J. Physiol. (1986), **377**, *pp.* 297–313
With 9 text-figures
Printed in Great Britain

ENTERIC NEURAL REGULATION OF SLOW WAVES IN CIRCULAR MUSCLE OF THE CANINE PROXIMAL COLON

BY KENTON M. SANDERS AND TERENCE K. SMITH

From the Department of Physiology, University of Nevada School of Medicine, Reno, NV 89957, U.S.A.

(*Received 3 September 1985*)

SUMMARY

1. The spontaneous electrical activities of circular muscle cells of the canine proximal colon were studied with intracellular micro-electrodes.

2. All circular muscle cells exhibited slow waves at frequencies ranging between 2·8 and 7·0 cycles/min. The slow waves consisted of an upstroke phase followed by a plateau phase of variable duration (2–40 s). Many cells displayed a slow diastolic depolarization, or 'pre-potential' between slow waves.

3. Slow waves spontaneously varied in duration and frequency in most preparations, creating distinctive slow wave patterns. Atropine, 2×10^{-6} M, decreased the durations of slow waves in many preparations and often changed the pattern to a series of relatively uniform slow waves. A further reduction in mean slow wave duration was produced by additional treatment with tetrodotoxin, 10^{-6} M. These results suggested that slow wave duration and pattern were affected by spontaneous discharge from both cholinergic and non-cholinergic excitatory nerves.

4. Transmural nerve stimulation caused a short latency increase in slow wave duration (up to 38 s) that was abolished by atropine. In the presence of atropine, transmural stimulation evoked inhibitory junction potentials that reduced the amplitude and duration of the subsequent slow wave. The slow wave of reduced amplitude was followed by a slow wave of increased duration. The increase in duration of the slow wave did not appear to be related to the size of the preceding hyperpolarization, suggesting it was mediated by the release from non-cholinergic excitatory nerves. All responses to transmural stimulation were blocked by tetrodotoxin.

5. Microejection of acetylcholine on to the muscle adjacent to the micro-electrode also produced an atropine-sensitive increase in slow wave duration.

6. Tissues that had been stored in the cold overnight to reduce intrinsic neural activity exhibited regular slow waves of short duration.

7. It is proposed that the basic myogenic pattern of spontaneous slow wave activity consists of regularly occurring slow waves of short duration (2–5 s). Intrinsic cholinergic and non-cholinergic excitatory nerves appear to modulate slow wave activity *in vitro*, producing distinctive slow wave patterns of variable instantaneous frequency and duration.

INTRODUCTION

Previous studies have shown that canine, feline, and porcine colonic circular smooth muscle cells exhibit rhythmic, myogenic depolarizations and repolarizations of the membrane potential or 'slow waves' (Christensen, Caprilli & Lund, 1969; El-Sharkawy, 1983; Huizinga, Diamant & El-Sharkawy, 1983; Chambers, Kingma & Bowes, 1984). In contrast to the slow waves in the small intestine and stomach, which appear to be generated within or near the longitudinal muscle and propagate into the circular muscle layer (Bortoff, 1965; Kobayashi, Nagai & Prosser, 1966; Connor, Prosser & Weems, 1974; Connor, Kreulen, Prosser & Weigel, 1977; Bauer, Publicover & Sanders, 1985), slow waves of colonic circular muscle appear to be generated in the circular layer (Christensen *et al.* 1969; Caprilli & Onori, 1972; El-Sharkawy, 1983; Huizinga *et al.* 1983) adjacent to the submucosal border (Caprilli & Onori, 1972; Chambers *et al.* 1984). Unlike the small bowel and stomach, the longitudinal and circular muscles of the colon exhibit different electrical activities (Christensen *et al.* 1969; El-Sharkawy, 1983; Huizinga *et al.* 1983). Circular muscle generates slow wave activity as described above, whereas longitudinal muscles of the dog and pig colon generate pre-potentials and action potentials that are not associated with slow waves but appear to be dependent on external stimuli such as stretch or acetylcholine (El-Sharkawy, 1983; Huizinga *et al.* 1983).

Christensen *et al.* (1969) reported that the duration of spontaneous slow waves of the circular muscle of the cat colon *in vitro* was variable (3–19 s). Others have shown that cholinergic agonists, substance P, pentagastrin and cholecystokinin-octapeptide increased the duration of slow waves (Weinbeck & Christensen, 1971; Huizinga, Chang, Diamant & El-Sharkawy, 1984*a*,*b*). Intrinsic cholinergic and non-cholinergic excitatory nerves, non-adrenergic inhibitory nerves, and nerves rich in neural peptides have been observed in colonic tissues (see Costa & Furness, 1982; Tange, 1983). These results suggest that slow wave duration, and possibly frequency, may be regulated by intrinsic nervous activity, but few studies have tested this hypothesis (Christensen, Anuras & Arthur, 1978; Holman & Smith, 1983). The present investigations attempted to: (i) characterize the spontaneous patterns of slow wave activity in the canine proximal colon, (ii) determine the effects of spontaneous neural discharge on slow wave activity, (iii) document the effects of transmural nerve stimulation on slow waves, and (iv) determine whether micropressure ejection of acetylcholine mimics neural stimulation. Intracellular experiments were conducted on colonic muscles *in vitro* to fully quantitate the effects on intrinsic nerves on resting membrane potential and the wave forms of slow waves.

METHODS

Mongrel dogs of either sex were anaesthetized with sodium pentobarbitone (30 mg/kg). The abdomen was opened along the mid line exposing the viscera, and a segment of the proximal colon, 6–14 cm from the ileocaecal sphincter, was removed. The colonic segment was then cut along the mesenteric border and faecal matter removed by washing with Krebs-bicarbonate solution. The segment of colon was pinned to the Sylgard floor of a dissecting dish and bathed in Krebs-bicarbonate solution that was continuously oxygenated throughout the dissection. A rectangle of muscle along the anti-mesenteric border was isolated from the middle of the segment. Strips of muscle

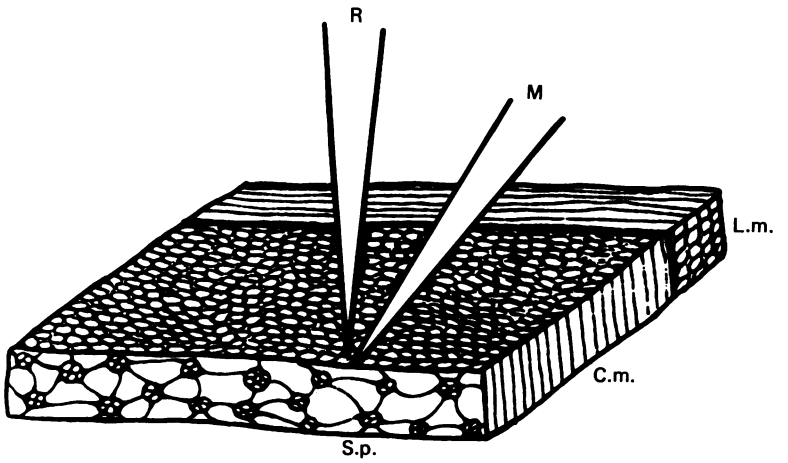


Fig. 1. A schematic representation of the cross-sectional preparation. Lettering denotes longitudinal muscle (l.m.), circular muscle (c.m.), recording micro-electrode (R) and 'spritz' pipette (M). The longitudinal and circular muscle fibres were orientated parallel and perpendicular to the long axis of the colon, respectively. Removal of the mucosa left the submucous plexus (s.p.) intact (see text for details of dissection).

(2 mm × 20 mm) were cut parallel to the longitudinal fibres and turned on-side to expose the entire cross-section of the muscularis. The mucosal layers were carefully removed by cutting along the inner-mucosal border. This technique left the submucous plexus intact as verified by histological examination. The muscles were transferred to a chamber designed for electrophysiological recording and again pinned to expose a cross-sectional view of the muscularis (Fig. 1). The recording chamber was constantly perfused with pre-warmed, pre-oxygenated Krebs solution. Temperature was monitored by a thermistor probe submerged in the bathing solution near the muscle and was maintained at 37·5 ± 0·5 °C. The muscles were allowed to equilibrate for at least 1 h before recordings were begun. Circular muscle cells within 0·2 mm of the submucosal edge of the muscle strip were studied. Cells were impaled with glass micro-electrodes filled with 3 M-KCl and having resistances ranging from 25 to 50 MΩ. Impalements were accepted when a sharp voltage drop of greater than 60 mV (inside negative) was observed, a steady resting potential persisted for at least 5 min, and spontaneous rhythmic depolarizations (slow waves) were observed.

Transmembrane potential was measured by a high input impedance electrometer (WPI M-707, WP Instruments Inc., New Haven, CT, U.S.A.), and the output was displayed on an oscilloscope (Tektronix 5111). Electrical signals were recorded on magnetic tape (Crown–Vetter). Data were analysed off-line, and records for publication were made by a chart recorder (Gould 2200) with sufficient frequency response to reproduce the data.

Two forms of stimuli were used: transmural nerve stimulation and micropressure ejection. Two chlorided silver plates (1·5 mm × 6 mm) were placed on either side of the muscle strip. A Grass S88 stimulator was connected to these plates via a stimulus isolation unit. One- to ten-second trains of biphasic pulses (0·3–1 ms duration; 30–100 V) were used to stimulate the intrinsic nerves. Transmural stimuli were routinely repeated in the presence of tetrodotoxin to confirm that the responses were blocked by this drug. Micropressure ejection was achieved by positioning a 'spritz' pipette within 0·5 mm of the recording site (see Fig. 1). This pipette was connected to the output of a picospritzer (General Valve) which was capable of delivering pressure pulses of 1–100 ms duration. Pipettes for pressure ejection were prepared by breaking back the tips of micro-electrodes to a diameter of approximately 100 μm.

Solution and drugs

The standard Krebs-bicarbonate solution used in this study contained (mM): Na^+, 137·4; K^+, 5·9; Ca^{2+}, 2·5; Cl^-, 134; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2; dextrose, 11·5. This solution had a pH of 7·3–7·4 at 37·5 °C when bubbled to equilibrium with 97 % O_2–3 % CO_2.

Stock solutions of atropine, 10^{-3} M, and tetrodotoxin (TTX), 10^{-3} M, were prepared in distilled water. These drugs were diluted to the desired concentrations with Krebs-bicarbonate solution. Acetylcholine (ACh) solutions, 10^{-3} M, used to fill the 'spritz' pipettes were prepared with Krebs-bicarbonate solution.

Analysis of data

All averaged data are expressed as means ± S.D. Student's *t* tests were used where appropriate to determine levels of significance. Differences in parameters such as slow wave duration, slow wave frequency and resting membrane potential (r.m.p.) recorded in Krebs solution, after the addition of atropine and TTX, were tested for significance by using a two-way analysis of variance test and the Student Newman–Keuls test. Slow wave rate-of-rise was measured using a differentiator amplifier (Gould: 13-4615-71) which records maximal upstroke velocity. Slow wave duration was measured at 90% of the repolarization phase of the slow wave.

RESULTS

Spontaneous electrical slow waves

Circular muscle cells within 0·2 mm of the submucosal border were impaled in forty-three muscles of forty-three dogs. Resting membrane potentials varied between −64 and −77 mV. Each preparation exhibited spontaneous slow waves which occurred at frequencies ranging between 2·8 and 7·0 cycles/min. Slow wave frequency varied in most preparations because of substantial variations in slow wave durations from moment to moment. The slow wave events consisted of at least two components: a rapid upstroke phase followed by a plateau phase of variable duration (2–40 s). The maximum rate-of-rise and amplitude of the slow wave upstroke phase was 0·9 V/s and 46 mV respectively. The maximum plateau amplitude was 45 mV. The maximum level of polarization or 'resting' membrane potential between slow waves in 81% of the cells studied was not constant. A slow depolarization, or 'pre-potential', with a maximum amplitude of 6 mV often preceded the rapid upstroke phase of each slow wave. The rate-of-rise and amplitude of these pre-potentials often varied as a function of time. Regions with and without pre-potentials could be observed in different areas of the same preparation and at different times in the same cell, suggesting that the sites of pace-maker activity may be labile.

Action potentials associated with slow waves were not observed in any preparation. However, small amplitude oscillations in membrane potential were superimposed upon the plateau phases of slow waves in 25% of the preparations.

Following the repolarization phase of some slow waves, a transient after-hyperpolarization of up to 5 mV was observed. These events were only observed on slow waves of duration greater than 15 s, and usually decreased the rate-of-rise of the subsequent slow wave.

The various components of membrane potential that could be recorded within a single slow wave cycle are depicted in Fig. 2. Several parameters of spontaneous slow waves were analysed quantitatively in five representative preparations. The average values are tabulated in Table 1.

Spontaneous slow wave patterns

Neither the durations of slow waves nor the intervals between events were constant in thirty-four, i.e. 79%, out of the forty-three muscles studied. This variability

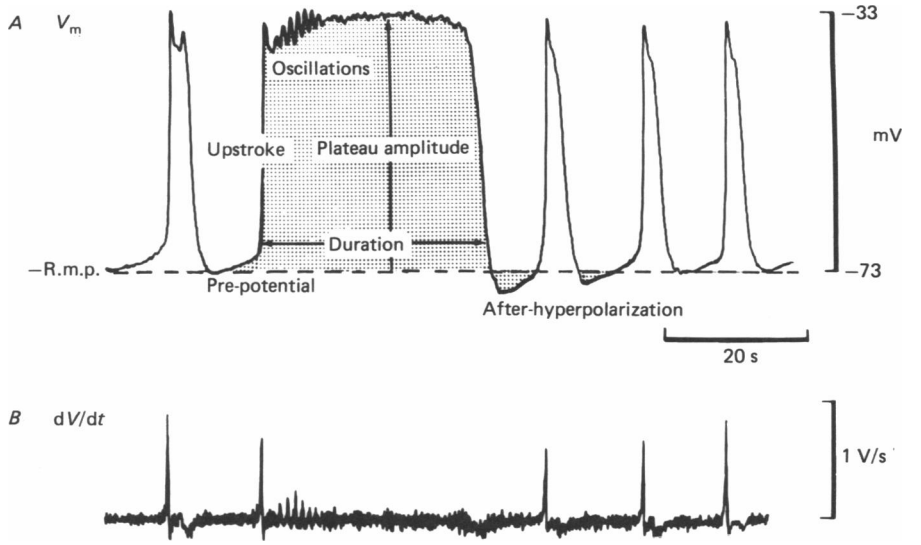


Fig. 2. Characteristics of colonic slow waves. *A*, changes in membrane potential (V_m) associated with a series of spontaneous slow waves, including a slow wave of large duration (stippled slow wave). *B*, the differential or rate-of-rise (dV/dt) of the slow waves in upper panel. Note the various features of membrane potential observed in the canine proximal colon (see stippled slow wave): resting membrane potential (r.m.p.), pre-potential, upstroke phase, plateau phase with oscillations and after-hyperpolarization following the repolarization of the plateau phase. The two slow waves following the slow wave of large duration and associated with the after-hyperpolarization were of reduced upstroke velocity compared to the first spontaneous slow wave in the trace.

TABLE 1. Slow wave parameters for the circular muscle of the canine proximal colon

Parameter	Mean ± S.D.	
R.m.p. (mV)	−70·7 ± 6·2	(n = 100 cells)
Upstroke amplitude (mV)	32·8 ± 4·1	(n = 100 slow waves)
Upstroke rate of rise (V/s)	0·65 ± 0·56	(n = 100 slow waves)
Plateau amplitude (mV)	30·7 ± 6·5	(n = 100 slow waves)
Duration (s)	8·2 ± 4·9	(n = 100 slow waves)
Pre-potential rate-of-rise (mV/s)	0·47 ± 0·22	(n = 50 slow waves)
Pre-potential amplitude (mV)	1·2 ± 0·6	(n = 50 slow waves)

(Data above from five preparations.)

Frequency (cycles/min) 4·2 ± 0·8 (n = 40 preparations).

produced several patterns of slow wave activity that were grouped into five classes (Fig. 3): (a) in nine, i.e. 21 %, of the muscles slow wave duration and the intervals between events were relatively constant. Slow waves in these preparations were among the shortest in duration (2–6 s) because of their brief plateau phases (Fig. 3*A*). (b) in seventeen, i.e. 39 %, of the muscles slow waves of large and irregular duration (10–40 s) were randomly interspersed with slow waves of significantly shorter duration (Fig. 3*B*). (c) in eleven, i.e. 26 %, of the muscles slow waves were rectangular in shape. These events were always longer in duration than those described in class A. The durations of these events were also variable (6–14 s), but were separated in

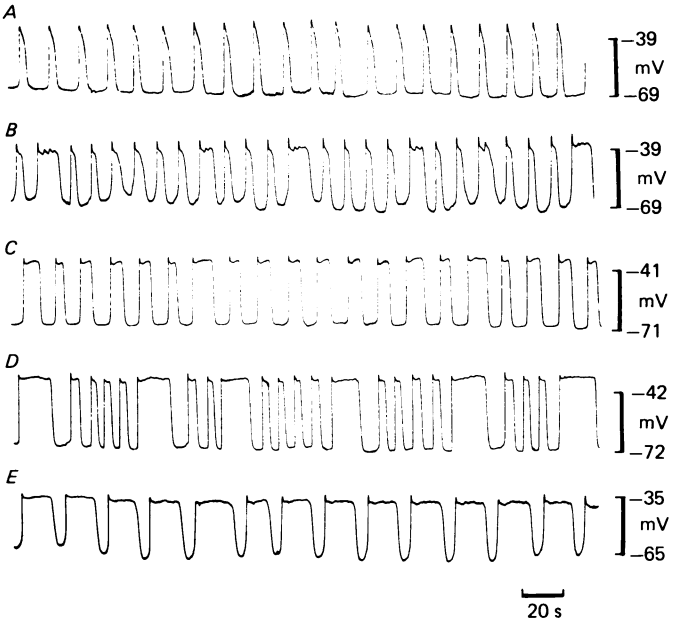


Fig. 3. Five classes of slow wave pattern observed in circular muscle of proximal colon. *A*, class A slow waves were of regular and minimum duration (2–6 s). *B*, slow waves of long duration (10–40 s) were randomly interspersed with slow waves of much shorter duration (class B). *C*, class C slow waves were rectangular in shape and of longer duration (6–14 s) than the class A slow waves. *D*, class D consisted of slow waves with large and regular duration (16–20 s) interspersed in repetitive patterns with short duration slow waves. *E*, class E slow waves were continuously long in duration (11–25 s).

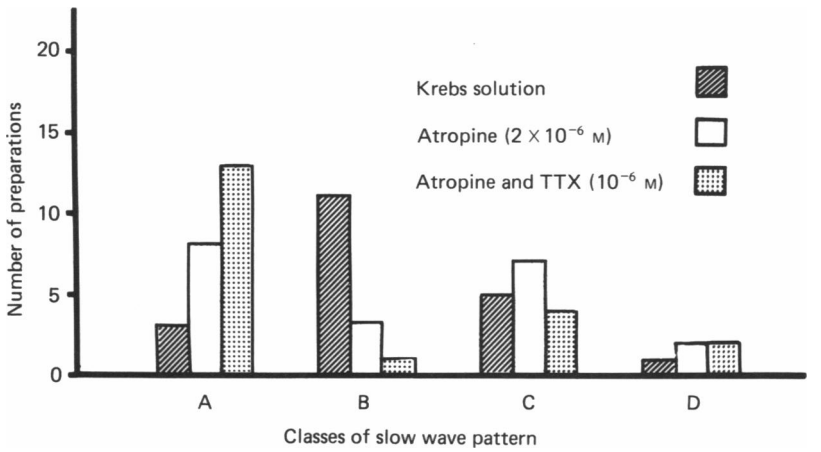


Fig. 4. Histogram of slow wave patterns observed in Krebs solution, after atropine, 2×10^{-6} M, and after atropine and TTX, 10^{-6} M. Preparations exhibiting class A pattern (2–6 s) increased after atropine and further increased after TTX. Preparations exhibiting class B slow wave pattern decreased after atropine and TTX.

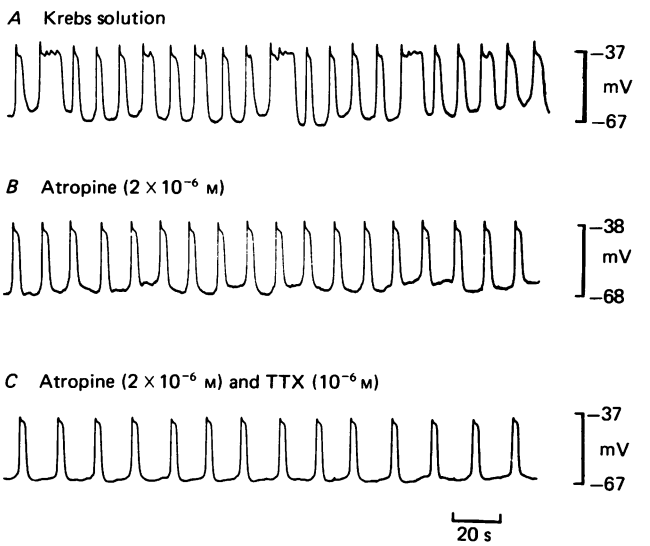


Fig. 5. Spontaneous slow wave activity. *A*, in Krebs solution slow wave pattern was of the class B type (see Fig. 3*B*). *B*, after atropine the slow wave pattern was converted to a class A pattern (see Fig. 3*A*). In this experiment further addition of TTX had no effect, because class A is the basic slow wave pattern.

time by regular intervals (Fig. 3*C*). (d) in four, i.e. 9%, of the muscles slow waves of large and regular duration (16–20 s) were interspersed in reproducible patterns with short duration slow waves (4–6 s) (Fig. 3*D*). (e) in two, i.e. 5%, of the preparations slow waves of long duration (11–25 s) were continuously recorded (Fig. 3*E*).

Effects of atropine and tetrodotoxin on slow waves

The effects of atropine and TTX on slow waves were studied in order to test whether the variability in slow wave durations and patterns described above resulted from spontaneous intrinsic neural activity. Slow wave activity was recorded from twenty preparations in Krebs solution. The muscles were then exposed to atropine, 2×10^{-6} M, for 30 min, while maintaining impalements in many of the preparations. Atropine significantly decreased slow wave duration (Fig. 4) but did not significantly affect resting membrane potential and slow wave frequency (Table 2). Perhaps the most interesting effect of the muscarinic antagonist was an increase in the number of preparations with class A slow wave patterns (from three to eight; a 167% increase). This increase in the occurrence of more regular, short-duration, slow waves was the result of a shift of four preparations from class B, 36% (Figs. 4 and 5) and one preparation of class C, 25%, to a class A pattern. There were three preparations that exhibited class A patterns in Krebs solution, and these were unaffected by atropine. Of the eleven class B preparations observed in Krebs solution eight were affected by atropine. As described, four of these preparations exhibited class A slow wave patterns after atropine, three were converted to class C patterns, and 1 was converted to a class D pattern. The number of preparations with class C and D

patterns were increased slightly by atropine (Fig. 4) from five to seven (40 % increase) and from one to two (100 % increase), respectively.

Atropine decreased the variability in slow wave patterns recorded from most preparations in Krebs solution and caused many preparations to produce regular slow waves of generally shorter duration. Yet despite atropine a significant number of

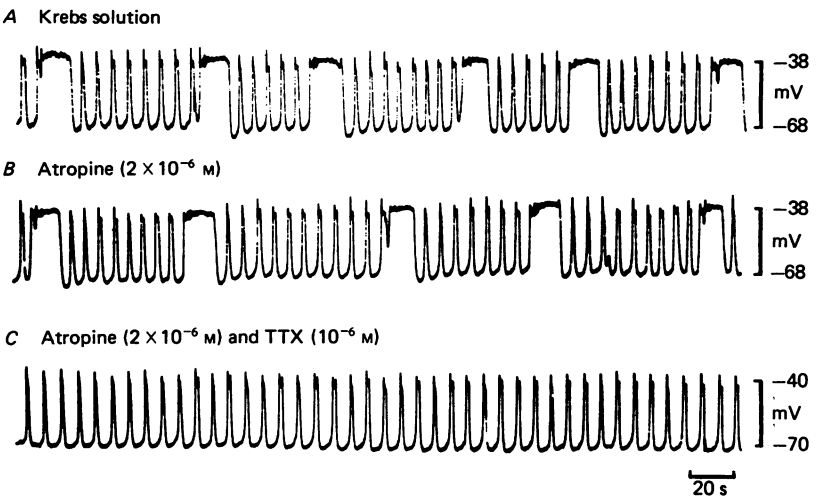


Fig. 6. Spontaneous slow wave activity. *A*, in Krebs solution slow wave pattern was of the class D type. *B*, atropine did not change the pattern of slow waves, but occurrence of long duration slow wave did decrease somewhat. *C*, further addition of TTX converted the slow wave pattern to a class A pattern. In Fig. 4 this class D slow wave pattern was replaced by a class C which became class D after the addition of TTX.

preparations still exhibited variable slow wave durations (see Fig. 6). This suggested that non-cholinergic nerves may also affect slow wave pattern and duration. Therefore, the twenty atropinized preparations were also exposed to TTX, 10^{-6} M, for 30 min after recordings in atropine were completed. TTX increased the number of preparations with the class A slow wave pattern from eight to thirteen (a 63 % increase; Figs. 4 and 6). TTX also reduced the number of preparations of the class B pattern from three to one (a 67 % reduction) and the number of class C pattern from seven to four (a 43 % reduction). The five preparations which had been converted to class A activity by atropine were also unaffected by TTX (Fig. 5). TTX did not significantly affect resting membrane potential or slow wave frequency (Table 2).

Effects of transmural nerve stimulation

These findings suggest that spontaneous release of neural transmitters affects slow wave duration and pattern. The next series of experiments were designed to test whether slow wave duration could be enhanced by transmural nerve stimulation. Three preparations were studied to determine the frequency–response relationship. Frequencies less than 5 Hz did not significantly enhance slow wave duration, and an optimum effect was observed at 20 Hz. The subsequent experiments determined the

TABLE 2. Slow wave parameters in Krebs, atropine and TTX

	Frequency (cycles/min)	R.m.p. (mV)	Duration (s)
Krebs	4·2 ± 0·79	−71·4 ± 0·4	8·29 ± 3·12
Atropine	4·2 ± 0·63	−71·6 ± 0·3	6·40 ± 1·56
Atropine + TTX	4·0 ± 0·72	−69·7 ± 0·9	5·75 ± 1·52

(n = 20 preparations; r.m.p. = four cells per preparation (n = 80))

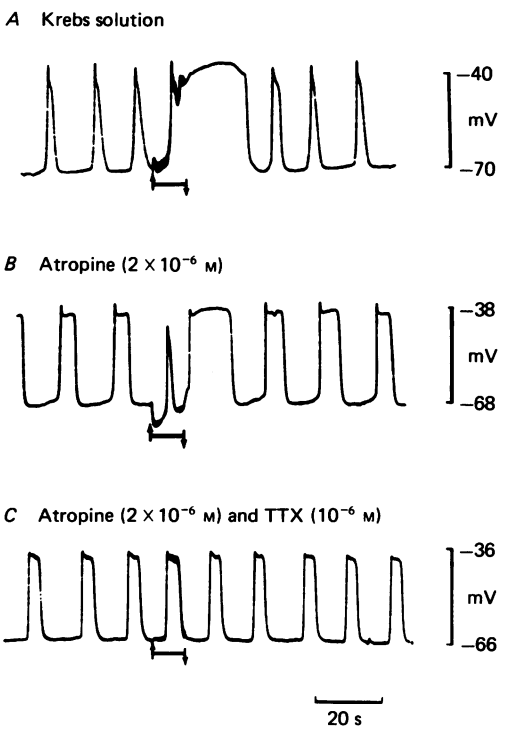
A two-way analysis of variance on these slow wave parameters in the twenty preparations studied showed that: (i) the frequencies and r.m.p.s in Krebs, atropine, and atropine plus TTX were not significantly ($P > 0·05$) different between the three groups (Krebs, atropine, and atropine plus TTX). However, the mean slow wave durations between all three groups were significantly different ($P < 0·05$). The more conservative Student Newman–Keuls test showed that the mean slow wave duration of all preparations observed in Krebs significantly differed ($P < 0·05$) from the slow wave duration in atropine and atropine plus TTX. However, there was no significant change ($P > 0·05$) in the average slow duration between all preparations in atropine and those in atropine plus TTX. This lack of significance between the atropine and atropine plus TTX slow wave duration groups was attributable to the inclusion of the class A preparations and the 35% of preparations with variable slow wave patterns in atropine that were not significantly ($P > 0·05$) altered in mean duration by the addition of TTX.

maximum increase in slow wave duration that could be elicited with maximal stimulus parameters. In these experiments quantitation of slow wave duration in response to nerve stimulation was confined to cells in which class A slow wave activity was observed. The rationale for selecting class A preparations was: (i) the class A pattern appeared to be the basic myogenic pattern. It was of interest to determine the contribution of neural stimulation upon the underlying myogenic activity. (ii) The other patterns of slow wave activity (classes B–D) were highly variable, so it was difficult to be sure that changes observed were due to neural stimulation and not due to spontaneous variation.

In ten preparations the average slow wave duration in these cells was 5·2 ± 0·5 s and the average plateau amplitude was 31 ± 1·2 mV. Following a 10 s train (20 Hz, 1 ms pulse duration, 80 V) the slow wave duration and plateau amplitude increased to an average of 23·8 ± 6·8 s (maximum 38 s) and by an average of 4·7 ± 1·3 mV, respectively (see Fig. 7*A*). These increases were significant at the $P < 0·05$ level. The latency (first pulse of stimulus train to upstroke peak of slow wave) of the subsequent slow wave of enhanced duration was 4·0 ± 0·8 s (twenty-four stimulations). If stimuli were applied early within the slow wave cycle it was possible to evoke premature slow waves. To standardize our protocol nerve stimuli were applied at approximately the same time within each slow wave cycle.

In four of the ten muscle strips studied, the slow wave of increased duration following the stimulus was preceded by a hyperpolarization of amplitude 3 ± 0·5 mV which appeared to be due to an underlying inhibitory junction potential (see below).

Atropine unmasked an inhibitory junction potential (i.j.p.). This i.j.p. was followed by a slow wave of reduced amplitude, and then by a slow wave of increased duration. This enhanced duration slow wave occurred after a much longer latency than similar responses in Krebs solution. An example of this complex response in shown in Fig. 7*B*. The amplitude, time-to-peak, duration and latency of onset of the i.j.p.



(stim.: 1 ms, 20 Hz for 10 s, 80 V)

Fig. 7. The effects of transmural nerve stimulation. *A*, in normal Krebs solution transmural nerve stimulation caused a short latency increase in slow wave duration and amplitude. *B*, in atropine, 2×10^{-6} M, stimulation caused an inhibitory junction potential which decreased the amplitude of the next slow wave. Following this depressed slow wave a slow wave of increased duration and amplitude occurred. *C*, all responses to stimulation were blocked by addition of TTX, 10^{-6} M.

(measured from the first stimulus in the train) were $5{\cdot}40 \pm 1{\cdot}17$ mV ($n = 24$ responses, five preparations), $815{\cdot}7 \pm 155{\cdot}3$ ms ($n = 14$ responses), $4{\cdot}3 \pm 1{\cdot}5$ s ($n = 11$ responses) and 359 ± 131 ms, respectively. The latency (first stimulus pulse to upstroke peak of slow wave) and duration of the broad slow wave following the slow wave of reduced amplitude was $14{\cdot}6 \pm 1{\cdot}5$ s ($n = 14$ responses) and $25{\cdot}2 \pm 5{\cdot}7$ s, respectively. Stimulus trains of 1 s duration at a frequency of 20 Hz often produced i.j.p.s with similar amplitudes to those described above but without significant increase in the duration of slow waves following repolarization of the i.j.p. All of the responses to transmural stimulation were blocked by TTX, 10^{-6} M (Fig. 7*C*).

The effects of transmural nerve stimulation were also tested on class B–D preparations, and were qualitatively similar to the responses of class A preparations just described. These data were not included in the quantification of slow wave parameters reported above because the variations in slow wave activity of these preparations made it difficult to discern evoked responses from spontaneous variation.

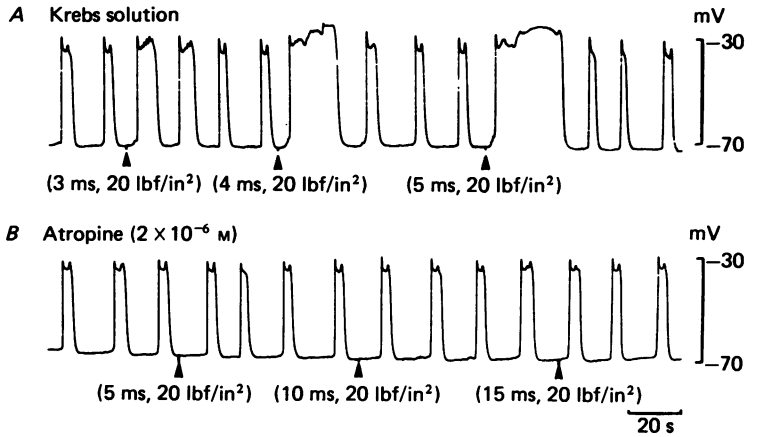


Fig. 8. Effects of microejection of ACh. *A*, ACh caused an increase in duration and amplitude of the slow wave following the stimulus. This effect was concentration dependent and increased when stimulus parameters (duration or pressure of pulse) were increased. The slow wave was evoked prematurely if the stimulus occurred early within the slow wave cycle. *B*, the responses to microejection of ACh were blocked by atropine. Traces *A* and *B* were from the same cell.

Effects of ACh on slow waves

As described above atropine and TTX reduced the duration of spontaneous slow waves in many preparations and increased the regularity of the slow wave pattern. Transmural nerve stimulation increased the duration of slow waves immediately following the stimuli, an effect blocked by atropine. These observations suggest that release of ACh from intrinsic nerve affects slow wave activity in proximal colonic circular muscles. Experiments were performed on class A–D preparations in order to test whether ACh could mimic the effect of transmural nerve stimulation on slow wave duration. In these experiments a 'spritz' micropipette was positioned near the recording electrode (Fig. 1) to micropressure-eject ACh onto the recording site. Topical ACh applied to ten preparations produced an increase in both the duration and plateau amplitude of slow waves immediately following the stimuli (Fig. 8*A*). ACh also caused slow waves to occur prematurely if the stimulus occurred early within the slow wave cycle. The effects of ACh were related to the duration of the pressure ejection pulse as shown in Fig. 8*A*, but it was not possible to tabulate this concentration–response data from preparation to preparation because: (i) 'spritz' pipette diameter varied; (ii) the position of the pipette tip in relation to the cell from which recordings were made could not be precisely controlled; (iii) maximal responses could not be obtained because large stimuli resulted in strong contractions which dislodged the micro-electrode. Therefore, 'percent of maximal response' normalization of the data was meaningless. The latency (onset of stimulus to upstroke peak of the evoked slow wave) was $4{\cdot}1 \pm 0{\cdot}7$ s ($n = 17$ responses). The latency was not significantly different ($P > 0{\cdot}05$) from the latency between stimulus and the long-duration slow wave induced by transmural nerve stimulation, as described in the section above. Atropine, 2×10^{-6} M, abolished the effect of the topical microejection of ACh (Fig. 8*B*).

All the long duration slow waves evoked by either transmural stimulation or microejection of ACh, as well as those occurring spontaneously, were often associated with strong contractions that tended to dislodge the micro-electrode.

Effects of cold storage on slow wave activity

The data in this study have suggested that intrinsic nerves regulate the wave forms and patterns of slow waves. After several hours of recording: (i) the durations of spontaneous slow waves decreased and became less variable, (ii) the intervals between

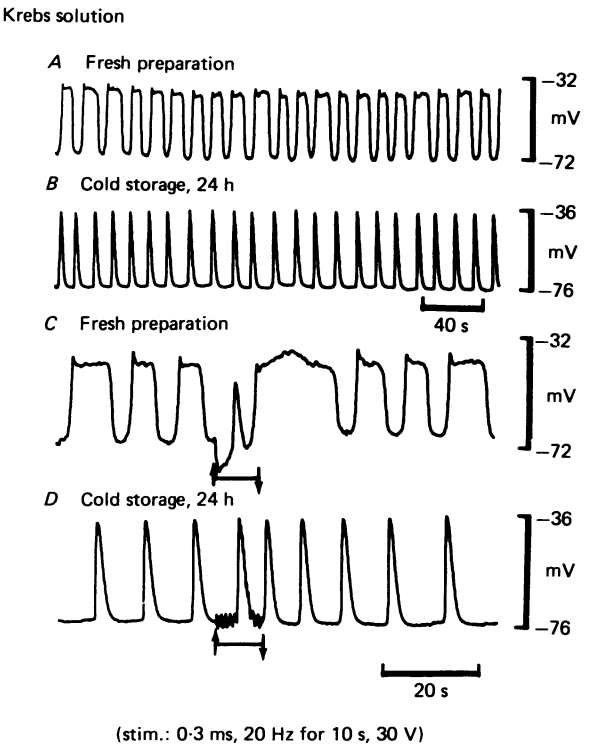


Fig. 9. Effects of cold storage. *A*, the fresh preparation exhibited slow waves of variable duration, class C. *B*, in contrast, slow waves recorded in the other half of the same muscle strip that had been placed in cold storage for 24 h were regular and of short duration, class A. *C*, in the fresh preparation transmural nerve stimulation (0·3 ms, 10 s train of biphasic pulses at 20 Hz, 30 V) evoked an inhibitory junction potential that was associated with a reduction in the amplitude of the next slow wave which in turn was followed by a slow wave of increased duration. *D*, the same stimulus applied to the muscle strip that had been in cold storage only caused a transient increase in slow wave frequency.

slow waves became more regular, and (iii) the responses to transmural nerve stimulation decreased. These observations might be explained by the decay in neural activity that occurs *in vitro* as a function of time (Kuriyama, Osa & Toida, 1967; Wood, 1970). To test this possibility muscles of five animals were studied before and after 24 h of storage at 4 °C. In Krebs solution, the fresh muscles exhibited variable duration, classes B and C slow wave activity. The average duration for slow waves

of this group was $6{\cdot}7 \pm 2{\cdot}2$ s. After storage in the cold the muscles were rewarmed and allowed to equilibrate for the normal period. Cold storage reduced the variability in slow wave duration and yielded class A slow wave patterns. The average duration of slow waves was reduced significantly to $3{\cdot}7 \pm 0{\cdot}5$ s after storage in the cold ($P < 0{\cdot}05$). Cold storage also abolished all response to transmural nerve stimulation in two preparations, and significantly depressed these responses in the other three preparations. Typical slow wave patterns and the responses to transmural stimulation before and after cold storage are shown in Fig. 9.

DISCUSSION

These studies have confirmed that the slow waves recorded from circular muscle cells of the canine proximal colon consist of two components, a relatively rapid upstroke depolarization followed by a plateau phase that can last more than 3 s (Christensen *et al.* 1969; El-Sharkawy, 1983). In contrast to these previous studies in which these investigators reported 'regular' slow waves (Durdle, Kingma, Bowes & Chambers, 1983) of 'remarkably regular frequency' (El-Sharkawy, 1983), our records show obvious spontaneous variations in slow wave duration and instantaneous frequency which together produce distinctive slow wave patterns (see Fig. 3). These complex slow wave patterns are identical to those of canine circular colonic muscles recorded in response to perfusion with excitatory agonists (Huizinga *et al.* 1984*a*,*b*), and suggest that tonic excitation, perhaps by intrinsic excitatory nerves, persists *in vitro*. This tonic excitatory input may be a basic underlying feature of colonic motility (Snape & Shiff, 1983) which is regulated *in vivo* by tonic sympathetic inhibition (Learmonth & Markowitz, 1929; Garry, 1933; deGroat & Krier, 1979). The excitatory drive on proximal colonic circular muscle appears to be the result of intrinsic neural activity, and may be mediated by cholinergic and possibly non-cholinergic transmitters. The results supporting this conclusion are as follows: (i) atropine decreased the duration of slow waves and converted the electrical pattern of many preparations to regular, short-duration slow waves (class A pattern); (ii) in atropinized muscles TTX further increased the occurrence of class A slow wave patterns; (iii) transmural nerve stimulation enhanced slow wave duration in Krebs solution and this effect was mimicked by localized application of ACh; (iv) a portion of the increase in slow wave duration by transmural stimulation was atropine insensitive; (v) cold storage of muscles decreased slow wave duration and increased the occurrence of class A slow wave patterns.

The observation that atropine, TTX, and cold storage decrease the durations of slow waves and change the pattern to one of regular, short-duration events (class A pattern) suggest that this is the basic myogenic pattern in the colon. The spontaneous fluctuations in slow duration (classes B–D) tend to suggest that intrinsic neural discharge is phasic. Spontaneously discharging neurones have been observed with extracellular and intracellular electrodes in the myenteric and submucous plexuses of the small intestine and colon of a number of animals (Wood, 1970; Ohkawa & Prosser, 1972; Wood & Mayer, 1978; Surprenant, 1984; Wade & Wood, 1985). It is possible that these neurones provide phasic motor output to the circular muscle. However, others have shown that constant perfusion of proximal colon strips with

low concentrations of several agonists, including ACh, substance P and carbachol, produced slow wave patterns in which durations are highly variable (Wienbeck & Christensen, 1971; Huizinga *et al.* 1984*a*,*b*). Therefore tonic release of excitatory transmitters could also conceivably produce the patterns observed. The mechanism by which steady-state agonist stimulation can produce an oscillation in slow wave duration is not understood. However, in the present study it was observed that the velocity of upstrokes immediately following a long slow wave were decreased. This suggests that a long-duration plateau potential produces a lasting conductance change which might affect the durations of subsequent slow waves.

The location of the motoneurones in the canine colon which regulate slow wave duration are unknown. Histological examination of the preparations used in these studies revealed that both the myenteric and submucous plexuses were intact. The motoneurones that regulate the motility of the small intestine are generally considered to be located in the myenteric plexus with the submucous plexus playing only a sensory role (Bulbring, Lin & Schofield, 1958). This is logical organization because, in the small bowel, slow waves are generated near the myenteric plexus (Bortoff, 1965; Kobayashi *et al.* 1966; Connor *et al.* 1974; Taylor, Daniel & Tomita, 1975). In contrast slow waves are generated in circular muscle in the colon, probably from cells immediately adjacent to the submucosa (Christensen *et al.* 1969; Caprilli & Onori, 1972; Durdle *et al.* 1983). Therefore, it seems logical that motoneurones which regulate slow wave generation and frequency might be situated in the submucous plexus, near the site of slow wave origin. Submucosal neurones may influence the electrical activity of circular muscle cells via innervation of the network of interstitial cells of Cajal which have been suggested as pace-maker cells in gastrointestinal muscles (see Thuneberg, 1982).

The mean slow wave duration in 35 % of the atropinized preparations with variable slow wave patterns was affected very little by the addition of TTX suggesting that: (i) there was tonic leak of a non-cholinergic excitatory transmitter from nerve terminals as demonstrated for cholinergic terminals in the small intestine (Paton, Vizi & Zar, 1971, (ii) TTX produced incomplete blockade of nervous conduction, perhaps because of a Ca^{2+} component of the action potential in the membrane of the cell soma (see Hirst, Holman & Spence, 1974; Surprenant, 1984).

In contrast to previous studies of the canine proximal colon (El-Sharkawy, 1983; Chambers *et al.* 1984) the majority of cells recorded from in the present study exhibited 'pre-potentials' or diastolic depolarizations between slow waves. The occurrence of pre-potentials may be limited to the site(s) of slow wave generation in these muscles since: (i) they are assumed to be associated with pace-maker activity (El-Sharkawy & Daniel, 1975); (ii) they are low amplitude events which would tend to decay over short distances; and (iii) they are recorded predominantly along the submucosal surface of the circular muscle and decay rapidly through the bulk of the circular layer (T. K. Smith, unpublished observations). Therefore previous studies may not have detected the presence of pre-potentials if the site of recording was not adjacent to the submucosa. In the present study cells within 0·2 mm of the submucosal border, which others have shown to be the most likely site of slow wave origin (Caprilli & Onori, 1972; Durdle *et al.* 1983), were selectively studied. The large proportion of these cells with pre-potentials and the variation in amplitude and

rates-of-rise of these depolarizations within the same cell suggests that pace-maker activity is labile and that the site of the dominant pace-maker may shift from moment to moment. The effects of refractoriness following slow waves, membrane potential, and agonists on the pre-potential depolarization are yet to be described.

Slow waves of long duration which occurred spontaneously or were evoked by microejection of ACh or transmural nerve stimulation were associated with strong contractions that often tended to dislodge the micro-electrode. The occurrence of these contractions was apparently not dependent upon generation of action potentials. These results suggest that excitation–contraction coupling can be achieved in the colon by the depolarization of the slow wave alone, as in the canine stomach and small intestine (Morgan & Szurszewski, 1980; Sanders, 1983). Previous studies using either extracellular pressure electrodes or the sucrose-gap apparatus have shown that ACh, carbachol, substance P, pentagastrin, and cholecystokinin-octapeptide (Huizinga *et al.* 1984*a*,*b*) increase slow wave duration and amplitude in a concentration-dependent manner. Although each of these agonists also increased the force of colonic contractions, they do not all necessarily elicit action potentials. Christensen *et al.* (1969) has also reported that the durations of contractions in the cat colon were directly proportional to slow wave duration. Taken together, these observations suggest that the slow wave event, besides serving in its classical role of timing phasic contractions in the gastrointestinal tract, also provides sufficient depolarization to achieve excitation–contraction coupling. This hypothesis, although in need of further testing, suggests that the canine colonic muscles and gastric muscles are similar in their mechanisms of excitation–contraction coupling (Morgan & Szurszewski, 1980).

This study was supported by research grants from the National Institutes of Health (AM32176) and from the National Institute for Alcohol Abuse and Alcoholism (AA05883). Dr Sanders was supported by NIH Research Career Development Award AM01209.

REFERENCES

BAUER, A. J., PUBLICOVER, N. & SANDERS, K. M. (1985). Origin and spread of slow waves in canine gastric circular muscle. *American Journal of Physiology* **249**, G800–806.

BORTOFF, A. (1965). Electrical transmission of slow waves from longitudinal to circular muscle. *American Journal of Physiology* **209**, 1254–1260.

BULBRING, E., LIN, R. C. & SCHOFIELD, G. (1958). An investigation of the peristaltic reflex in relation to anatomical observations. *Quarterly Journal of Experimental Physiology* **43**, 26–37.

CAPRILLI, R. & ONORI, L. (1972). Origin, transmission and ionic dependence of colonic electrical slow waves. *Scandinavian Journal of Gastroenterology* **7**, 65–74.

CHAMBERS, M. M., KINGMA, Y. J. & BOWES, K. L. (1984). Intracellular electrical activity in circular muscle of canine colon. *Gut* **25**, 1268–1270.

CHRISTENSEN, J., ANURAS, S. & ARTHUR, C. (1978). Influence of intrinsic nerves on the electromyogram of cat colon 'in vitro'. *American Journal of Physiology* **234(6)**, E641–647.

CHRISTENSEN, J., CAPRILLI, R. & LUND, G. F. (1969). Electrical slow waves in the circular muscle of cat colon. *American Journal of Physiology* **217**, 771–776.

CONNOR, J. A., KREULEN, D., PROSSER, C. L. & WEIGEL, R. (1977). Interaction between longitudinal and circular muscle in intestine of cat. *Journal of Physiology* **273**, 665–689.

CONNOR, J. A., PROSSER, C. L. & WEEMS, W. (1974). A study of pace-maker activity in intestinal smooth muscle. *Journal of Physiology* **240**, 671–701.

COSTA, M. & FURNESS, J. B. (1982). Nervous control of intestinal motility. In *Mediators and Drugs in Gastrointestinal Motility*, ed. BERTACCINI, G., pp. 280–381. New York: Springer-Verlag.

DEGROAT, W. C. & KRIER, J. (1979). The central control of the lumbar sympathetic to the large intestine of the cat. *Journal of Physiology* **289**, 449–468.

DURDLE, N. G., KINGMA, Y. J., BOWES, K. L. & CHAMBERS, M. M. (1983). Origin of slow waves in the canine colon. *Gastroenterology* **84**, 375–382.

EL-SHARKAWY, T. Y. (1983). Electrical activities of the muscle layers of the canine colon. *Journal of Physiology* **342**, 67–83.

EL-SHARKAWY, T. Y. & DANIEL, E. E. (1975). Electrical activity of small intestinal smooth muscle and its temperature dependence. *American Journal of Physiology* **229(5)**, 1268–1276.

GARRY, R. C. (1933). The nervous control of the caudal region of large bowel. *Journal of Physiology* **77**, 422–431.

HIRST, G. D. S., HOLMAN, M. E. & SPENCE, I. (1974). Two types of neurones in the myenteric plexus of duodenum in the guinea-pig. *Journal of Physiology* **236**, 303–326.

HOLMAN, M. E. & SMITH, T. K. (1983). Effect of distension on the slow wave activity of the guinea-pig distal colon. *Proceedings of the International Union of Physiological Sciences (XXIXth Congress)* **XV**, 523.16.

HUIZINGA, J. D., CHANG, N. E., DIAMANT, N. E. & EL-SHARKAWY, T. Y. (1984*a*). Electrophysiological basis of excitation of canine colonic circular muscle by cholinergic agents and substance P. *Journal of Pharmacology and Experimental Therapeutics* **231**, No. 3, 692–699.

HUIZINGA, J. D., CHANG, N. E., DIAMANT, N. E. & EL-SHARKAWY, T. Y. (1984*b*). The effects of cholecystokinin-octapeptide and pentagastrin on electrical and motor activities of canine colonic circular muscle. *Canadian Journal of Physiology and Pharmacology* **62**, 1440–1447.

HUIZINGA, J. D., DIAMANT, N. E. & EL-SHARKAWY, T. Y. (1983). Electrical basis of contractions in the muscle layers of the pig colon. *American Journal of Physiology* **245**, G482–491.

KOBAYASHI, M., NAGAI, T. & PROSSER, C. L. (1966). Electrical interaction between muscle layers of cat intestine. *American Journal of Physiology* **211(6)**, 1281–1291.

KOTTEGODA, S. R. (1970). Peristalsis of the small intestine. In *Smooth Muscle*, ed. BULBRING, E., BRADING, A. F., JONES, A. W. & TOMITA, T., pp. 525–541. London: Arnold.

KURIYAMA, H., OSA, T. & TOIDA, N. (1967). Nervous factors influencing the membrane activity of intestinal smooth muscle. *Journal of Physiology* **191**, 257–270.

LEARMONTH, J. R. & MARKOWITZ, J. (1929). Studies of the function of the lumbar sympathetic outflow. *American Journal of Physiology* **89**, 686–691.

MORGAN, K. G. & SZURSZEWSKI, J. H. (1980). Mechanisms of phasic and tonic actions of pentagastrin on canine gastric smooth muscle. *Journal of Physiology* **301**, 229–242.

OHKAWA, H. & PROSSER, C. L. (1972). Functions of neurones in the enteric plexuses of cat intestine. *American Journal of Physiology* **222**, 1420–1426.

PATON, W. D. M., VIZI, E. S. & ZAR, M. A. (1971). The mechanism of acetylcholine release from parasympathetic nerves. *Journal of Physiology* **215**, 819–848.

SANDERS, K. M. (1983). Excitation–contraction coupling without Ca^{2+} action potentials in small intestine. *American Journal of Physiology* **244**, C356–361.

SNAPE, W. J. & SHIFF, S. (1983). Neurohumoral control of colonic motility in the rabbit. *American Journal of Physiology* **245(8)**, G582–588.

SURPRENANT, A. (1984). Two types of neurones lacking synaptic input in the submucous plexus of guinea-pig small intestine. *Journal of Physiology* **351**, 363–378.

TANGE, A. (1983). Distribution of peptide-containing endocrine cells and neurons in the gastrointestinal tract of the dog: immunohistochemical studies using antisera to somatostatin, substance P, vasoactive intestinal polypeptide, met-enkephalin, and neurotensin. *Biomedical Research* **4(1)**, 9–24.

TAYLOR, G. S., DANIEL, E. E. & TOMITA, T. (1975). Origin and mechanism of intestinal slow waves. In *Proceedings of the 5th International Symposium on Gastrointestinal Motility*, ed. VANTRAPPEN, G., pp. 102–106. Herentals, Belgium: Typoff Press.

THUNEBERG, L. (1982). Interstitial cells of Cajal: intestinal pace-maker cells. *Advances in Anatomy, Embryology and Cell Biology* **71**, 1–130.

WADE, P. R. & WOOD, J. D. (1985). Intracellular study of electrical activity of myenteric neurons in guinea-pig distal colon. *Federation Proceedings* **44(5)**, 1719.

WEINBECK, M. & CHRISTENSEN, J. (1971). Effects of some drugs on electrical activity of the isolated colon of the cat. *Gastroenterology* **61(4)**, 470–478.

WOOD, J. D. (1970). Electrical activity of single neurons in Auerbach's plexus. *American Journal of Physiology* **219**, 159–169.

WOOD, J. D. & MAYER, C. J. (1978). Intracellular study of electrical activity of Auerbach's plexus in guinea-pig small intestine. *Pflügers Archiv* **374**, 265–275.