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MEMBRANE PROPERTIES AND INHIBITORY INNERVATION OF THE CIRCULAR MUSCLE CELLS OF GUINEA-PIG CAECUM

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SUMMARY

1. The membrane properties of the circular muscle cells of guinea-pig caecum and nervous factors influencing the muscle activity were studied with micro-electrodes using partition and field stimulating methods.

2. The mean membrane potential was −52 mV. Spontaneous discharges appeared as regular bursts between silent periods, as regular spikes without silent period, or as regular slow potential changes with superimposed spikes.

3. Spontaneous spikes with overshoot were frequently observed. The mean maximum rate of rise was 5·2 V/sec. The mean conduction velocity of evoked spikes was 5·4 cm/sec.

4. The amplitude of the electrotonic potential was linearly proportional to the current applied by the partition stimulating method. The spatial decay of the electrotonic potential along the tissue was exponential, with a mean length constant of 1·7 mm.

5. The time constants of the membrane calculated from the electrotonic potential, and from the conduction velocity, length constant and time course of the foot of the spike were about 200 and 100 msec respectively. These results indicate that the circular muscle of guinea-pig caecum possesses cable like properties.

6. Field stimulation (0·3 msec pulse duration) to the circular muscle evoked three different responses successively, i.e. initial depolarization (initial excitatory junction potential) with or without spike, hyperpolarization (inhibitory junction potential) and delayed depolarization (delayed excitatory junction potential) with or without spikes.

7. These three different potential changes were completely blocked by treatment with tetrodotoxin (5×10^{-6} g/ml.), and both the initial and late excitatory junction potentials were blocked by treatment with atropine (5×10^{-5} g/ml.).

8. The distribution of inhibitory nerves in the circular muscle cells was investigated. The results indicate that inhibitory nerves arise from Auerbach's plexus situated just beneath the taenia coli and the nerve branches spread over the whole distance from one taenia coli to the next along the circular muscle cells, a width of about 3 mm.

9. The mean conduction velocity of excitation of the inhibitory nerves was 16·0 cm/sec. Hexamethonium, in a concentration of 5×10^{-6} g/ml. depolarized the circular muscle membrane and lowered the rate of rise and fall of spike, but did not block the generation of inhibitory junction potentials.

INTRODUCTION

The muscular wall of the guinea-pig caecum is composed of (*a*) a circular layer which covers the whole surface and (*b*) three discrete bundles of longitudinal muscle. Although much work has been done on the physiology, pharmacology and innervation of the longitudinal muscle of guinea-pig caecum, i.e. the 'taenia coli', little is known of the electrical properties and innervation of the circular muscle. Abe & Tomita (1968) systematically analysed the cable properties of the taenia coli by intracellular recording of electrotonic potentials produced by square current pulses applied with external electrodes. They concluded that the cable equation originally derived for nerve and skeletal muscle is applicable for analysis of the membrane properties of the taenia coli. As well as taenia coli, the passive electrical properties of various other visceral smooth muscles, namely, many regions of alimentary canal, ureter, urinary bladder, uterus and vas deferens, have been investigated extensively in the last few years (see reviews, by Holman, 1968; Kuriyama, 1968; Tomita, 1970).

Nervous factors have a marked influence on the motility of visceral smooth muscle cells. For example, in the alimentary canal, cholinergic excitatory nerves, adrenergic inhibitory nerves, non-adrenergic inhibitory nerves ('purinergic' nerves) and non-cholinergic excitatory nerves are thought to be distributed within the muscle layers (see review by Burnstock, 1972).

The aims of the present experiments were to investigate the membrane properties of the smooth muscle cells of the circular muscle of guinea-pig caecum and to determine whether or not the tissue possesses cable properties. In addition the nervous factors influencing the muscle membrane activity were investigated.

The results led to the conclusion that the smooth muscle cells of the circular muscle of guinea-pig caecum show similar electrical activity to that observed in other regions of the alimentary canal, and the tissue possesses cable properties. It was also concluded that the tissue contains

at least three nervous elements, which evoke an early excitatory junction potential, an inhibitory junction potential and a late excitatory junction potential.

A preliminary report of this work has already been given to the Physiological Society (Ito & Kuriyama, 1972).

METHODS

Guinea-pigs of either sex, weighing 180–200 g, were used. The animals were stunned and bled. The caecum was dissected from the abdomen and was opened by an incision from the ileocaecal junction to the terminal region of caecum along the taenia coli. Two types of isolated preparation of the circular muscle were used. To analyse the electrical membrane properties of the circular muscle cells, a piece was dissected along the circular direction and the mucosal layer was removed carefully under a binocular microscope. The tissue was cut into segments, 1·5–3·0 cm in length and 0·5–0·8 cm in width. The tissue was then mounted in an organ bath through which a solution at a temperature of 30–32° C flowed continuously driven by a thermostatically controlled pump. The method of application of square current pulses was based on the partition stimulating method described by Abe & Tomita (1968).

To investigate the nervous elements, a piece of the circular muscle preparation of about 2·5–3·5 cm in length and 0·5 cm in width which contained two taenia coli bundles at each end (see inset to Fig. 7, left) was pinned to a rubber plate. The organ bath was divided, by two thin insulating plastic partitions, into three compartments, the central one for recording and the other two for stimulating. Field stimulation was applied to the tissue through a silver needle electrode coated except for the tip (diameter 0·3 mm) with Araldite (CIBA Ltd), and an indifferent electrode (diameter 1 mm) placed at a distance of 1·0–1·5 cm from the tissue. Intracellular recordings were made with micro-electrodes filled with 3 M-KCl. The resistance of the micro-electrode was between 50 and 80 MΩ. The distance of the micro-electrode from the stimulating electrode was measured through a binocular microscope.

A modified Krebs solution (hereafter referred to as Krebs) 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_2–3 % CO_2.

RESULTS

Membrane activity of the circular muscle of guinea-pig caecum

The membrane potential of circular muscle of guinea-pig caecum ranged between −48 and −65 mV. The mean value of the membrane potential was −52 mV (S.D. = ±4·5 mV, n = 56). This value agreed with that of longitudinal muscle, i.e. the taenia coli. In the circular muscle spontaneous electrical activity could be observed. The spike discharges were classified into three different types, i.e. (i) regular bursts of short trains of spikes (3–5/sec and lasting about 10–25 sec) alternating with silent periods (lasting 6–25 sec, Fig. 11); (ii) continuous regular spike discharges (2–4/sec) and (iii) regular slow depolarizing waves (25–60/min) with superimposed spikes (Fig. 1). However, spontaneous generation of

slow waves was rather rare in this tissue, and types i and ii were the usual patterns. The spontaneously generated spikes usually showed overshoot potentials, and the maximum amplitude of the spike observed was 65 mV at a membrane potential of −54 mV. Tetrodotoxin (5×10^{-6} g/ml.) did not abolish the spontaneous spike activity as shown in Fig. 6.

An action potential with overshoot also could be generated by outward current applied extracellularly. The mean values of the maximum rates of rise and fall of the spike evoked by electrical stimulation were 5·2 V/sec (S.D. = ±1·7, n = 10) and 5·6 V/sec (S.D. = 1·8, n = 10) respectively.

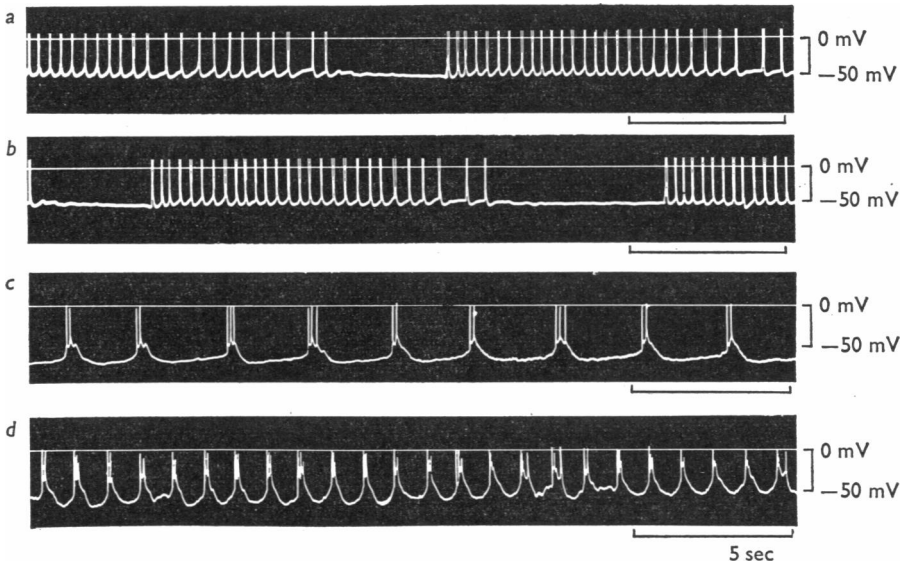

Fig. 1. Spontaneous electrical activity recorded from the circular muscle of guinea-pig caecum. *a* and *b*, regular bursts of short train discharges of spikes alternating with silent periods. *c* and *d*, regular slow waves with spikes of low (25/min) and high (60/min) frequency respectively.

These values of membrane potential, amplitude of the spike and maximum rates of rise and fall were nearly the same as those of the taenia coli (Bülbring & Kuriyama, 1963).

Characteristic constants of the circular muscle of guinea-pig caecum

In order to measure the various electrical properties of the circular muscle of guinea-pig caecum, square pulses of 1 sec duration were applied to the tissue by the partition stimulating method. Fig. 2 shows the current–voltage relationship recorded at three different distances from the stimulating electrode. A roughly linear relationship between the applied inward

current intensity and the amplitude of the electrotonic potential up to about 40 mV of hyperpolarization could be observed.

If the cable equations are applicable to this tissue, the relationship between the amplitude of electrotonic potentials plotted on a log scale and the distance from the stimulating electrode should be linear. In fact a linear relationship was observed in the circular muscle for any given

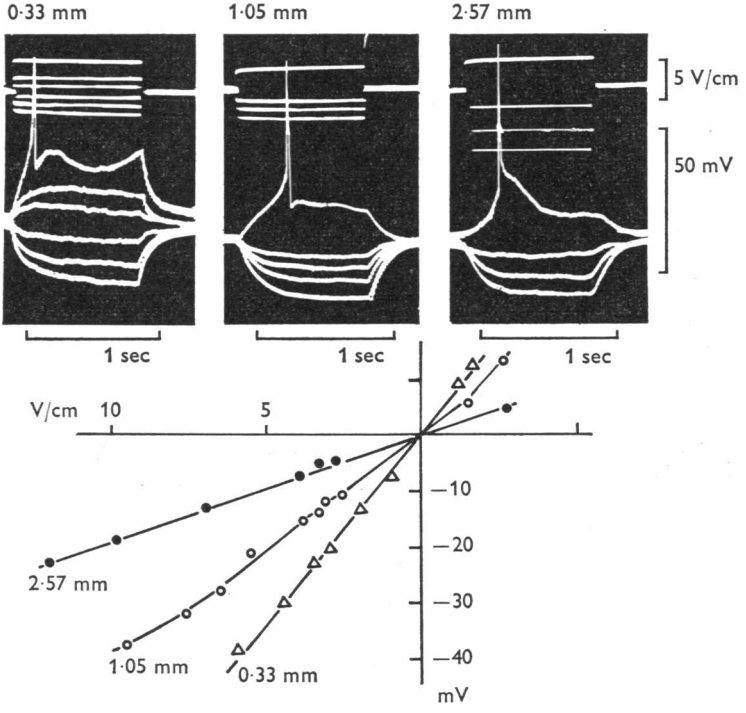

Fig. 2. Example of the current–voltage relation measured at three different distances from the stimulating partition (0·33, 1·05 and 2·57 mm). Electrotonic potentials and spikes evoked by in- and outward current pulses are also shown above. Ordinate: displacement of intracellularly recorded potential. Abscissa: current intensity expressed in terms of the potential field in the stimulating chamber.

current intensity. From the slope of the decay with distance, the length constant (λ) can be calculated. In Fig. 3, two examples are demonstrated. The mean value of the space constant, that is the distance at which the electrotonic potential decays to 1/e, was 1·7 mm (S.D. = $\pm$ 0·2, n = 6).

In order to measure the time constant of the membrane (τ_m), the time to reach half of the final steady amplitude of the electrotonic potential was plotted against distance from the stimulating electrode. A linear

relationship was found from four different preparations. From the cable equation, the slope can be expressed as $\tau_m/2\lambda$, where τ_m is the time constant of the membrane at erf 1 and λ is the length constant of the membrane. The time constant of the circular muscle membrane was found to be 205·8 msec (S.D. = ± 25·9, n = 4). An alternative method of calculating the time constant of the membrane (τ_m) is to make use of the cable equation

$$T = \frac{2\lambda^2}{\theta^2 (\tau_m + \sqrt{\tau_m{}^2 + 4\lambda^2/\theta^2})},$$

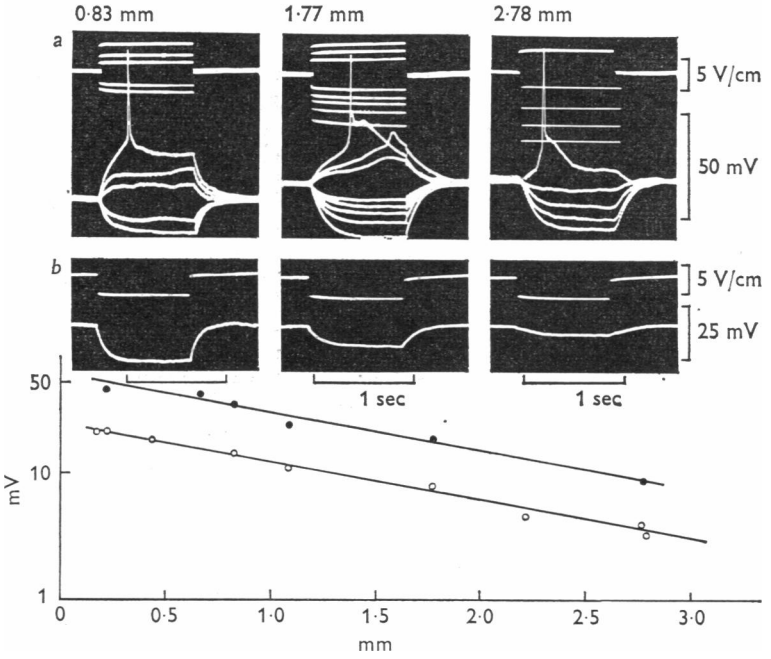

Fig. 3. Spatial decay of the amplitude of the electrotonic potential of the circular muscle of guinea-pig caecum. Points (○ and ●) were obtained from the steady-state amplitude of electrotonic potentials evoked by two different constant current intensities at different distances. Ordinate: the amplitude of the electrotonic potential (log scale). Abscissa: the distances from the stimulating electrode. *a* record of electrotonic potentials and spikes with overshoot (indicating satisfactory impalement with the micro-electrode). *b* electrotonic potentials evoked by a constant current intensity (3·2 V/cm) at three different distances.

where T is the time constant of the foot of the propagated spike, θ the conduction velocity and λ the length constant. To calculate the time constant of the membrane with the aid of this equation, the time constant of the foot of the propagated spike and the conduction velocity of the

spike were measured. The interval between the stimulus artifacts and the point of onset of the maximum rising phase of the action potential produced by a short outward current pulse (10 msec duration) was plotted against the distance from the stimulating electrode. Fig. 4 shows the relationship observed. The interval was found to increase linearly with the distance from the stimulating electrode. From the straight line the conduction velocity was calculated to be 8·3 cm/sec in the case of Fig. 4. The mean value of the conduction velocity of the action potential was 5·4 cm/sec (S.D. = ± 1·3, n = 6, range 4·3 – 8·3 cm/sec). The foot of the propagated spike was found to rise exponentially, and the time constant

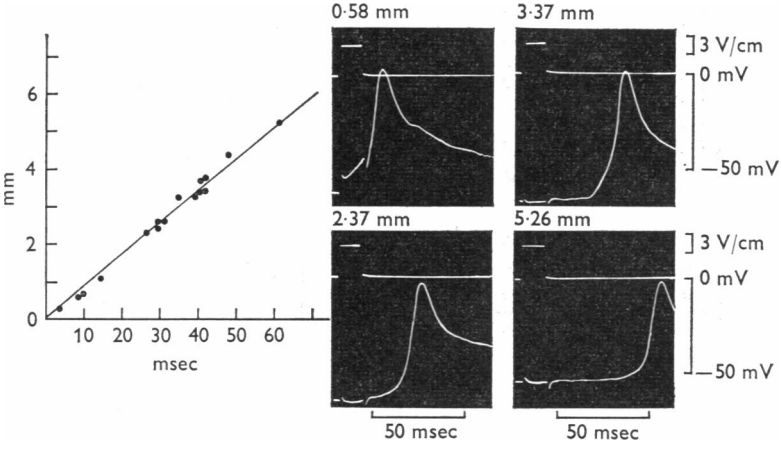

Fig. 4. Relationship between time from the stimulus to the maximum rate of rise of the action potential and distance of micro-electrode from the stimulating partition. Individual records from four cells at 0·58, 2·37, 3·37 and 5·26 mm are also shown. The stimulating pulse was of 10 msec duration.

of the foot was calculated as 10·5 msec (S.D. = ± 1·3, n = 5). Time constant of the membrane (τ_m), was therefore calculated as 110 msec. This value does not agree with the time constant obtained from the electrotonic potentials (about 200 msec).

Responses of the circular muscle of caecum to field stimulation

In response to the field stimulation (0·3 msec duration) a complex mixture of excitatory and inhibitory junction potentials was evoked in the circular muscle. These were (*a*) the initial excitatory junction potential (depolarization) with or without spike, (*b*) an inhibitory junction potential (hyperpolarization), and (*c*), a delayed excitatory junction potential (depolarization) again with or without spike. These responses resemble those of the

longitudinal smooth muscle of guinea-pig rectum to field stimulation (Ito & Kuriyama, 1971). However, all these potential changes did not appear consistently following field stimulation of the circular muscle, but as various combinations, i.e. (i) initial excitatory junction potential followed by both an inhibitory junction potential and late excitatory junction potential, (ii) inhibitory junction potential followed by late excitatory junction potential and (iii) only the inhibitory junction potential. Both of the excitatory junction potentials could cause the generation of spikes, but this again was not consistent.

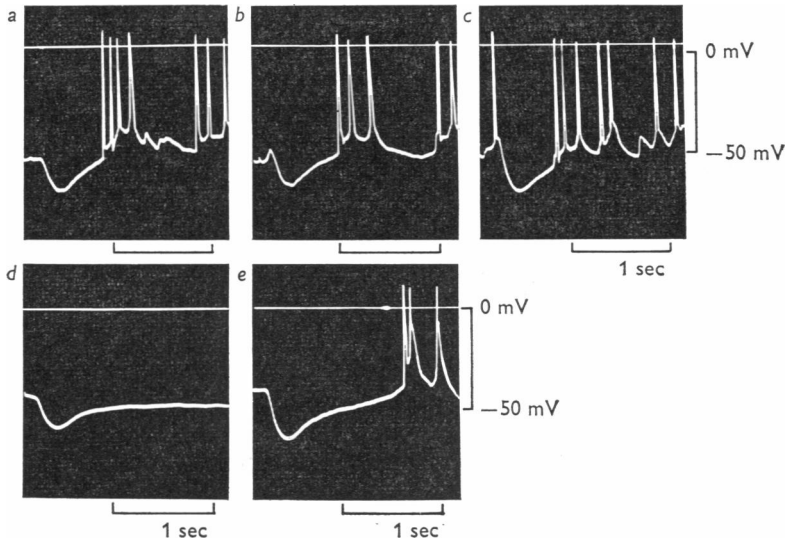

Fig. 5. The responses of the circular muscle of guinea-pig caecum to field stimulation (0·3 msec pulse duration). *a*, *b* and *c*: responses from the same cell evoked by field stimulation of increasing intensity, showing the initial excitatory junction potential, inhibitory junction potential and late excitatory junction potential with spikes. Note the irregular time course of the late excitatory junction potential. *d*: inhibitory junction potential recorded after 3 hr. *e*: inhibitory junction potential followed by spikes recorded after 3 hr with stronger intensity of stimulation than *d*.

These three different potential changes were completely blocked by treatment with tetrodotoxin (5×10^{-6} g/ml.), and both the initial and late excitatory junction potentials were blocked by treatment with atropine (5×10^{-5} g/ml.). Therefore at least two or three different nervous elements are thought to be distributed within the whole region of the circular muscle.

As described previously, the initial and late excitatory junction potentials were not observed consistently. On the other hand the inhibitory junction potential could be recorded from most cells in response to field

stimulation when impalement with the micro-electrode was satisfactory (as judged by a steady resting potential and a full size of the spontaneous spike). In Fig. 5, the records *a*, *b* and *c* were taken from the same cell and the intensity of the field stimulation was increased gradually from *a* to *c*. Only when the intensity of the field stimulation was increased was the initial excitatory junction potential elicited. The relative intensities needed to activate the inhibitory and late excitatory nerves were lower than for the initial excitatory nerve.

The inhibitory junction potential could be separated from the excitatory junction potentials by prolonged exposure of the tissue to Krebs solution (exceeding 3 hr) (*d* and *e* in Fig. 5), or by treatment with atropine as described previously. The minimum latency of the excitatory junction potential was 6 msec and it was 38 msec for the inhibitory junction potential. Neither increased intensity of stimulation nor decrease in distance from the stimulating electrode could reduce the latency of the inhibitory junction potential.

The most typical pattern of spontaneous spike discharges consisted of regular train discharges separated by silent periods. The effects of repetitive field stimulation applied during regular train discharges were examined. When stimulation was applied, inhibitory junction potentials followed by spikes were evoked even during the train discharges. However, the pattern of spontaneous electrical activity was changed from the control pattern (*c* in Fig. 6). When the effects of tetrodotoxin (5×10^{-6} g/ml.) on the inhibitory junction potentials and spontaneous spike activity were tested, not only was the inhibitory junction potential blocked as shown in Fig. 6(*d* and *e*) but also the pattern of the spontaneous spike discharges was sometimes completely changed from regular trains alternating with silent periods to a continuous discharge. In three out of five fresh preparations, tetrodotoxin (10^{-7} g/ml.) conversed the regular train discharges to a continuous action potential discharge.

Distribution of the inhibitory nerves along the circular muscle layer

To investigate the distribution of the inhibitory nerves, a preparation of about 5 mm width which contained two bundles of the taenia coli with the circular muscle was used. The sites of stimulation and recording of the inhibitory junction potentials were arranged in the following way. The micro-electrode was inserted into the circular muscle cells in the same muscle bundle by the aid of a binocular microscope, moving along the circular direction. Two stimulating electrodes were placed on the circular muscle just beneath the two bundles of the taenia coli and the indifferent electrode was placed in another part of the bath. Field stimulation was applied alternately on the left- and right-hand sides.

As shown diagrammatically in Fig. 7, inhibitory junction potentials could be evoked by field stimulation applied through the electrodes on both sides if the recording electrode was inserted into a circular muscle cell located between the two stimulating electrodes and at right angles to the taenia coli. The inhibitory junction potentials shown in the left and right columns

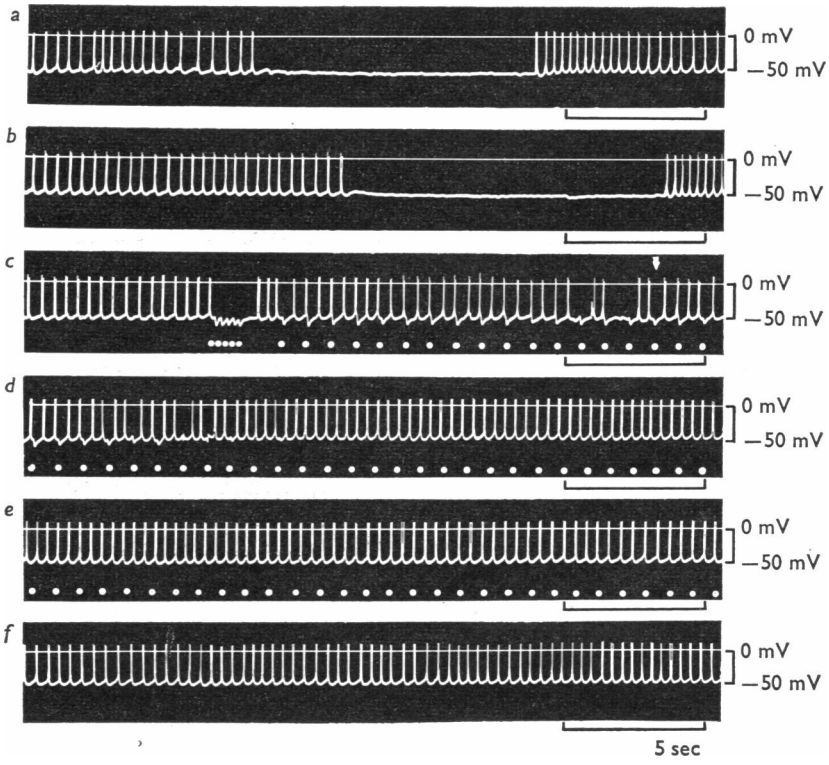

Fig. 6. The effects of tetrodotoxin (5×10^{-6} g/ml.) on the inhibitory junction potentials and spontaneous spike activity. *a* and *b*: regular bursts of spikes alternating with silent periods. *c*: inhibitory junction potentials were evoked by filed stimulation. Tetrodotoxin (5×10^{-6} g/ml.) was applied to the tissue at arrow. *d* and *e*: tetrodotoxin blocked the inhibitory junction potential but not spontaneous spike activity. *f*: the pattern of the spontaneous spike activities was changed from the normal pattern of *a* and *b* to regular discharges by the application of tetrodotoxin.

of Fig. 7*A*, *B*, *C* were elicited by field stimulation applied to the circular muscle just beneath the taenia coli on the two sides. Presumably the inhibitory nerves arise from ganglion cells situated in Auerbach's plexus just beneath the taenia coli, and these axons run in both directions over the whole distance from one taenia coli to the next along the circular

muscle cells. In order to calculate the conduction velocity of the inhibitory nerves in both directions, the latencies between stimulus artifact and the onset of the inhibitory junction potential obtained from the records were plotted against the distances from the stimulating electrode. A roughly linear relationship was obtained, and when the latencies to stimulation of each side were plotted on the same graph against the distances from the stimulation electrodes, the lines crossed at the approximate mid point of

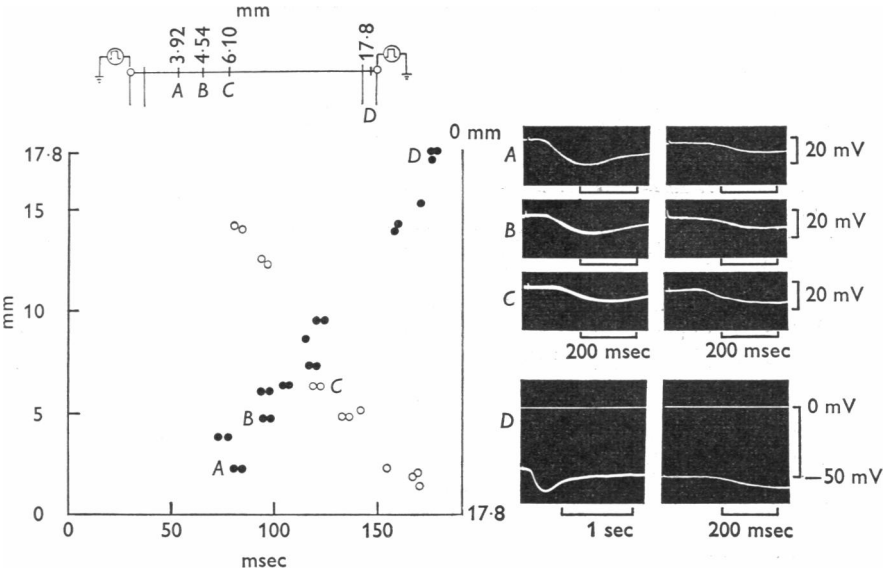

Fig. 7. Relationship between intervals from stimulus to the onset of inhibitory junction potential and the distance of microelectrode from the stimulating electrodes. As shown schematically in the Figure, two stimulating electrodes were placed on the circular muscle just beneath the bundles of the taenia coli. Field stimulation was applied alternately at the left- and right-hand sides (filled and open circles respectively). Records from four individual cells are also shown. *A*, *B* and *C*: inhibitory junction potentials evoked by left and right stimulating electrodes at distances of 3·92, 4·54 and 6·10 mm from the left electrode. The left column was recorded with stimulation of the left side and the right column with stimulation of the right. *D*: inhibitory junction potential recorded at a distance of about 1·8 cm from the left stimulating electrode at a position just above taenia coli of the other side. Two different film speeds are illustrated.

the circular muscle. The conduction velocities calculated from the slopes of these lines were 15·0 cm/sec (left to right) and 14·5 cm/sec (right to left). The mean conduction velocity of the inhibitory nerves was 16·0 cm/sec (S.D. = $\pm$ 0·34, n = 5).

Fig. 8 shows the responses of the circular muscle at two different distances in the transverse direction from the line joining the stimulating electrodes (i.e. the micro-electrode was moved parallel to the taenia coli). In the example, at 1·84 mm, no inhibitory junction potential was evoked. When the recording electrode was placed at various distances in the transverse direction, the maximum distance at which the inhibitory junction potential could be recorded was 1·5 mm.

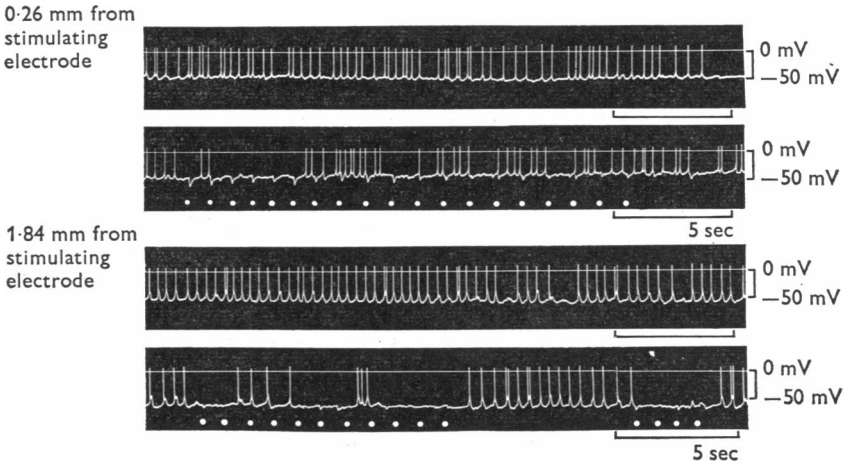

Fig. 8. The inhibitory junction potentials recorded from two cells at distances of 0·26 and 1·84 mm from the stimulating electrode in a direction transverse to the circular muscle. Dots indicate the application of field stimulation.

Another interesting feature of the inhibitory junction potentials recorded from the circular muscle was the shapes of the potential change recorded at various distances from the stimulating electrode. As shown in Fig. 7, when the recording electrode was moved to various distances in steps from the stimulating electrode, the latencies for onset of the inhibitory junction potential became greater as described previously. The rate of rise and the amplitude of the inhibitory junction potential were reduced. These changes were unlikely to be due to the electrotonic decay of the inhibitory junction potential generated close to the taenia coli, since the length constant of the muscle tissue was 1·7 mm, and the junction potential could be recorded at a distance of more than 15 mm. The reduction of the amplitude of the inhibitory junction potential was therefore less than that expected from the cable like structure (at 3·9 mm distant from the taenia coli, the amplitude was reduced to 65 %). Furthermore, increased intensity of stimulation enhanced the amplitude and rate of rise of the inhibitory junction potential.

To investigate whether or not ganglia are distributed in the circular muscle tissue, the ganglion blocking agent, hexamethonium, was used. Fig. 9 shows the effects of field stimulation on the circular muscle in the presence of hexamethonium (5×10^{-6} g/ml.). In a concentration of 5×10^{-6} g/ml., hexamethonium depolarized the membrane from -51 to -45 mV, and lowered the maximum rates of rise and fall of the spike. When the tissue was bathed in the hexamethonium containing solution for more than 30 min, the spike activity deteriorated. Even under these conditions, the inhibitory junction potential was evoked by field stimulation.

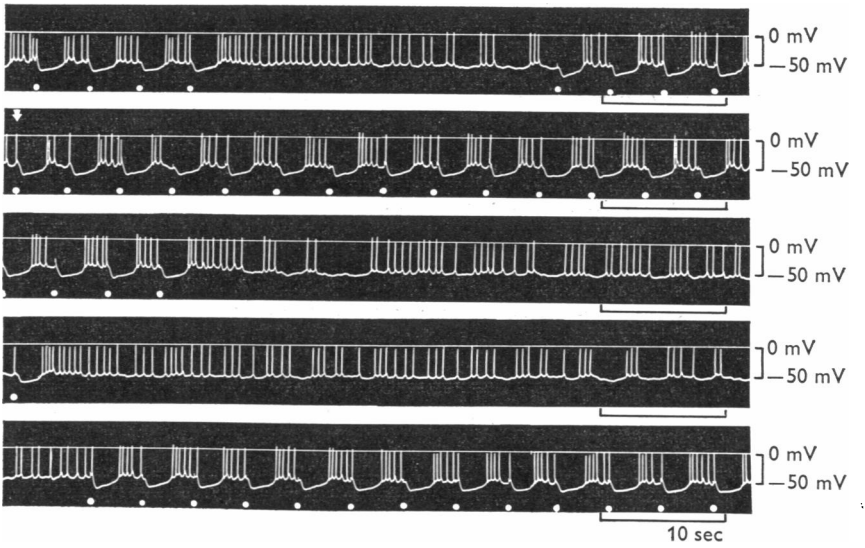

Fig. 9. Effects of hexamethonium (5×10^{-6} g/ml.) on the inhibitory junction potential and on membrane activity. The drug was applied at the arrow (second record). Dots indicate the application of field stimulation. Distance between micro-electrode and stimulating electrode was 4·2 mm.

DISCUSSION

Membrane properties of the circular muscle

The circular muscle of guinea-pig caecum had an average membrane potential of -52 mV, a spike with overshoot potential (maximum rate of rise and fall were 5·2 and 5·6 V/sec respectively), and a conduction velocity of 5·4 cm/sec. These values were almost the same as those obtained in the taenia coli. Furthermore, the electrical characteristic constants of the circular muscle of guinea-pig caecum were similar to these observed in the taenia coli (Abe & Tomita, 1968). For example, the length constant

of the circular muscle was 1·7 mm and that of the taenia coli was 1·5 mm; further the conduction velocities of the excitation in the circular muscle and taenia coli were 5·4 and 6 cm/sec respectively. In this tissue the time constant of the membrane calculated from $\tau/2\lambda$ was about 200 msec which was almost 2 times larger than that of taenia coli. However the time constant of the membrane of this circular muscle calculated from the conduction velocity of the excitation and the time constant of the foot of propagated spike was about 100 msec. The discrepancy of the time constant obtained by two different methods was also observed in the rectal smooth muscle of guinea-pig (Kuriyama & Mekata, 1971), but not in the stomach and taenia coli (Kuriyama, Osa & Tasaki, 1970; Abe & Tomita, 1968). This may mean that there are two capacitative components in the circular muscle cells of guinea-pig caecum as described in skeletal muscle (Falk & Fatt, 1964). In circular muscle of the caecum the vesicles distributed under the plasma membrane might possibly behave like the tubular system in skeletal muscle.

Distribution and nature of the inhibitory nerves

Field stimulation applied to the circular muscle evoked three potential changes, i.e. initial excitatory junction potential, inhibitory junction potential and late excitatory potential. These potential changes were abolished by tetrodotoxin and both the initial and late excitatory junction potentials were also blocked by atropine. From the present experiments, at least cholinergic excitatory and non-adrenergic ('Purinergic') inhibitory nervous elements are thought to be distributed in the circular muscle of the guinea-pig caecum. In the alimentary canal, the intrinsic nerve plexus is known to distribute between circular and longitudinal muscle layers. It is therefore probable that axons from the intrinsic nerves situated in Auerbach's plexus just beneath the taenia coli spread over the whole distance from one taenia coli to the next in the circular muscle layers at a width of about 3 mm.

While inhibitory junction potentials could be recorded from most of the cells, only a few cells showed excitatory junction potentials. The smooth muscle cells of taenia coli are known to have an extensive intramural inhibitory innervation, but a sparse sympathetic inhibitory and cholinergic excitatory innervation (Bennett & Rogers, 1967). Presumably the same may obtain in the circular muscle.

While the effect of tetrodotoxin on the spontaneous spike activity might indicate that this is myogenic, the present results suggest that modulation of the electrical activity (burst discharges separated by silent periods. may be partly due to the activity of the intrinsic inhibitory nerves.

Generation of non-adrenergic inhibitory junction potentials has been

described in the various regions of the alimentary canal. The inhibitory junction potentials recorded from the circular muscle of guinea-pig caecum has nearly the same amplitude and time course as those recorded from other regions of the alimentary canal (see reviews by Holman, 1970; Burnstock, 1972). However, the distribution of the inhibitory nerve in the circular muscle is thought to be rather different from the distribution in taenia coli. Bülbring & Tomita (1967) investigated the properties of the inhibitory nerves in the taenia coli and concluded that the length of the nerve fibre is only a few mm, the length constant is in the order of 0·1 mm and the conduction velocity 0·1–0·2 m/sec. The conduction velocity of the inhibitory nerve distributed in the circular muscle layer was found to be about 0·15 m/sec, but the length of the nerve fibre is more than 15 mm. No synaptic transmission would seem to occur within the circular muscle tissue, since hexamethonium did not suppress the inhibitory junction potential.

It is also probable that the inhibitory nerve may not innervate individual smooth muscle cells, and the chemical transmitter released from the varicosities and nerve terminals might modulate the smooth muscle activity either directly or indirectly. The time courses of the inhibitory junction potentials recorded at various distances from the taenia coli and also the effects of hexamethonium indicate that the density of innervation, or of sites of release of the inhibitory chemical transmitter, is highest close to the taenia coli.

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