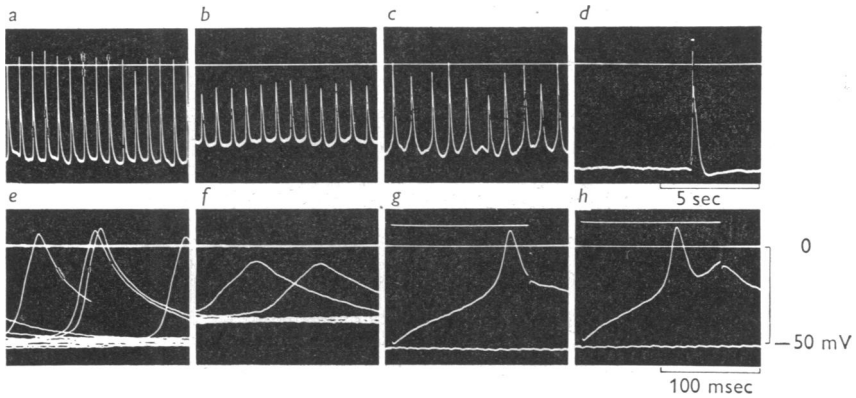

Fig. 7. Effects of reducing Na after the preceding exposure to low Ca. Intracellular recordings (*a*) and (*e*): spontaneous spikes in normal Krebs solution, A, at two different sweep speeds; (*b*) and (*f*): in 0·5 mM-Ca solution containing normal Na; (*c*): 5 min and (*d*, *g*, *h*): 30 min in 15 mM-Na and 0·5 mM-Ca. Note recovery of amplitude and rate of rise of the spike by reducing Na concentration in the presence of low Ca.

the Ca concentration was reduced to 0·5 mM in Krebs solution containing the normal Na concentration, the membrane was depolarized by about 10 mV and the spike became small (Fig. 7*b*, *f*) as previously reported (Holman, 1958; Bülbring & Kuriyama, 1963). Thus, the effect of reducing Ca was much greater in the presence of the normal Na concentration than with low Na (see Fig. 6). Moreover, when the external Na concentration was now reduced to 15 mM keeping the Ca concentration low, the membrane was gradually repolarized, the spike frequency became low and the amplitude of the spike was increased (*c*). The spontaneous activity finally stopped after further repolarization (*d*). In this condition, a spike with nearly normal amplitude and rate of rise could be evoked by depolarizing current (*g*, *h*).

The records shown in Fig. 8 were obtained with external recording using the double sucrose-gap method (Bülbring & Tomita, 1969*a*). In this experiment depolarizing and hyperpolarizing current pulses of constant intensity were applied throughout. Therefore, a change in the size of the

electrotonic potential indicated a change in the membrane resistance. The tissue had been set up in Locke solution, B. When it was exposed to zero Ca solution, but maintaining the normal Na concentration, the membrane was depolarized, it lost all activity, and the membrane resistance became very low (Bülbring & Tomita, 1968*b*, 1969*b*). This condition is shown in the

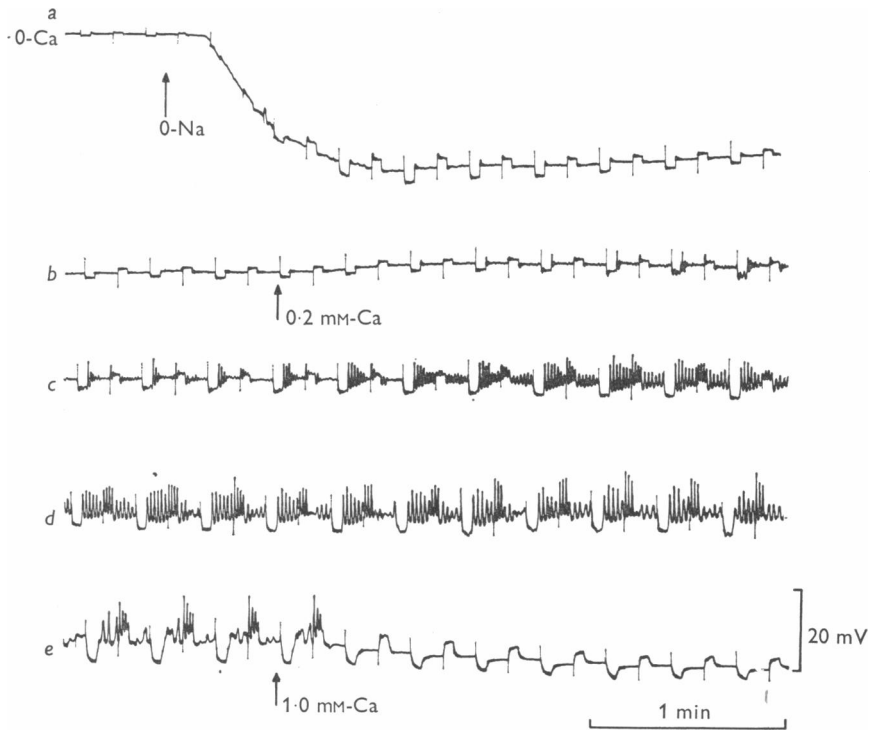

Fig. 8. External recording with double sucrose-gap method. Constant current pulses with alternating polarities (3 sec duration, every 10 sec) applied throughout. (*a*): state of depolarization and low membrane resistance after 15 min exposure to Locke solution containing zero Ca. At arrow, removal of Na (sucrose substitution, i.e. solution E, but without $CaCl_2$) causing hyperpolarization and recovery of membrane resistance. (*b*): after 7 min in zero Na and zero Ca, at arrow: 0·2 mM-Ca added. (*c*) and (*d*): continuous records following (*b*). (*e*): after 20 min in 0·2 mM-Ca and zero Na, at arrow, 1 mM-Ca added. Note recovery of spike activity by 0·2 mM-Ca but suppression of spikes by 1 mM-Ca in zero Na.

beginning of the top record (*a*). When now Na was also removed (at arrow replacement of NaCl with sucrose, K-buffer), the membrane was repolarized and the membrane resistance was increased as seen in the larger electrotonic potentials (*a*) even though the solution still contained zero Ca. In (*b*), after addition of only 1/10 of the normal Ca concentration, still in the absence of Na in the external solution, spike activity returned (*b* to *e*) and

the membrane resistance increased. Further increase of the Ca concentration to 1 mM already suppressed the spontaneous activity (*e*) and, as the membrane was hyperpolarized, the electrotonic potential became smaller. (This stabilizing effect was similar to that produced by lowering the Na concentration in a solution containing the normal Ca concentration.) If either a strong depolarizing current pulse or conditioning depolarization was applied, the spike could be evoked even though no Na was present (Bülbring & Tomita, 1969 *a*).

Effects of Mn

Using external recording, Nonomura *et al.* (1966) have shown that 0·5 mM-Mn abolishes the spontaneous spike activity very rapidly, i.e. within seconds. Bülbring & Tomita (1968*b*, 1969*b*), also using external recording, have reported that the onset is somewhat slower: 0·5 to 1 mM-Mn blocked the spontaneous spikes within 1 min, but a spike could be evoked by an outward current pulse.

In the present experiments, using intracellular recording, it was found that the block of the spontaneous spike was much slower than that observed by external recording. This difference may be partly due to differences in experimental conditions, e.g. the degree of stretch of the tissue, and the speed at which the final Mn concentration was reached. Desynchronization of the spikes in fibre bundles of the tissue may be another factor reducing activity when it is recorded externally.

Figure 9 shows the effects of 0·5 mM-Mn in tris-buffered Krebs solution, G. Normal spontaneous activity is shown in (*a*). When Mn was applied, the first effect was an increase in spike duration and slowing of the frequency of discharge (*b*, *c*). The membrane was gradually depolarized by 10–15 mV, the slow depolarization (pace-maker potential) between the spikes developed clearly, but the activity became irregular, the spikes being blocked occasionally (*d*, *e*). Usually, it took more than 20 min for the spontaneous activity to stop. However, spikes could be evoked by applying depolarizing or hyperpolarizing current, (*f*), although the amplitude and the rate of rise of the spike were less than in normal solution.

With a higher Mn concentration (1 mM), the block of spontaneous activity appeared more quickly (within 10–15 min). However, the spike could still be evoked by depolarizing current, especially during conditioning hyperpolarization (Fig. 10). If the concentration was further increased to more than 2 mM, the evoked spike was also abolished.

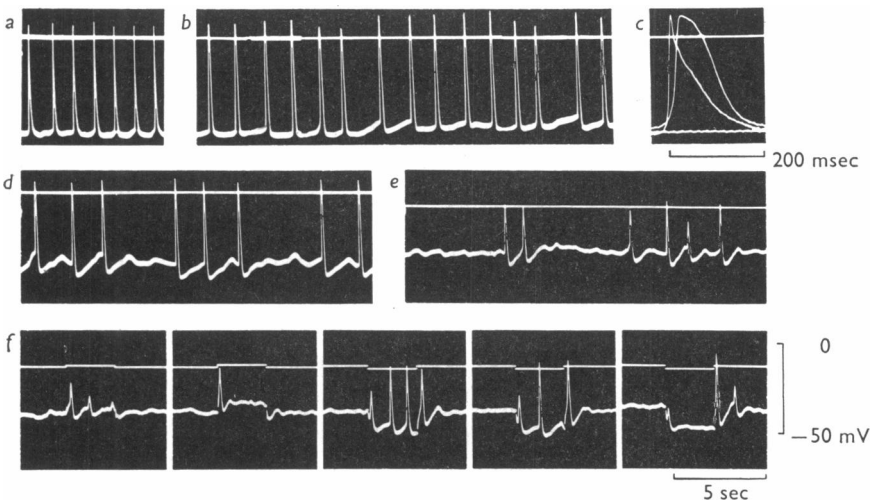

Fig. 9. Effect of Mn (0·5 mM). Intracellular records. (*a*): spontaneous activity in tris-buffered Krebs solution, G; (*b*): after 8 min exposure to 0·5 mM-Mn; (*c*): superimposed spikes of (*a*) and (*b*) with fast sweep; (*d*): after 15 min.; (*e*): after 25 min exposure to 0·5 mM-Mn; (*f*): block of the spontaneous spike after 40 min exposure, but evoked activity by depolarizing and hyperpolarizing current pulses. Note gradual change in spike shape and frequency in Mn solution.

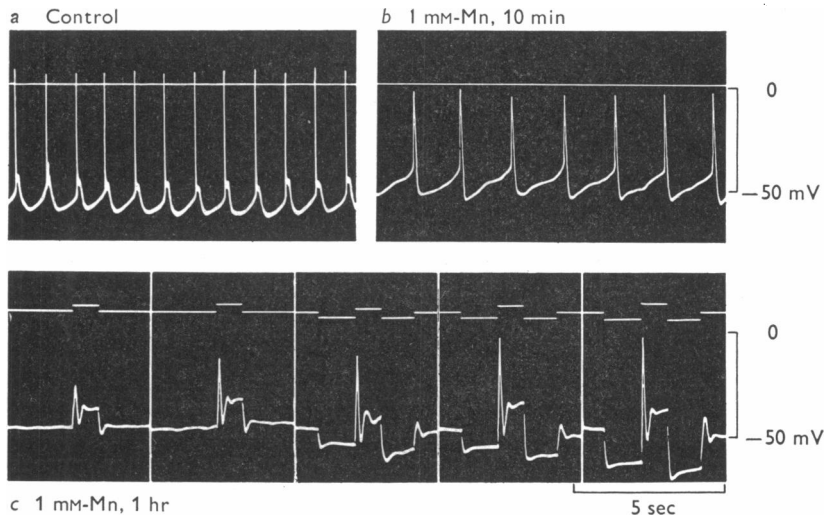

Fig. 10. Effects of Mn (1·0 mM). Intracellular records. (*a*): spontaneous activity in normal Locke solution, B; (*b*): after 10 min exposure to 1 mM-Mn; (*c*) spikes evoked by current pulses, with and without conditioning hyperpolarization, after 1 hr exposure to 1 mM-Mn.

DISCUSSION

In previous experiments the effects of Na deficiency have been studied on the spontaneous spike activity with either choline-chloride (Holman, 1958) or tris-chloride (Bülbring & Kuriyama, 1963) as substitutes for NaCl. In choline chloride solution (5–20 mM-Na), the spontaneous activity was maintained at normal frequency for 100 min, the amplitude and the rate of rise both being reduced. In tris-chloride solution (14 mM-Na), the membrane was slightly depolarized after an initial hyperpolarization, the spike amplitude was also slightly decreased, and the maximum rate of rise was reduced from 8 to 5 V/sec. Spontaneous activity was maintained for 30–60 min.

In the present experiments NaCl was substituted with sucrose. The membrane was hyperpolarized, spontaneous activity stopped, and the amplitude and rate of rise of the evoked spike was increased for more than 100 min in the presence of low Na (10 to 15 mM).

Since replacement of Cl with a large anion depolarizes the membrane (Kuriyama, 1963), the hyperpolarization in sucrose solution is not due to a lack of Cl. It is more likely due to a lack of Na, or to a loss of water causing an increase of the internal K concentration, or to a reduction of the ionic strength, which might increase Ca binding in the membrane (Lüttgau & Niedergerke, 1958).

The block of spontaneous activity in low Na (sucrose) solution is probably a result of the membrane hyperpolarization, not a direct result of the Na-deficiency, because depolarization produced either by current application or by excess K initiated repetitive firing of the spike.

A definite conclusion regarding the spike mechanism can as yet not be drawn from the present experiments. There are still two possibilities to explain the results, although the first is more likely. First, there is the possibility that the Na-contribution to the spike is not substantial and the normal spike is due to Ca-influx. Choline and tris have an effect of their own reducing the amplitude and the rate of rise of the spike, since this is not seen in sucrose solution. It is possible that spontaneous activity stops in low Na solution because the slight hyperpolarization affects the spike configuration and thus delays or blocks the spike propagation at the branching of the muscle bundles (Tomita, 1967).

Secondly, there is the possibility that Na carries part of the total current during the spike. When sucrose is used as Na substitute, Ca in the membrane is increased and potentiates the regenerative process of the spike, as in excess Ca solution (Frankenhaeuser & Hodgkin, 1957). This potentiation overcomes the effect of Na deficiency.

Nevertheless, the Na contribution to the spike is, if any, probably rather

small, because not only are the overshoot and the rate of rise of the spike actually increased in low Na solution, but the spike can also be evoked in zero Na solution containing Ca. These observations suggest strongly that the spike in the taenia is due to Ca entry. Bennett (1967) has also reached the conclusion that in the guinea-pig vas deferens part of the current responsible for the rising phase of the spike is carried by Ca, as a result of observing the effects of Na and Ca on the spike. It is not possible to estimate the free intracellular Ca^{2+} in smooth muscle, but, if it is similar to other muscles, it is probably less than 10^{-6}–10^{-7} M (Caldwell, 1968). The calcium equilibrium potential in normal solution (2·5 mM) would then be over 100 mV.

There is considerable evidence supporting the idea that Ca is the main ion involved in the spike mechanism in the taenia.

(1) In low Na solution (10 mM), the intracellular Na concentration is only reduced from 35 to 24 mM, thus reversing the equilibrium potential from +34 to −22 mV, while the overshoot is increased from +10 to +20 mV. It is likely that some intracellular Na is bound, so that the free Na ion concentration in the cell is actually lower than 24 mM. However, it is unlikely that external Na reduction decreases the intracellular Na so much that the concentration gradient becomes actually larger than before and causes an increase of the overshoot of the spike.

(2) In low Na solution (which increases the spike amplitude and rate of rise) Ca deficiency causes only a small depolarization but decreases the amplitude and rate of rise of the spike considerably, suggesting an increase in Ca-permeability during the spike. The results of the present experiments are not sufficient to analyse the parameters of the spike quantitatively in relation to the external Ca concentration. The increase in the spike amplitude and the rate of rise in low Na solution, and the decrease in low Ca solution, are more than can be explained by the shift of the membrane potential, because conditioning depolarization or hyperpolarization have much less effect.

(3) The following observations are similar to those made in crustacean muscle (Fatt & Ginsborg, 1958; Hagiwara & Naka, 1964; Hagiwara & Nakajima, 1966) whose spike is due to Ca entry: (*a*), ineffectiveness of tetrodotoxin on the spike (Kuriyama *et al.* 1966; Nonomura *et al.* 1966) although it blocks the electrical response produced by nervous elements in the muscle (Bülbring & Tomita, 1967), (*b*) spike generation in Ca-free solution containing Ba (Hotta & Tsukui, 1968; Bülbring & Tomita, 1968*b*, 1969*c*), (*c*) suppression of the spike by Mn (Nonomura *et al.* 1966; Bülbring & Tomita, 1968*b*, *e*).

The effect of reducing the external Na concentration is similar to that of increasing the external Ca concentration on the membrane potential and

on the spike. The degree of depolarization in low or zero Ca solution depends on the external Na concentration, suggesting an increase of Na conductance of the membrane in low Ca solution. Therefore, it is likely that Na influences the spike activity indirectly through competition with Ca as in the cardiac muscle (Lüttgau & Niedergerke, 1958; Niedergerke & Orkand, 1966); or by competing with Ca in the control of the membrane potential.

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REFERENCES

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

BENNETT, M. R. (1967). The effect of cations on the electrical properties of the smooth muscle cells of the guinea-pig vas deferens. *J. Physiol.* **190**, 465–479.

BRADING, A. F. & TOMITA, T. (1968). The action potential of the guinea-pig taenia coli in low sodium solution. *J. Physiol.* **197**, 30–31 *P*.

BÜLBRING, E. & KURIYAMA, H. (1963). Effects of changes in the external sodium and calcium concentrations on spontaneous electrical activity in smooth muscle of guinea-pig taenia coli. *J. Physiol.* **166**, 29–58.

BÜLBRING, E. & TOMITA, T. (1967). Properties of the inhibitory potential of smooth muscle as observed in the response to field stimulation of the guinea-pig taenia coli. *J. Physiol.* **189**, 299–315.

BÜLBRING, E. & TOMITA, T. (1968*a*). The effect of catecholamines on the membrane resistance and spike generation in the smooth muscle of guinea-pig taenia coli. *J. Physiol.* **194**, 74–76*P*.

BÜLBRING, E. & TOMITA, T. (1968*b*). The effects of Ba^{2+} and Mn^{2+} on the smooth muscle of guinea-pig taenia coli. *J. Physiol.* **197**, 137–139*P*.

BÜLBRING, E. & TOMITA, T. (1969*a*). Increase of membrane conductance by adrenaline in the smooth muscle of guinea-pig taenia coli. *Proc. R. Soc.* B. (In the Press.) **172**, 89–102.

BÜLBRING, E. & TOMITA, T. (1969*b*). 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.

BÜLBRING, E. & TOMITA T. (1969*c*). Suppression of spontaneous spike generation by catecholamines in the smooth muscle of the guinea-pig taenia coli. *Proc. R. Soc.* B. **172**, 103–119.

CALDWELL, P. C. (1968). Factors governing movement and distribution of inorganic ions in nerve and muscle. *Physiol. Rev.* **46**, 1–64.

CASTEELS, R. & KURIYAMA, H. (1965). Membrane potential and ionic content in pregnant and non-pregnant rat myometrium. *J. Physiol.* **177**, 263–287.

FATT, P. & GINSBORG, B. L. (1958). The ionic requirements for the production of action potentials in crustacean muscle fibres. *J. Physiol.* **142**, 516–543.

FRANKENHAEUSER, B. & HODGKIN, A. L. (1957). The action of calcium on the electrical properties of squid axons. *J. Physiol.* **138**, 218–244.

HAGIWARA, S. & NAKA, K. (1964). The initiation of spike potential in barnacle muscle fibers under low intracellular Ca^{++}. *J. gen. Physiol.* **46**, 141–162.

HAGIWARA, S. & NAKAJIMA, S. (1966). Differences in Na^{+} and Ca^{++} spikes as examined by application of tetrodotoxin, procaine and manganese ions. *J. gen. Physiol.* **49**, 793–805.

HOLMAN, M. E. (1958). Membrane potentials recorded with high-resistance micro-electrodes; and the effects of changes in ionic environment on the electrical and mechanical activity of the smooth muscle of the taenia coli of the guinea-pig. *J. Physiol.* **141**, 464–488.

HOTTA, Y. & TSUKUI, R. (1968). Effect on the guinea-pig taenia coli of the substitution of strontium or barium ions for calcium ions. *Nature, Lond.* **217**, 867–869.

KURIYAMA, H. (1963). The influence of potassium, sodium and chloride on the membrane potential of the smooth muscle of taenia coli. *J. Physiol.* **166**, 15–28.

KURIYAMA, H., OSA, T. & TOIDA, N. (1966). Effect of tetrodotoxin on smooth muscle cells of the guinea-pig taenia coli. *Br. J. Pharmac. Chemother.* **27**, 366–376.

LÜTTGAU, H. C. & NIEDERGERKE, R. (1958). The antagonism between Ca and Na ions on the frog's heart. *J. Physiol.* **143**, 486–505.

NIEDERGERKE, R. & ORKAND, R. K. (1966). The dependence of the action potential of the frog's heart on the external and intracellular sodium concentration. *J. Physiol.* **184**, 312–334.

NONOMURA, Y., HOTTA, Y. & OHASHI, H. (1966). Tetrodotoxin and manganese ions: Effects on electrical activity and tension in taenia coli of guinea-pig. *Science, N.Y.* **152**, 97–99.

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

TOMITA, T. (1967). Spike propagation in the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **191**, 517–527.