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SOME MEMBRANE PROPERTIES OF THE CIRCULAR MUSCLE OF CHICKEN RECTUM AND ITS NON-ADRENERGIC NON-CHOLINERGIC INNERVATION

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SUMMARY

1. Membrane properties and innervation of the circular muscle of chicken rectum were investigated by recording intracellularly electrotonic potentials evoked by passing current, and excitatory and inhibitory junction potentials (EJPs and IJPs) evoked by electrical stimulation of the extrinsic or intrinsic nerves.

2. The membrane potential was $-55 \pm 0{\cdot}6$ mV ($n = 95$). Action potentials of long duration (1·2–4·0 s) discharged spontaneously, or were generated when the membrane depolarization due to either electrotonic potential or EJP reached the threshold. The drug D600 blocked the generation of action potentials.

3. Electrotonic potentials spread fairly well in the longitudinal direction of the muscle fibres but not in the transverse direction. The longitudinal space constant was $1{\cdot}7 \pm 0{\cdot}2$ mm ($n = 10$) and the membrane time constant was 205 ± 21 ms ($n = 10$).

4. Field stimulation of intramural nerves evoked an EJP followed by a long-lasting IJP (3–12 s in total duration) in most cells, and an EJP alone or an IJP alone in a small number of cells. The EJP and IJP were preserved in the simultaneous presence of atropine and guanethidine, but abolished with tetrodotoxin.

5. Stimulation of Remak's nerve trunk or its branches produced EJPs which were atropine resistant and guanethidine resistant just like the EJP elicited by intramural nerve stimulation. The extrinsic nerve stimulation was ineffective in eliciting IJPs.

6. The EJP amplitude declined in a linear manner as the distance from the stimulating site of intramural nerves was increased. The decline was much greater along the transverse axis than the longitudinal axis of circular muscle fibres.

7. The reversal potential for the EJP was estimated by extrapolation to be about $-15{\cdot}3 \pm 0{\cdot}3$ mV ($n = 7$).

8. Apamin did not inhibit the IJP. During the hyperpolarization of a single IJP or summed IJPs, electrotonic potentials remained unchanged or slightly decreased in amplitude.

INTRODUCTION

The rectum of the chicken is an organ which functions to store the content and to expel it when the intraluminal pressure reaches a threshold for initiation of

contractions (Hukuhara, Naitoh & Kameyama, 1974). The physiological functions may depend on a number of factors, including the electrical membrane properties of the muscle as well as its innervation. The rectal region of the chicken intestine receives a strong innervation via the nerve of Remak, a large and ganglionated nerve trunk running along the alimentary canal from the cloaca to the duodenum (Watanabe, 1972; Akester, 1979).

The active and passive membrane properties of the longitudinal muscle of chicken rectum have been investigated. The muscle presents the same cable-like properties as those of other intestinal smooth muscles of many species and readily discharges action potentials of relatively brief duration (10 ms or so) on depolarization in response to applied outward current and stimulant substances, or an excitatory junction potential following nerve stimulation (Komori, Ohashi & Takewaki, 1980; Komori & Ohashi, 1982, 1984). The innervation of the longitudinal muscle of the chicken rectum has been described in several papers (Bartlet & Hassan, 1971; Bartlet, 1974; Takewaki, Ohashi & Okada, 1977; Komori & Ohashi, 1984). Non-adrenergic, non-cholinergic neurones whose cell bodies are located in Remak's ganglia (Kanazawa, Ohashi & Takewaki, 1980) are mainly responsible for the excitatory innervation, and excitatory junction potentials are recorded intracellularly from the smooth muscle cells (Takewaki & Ohashi, 1977; Komori & Ohashi, 1982). Adrenergic nerve fibres innervating the muscle have been also demonstrated in the electrophysiological studies, and electrical stimulation of these fibres at a relatively high frequency produces membrane hyperpolarization, inhibits discharges of the action potentials and in turn causes relaxation (Komori & Ohashi, 1987). Although the circular muscle of chicken rectum has been suggested to play an essential role in storing and expelling the contents, little is known about the electrical membrane properties or about its innervation.

The present study has attempted to investigate the excitatory and inhibitory innervation of the circular muscle of chicken rectum by non-adrenergic, non-cholinergic nerves and some of its membrane properties which might provide insight into mechanisms of the neuronal control of this effector. The muscle discharges action potentials of long duration (1·2–4·0 s). The spread of electrotonic potentials is comparable to that of the longitudinal muscle in the longitudinal direction of the circular muscle fibres, but it is very limited in the transverse direction. The muscle receives a non-adrenergic, non-cholinergic excitatory innervation via Remak's nerve and an inhibitory innervation of non-adrenergic, non-cholinergic neurones arising in the enteric plexuses. Such inhibitory neurones do not appear to be driven by stimulation of nerve fibres in Remak's nerve.

METHODS

White Leghorn chickens (*Gallus domesticus*) of either sex, aged more than 100 days, weighing about 0·8–2·0 kg, were stunned and bled to death. The rectal region of the intestine was removed with attached Remak's nerve and flushed clean with Tyrode solution. Strips of the intestinal wall (about 2 mm wide and about 20 mm long), which were obtained by cutting at right angles or parallel to the longitudinal axis of the isolated rectum, were dissected free of adhering tissues as well as the mucosa. Excess tissue was then trimmed away and strips of circular muscle were cut parallel to the longitudinal axis or transverse axis of the circular muscle fibres (1·0–1·5 mm wide

and about 20 mm long) to which the longitudinal muscle layer remained attached. Wider longitudinal strips of circular muscle (about 10 mm wide and about 20 mm long) with Remak's nerve supply were obtained and dissected free of the mucosa.

Longitudinal strips (1·0–1·5 mm wide and 20 mm long) of circular muscle were mounted mucosal side up in an organ bath (2 ml) consisting of two chambers, one for recording and the other for stimulating, as described by Abe & Tomita (1968). The organ bath was filled and perfused at a flow rate of 2–3 ml/min with Tyrode solution pre-heated to 33 ± 1 °C. Electrotonic potentials in response to current pulses of varied durations applied to the tissue through the large stimulating plates were recorded intracellularly from circular muscle cells in the extrapolar region at various distances from the stimulating plate between the two chambers. Transverse strips of circular muscle were used for testing passive potential spread in the transverse direction of the circular muscle layer.

Strips of circular muscle with attached Remak's nerve were mounted mucosal side up in an organ bath (2 ml) by pinning out flat on a rubber board with special care being taken not to compress the mesentery in which Remak's nerve runs. The organ bath was filled and perfused with Tyrode solution pre-heated to 33 ± 1 °C. For electrical stimulation, a 5 mm length of the cut anal end of Remak's nerve trunk or the cut peripheral end of its nerve branches extending toward the muscle layer was detached from the mesentery and introduced into a bipolar suction electrode. For electrical stimulation of intramural nerves of the tissue, a pair of Ag wire electrodes (1 mm in diameter) was used. One electrode, which was insulated with Araldite except for the tip, was placed in contact with the circular muscle layer and the other electrode, which was uninsulated, was placed in the bathing solution. To record both junction potential and electrotonic potential from the same cell, one electrode for nerve stimulation was placed at 1–1·5 mm from the partition plate and the recording site was restricted to the region between the nerve-stimulating electrode and the plate. Membrane potentials of the circular muscle were recorded intracellularly. Glass microelectrodes filled with 3 M-KCl solution, whose resistance varied from 40 to 60 MΩ, were advanced from the mucosal side of the tissue. Changes in the membrane potential were displaced on an oscilloscope and photographed. Nerve stimulation was achieved with rectangular pulses which were delivered by a Nihon-Kohden MSE-3 stimulator.

Tyrode solution of the following compositon was used (mM): NaCl, 137·0; KCl, 2·7; NaH_2PO_4, 0·4; $NaHCO_3$, 12·0; $MgCl_2$, 1·0; $CaCl_2$, 1·8; glucose, 5·6; bubbled with air in the perfusing reservoir. Drugs used were atropine sulphate (Merck), guanethidine sulphate (Ciba Geigy), tetrodotoxin (TTX, Sankyo), tetraethylammonium chloride (TEA, Daiichi), methoxyverapamil hydrochloride (D600, Knoll) and apamin (Serva).

The experimental values obtained were expressed as mean ± S.E.M. Regression lines were calculated using the least-squares method. Statistical significance was tested using Student's t test, and probabilities of less than 0·05 ($P < 0·05$) were considered significant.

RESULTS

Electrical activities

All preparations were first equilibrated for at least 30 min in Tyrode solution before starting the experiment. The resting membrane potential ranged between −42 and −68 mV, giving a mean of $-55·0 \pm 0·6$ mV ($n = 95$). This value was somewhat larger than that of the longitudinal muscle of the chicken rectum (Komori & Ohashi, 1987). Some preparations (70 %) were spontaneously active and exhibited spontaneous membrane depolarizations in the early period of each experiment, during which the resting membrane potential was −55 mV or more. The spontaneous depolarizations presented characteristics of action potentials (see below) and hereafter they are referred to as slow action potentials. The slow time course and lack of pre-potential-like depolarization and after-hyperpolarization were characteristic of the action potentials. The discharge interval was irregular in most preparations, and varied from 10 s to a few minutes. In some preparations it was relatively

constant (6–10 s). The amplitude and rate of rise of the action potentials recorded from one preparation were more or less constant, but they varied from one preparation to another. The amplitude ranged from 15 to 45 mV in thirty-four cells of twenty-two different preparations, giving a mean of $28{\cdot}9 \pm 0{\cdot}4$ mV. It was found that there is a positive correlation between the amplitude (E) and the resting potential (E_r) (correlation coefficient, $r = 0{\cdot}603$, $n = 27$, $P < 0{\cdot}01$; regression line, $E = -54{\cdot}3 - 1{\cdot}34\, E_r$). The time taken to reach 90% from 10% of the peak

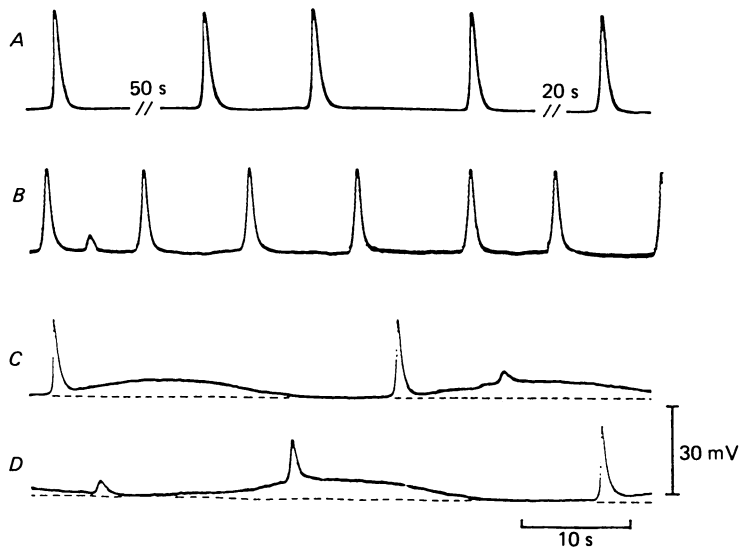

Fig. 1. Variations in discharge of spontaneous action potentials recorded intracellularly in the circular muscle strip of chicken rectum. *A*, discharges at varied intervals ranging from 6 s to a few minutes; *B*, discharges at a relatively regular interval of 6–10 s; *C* and *D*, continuous records, discharges with a slower and smaller depolarizing wave. The record in *A* was interrupted for 50 s and 20 s as indicated. Dashed lines indicate resting level of membrane potential in the records. In this and subsequent Figures atropine (0·5 μM) and guanethidine (2 μM) are present in the bathing solution.

depolarization varied from 0·2 to 0·8 s and the maximum rate of rise was calculated to be 0·02–0·20 V/s, with a mean value of $0{\cdot}08 \pm 0{\cdot}01$ V/s (twenty-five cells). The half-decay time and total duration were 0·3–0·8 s and 1·2–4·0 s, respectively. On rare occasions, smaller and slower depolarizations (less than 10 mV in amplitude and 30 s or so in duration) were observed. The different types of spontaneous changes in membrane potential are presented in Fig. 1. All these spontaneous activities remained unchanged after simultaneous application of atropine (1 μM) and guanethidine (2 μM) or after application of TTX (0·5 μM), and they appeared to be of myogenic origin. Even in quiescent preparations with the membrane potential higher than −55 mV, a slow action potential, which was very similar in configuration to the spontaneous one, was evoked by application of outward current pulses of 0·8 or 1·0 s duration when membrane depolarization reached the threshold (Fig. 2*A*).

Application of D600 (10 μM) resulted in abolition of the spontaneous activities. As shown in Fig. 2*B*, the generation of the response to current pulses was supressed after 10 μM-D600, a concentration which decreased the resting membrane potential by less than 8 mV but caused no apparent change in membrane resistance (five preparations). Electronic potentials in response to inward current pulses of 300 ms duration were decreased in amplitude during the rising phase of the slow action potential. Thus, the electrical activity, which is spontaneous or evoked by the application of current pulses, is assumed to be generated by an increase in membrane conductance to Ca^{2+} through activation of potential-dependent Ca^{2+} channels.

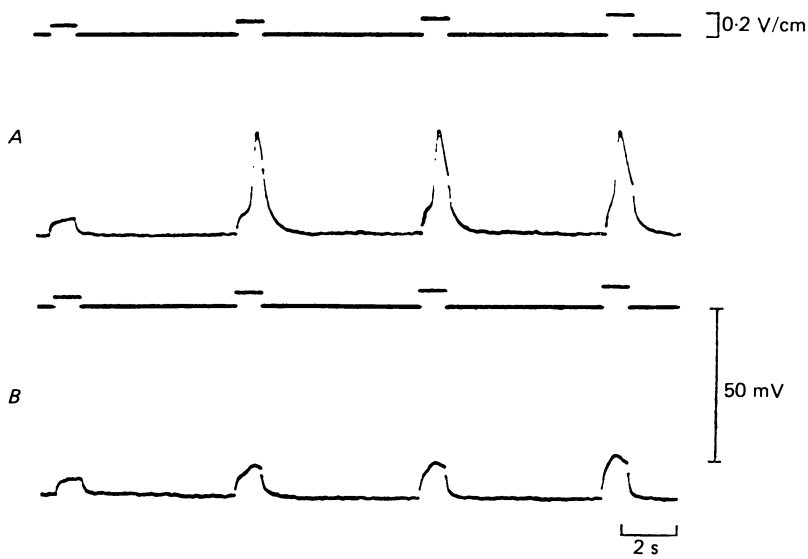

Fig. 2. The effect of D600 on action potentials. *A*, control responses evoked by applying outwards current pulses of 1·0 s duration at four different strengths; *B*, 15 min after application of D600 (10 μM). Upper trace in *A* and *B*, zero potential level of the cell and applied current strength; lower trace, potential records.

Spatial decay of the steady-state electrotonic potential

Figure 3 illustrates electrotonic potentials in response to current pulses of 1 s duration at different strengths recorded at a distance of 0·4 mm from the stimulating partition, the graph showing an approximately linear relationship between the applied current intensity and the amplitude of the electrotonic potential by up to 35 mV of hyperpolarization. With a depolarization larger than 8 mV, the input resistance occasionally decreased, which may be due to normal rectification of the membrane. The size of hyperpolarizing electrotonic potentials recorded at any distance also increased linearly up to a limit (about 40 mV) as the current intensity was increased (see Fig. 9*B*). To avoid complicated calculations, electrotonic potentials of less than 40 mV were used for measurements of the membrane parameters. Figure 4*A* and *B* shows electrotonic potentials produced by a given

current intensity and recorded at five different distances from the stimulating partition and the semilogarithmic plot of the size against the distance. This relation was roughly linear in all preparations for longitudinal strips. The electrotonic potential decayed exponentially along the tissue in the longitudinal direction of the circular muscle, as in other smooth muscles. The space constant (λ), the distance at which the size of electrotonic potentials decayed by 63% ($1-1/e$), was measured.

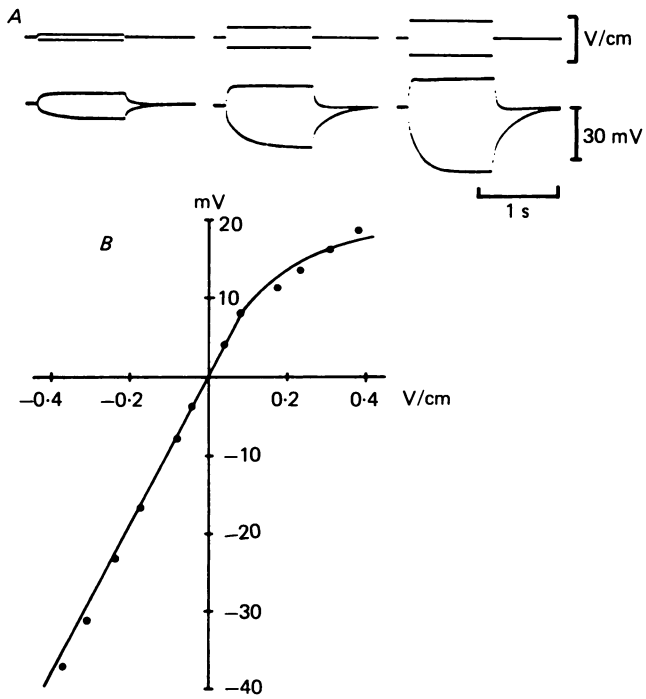

Fig. 3. The current–voltage relation in the circular muscle. *A*, electrotonic potentials evoked by applying current pulses of 1 s duration at three different strengths. Upper trace, strength in terms of V/cm and direction of current pulses. In this and subsequent Figures outwards current is upwards, inwards current downwards. Lower trace, potential records. *B*, a current–voltage plot of steady-state electrotonic potentials.

The mean value for λ obtained from ten tissues was $1{\cdot}7 \pm 0{\cdot}2$ mm with a range from 1·3–2·3 mm. The characteristics of the electrotonic potential strongly suggest that the tissue has cable properties. In this case, the relation of the time taken for the electrotonic potential to reach its 50% amplitude to the distance from the current-passing plate is expected to be linear, as shown in Fig. 4*C*, and the slope of the line is determined by the membrane time constant (τ_m) and space constant (λ) ($\tau_m/2\lambda$; Hodgkin & Ruston, 1946; Katz, 1948). The time constant, τ_m, was obtained since λ was known. The average time constant obtained from ten different preparations was 205 ± 21 ms with a range from 160–270 ms. The mean values for λ and τ_m are comparable with those in the longitudinal muscle of the rectum of chicken (Komori *et al.* 1980; Komori & Ohashi, 1982).

In order to obtain evidence for the spatial decay of the electrotonic potential in the transverse direction of the circular muscle, transverse strips of the circular muscle (see Methods) were used. In all strips of seven different preparations, no apparent electrotonic potential was recorded even at a distance of 0·2 mm from the stimulating partition. In four out of the seven preparations a pair of longitudinal and transverse strips was taken from each preparation, and experiments were followed in which the

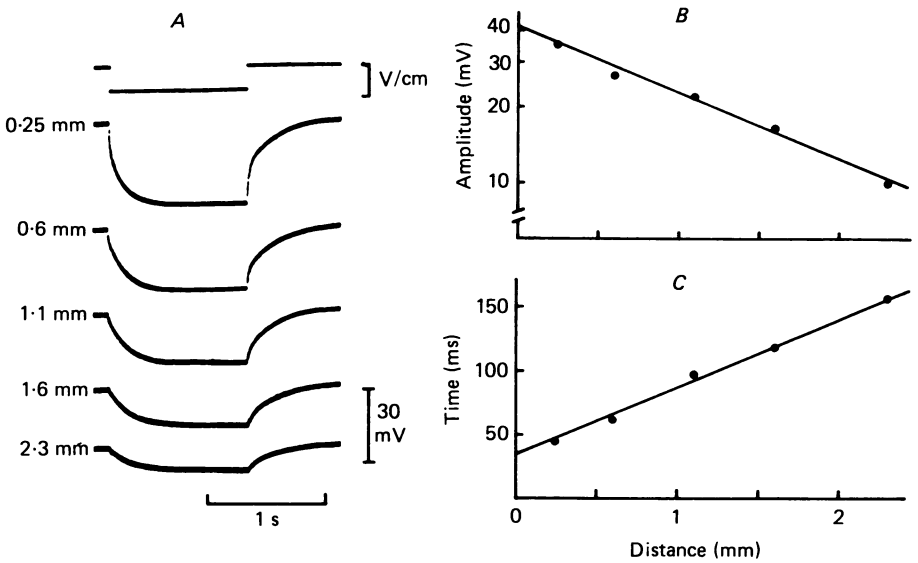

Fig. 4. Current spread in the longitudinal direction of the circular muscle. *A*, potential records at five different distances from the stimulating plate at which a constant current pulse of 1·2 s duration was applied; *B*, a semilogarithmic plot of electrotonic potential amplitude against distance; *C*, a plot of the time required to reach half of the maximum potential against distance. Lines fitted to the data points by eye. The space constant (λ) in the longitudinal direction of this tissue is found to be 1·63 mm from the slope of the line in *B*, and the membrane time constant (τ_m) is calculated to be 174 ms from the slope of the line in *C* (slope = $\tau_m/2\lambda$).

longitudinal strip was used to test for recording electrotonic potentials. In all of the longitudinal strips, electrotonic potentials were recorded and the spatial decay was not different from that in the longitudinal strips mentioned above. These results strongly suggest that the passive current spread in the transverse direction across the circular muscle is very limited, probably by the high effective coupling resistance.

Innervation of intramural nerves

Responses to field stimulation of intramural nerves were recorded from circular muscle cells at a distance of 1–2 mm along long axes of the muscle fibres from the stimulating electrode, unless otherwise stated. Field stimulation with single pulses of durations of 0·08–0·2 ms elicited a transient and small membrane depolarization followed by a hyperpolarization. Both electrical responses were mediated by

intramural nerves since they were abolished with TTX (1 μM), being EJPs and IJPs. Both EJPs and IJPs varied in amplitude from barely detectable levels to several millivolts in different preparations so that two extremes in configuration, an EJP alone and an IJP alone, were recorded from a small number of cells. Biphasic junction potentials in which the constituent EJP was predominant were usually recorded. The three types of junction potentials are presented in Fig. 5*A*. The

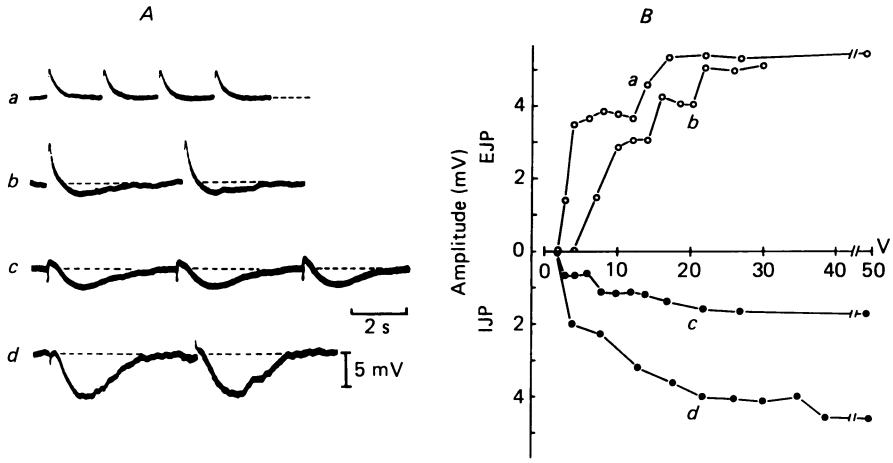

Fig. 5. Variations in junction potentials and changes in the amplitude with stimulus voltage. Square-wave pulses of 0·2 ms duration were used for intramural nerve stimulation. *A*, potential records; *a*, EJPs; *b* and *c*, EJPs with IJPs; *d*, IJPs evoked by two single stimuli with different polarities. Dashed lines indicate the resting level in membrane potential. *B*, plots of junction potential amplitude against stimulus voltage; *a*, for EJPs in biphasic junction potentials; *b*, for EJPs; *c*, for IJPs in the same biphasic junction potentials as in *a*; *d*, for IJPs. The EJP amplitude is upwards, IJP amplitude downwards. Each point represents the mean of three or four experiments.

average temporal parameters of the EJP and IJP evoked by field stimulation are summarized in Table 1. The values for EJPs were substantially the same as those for EJPs in the longitudinal muscle under the sufficiently comparable conditions (Komori & Ohashi, 1982). The total duration of the IJP varied from 3 to 12 s in different preparations. The variation resulted mainly from the difference in the time course of the decay phase of IJPs. The time taken for the EJP to reach its peak after field stimulation was about 80 ms, being about 1300 ms shorter than that for the IJP. There was no significant difference between the average times for the constituent EJP of biphasic junction potentials and the EJP alone, indicating that the preceding EJP of the biphasic responses was not truncated by the following IJP before it had reached its peak. The EJP and IJP were preserved in the presence of atropine (up to 1 μM) and guanethidine (up to 4 μM), and they are mediated by non-adrenergic and non-cholinergic nerves stimulated by field stimulation. The amplitude of EJPs and IJPs increased as the stimulus voltage was increased, as shown in Fig. 5*B*. The curves revealed that the minimum effective voltage for eliciting the EJP and

IJP is not appreciably different, but the voltage for attaining the maximum amplitude is slightly lower for the EJP than the IJP. This was the case for all preparations. Occasionally, distinct steps appeared in EJPs as well as IJPs when the stimulus voltage was varied. The step-like amplitude changes may reflect participation of excitatory and inhibitory nerve fibres of higher threshold by which unit junction potentials with different sizes are elicited. These phenomena have been taken as evidence for multiple innervation of single smooth muscle cells or functional muscle bundles.

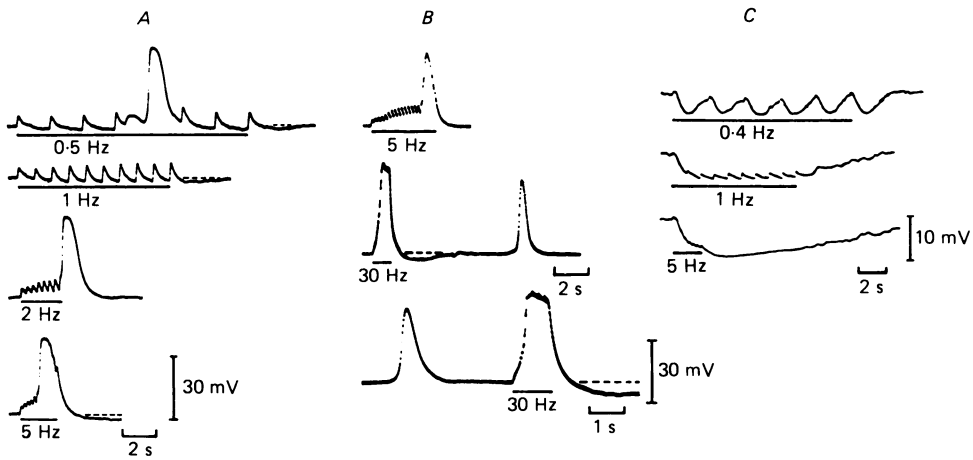

Fig. 6. Facilitation or summation of junction potentials following repetitive stimulation of intramural nerves. Nerve stimulation with square-wave pulses of 0·2 ms duration at varied frequencies. Bars with figures, stimulus period at the frequency (Hz) as indicated. Dashed lines, the resting level of membrane potential in the records. *A*, potential records from a cell which responds by a large EJP with a small IJP to a single stimulus; *B*, records from a cell which responds by an EJP alone to a single stimulus; *C*, records from a cell which responds by a large IJP with a small EJP to a single stimulus. See text.

In cells from which EJPs were predominantly recorded, repetitive nerve stimulation at frequencies of 0·5–1 Hz caused facilitation of EJPs and a gradual increase in the amplitude and, at frequencies higher than 2 Hz, summation in addition to facilitation occurred, as illustrated in Fig. 6*A* and *B*. If the summed EJPs reached a critical depolarization (5–15 mV), then a regenerative potential just like the spontaneously discharged depolarization was generated (Fig. 6*A* and *B*). Since the time required for reaching the threshold depolarization was shortened as the stimulus frequency was increased, at frequencies higher than 20 Hz, a regenerative potential was evoked immediately after starting stimulation. During nerve stimulation at high frequencies, the membrane depolarization exceeded by 10 mV or so the peak of the action potential, and the level of membrane potential was maintained over the period of nerve stimulation (Fig. 6*B*).

No facilitation was shown by IJPs so that there was no detectable difference in amplitude between the first IJP and tenth IJP evoked by repetitive stimulation at 0·3 Hz. At higher frequencies up to 5 Hz, successive IJPs summed up to give a

long-lasting hyperpolarization on which small EJPs were superimposed (Fig. 6*C*). When the stimulus frequency was increased to 10 Hz or more (up to 40 Hz), a transient depolarization of the sum of EJPs occurred before the membrane was hyperpolarized. This was true of cells in which no contribution of any EJP to the junction potential with single stimuli was apparently observed. The preceding depolarization was frequently larger than the level at which an action potential was generated in the case of current pulses, and it did not necessarily trigger an action potential. Maximal hyperpolarizations resulting from summation of IJPs rarely exceeded 10 mV, and their half-decay times varied from 3 to 10 s. Occasionally, the hyperpolarization was followed by a rebound depolarization.

TABLE 1. Temporal parameters of the EJP and IJP in the circular muscle of chicken rectum

Stimulation	Response	Latency (ms)	Time to peak (ms)	Half-decay time (ms)	Total duration (ms)	*n*
Intramural nerves	EJP	10[a]	80±5 (12)	188±12 (12)	905±27 (12)	9
	IJP	156±7 (5)	1300±100 (9)	1800±200 (9)	6500±1100 (9)	5
	Biphasic JP	10[b]	—	—	6400±600 (22)	11
	EJP[c]	—	82±5 (12)	—	—	
	IJP[d]	—	140±100 (22)	2000±300 (22)	—	
Remak's nerve	EJP	40±5 (5)	98±5* (11)	205±10 (11)	980±31 (11)	5

Each value represents mean±S.E.M. Number of measurements are given in parentheses. *n*: number of preparations. a, b: not accurately measured. c, d: constituent EJP and IJP of biphasic JPs, respectively. * Significantly different from the value for EJP evoked by intramural nerve stimulation ($P < 0.02$).

EJPs in response to stimulation of Remak's nerve

When electrical stimulation was applied to Remak's nerve trunk or its branches, EJPs were recorded from some cells in each preparation, but no IJP was recorded from any of ninety cells in eight preparations. This was true of cells from which IJPs were recorded in response to intramural nerve stimulation. The EJP was smaller in amplitude (3 mV or less) and longer in latency compared to the EJPs elicited by field stimulation of the intramural nerves (Table 1). The longer latency seemed to be due to a longer conduction of nerve impulse (Komori & Ohashi, 1982). It was found that TEA (1 or 3 mM) increased the EJP amplitude, and the number of cells from which an EJP is recorded was increased (see Fig. 7*C*). Repetitive stimulation at frequencies higher than 2 Hz caused facilitation and summation of the EJPs to reach the threshold for initiation of a slow action potential (Fig. 7*A*). In most cells from which EJPs were elicited, IJPs were also evoked if the stimulating site was switched from the extrinsic nerve to the intramural nerves. This indicates that the cells receive excitatory innervation of Remak's nerve and inhibitory innervation of the intramural neurones. In order to obtain further evidence for the only excitatory innervation of the extrinsic nerve, repetitive stimulation of Remak's nerve at its

trunk or branches was followed by repetitive stimulation of the intramural nerves and the responses were recorded from the same cell, as shown in Fig. 7. No membrane hyperpolarization was observed in the case of stimulation of Remak's nerve. The results suggest that stimulation of Remak's nerve may not activate any inhibitory nervous elements producing IJPs.

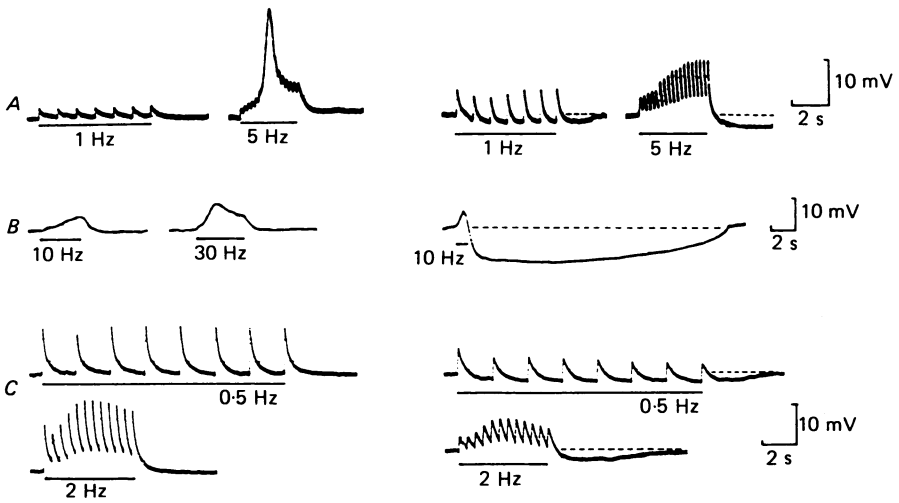

Fig. 7. Comparison of responses recorded from the same cell to repetitive stimulation of Remak's nerve (left) and intramural nerves (right). Remak's nerve stimulation with square-wave pulses of 0·8 ms duration, intramural nerve stimulation with square-wave pulses of 0·2 ms duration. Bars with figures, stimulus period at the frequency (Hz) as indicated. Dashed lines, the resting level of membrane potential in the records. Remak's nerve stimulation at the trunk in *A* and *B*, and at the branches in *C*. The experiment in *C* in the presence of TEA (3 mM). Notice no membrane hyperpolarization in the case of stimulation of Remak's nerve.

Distribution of the excitatory nerves

The experiments were performed in the presence of TEA (1 or 3 mM) which served to increase the EJP amplitude with no appreciable change in membrane resistance. The EJPs were recorded from cells located at various distances along the longitudinal or transverse axes of the circular muscle fibres from the point at which the electrode for intramural nerve stimulation was placed. Figure 8 shows an example of the results. In this preparation, the average amplitude of EJPs was $5{\cdot}9 \pm 0{\cdot}3$ mV in eleven cells at 1 mm in the longitudinal direction. The EJP amplitude did not change significantly when the distance was increased to 2·5 mm. However, as the distance was increased further, the EJP amplitude declined in an approximately linear manner with the distance. In fact EJPs were still recorded at 11 mm but their amplitude was about 15% of the EJP amplitude at 1 mm (Fig. 8, ●). In the transverse direction the average EJP amplitude was $5{\cdot}5 \pm 0{\cdot}3$ mV ($n = 10$) at 0·5 mm. If the distance was increased by 1·0 mm the EJP amplitude declined to about 15%. No EJP was recorded at 2 mm (Fig. 8, ○). In seven other preparations substantially

similar results were obtained. In three out of the eight preparations, similar measurements were performed for the IJP and the results suggest that the IJP decreased in amplitude with the distance in the same manner as the EJP.

Reversal potential for the junction potential

In order to know the reversal potential for the EJP, EJPs were elicited at different membrane potentials which were achieved by applying inward or outward current

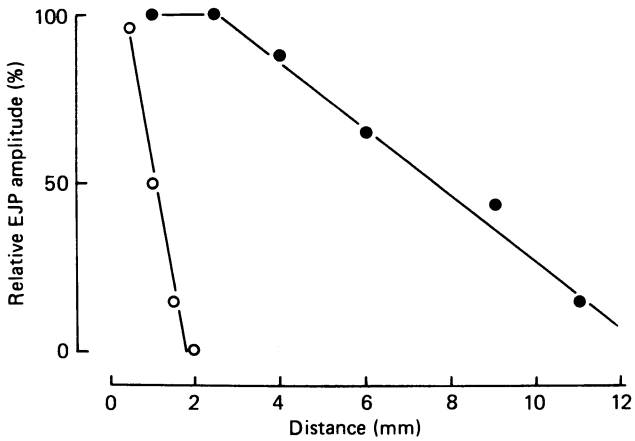

Fig. 8. A plot of changes in amplitude of EJPs evoked by intramural nerve stimulation against distance from the stimulating point. The experiment in the presence of TEA (1 mM). Abscissa: distance from the stimulating point along the longitudinal (●) or transverse (○) axes of circular muscle fibres. Ordinate: percentages of mean EJP amplitudes (the mean EJP amplitude at 1 mm in the longitudinal direction was taken as 100%). Each point represents the mean EJP amplitude of six to eleven different cells. Lines fitted by eye to the data points. See text.

pulses of 2 s duration. Figure 9*A* shows a typical example of the experiments. The relationship between the EJP amplitude and membrane potential is approximately linear in the range of membrane potential from −40 to −85 mV. The input resistance remains constant over this range of membrane potential as measured by the linear current–voltage relationship (Fig. 9*B*). In this case, the reversal potential for the EJP is given as −15·7 mV by extrapolating the regression line showing the EJP amplitude–membrane potential relationship to zero EJP (Fig. 9*C*). In seven different preparations one measurement for each preparation was made and the average reversal potential was estimated to be −15·3±0·3 mV with a range of −8 to −28 mV. In three preparations similar experiments were performed for the IJP. Inward current pulses of 8 or 10 s duration were used for displacement of membrane potential. The IJP amplitude remained almost unchanged even when the membrane was hyperpolarized by up to 30 mV, as shown in Fig. 10. Larger displacements to −90 to −105 mV resulted in abolition or a profound decrease in the amplitude of the IJP. On the other hand, the EJP amplitude was again linearly related to the level of membrane potential, and a reversal potential of −13 to −25 mV was estimated.

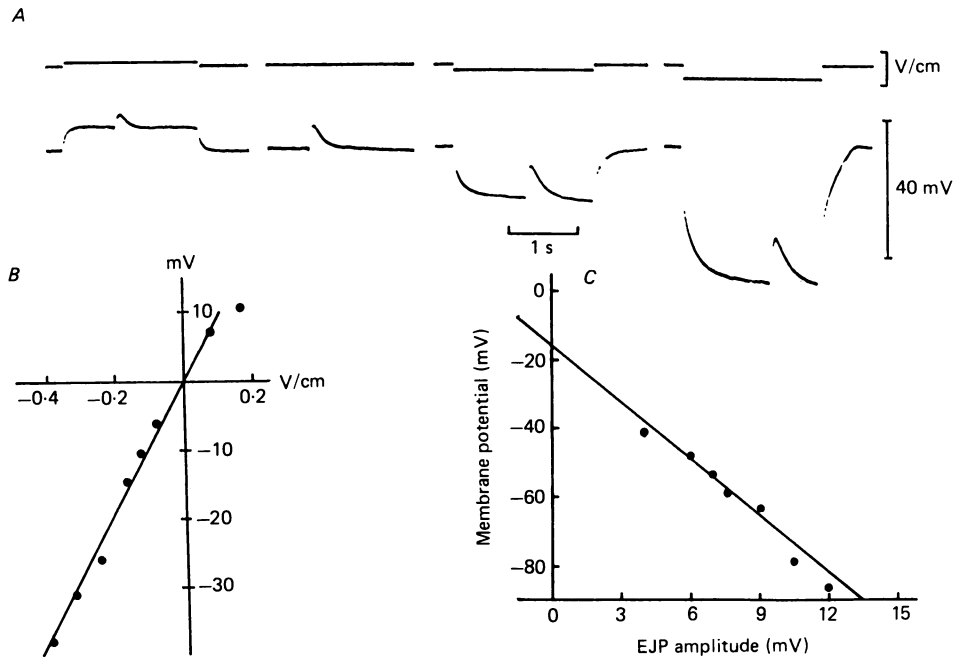

Fig. 9. Relationship between EJP amplitude and membrane potential. *A*, EJPs evoked at four different membrane potentials displaced by applying current pulses of 2 s duration. Intramural nerve stimulation with square-wave pulses of 0·1 ms duration. Upper trace, current monitor; lower trace, potential records. *B*, the current–voltage relationship for the cell membrane. *C*, a plot of changes in the EJP amplitude against membrane potential. Membrane potential was displaced by applying current pulses of 2 s duration and the EJPs were evoked by intramural nerve stimulation (square-wave pulses of 0·1 ms duration). *A*, *B* and *C*, obtained from records in one cell. In *C*, the calculated regression line is given as $Y = -5{\cdot}8X - 15{\cdot}7$, where Y is membrane potential and X is EJP amplitude.

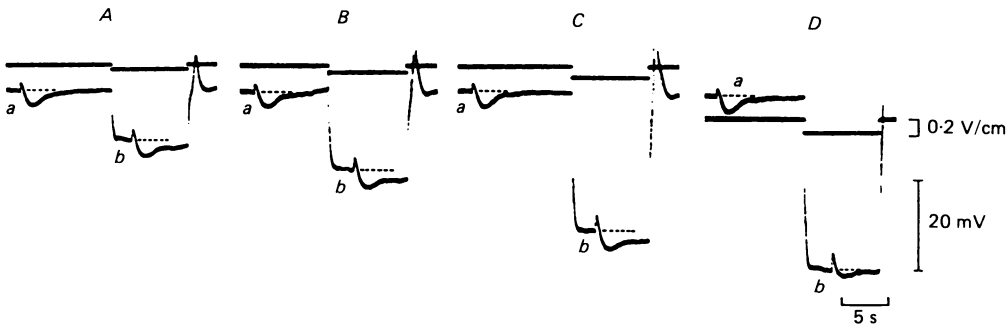

Fig. 10. Effect of displacement of membrane potential on IJP amplitude. Membrane potential displaced from the resting level (−55 mV) to −66 mV (*A*), −72 mV (*B*), −85 mV (*C*) and −93 mV (*D*) by passing inward current (8 s duration). The IJPs evoked by intramural nerve stimulation (square-wave pulses of 0·2 ms duration). *a* in *A*, *B*, *C* and *D*, control; *b*, at the displaced membrane potential. *A*, *B* and *C*, records in one cell; *D*, in another cell. Each horizontal line, current monitor. Dashed lines, the basal level of membrane potential. Notice the non-linear reduction in IJP amplitude with prolonged decay phase.

Effect of apamin on the IJP

Apamin at 0·25 or 0·5 μM had no inhibitory effect on the IJP, but slightly increased the amplitude and duration, as shown in Fig. 11. The EJP and resting membrane potential remained almost unchanged in the presence of apamin ($n = 5$). Tetrodotoxin (0·5 μM), which was applied additively to the preparation, abolished both IJP and EJP (Fig. 11*B*). The concentration (0·25 μM) of apamin used has been shown to abolish the IJPs in the circular muscle of guinea-pig ileum (Niel, Bywater & Taylor, 1983; Bywater & Taylor, 1986). We confirmed this before use of apamin in the present experiment. Thus, the IJP in the circular muscle of chicken rectum appeared to be resistant to apamin.

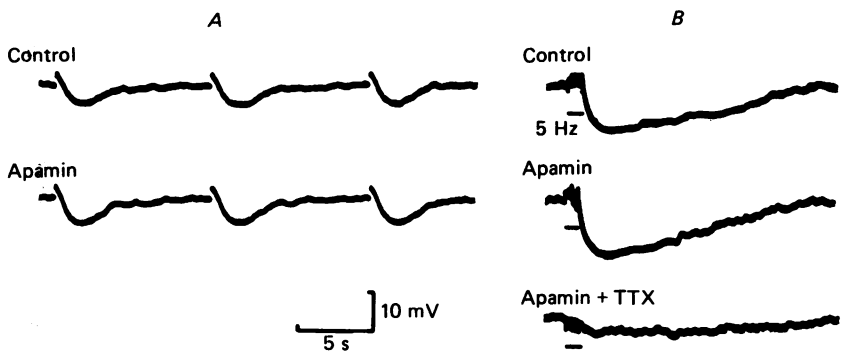

Fig. 11. Effect of apamin on the IJP. The IJPs evoked by intramural nerve stimulation with square-wave pulses of 0·2 ms duration at 0·1 Hz in *A* and 5 Hz in *B* (continuous line). In *A*, upper trace, control; lower trace, 15 min after application of apamin (0·5 μM). In *B*, uppermost trace, control; middle trace, 15 min after apamin (0·5 μM); bottom trace, 3 min after additional application of TTX (0·5 μM). Notice no inhibitory effect of apamin on the IJP.

Change in membrane conductance during the IJP

Inward current pulses of 300–800 ms were applied before and during the IJP. Electrotonic potentials remained unchanged or slightly decreased in amplitude (by up to 20%) during the IJP in all six preparations used. Substantially similar results were obtained for summed IJPs (Fig. 12). In addition, there was no correlation between the amplitude of IJP-induced hyperpolarizations (3–9 mV) and the percentage reduction of electrotonic potentials (0–25%) ($r = 0·147$, $n = 9$, $P < 0·05$).

DISCUSSION

The membrane depolarizations (referred to as slow action potentials in the Results), which are spontaneous or electrically evoked, in the circular muscle of chicken rectum are similar in temporal parameters and amplitude to the slow waves in other gastrointestinal smooth muscles of many species (see Tomita, 1981). The slow depolarization is unlikely to be electrotonic spread from the longitudinal

muscle, because such slow changes in membrane potential have not been observed in the longitudinal muscle of chicken rectum (Komori *et al.* 1980; Komori & Ohashi, 1987). The effectiveness of D600 in suppressing discharge of the slow depolarization can be interpreted as an indication for involvement of a Ca^{2+} inward current in the generation. In contrast to other smooth muscles, no spike activity appears on top of the depolarization. In most smooth muscles, the spike activity, rather than the slow depolarization, is related to the strength of the mechanical response. A certain voltage might be required for a rise in tension of the circular muscle of chicken rectum, as described for the circular muscles of canine antrum and corpus (Morgan & Szurszewski, 1980). The question of how excitation–contraction coupling takes place in this muscle remains to be resolved.

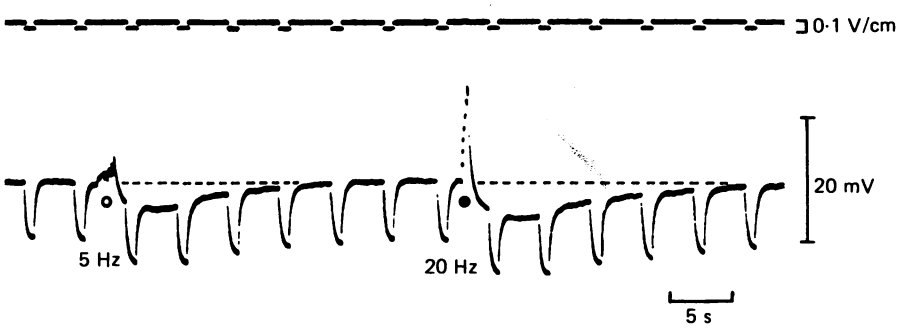

Fig. 12. Change in membrane conductance during hyperpolarization produced by the IJP. The IJPs evoked by intramural nerve stimulation with a train of seven stimuli at 5 Hz (○) or a train of seven stimuli at 20 Hz (●) (square-wave pulses of 0·2 ms duration). Upper trace, current monitor; lower trace, potential records. Dashed line, the resting level of membrane potential in this record. Notice no apparent change in amplitude of electrotonic potentials during the membrane hyperpolarizations produced by the IJPs.

The observations on the spatial decay of the electrotonic potential along the long axes of the muscle fibres suggest that the functional structure of the rectal muscle has cable-like properties. The longitudinal space constant of 1·7 mm and the membrane time constant of 205 ms are comparable to those in the longitudinal muscle of chicken rectum observed by Komori & Ohashi (1982). The present results showing that no apparent electrotonic potential is recorded in the transverse direction lead us to believe the transverse space constant of less than 0·1 mm. Cells in the circular muscle layer may be similar in form and orientation to those in other intestines, and the cell length may be 20–100 times longer than the cell width (2–6 μm) (Burnstock, 1970). The junctions with an electrical coupling between cells may be randomly distributed and may cover a relatively small part of the cell membrane (Burnstock, 1970). In this case, the coupling resistance is expected to increase much more with distance in the transverse direction than the longitudinal direction. This is the most plausible explanation for the much shorter length constant in the transverse direction. The present results also suggest that the circular muscle cells may form a functional ring of the same radius as that of the intestine and of a width of less than

0·2 mm, and that the unitary rings may be arranged together parallel to each other to constitute the circular muscle coat.

The present results provide evidence that all circular muscle cells receive two kinds of non-adrenergic, non-cholinergic innervation, one producing an EJP and the other producing an IJP, but that the relative contribution to the junction potential evoked by field stimulation varies greatly in different cells. For example, even in cells from which the IJP alone was recorded, two components of the IJP and EJP could also be demonstrated during repetitive nerve stimulation at higher frequencies. It is of interest that the inhibitory nerves cannot be activated by Remak's nerve stimulation. The inhibitory nerves mediating the IJP may originate from neurones in the myenteric plexus and the neurones may not have synaptic connections in such a way that they can be driven by the fibres in Remak's nerve. In cells in the longitudinal muscle, non-adrenergic, non-cholinergic nerve activation by field stimulation does not produce any IJP (Takewaki & Ohashi, 1977; Ohashi, Naito, Takewaki & Okada, 1977; Komori & Ohashi, 1982, 1984). Therefore, the inhibitory innervation is one of the striking features of the circular muscle and may be involved in a physiological function in which the muscle plays an important part. In the guinea-pig colon and rabbit colon, non-adrenergic, non-cholinergic inhibitory nerves are suspected to function as an efferent arc of intrinsic reflexes controlling the mechanical activity (Furness, 1969).

As demonstrated by field stimulation, all cells received excitatory innervation, but only in some cells was the EJP evoked by Remak's nerve stimulation. This finding is very different from the fact that following Remak's nerve stimulation the EJP is recorded with similar amplitude in every cell in the longitudinal muscle (Takewaki & Ohashi, 1977; Komori & Ohashi, 1982). The TEA-induced increase in the number of cells from which EJPs were recorded suggests that many cells may be placed under the control of the extrinsic nerve fibres excited by Remak's nerve stimulation. The discrepancy would result from unknown factors which are weakened by TEA.

The fact that the amplitude of the EJP or IJP evoked by field stimulation decreased with the distance from the stimulating point is due not solely to the spatial current spread through electrical coupling between cells in either direction, but rather reflects the extent of spread of the nerve fibres mediating the junction potentials. The linear relationship between the EJP amplitude and the distance serves to support this aspect. The distances at which the EJP amplitude decreased to 15% of the standard amplitude are 11 mm in the longitudinal direction and 1·5 mm in the transverse direction. The former distance corresponds to about half of the circumference of the rectum (about 20 mm) and the latter distance corresponds to several widths of the possible unitary ring.

The decrease in the EJP amplitude with lowering the membrane potential in a linear manner suggests that the EJP is produced by an increase in membrane conductance. From the linear relationship, the reversal potential for the EJP was found to be -15 mV which is very similar to the reversal potential for the cholinergic EJPs (Hidaka & Kuriyama, 1969; Komori & Suzuki, 1986) and acetylcholine (Bülbring & Kuriyama, 1963; Bolton, 1972, 1981) in other gastrointestinal muscles. However, as atropine has no effect, the transmitter responsible for the EJP cannot be acetylcholine. It is highly probable that the excitatory transmitter mediating the

EJP may increase the ionic permeability of the membrane non-selectively to cations, preferentially to Na^+, as does acetylcholine (Bolton, 1972). It is conceivable that activation of different receptor types leads to operation of a common transduction (signalling) mechanism by which the membrane is altered to be non-selectively permeable to cations. The transmitter in chicken rectum is not known yet, although recently ATP (Meldrum & Burnstock, 1985) and chicken neurotensin (Komori, Fukutome & Ohashi, 1986) have been proposed as candidates.

Apamin is considered to be a Ca^{2+}-dependent K^+ channel blocker (Banks, Brown, Burgess, Burnstock, Claret, Cooks & Jenkinson, 1979). In smooth muscles of the guinea-pig stomach, ileum and caecum, the IJPs are prevented by apamin and considered to be due to an increase in K^+ permeability (Shuba & Vladimirova, 1980; Maas, 1981; Bauer & Kuriyama, 1982; Niel *et al.* 1983; Komori & Suzuki, 1986). The IJP in the chicken rectum is different from these IJPs in that it was apamin-resistant, slower in time course and smaller in amplitude. Similar apamin-resistant responses to stimulation of intramural nerves have been observed in the circular muscle of the guinea-pig ileum (Niel *et al.* 1983; Bywater & Taylor, 1986), which are also relatively long in duration and small in amplitude. Therefore IJPs in intestinal smooth muscles could be mediated by more than one transmitter. The question of how the apamin-resistant IJP in chicken rectum is generated remains to be answered, but the present data provided some characteristics of the IJP which would be useful for elucidation of the underlying mechanism. (1) The IJP is longer in duration and smaller in amplitude than the IJPs sensitive to apamin, (2) the IJP does not undergo change in amplitude in a linear relation to the level of resting potential but the amplitude is markedly decreased at a displaced potential close to the K^+ equilibrium potential in intestinal smooth muscles (Casteels, 1970), and (3) the IJP is not associated with any consistent change in membrane conductance.

From the present results it seems likely that non-adrenergic, non-cholinergic excitatory or inhibitory nerves seem to run circumferentially in the intestinal wall and innervate several of the possible unitary rings of the circular muscle. Physiologically, this nerve arrangement would be of benefit since it would serve to limit the area of contraction or relaxation of the muscle layer depending on the physiological demand.

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