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ELECTROPHYSIOLOGICAL STUDY OF THE INTESTINAL SMOOTH MUSCLE OF THE GUINEA-PIG

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SUMMARY

The membrane properties of single cells of intestinal smooth muscle of duodenum, jejunum, ileum, caecum and rectum of guinea-pig have been studied.

1. The membrane potentials of longitudinal muscles of the duodenum, jejunum, ileum, caecum and rectum varied from 54 to 56 mV and those of circular muscles of jejunum, ileum, caecum and rectum varied from 57 to 60 mV. The ablated longitudinal muscle had a slightly lower value (50 mV) than the intact one.

2. The longitudinal muscle generated spontaneous discharges but these were rare in the circular muscles of the intestine except for the caecum. Overshoot potentials could be observed in all regions of the intestine. The maximum rate of rise of the spontaneously discharging longitudinal muscles varied from 11 to 18 V/sec.

3. Not all of the slow potential changes (but at least some) were generated by the nervous elements distributed between the muscle layers and in them.

4. The conduction velocities measured from the longitudinal muscles of jejunum and rectum in the presence of tetrodotoxin were 2·1 cm/sec and 4·0 cm/sec respectively.

5. Chronaxies of the longitudinal muscles of jejunum and rectum were 2–5 msec and 5–18 msec respectively.

6. Intracellular stimulation of the single cells of the duodenum and caecum could trigger a spike, similar to that observed in the taenia coli. The spikes were mostly graded ones; a spike of full size was rarely elicited. When the spikes were triggered, the after-hyperpolarization appeared consistently presumably caused by the increased potassium conductance.

7. The effective membrane resistance and the time constant were measured for the longitudinal muscles of the jejunum and rectum. When spikes were generated by intracellular stimulation the observed values were

40–50 MΩ and 3–5 msec in both tissues. These values were the same as those observed in the taenia coli.

8. When the time constant of the membrane was measured by the extracellular polarizing method, the longitudinal muscles of the jejunum especially and the rectum had smaller time constants than the taenia coli.

9. The differences of conduction velocity and chronaxie of the different regions of the intestine are discussed in relation to the cable properties of the tissues which are directly influenced by the morphological arrangements of the tissues.

INTRODUCTION

Despite continued efforts, our knowledge of the electrical properties of smooth muscle is far behind that of striated muscle. The difficulties of the investigation on this tissue are not only due to the small cell dimensions and the complicated arrangement of the cells but also to the complicated innervation.

Recently, the method of intracellular stimulation has been successfully applied to smooth-muscle cells (cat circular intestinal muscle: Nagai & Prosser, 1963*b*; Sperelakis & Tarr, 1965; guinea-pig taenia coli: Kuriyama & Tomita, 1964, 1965; guinea-pig vas deferens: Hashimoto, Holman & Tille, 1966; guinea-pig ureter: Kuriyama, Osa & Toida, 1967*a*), and has enabled the passive membrane properties to be compared with other excitable cells.

The electrophysiological studies of intestinal smooth muscle by microelectrodes have been mainly done on the guinea-pig taenia coli by Bülbring and her co-workers (Bülbring, 1955, 1957; Bülbring, Burnstock & Holman, 1958; Bülbring & Kuriyama, 1963) and on the cat small intestine by Prosser and his co-workers (Burnstock & Prosser, 1960; Prosser, 1962; Prosser, Burnstock & Kahn, 1960; Prosser & Sperelakis, 1956). Only few experiments have been carried out on other parts of the intestine. In the present experiments, therefore, an electrophysiolgical study of various parts of the intestinal tract was undertaken, including the longitudinal and circular muscle of the guinea-pig duodenum, jejunum, ileum, caecum and rectum. For the measurement of the passive membrane properties, the longitudinal muscles of the jejunum and the rectum were chosen, and compared with those of the longitudinal muscle of the caecum (taenia coli), which have been studied by Kuriyama & Tomita (1965) and Tomita (1966*a*, *b*). The results indicate that the individual smooth muscle cells of the whole intestine have very similar membrane properties. However, differences are observed in the conduction velocity and the chronaxie; these may be due to differences in the size and shape of the functional muscle bundles, in their turn caused by differences in the arrangements of the cells.

METHODS

The smooth muscle of isolated intestine of the guinea-pig was used. Guinea-pigs weighing 250–350 g were stunned and bled. The intestine was removed from the abdomen. For the study of the longitudinal muscle of the duodenum, jejunum, ileum, caecum and rectum, small pieces were dissected gently to a size of about 10 × 5 mm. For the study of the circular muscle, the longitudinal muscle layer was ablated from the intestinal tube. This required practice: if it was too gentle, Auerbach's plexus remained attached to the circular muscle, and, if it was too rough, the circular muscle was damaged, which resulted in a low membrane potential and low spike amplitude. Auerbach's plexus could easily be located under a dissecting microscope. The ablation of the longitudinal muscle from the duodenum and region of the rectocaecal junction was more difficult. The pieces were placed in an organ-bath through which solution flowed continuously at a constant temperature of 35–36° C.

A modified Krebs solution of the following composition was used (mM): Na^+ 137·4; K^+ 5·9; Ca^{2+} 2·5; Mg^{2+} 1·2; Cl^- 134·0; HCO^-_3 15·5; $H_2PO^-_4$ 1·2 and glucose 11·5; equilibrated with 97 % O_2 + 3 % CO_2.

For extracellular stimulation of the tissue two different arrangements were used: (i) Two Ag–AgCl electrodes were used (diameter 1 mm), one electrode being placed on the tissue and the other at a distance of 3–4 cm from the tissue, (ii) two Ag–AgCl electrode rings were embedded 2 mm apart in insulating Araldite and the tissue was pulled through the rings.

For intracellular stimulation a single microelectrode was used for electrical recording as well as for stimulating by means of the Wheatstone bridge method as described previously by Kuriyama & Tomita (1965). The resistance of micro-electrodes filled with 3 M-KCl varied between 40 and 80 MΩ.

RESULTS

Membrane potentials. Table 1 shows the membrane potentials measured from longitudinal and circular muscles of duodenum, jejunum, ileum, caecum and rectum. As mentioned under Methods, the complete ablation of the longitudinal muscle from the duodenum was rather difficult, therefore the membrane potential of the circular muscle of the duodenum was not measured. In various parts of the intestine the membrane potential varied from 54 to 60 mV and was slightly higher in the circular muscles, though this was not significant. In the caecum, in which the circular muscle cells could be penetrated without the ablation of the longitudinal muscle, the membrane potential of the circular muscle was still slightly higher. In the ablated longitudinal muscle of ileum, the mean value was 50·4 mV (S.D. = ± 5·8, n = 30), i.e. slightly lower than that of the intact one. Although, therefore, the circular muscle might be slightly damaged by the ablation procedure it had, nevertheless, a higher membrane potential.

Spontaneous membrane activity. Table 2 shows the amplitude, the half duration and the maximal rate of rise of the spontaneously generated spikes recorded from various parts of the intestine. For these measurements only monophasic spikes with high amplitude, and overshoot potentials were chosen. Therefore, the values do not indicate the over-all values.

An overshoot potential was recorded from all parts of the intestine, sometimes exceeding 15 mV. Typical patterns of the membrane activity of the longitudinal muscle of the jejunum are shown in Fig. 1.

TABLE 1. Membrane potentials recorded from various parts of the intestine

	Duodenum	Jejunum	Ileum	Caecum	Rectum	Ileo-caecal junction area	Caeco-rectal junction area
			Longitudinal muscle				
M.P. (mV)	54·3 $n = 115$	56·0 $n = 120$	54·0 $n = 120$	55·0* $n = 240$	55·6 $n = 120$	54·6 $n = 60$	55·5 $n = 30$
S.D.	4·3	4·1	4·3	—	5·0	4·2	4·8
S.E.	0·28	0·26	0·28	0·5	0·32	0·38	0·62
			Circular muscle				
M.P. (mV)	—	60·4 $n = 60$	57·4 $n = 120$	57·8 $n = 120$	56·6 $n = 60$	—	—
S.D.	—	6·2	5·9	5·8	5·9	—	—
S.E.	—	0·56	0·39	0·37	0·54	—	—

M.P. = membrane potential. * Bülbring & Kuriyama (1963)

TABLE 2. Parameters of spontaneous discharges recorded from the intestine

	Longitudinal muscle ($n = 30$)					Circular muscle ($n = 30$)
	Duodenum	Jejunum	Ileum	Caecum	Rectum	Caecum
Amplitude (mV)	62·5	64.2	61·8	63·2	60·8	64·3
S.D.	3·2	3·1	4·1	3·0	3·4	3·8
Half duration (msec)	6·2	5·4	6·6	7·2	7·6	7·1
S.D.	0·4	0·4	0·6	0·5	0·5	0·4
Maximum rate of rise (V/sec)	16·1	18·2	14·0	11·6	12·2	13·4
S.D.	1·3	1·5	1·6	1·4	1·3	1·2

Monophasic spikes of high amplitude with overshoot were chosen. Therefore, the values of the parameters described in the table do not indicate the overall mean values.

The spikes appeared either as a burst of discharge between silent periods (*a*), or the spike discharges appeared on a slow potential (*b*), as rhythmic discharge of spikes with slow components (*c*) or the spikes were followed by after-hyperpolarization (*d*). No characteristic pattern of the membrane activity was recorded from any part of the intestine. However, the pattern of the membrane activity was closely related to the degree of stretch of the tissue, and to the time after the immersion of the tissue in the Krebs solution. When it was exposed to the Krebs solution, at first, the membrane activity consisted of bursts of discharges, later it became continuous without silent periods, and often the amplitude of the slow potential increased and the maximum rate of rise decreased. When the

tissue was stretched, these changes of spike configuration appeared earlier. The main reason for these changes of the spike configuration was not due to fatigue of the tissue, because the membrane activity of the longitudinal muscle of the jejunum was completely changed by treatment with atropine (10^{-5}/ml.). Treatment with atropine lowered the spike frequency, slightly raised the membrane potential, and increased the amplitude and the maximum rate of rise of the spikes. Therefore, the deterioration of the membrane activity might be partly due to the spontaneous release of acetylcholine from the tissue. More detail will be described in the following paper (Kuriyama, Osa & Toida, 1967*b*, especially figs. 1, 2 and 4.

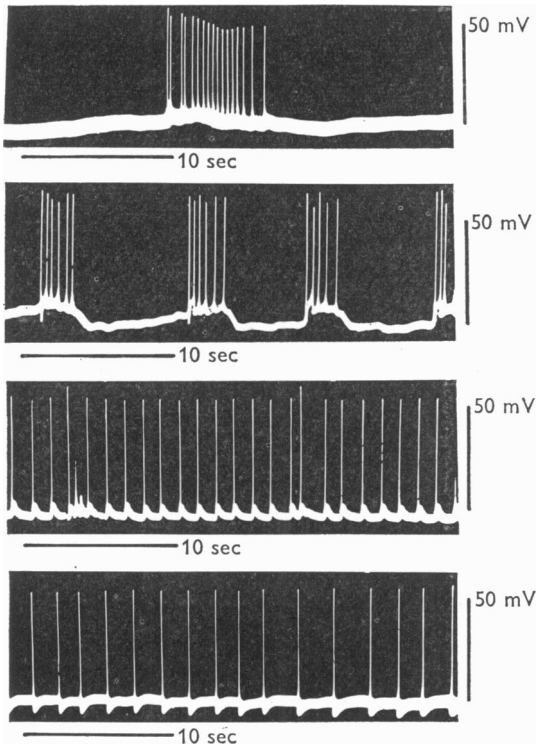

Fig. 1. Various patterns of the spontaneous generated spikes recorded from the smooth muscle cells of jejunum.

Figure 2 shows the membrane activities recorded from an intact and an ablated longitudinal muscle of the jejunum. They differed in the spike configuration. The ablated muscle never generated a burst of discharges, and the spike amplitudes and frequency were more irregular than in the intact muscle. The amplitudes of the slow depolarization and of the delayed

hyperpolarization following the electrically evoked spike were reduced in the ablated muscle (*c*, *d*), especially that of the slow depolarization.

The circular muscles were quiescent and a spike appeared only in response to electrical or chemical stimulation. Exceptionally, however, spontaneous discharge was seen in the caecum. Figure 3 shows the membrane activity recorded from the longitudinal and the circular muscle of the caecum, generated spontaneously as well as evoked by electrical stimulation. Since, in the caecum, the circular muscle is on the serosal surface, it was excised without damage. It never showed periodical bursts of discharges.

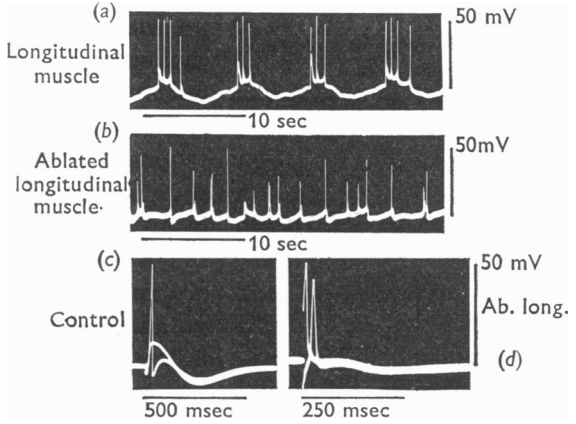

Fig. 2. Spontaneous discharges (*a*, *b*) and electrically evoked membrane activities (*c*, *d*) recorded from the intact (*a*, *c*) and ablated (*b*, *d*) longitudinal muscles of jejunum.

The spontaneously generated slow potential changes. The spontaneous generation of slow potential changes in smooth muscle has been observed by many investigators in many species and tissues (intestinal smooth muscle: Bülbring, 1957; Bülbring, Burnstock & Holman, 1958; Prosser, 1962; Bortoff, 1961; Gillespie, 1962; Bülbring & Kuriyama, 1963; uterus: Marshall, 1962; Goto, Kuriyama & Abe, 1961; Kuriyama, 1962; Csapo & Kuriyama, 1963; ureter: Kobayashi & Irisawa, 1964; Kuriyama *et al.* 1967*a*).

In early experiments, Bozler (1942) observed slow oscillatory potentials of the ureter using extracellular recording. He thought that these might be generator potentials. Figure 4 shows slow potential changes of the intestinal smooth muscle cell membrane; *a*, *b* and *c* were recorded from the jejunums of different animals. The duration of the spontaneous slow potentials was longer than 5 sec and often exceeded 10 sec. The amplitude of the slow potential successively increased and, when it reached the firing level, a burst of discharges was triggered (*a*). In some preparations, the duration

of the slow potential was 1–2 sec and also triggered spikes (*b*). When short pulses (0·5 msec) were successively applied (*c*) during the silent period (0·5 c/s) slow depolarization with or without triggering the spike, and delayed hyperpolarization, could be evoked. After the cessation of the

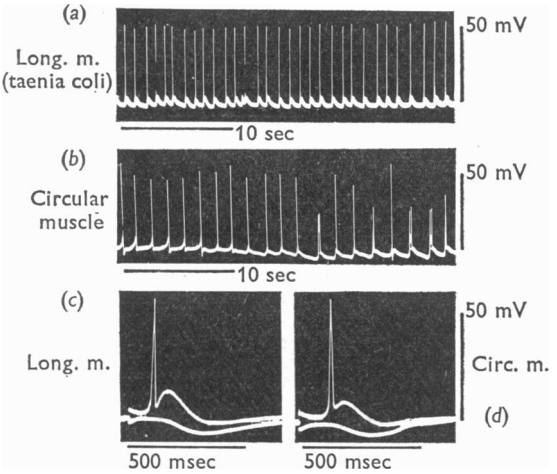

Fig. 3. Spontaneous discharges (*a*, *b*) and electrically evoked membrane activities (*c*, *d*) recorded from the longitudinal (*a*, *c*) and circular (*b*, *d*) muscle of colon.

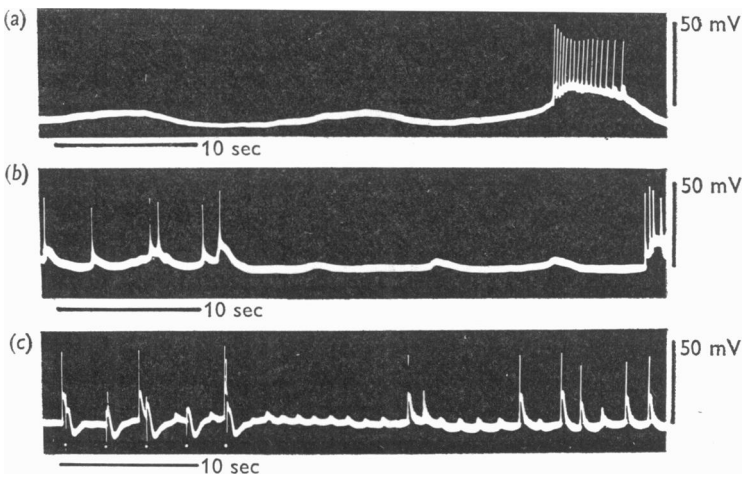

Fig. 4. Relation between the slow potentials and the spikes recorded from the longitudinal muscle of jejunum. (*a*) The slow potential appeared with a duration of about 10 sec and triggered the burst of discharges on it. (*b*) The slow potentials appeared more frequently but of shorter duration. (*c*) The slow potentials appeared after electrical stimulation (0·2 c/s) and after the cessation of the stimulation generated repetitively slow potentials.

stimulation, within 1 sec spontaneous small slow potentials were generated successively and, at a certain amplitude, triggered a spike (*c*).

If these slow potential changes were related to the automaticity of the smooth muscle cells, there would be two types of generator potentials in the smooth muscle, the first being the above slow potential and the second one being the prepotential which is analogous to that of the cardiac pacemaker cell. The former might be related to nervous activity and the latter might be myogenic, because, by treatment with tetrodotoxin (10^{-7} g/ml.), the first type is abolished (see subsequent paper (Kuriyama *et al.* 1967*b*), figs. 4 and 7) but the second type is not.

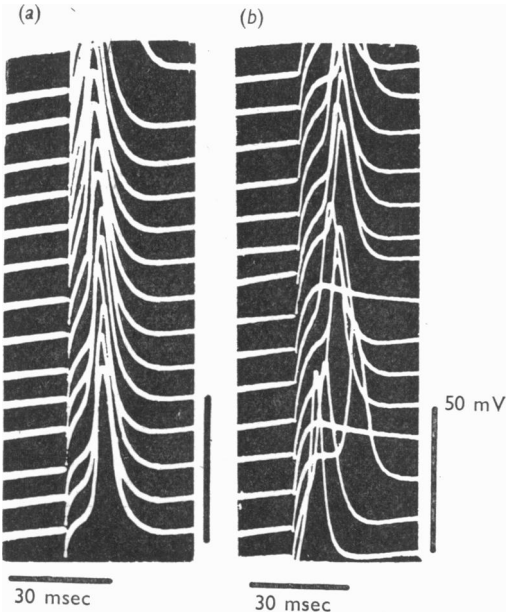

Fig. 5. The effect of repetitive stimulation on the longitudinal muscle of jejunum. (*a*) A group of almost synchronized spike generations. (*b*) Responses with various latencies. For fuller details see text.

Extracellular short pulse stimulation. It was difficult to measure the conduction velocity in the longitudinal muscle because of the spontaneous discharges and because, even during a quiescent period, the same intensities of electrical stimulation generated spikes with different latencies.

Figure 5 shows the effect of the repetitive stimulation (5 msec pulse, 1 c/s) on the longitudinal muscle of the jejunum, the stimulating and recording electrodes being fixed in the same place (1 mm apart). The shapes of the spike were not always the same. Especially when the extra-

cellular stimulation triggered the slow potential changes, the latency of the triggered spikes varied remarkably (*b*).

Conduction velocities of the excitation were measured from the longitudinal and circular muscles of the jejunum and the rectum. A typical pattern is demonstrated graphically in Fig. 6. The stimulating electrode was fixed in a certain place and the micro-electrode was moved along the same line to various distances from the stimulating electrode. When the

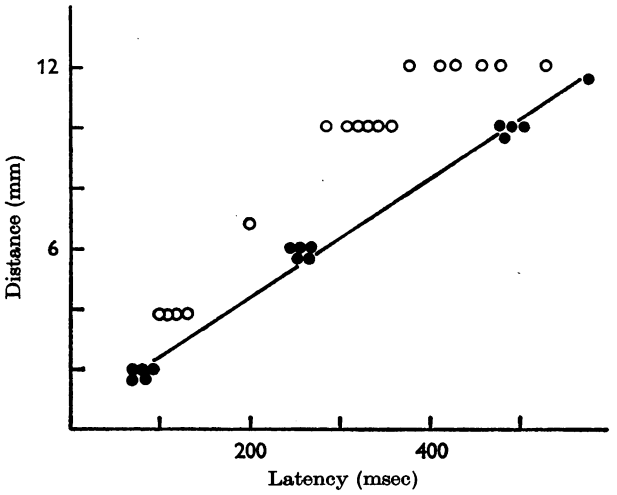

Fig. 6. Relation between the latencies of the spike and the distances from the site of stimulation in the longitudinal muscle of jejunum. Open circles represent the values measured before treatment with tetrodotoxin (10^{-7} g/ml.), filled circles represent the values after treatment. Continuous line indicates the conduction velocity.

micro-electrode was impaled in the cell which was placed 12 mm from the stimulating electrode, the latencies varied from 370 to 530 msec. However, when tetrodotoxin (10^{-7} g/ml.) was added to the solution the ranges of variation of the measured latency became very narrow, which enabled us to measure the constant value of the conduction velocity. The slow depolarizations elicited by extracellular electrical stimulation might be caused by nervous excitation, because tetrodotoxin (10^{-7} g/ml.) abolished the generation of the slow potential but it had no effect on the membrane properties such as membrane potential, the passive properties of the membrane and the spike amplitude (Kuriyama, Osa & Toida 1967*c*).

The conduction velocity of the tissues was always faster in the absence of tetrodotoxin. This observation agreed with that resulting from a study of the mechanical contraction in the whole small intestine by Yokoyama & Greven (1966). The mean values of the conduction velocities recorded from

the longitudinal muscle of the jejunum and rectum were 3·2 cm/sec and 4·8 cm/sec respectively, and in the presence of 10^{-7} g/ml. tetrodotoxin they were 2·1 cm/sec and 4·0 cm/sec respectively.

The intensity–duration relation in triggering the spike was also investigated in the presence of tetrodotoxin (10^{-7} g/ml.). The chronaxie of the jejunum was 2–5 msec and the variation of the measured values was small. In the rectum, the chronaxie varied from 5 msec to 18 msec. The chronaxie of the longitudinal muscle of the caecum (taenia coli) was about 20 msec (Tomita, 1966*a*). Compared with the taenia coli the jejunum had a very short chronaxie.

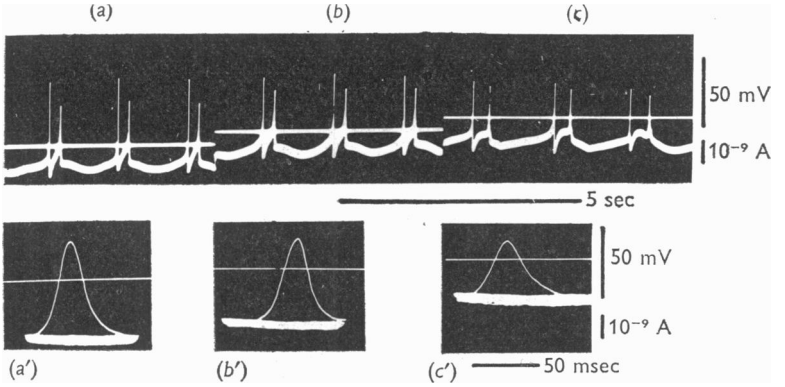

Fig. 7. Effects of intracellular polarization on the spontaneous discharges recorded from the longitudinal muscle of jejunum. Continuous lines indicate the level of applied current. Top: spikes are recorded with slow movement of the film. Bottom: faster records of the spikes. For detailed description see text. *a*, *a*', during hyperpolarization; *b*, *b*' control; *c*, *c*' during depolarization.

Intracellular stimulation. In the taenia coli and the ureter, intracellular polarization modified the amplitude and the maximum rate of rise of the spike. Similar effects could be seen on the rectum and the jejunum, i.e. inward current (10^{-9}–10^{-10}A) hyperpolarized the membrane and increased the spike amplitude and the maximum rate of rise, while outward current had the reverse effect. As shown in Fig. 7, in the longitudinal muscle of the jejunum the cell generated spontaneous twin spikes, the second spike appearing on the slow depolarization which followed the first spike. The second spike was probably triggered by the slow depolarization, for the spike wiped out the falling phase. In the control, the amplitude of the first spike was always higher than that of the second one. Intracellular polarization predominantly influenced the spike component more than the slow potential. Consequently, when depolarizing current was applied to the tissue (5×10^{-10}A), the amplitudes of the first and second spikes became

similar, and the interval between the first and the second spikes became longer owing to the slight reduction of the amplitude of the slow potential. This effect of depolarizing current was slightly different from that observed in the taenia. However, intracellularly applied current did not modify the spontaneous spike frequency. Changes of the maximum rate of rise of the spike and the spike amplitude are also illustrated in Fig. 9. The relation between the spike amplitude and the maximum rate of rise of the spike during intracellular polarization in rectum and jejunum agreed with that observed in the taenia coli and in the ureter (Kuriyama & Tomita, 1965; Kuriyama *et al.* 1967*a*).

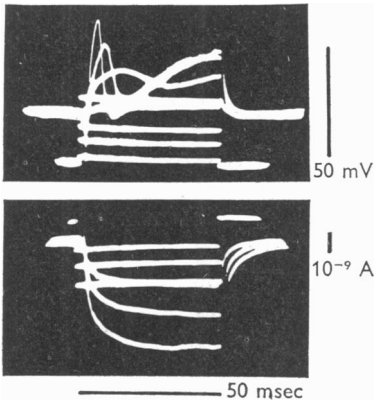

Fig. 8. Effects of intracellular polarization on a smooth muscle cell of jejunum. Top: increased depolarizing currents trigger the local potential, graded response and spike. Bottom: effects of hyperpolarizing current on the resting membrane.

Intracellular depolarizing current could trigger a full size spike, but mostly gave graded responses. Figure 8 shows the typical spike triggered from the longitudinal muscle of the jejunum. A typical feature of the triggered spike appeared as an undershoot potential during the falling phase which might be due to the increased potassium conductance.

Success in triggering a spike might be a criterion by which to judge a good penetration and adequate intracellular stimulation without damaging the cell. Therefore, the passive membrane properties were only measured in these cells by applying weak hyperpolarizing currents (10^{-10}–10^{-9}A).

Effective membrane resistance, capacitance and time constant were measured from the voltage change of the membrane caused by intracellular polarization. The mean values of the effective resistance was 45 MΩ and the time constant was 3·5 msec in the longitudinal muscle of the jejunum, whilst the effective resistance was 40 MΩ and the time constant was 3·5 msec in the longitudinal muscle of the rectum. The values

obtained from the jejunum and the rectum were nearly the same and these values also agreed with the values observed in the taenia coli.

The specific resistance and capacitance were calculated with various assumptions which were the same as those described by Kuriyama & Tomita (1965) and Kuriyama *et al.* (1967*a*), i.e. employing, with some modification, the equations used for the crustacean muscle fibre (Fatt & Ginsborg, 1958). The calculated values of the characteristic constants of the membranes in the jejunum and the rectum are given in Table 3, including, for comparison, the calculated values of the taenia coli.

TABLE 3. Various characteristic constants of intestinal smooth-muscle membranes

	Intracellular polarization				
	R_e (MΩ)	τ_m (msec)	R_m (Ω cm²)	C_m (μF/cm²)	λ (mm)
Jejunum	45	3–4	650	4·5	0·19
Rectum	40	3–4	600	5	0·18
Taenia coli	40	3–4	600	5	0·18

R_e = effective membrane resistance, R_m = specific resistance, C_m = specific membrane capacitance, λ = space constant, and τ_m = time constant.

Equations used: $(2\lambda/\lambda) \coth (l/2l) = (\pi d^2 R/R_i l)$,

$$R_m = (\pi^2 d^3 R^2/R_i) \tanh^2 (l/2\lambda)$$

(Fatt & Ginsborg, 1958; Kuriyama & Tomita, 1965).

Assumptions: R_i (internal specific resistance), 250 Ω cm; l (muscle cell length), 100 μ; d (muscle cell diameter), 5 μ.

Extracellular long pulse stimulation. It was interesting that the membrane characteristic constants of the jejunum, the caecum and the rectum were nearly the same, but that the conduction velocities and the chronaxies differed for various regions of the intestine. The conduction velocity and the chronaxie calculated from the equations, employed by Hodgkin & Rushton (1946) and Katz (1948), using the time constant of the membrane measured by the extracellular polarizing method, agreed with the measured values (taenia coli: Tomita, 1966*a*; ureter: Kuriyama, Osa & Toida 1967*a*). Therefore, the responses to extracellular long pulse stimulation were examined.

Figure 9 shows the effects of extracellular polarization on the longitudinal smooth muscles of the jejunum (*b*, *c*), and that of intracellular polarization to a single cell (*a*).

Extracellular application of depolarizing currents triggered the spike. Increased intensities reduced the latencies and slightly enhanced the spike amplitude. An undershoot potential (after-hyperpolarization) during the falling phase was observed (*b*). When hyperpolarizing currents were applied a weak current evoked an abortive potential with a long latency

but higher intensities reduced the amplitude of the abortive potentials further, though shortened the latency as observed in taenia coli (Tomita, 1966*a*). Hyperpolarizing current of strong intensity triggered the off-response (anodal break excitation) with a full-size spike which was followed by an after-hyperpolarization (*c*). Such an abortive spike potential and an anodal break excitation were not always observed, but only an electro-

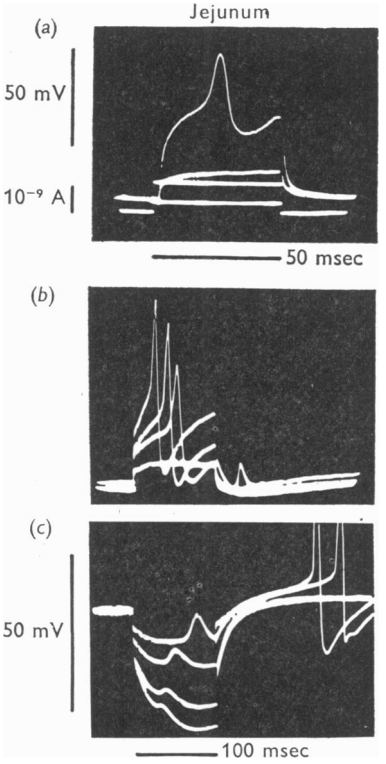

Fig. 9. Effects of intracellular (*a*) and extracellular (*b*, *c*) polarizations on the smooth muscle cells of jejunum. Top: intracellular depolarizing current triggers the spike. Middle: extracellular depolarizing current evoked the spike. Bottom: extracellular hyperpolarizing currents evoked graded responses and anodal break excitations. For detailed description see text.

tonic potential. The time constant of the longitudinal muscle of the jejunum measured by this method varied from 14 to 20 msec, and in the longitudinal muscle of the rectum it varied from 20 to 100 msec. The values were smaller than those observed in the taenia coli in modified Krebs solution as well as in hyperosmotic solution (twice the normal osmolarity), and the jejunum had a much smaller value than the rectum. Wide variations of the time constant measured in the rectum might be due to the inhomogeneous distribution of the longitudinal muscles around the

alimentary tract. In contrast to the different values found by extracellular polarization of the tissues, the time constants measured by the intracellular polarizing method were the same in all tissues.

DISCUSSION

The longitudinal and circular muscles of all the regions of the intestine had membrane potentials below 60 mV and the measured effective membrane resistances were higher than those of skeletal and cardiac muscle. The low membrane potentials might be explained by the low K permeability and a relatively high ratio of Na permeability to K permeability (Casteels & Kuriyama, 1965; Tomita 1966*b*). The slightly higher membrane potentials of the circular muscles compared with those of the longitudinal muscles might be related to the lack of the automaticity of the membrane, since no spontaneous activity was observed in the circular muscles.

The patterns of the spike discharges recorded from the various longitudinal muscles were nearly the same. In general, long silent periods alternated with long trains of discharges. In some cells slow depolarizations appeared without spikes, and in some cells spikes appeared as train discharges without the generation of a slow potential. Comparing the membrane activities recorded from the intact longitudinal muscle with the ablated muscle, the amplitude and the pattern of the spike discharges are more regular in the intact muscle even though the membrane potentials recorded from both tissues are nearly the same. The destruction of Auerbach's plexus in ablated longitudinal muscle may cause the removal of an intrinsic reflex which modifies membrane activity in intact tissue.

The conduction velocity of the excitation wave in the intestine has been measured by many investigators (in cat small intestine, longitudinal direction: 4·1 cm/sec, Prosser & Sperelakis, 1956; longitudinal muscle: 7·6 cm/sec, circular muscle: 4 cm/sec, Nagai & Prosser, 1963*a*; rabbit longitudinal muscle: 6–7 cm/sec, Yokoyama & Greven, 1966; guinea-pig taenia coli: 6·7–8·8 cm/sec, Bülbring, *et al.* 1958; 7·3 cm/sec, Tomita 1966*a*: 5·6 cm/sec, Kuriyama *et al.* 1967*a*). The observed values never exceed 10 cm/sec.

The present results show slower conduction velocity in the jejunum than in the taenia coli which is similar to the rectum. In the presence of tetrodotoxin or atropine the conduction velocities are lower and the variation less. Therefore a nervous factor influencing the conduction velocity cannot be ruled out.

The chronaxie of smooth muscle has also been measured by many investigators (cat intestinal circular muscle: 50 msec, Barr, 1961; guinea-pig taenia coli: 19 msec, Tomita, 1966*a*). From previous work it appears that the value for chronaxie is higher when tension development is taken

as indicator than when it is measured from the spike generation. In the guinea-pig vas deferens, chronaxie is 10 msec (Burnstock & Holman, 1961) and that of ureter is 30–60 msec (Kuriyama *et al.* 1967*a*) instead of 50 msec for cat ureter and 200 msec for rabbit ureter (Bozler, 1938). The chronaxies measured in this experiment show lower values in the jejunum (2–5 msec) than the taenia coli (19 msec).

It has already been suggested by Bozler (1948) that the visceral smooth muscle behaves as a functional syncytium and many later observations support this hypothesis (Burnstock & Straub, 1958; Burnstock & Prosser, 1960; Prosser, Burnstock & Kahn, 1960; Bülbring *et al.* 1958). However, it is difficult to obtain direct evidence of an electrotonic potential spreading from cell to cell (Sperelakis & Tarr, 1965; Kuriyama & Tomita 1965). The discrepancy between the results obtained by extracellular and intracellular stimulation might be explained by a three-dimensional spread of the electrical current (Tomita, 1966*a*, *b*).

The three-dimensional connexions of the cells in the taenia coli, called nexus, have been observed by the electron-microscopic technique (Dewey & Barr, 1964). Bülbring (1955) also reported that many fibres, connected in series and in parallel, are aggregated in bundles of large diameter. The cells within a bundle are closely packed, the space between cell membranes being from 200 to 300 Å. In the guinea-pig taenia coli the bundles might act as functional units of 30 μ diameter, with a space constant of 1·6 mm.

The conception of a functional bundle was also suggested by Burnstock & Prosser (1960) and Nagai & Prosser (1963*b*), i.e. when the tissue is dissected with a diameter of less than 100 μ, or if the stimulating electrode has a diameter of less than 50 μ, the generation of the spike becomes graded. In smooth muscle, conduction may be carried by a local current in the functional bundle with cable properties. In the taenia the measured conduction velocity is 5–8 cm/sec and in the ureter it is 6·3 cm/sec. The low conduction velocities in the jejunum (3·2 cm/sec) and rectum (4·8 cm/sec) which in fact, are even lower in the presence of tetrodotoxin (2·1 cm/sec and 4·0 cm/sec respectively), might be due to the small radius of the functional bundle, rather than to a large parallel capacitance, since the longitudinal muscle of the jejunum is very thin.

The theoretical value for the chronaxie was calculated using the same method as in the previous paper (Kuriyama *et al.* 1967*a*) to be equal to 0·22 τ.

Tomita (1966*a*) compared the measured chronaxie (20 msec) and the calculated value (11–22 msec) in the taenia coli and both values agreed well. In the ureter, Kuriyama *et al.* (1967*a*) also found similar values with both methods (measured chronaxie was 30–60 msec, calculated value was 44–97 msec).

In the jejunum the time constant is small (14–20 msec), therefore, the calculated chronaxie (0·22 τ) is 3·1–4·4 msec and this value is in good agreement with the value measured experimentally (2–5 msec). In the rectum the chronaxies measured by experiment (5–18 msec) and calculated theoretically (4·4–22 msec) also agreed well.

The above results obtained from the intestinal smooth muscle might be summarized as follows: the individual longitudinal muscle cells of the jejunum, caecum and rectum have similar passive membrane properties; however, the number and the arrangement of the cells constituting the functional bundles are different in various regions of the intestine, and this might cause the differences of the time constants measured by extracellular stimulation and of chronaxies and conduction velocities in the different regions of the intestine.

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