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Electrical correlate of circumferential contractions in human colonic circular muscle

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SUMMARY The role of myogenic electrical activity in the coordination of circumferential contraction of the human colon circular muscle was investigated. Five suction electrodes were placed (5–7 mm apart) on isolated rings of human colon and simultaneously electrical and motor activities were measured. In normal Krebs solution, the slow waves were not synchronised in most preparations studied. The electrical activities at the different recording sites were different with respect to slow wave frequency and amplitude, and amount of spiking activity. This resulted in irregular contractile activity. Cholinergic stimulation resulted in the development of a specific pattern of electrical activity: periodic slow wave activity with superimposed spiking activity which was synchronised over the length of the segment studied. This synchronised electrical activity resulted in regular phasic contractions at the frequency of the bursts of electrical activity (~1/min). The response to carbachol was mediated by muscarinic receptors since it was blocked by atropine. The periodic activity in the continuous presence of carbachol was not the result of periodic input of neural activity as it occurred in the presence of TTX. Intrinsic properties of the muscle cells were responsible for the carbachol induced pattern of activity. The present study presents evidence that the electrical correlate of circumferential contractions is different in man compared with the most commonly studied animal models. It is a specific, stimulus induced pattern of myogenic activity. Its characteristics closely resemble those of a particular pattern of *in vivo* recorded activity referred to as the 'long spike bursts'.

In a review on colonic motility, Christensen[1] stated that 'it is no longer satisfactory simply to look for an increase or a decrease in activity. Patterns of movement are important. These patterns must be defined and methods developed to allow them to be observed'. In the small intestine, different patterns of activity have been characterised, recently reviewed by Wingate[2] and Vantrappen.[3] In the colon, patterns of activity are less well defined and their relationship to transit less well understood. Recordings of spiking in the human colon *in vivo*, however, revealed certain patterns of activity. Of particular interest are the 'long spike bursts', 14–30 sec in duration.[4,5] These were seen to propagate over long segments of the colon[6,7] and were associated with transit of colonic contents.[8] The long spike bursts were particularly prevalent after stimulation by a meal or by neostigmine.[6,7] Regular appearance of such spike bursts is not commonly observed in *in vitro* preparations under unstimulated conditions. We recently observed that cholinergic stimulation could induce bursts of spiking activity similar to those described *in vivo*,[9] but their propagation characteristics were not examined. This study set out to test the hypothesis that cholinergic stimulation of human colonic circular muscle induces a specific pattern of activity that is synchronised over a segment of bowel in a pattern similar to neostigmine and meal induced activity *in vivo*. Furthermore, it was to be investigated whether such a pattern was a consequence of periodic neural input or because of intrinsic properties of the muscle cells. Furthermore, the relationship was to be

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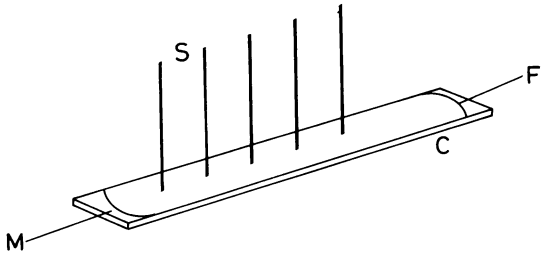

Fig. 1 *Diagrammatic drawing of the recording set up. Segment of colon (5×30 mm circular muscle cells facing up with their long axis parallel to the long axis of the muscle segment) is indicated by a C. M=micromanipulator to adjust stretch. F=force transducer to record tension. S=suction electrodes to record electrical activity, placed 5 mm apart. One reference electrode was placed in the organ bath solution (not shown).*

explored between spike bursts and the slow wave activity of the human colon.

Methods

TISSUE PREPARATION

A circumferential ring of colon (1·0 cm wide) was removed from 10 patients undergoing surgery for cancer. The muscle was taken from the distal colon at least 5 cm away from the site of cancer and was histologically free of tumor and inflammation. The segments were immediately put into oxygenated Krebs solution and sent to the laboratory for preparation. The ring was opened and with the mucosal side up the tissue was pinned flat to the Sylgard bottom of a dish containing continuously oxygenated Krebs solution. The mucosa was removed by sharp dissection. A thin layer of submucosa remained attached to the circular muscle layer.

RECORDING SET UP

The tissue segments (5 mm × 50 mm) with the circular muscle bundles visually in line with the long axis of the preparation, were mounted in an organ bath by means of silk threads, with circular muscle facing up. Electrical and mechanical activities were recorded using monopolar suction electrodes, placed on the surface of the circular muscle layer, and force displacement transducers (Grass FT.03C, Grass Medical Instruments, Quincy, Mass, USA) respectively (Fig. 1). Both electrical and mechanical activities were recorded on an eight channel ink writing recorder (Gould). The strips were allowed to equilibrate without stretch at 37°C for 45 minutes. Thereafter, they were stretched up to the maximal length that did not produce any measurable tension. Studies were done at 150% of this length (L_1) which was found to be the length at which the spontaneous contractions were greatest – that is, the optimal length (L_0) for these preparations. Activity measured under these circumstances was called spontaneous activity. This term should not be regarded as a synonym of either myogenic or unstimulated activity as stretch and/or activity of enteric neurones may contribute to the activity recorded under these conditions. Four or five electrodes were placed with a distance of 5–7 mm between each two electrodes (Fig. 1). The electrical signals were AC amplified with a time constant of 1 sec.

SOLUTIONS AND DRUGS

All experiments were carried out at 37°C. The composition of the Krebs solutions was (mM): NaCl, 120·3; KCl, 5·9; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 15·4; NaH_2PO_4, 1·2 and glucose, 11·5. The solution was equilibrated with 95% O_2–5% CO_2. The pH of the solution was 7·3–7·4. The following drugs were used: tetrodotoxin (TTX), carbachol (carbamylcholine chloride) and atropine, from Sigma Chemical Co, St Louis, MO, USA. Tetrodotoxin was dissolved in water and stored at −20°C.

TERMINOLOGY

Synchronisation: the phenomenon that slow waves or spikes occur at the same time or with a constant delay at separate recording sites. The term 'spike' or 'spiking activity' is used for the fast transient electrical depolarisations of 50–100 ms duration. The term 'slow wave' is used for the much slower transient depolarisations (1–5 s duration) on which the spikes are superimposed. Although the term 'slow wave' was chosen, we want to emphasize the differences between the slow waves observed in the human colon and the slow wave activity recorded from the stomach and the small intestine.[9 10]

ANALYSIS

Five recordings of electrical activity were obtained simultaneously at five different sites. Activities were said to be synchronised when they occurred simultaneously or with the same period frequency. It was quantified as the percentage of recording sites synchronised (four of five equals 80%). Synchronisation was measured in a 5 min period in Krebs solution and in different concentrations of carbachol. Differences were measured with the *t* test. Although synchronisation, as defined above, could be measured and quantified accurately, determination of phase shift could not be done because of the nature of the activity. A period of slow waves started with oscillations which were not always clearly distinguishable from noise because of their low

amplitude, and hence, the exact starting point of the periods of activity could normally not be measured.

Results

The electrical activity of the circular muscle of the human colon consisted of slow wave activity at variable frequency and superimposed spiking activity (Fig. 2a). Three patterns of slow wave activity could be distinguished: (a) continuous activity at a frequency varying in time, (b) continuous electrical activity at a constant frequency (Fig. 2a), and (c) periods of slow wave activity (15–60 s duration) alternating with periods of relative electrical quiescence (Fig. 2b).

PATTERNS OF SPONTANEOUS ACTIVITY

The predominant pattern of activity, observed without intrinsic stimulation, was the one described above first. The activity is represented by Fig. 2a and occurred in six of nine segments of tissue. The slow wave frequencies ranged from 8–30 cpm (mean= 17·1±2·0). The contractile activity associated with this type of electrical activity was irregular in shape and frequency (Fig. 2a). Synchronisation was 9±10%.

In two of the nine segments regular periodic slow wave activity was observed (Fig. 3a). This type of activity showed a high level of synchronisation (90±5%). The slow wave frequency was 23±1 cpm, the burst frequency 1·1±0·3/min and the burst duration 25±15 sec. Regular phasic contractile activity occurred only with this type of electrical activity. This spontaneous activity was not blocked by atropine (10^{-6}M).

In one other of the nine segments, continuous slow wave activity was observed at 12 cpm at all recording sites (Fig. 4a), and showed 80% synchronisation. The individual slow waves occurred without time lag at the different recording sites. This activity was associated with a tonic contraction with superimposed smaller contractions at the frequency of the slow waves.

Characteristics of the synchronisation of activity did not change significantly when intrinsic nerve conduction was blocked with tetrodotoxin (5×10^{-7}M, which blocked field stimulation induced relaxation in this tissue). Synchronisation of activities amounted to 41±9% (n=9) in TTX compared with 32±16% without TTX ($p>0.05$). When regular periodic activity occurred in the presence of TTX, 100% synchronisation of electrical activity was seen (n=3). In these preparations, the slow wave frequency, was 23·3±5·2 cpm, the burst frequency 0·7±0·2 per min, and the burst duration 46±17 sec (n=3).

Stretch was a necessary condition for electrical activity to occur (Fig. 4a). With blockade of neural activity, stretch was probably the predominant stimulus present under our *in vitro* conditions. Changes in stretch did not give consistent changes in the patterns of activity possibly related to limitations of movements of the muscle segment by suction electrodes.

PATTERNS OF ACTIVITY WITH CARBACHOL STIMULATION

Cholinergic stimulation with low concentrations of carbachol (5×10^{-8}M; n=9), resulted in a constant slow wave frequency at all recording sites in seven of nine preparations (24·4±1·9 cpm). At this concentration, however, periodic activity developed which was irregular in duration and different at most recording sites which resulted in synchronisation of 28±14%.

With carbachol, 2×10^{-7}M, the slow wave frequency was the same at all recording sites (22·2±1·9 cpm; n=9). In six of nine segments, regular periodic activity occurred (Figs. 2b, 3b) at 1·1±0·3/min. The duration of a period of activity was 37·1±6·0 s. In preparations which spontaneously exhibited periodic activity, carbachol increased the period frequency (Fig. 3b). Synchronisation was 92±5% ($p<0.01$ compared with control) and the periods of activity occurred simultaneously around the circumference. In three of nine segments, regular periodic activity did not develop with this concentration of carbachol and synchronisation was 46±7%.

With carbachol, 10^{-6}M, (n=4) synchronisation reached 96±5% ($p<0.01$ compared with control). All preparations showed regular periodic activity at 1·9±0·1/min. The duration of a period of activity decreased to 13·8±1·7 sec ($p<0.02$). Carbachol induced increase in intensity of spiking activity. Occasionally, the slow wave-spike activity merged into prolonged bursts of intense spiking activity.

Carbachol induced activity was blocked by atropine (10^{-6}M). Development of synchronisation was caused by carbachol and not by a time dependent phenomenon. Activity observed in Krebs solution for one hour did not change significantly in two subsequent hours (n=3). Stretch could modify responses to carbachol. When irregular periodic activity occurred in the presence of carbachol (2×10^{-7}M), release of some stretch could increase the regularity of the periodic activity (Fig. 4b, c).

Discussion

In order to obtain a propagating circumferential ring contraction of the colon, thousands of circular muscle cells have to act in synchrony. Circumferential coupling mechanisms have been studied in the cat[11] and

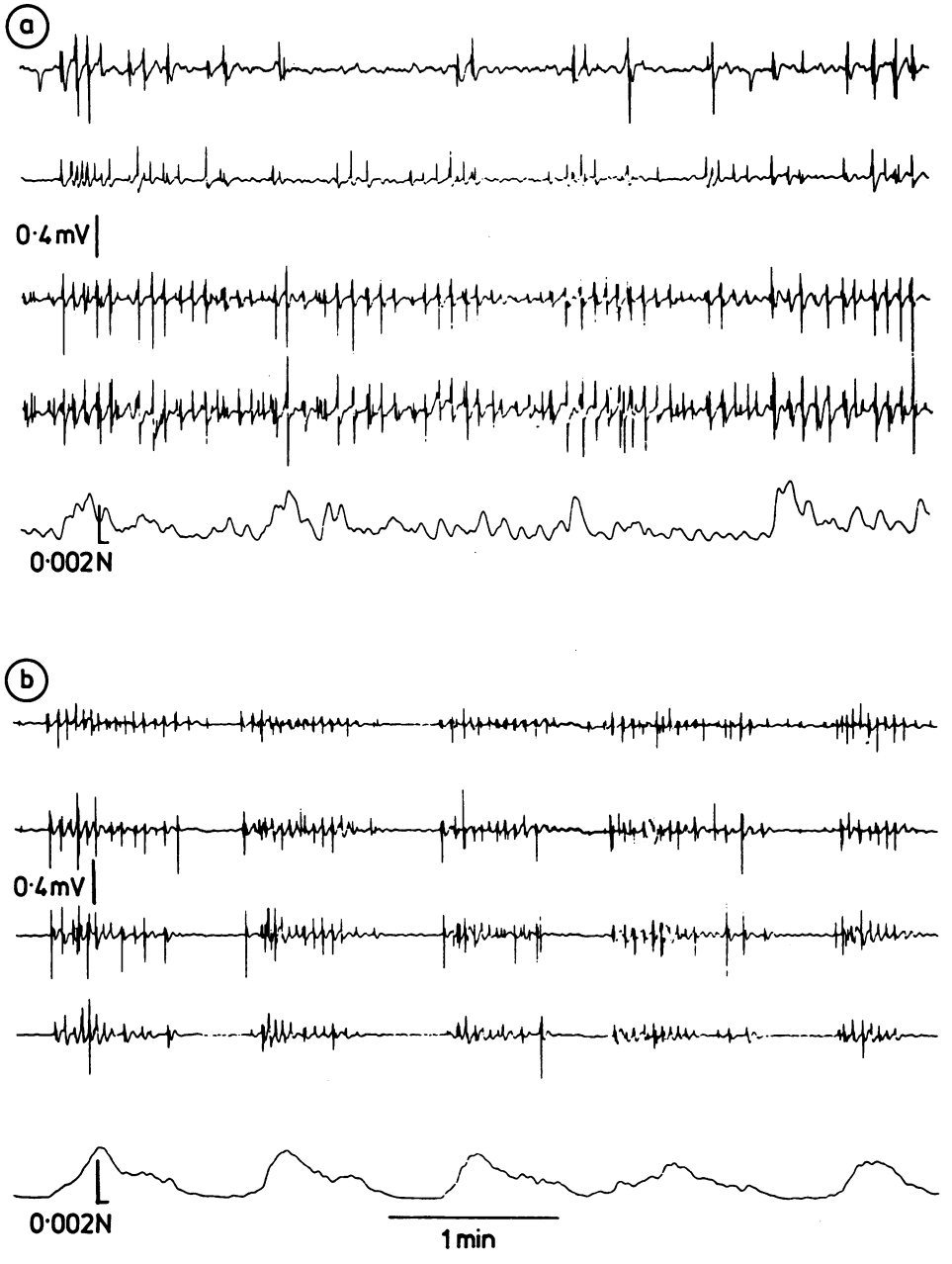

Fig. 2 *Development of synchronisation of electrical activity with cholinergic stimulation. Top four tracings in each panel show simultaneous measurements of electrical activities at four different sites in human colonic circular muscle. Electrodes were placed circumferentially and the distance between each pair of electrodes was 5 mm. Bottom tracing shows simultaneously measured contractile activity.* (a) *In normal Krebs solution, the electrical activities were largely uncoordinated. Different slow wave frequencies were observed at adjacent sites (see top three tracings). Result was an irregular pattern of contractions.* (b) *With the addition of carbachol (10^{-7}M) the electrical slow wave frequency became uniform and the activity became synchronised. Electrical activity became periodic with periods of relatively high amplitude slow waves with spiking activity alternating with periods of relative quiescence. Activity became synchronised and the resulting contractions were phasic and regular.*

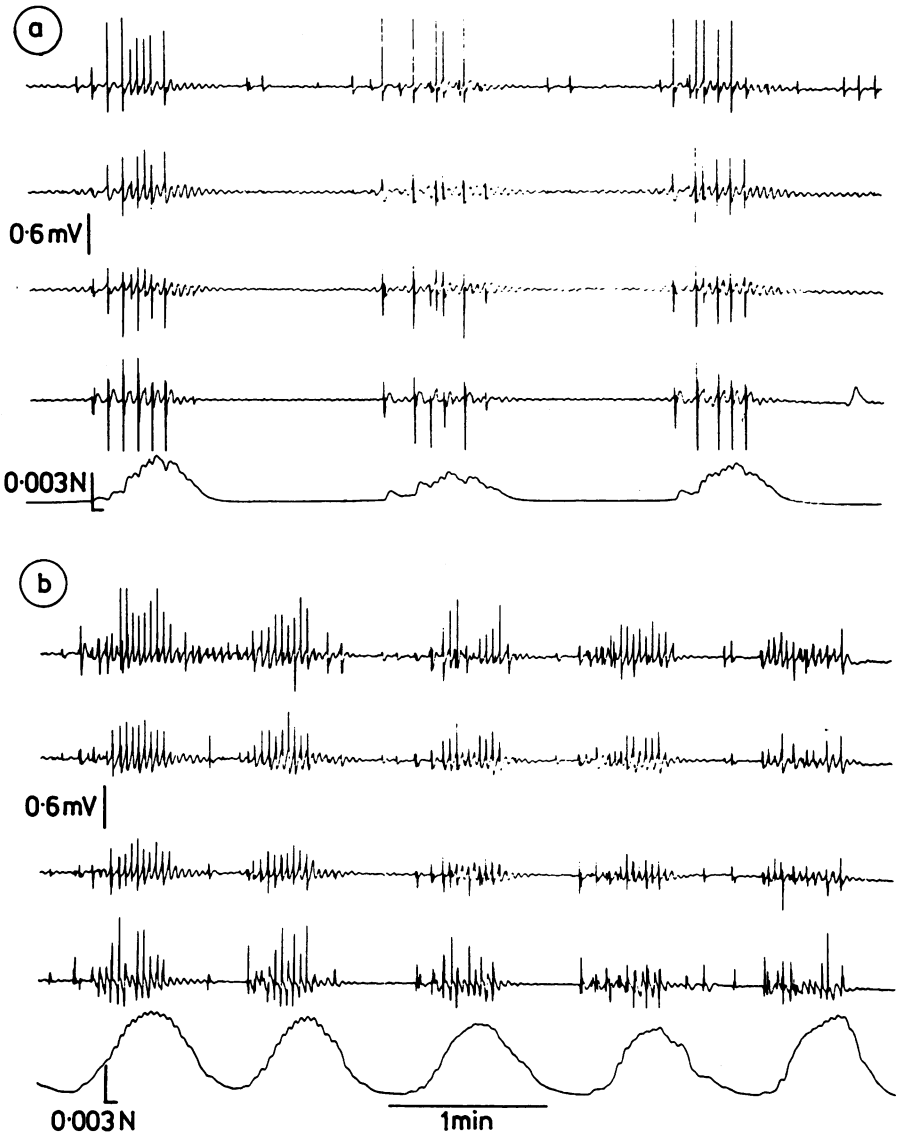

Fig. 3 *Periodic synchronised activity in normal Krebs in the presence of the nerve conduction blocker tetrodotoxin (TTX) and effect of cholinergic stimulation on periodic activity.* (a) *Periodic activity in normal Krebs solution in the presence of TTX ($5\times10^{-7}M$). Periods of activity occurred simultaneously at different recording sites.* (b) *Addition of carbachol ($10^{-7}M$) caused increase in the frequency of the periods of activity, the slow wave frequency and the amount of spiking activity. Consequently, the frequency and force of contractions were increased.*

dog[12] colon. The electrical control activity of the human colon differs substantially from that of animal models, however.[9 10 13–15] Slow waves occur at variable amplitude and in a wide frequency range. In addition, unlike slow waves of cat and dog colon, human colonic slow waves are dependent on stretch and are blocked by stimulation of adrenergic receptors.[9] The slow waves in the human colon do provide control function in that they determine the occurrence of spiking activity and the pattern of contractile activity.

The present study shows that in normal Krebs solution slow waves are not synchronised, comparing

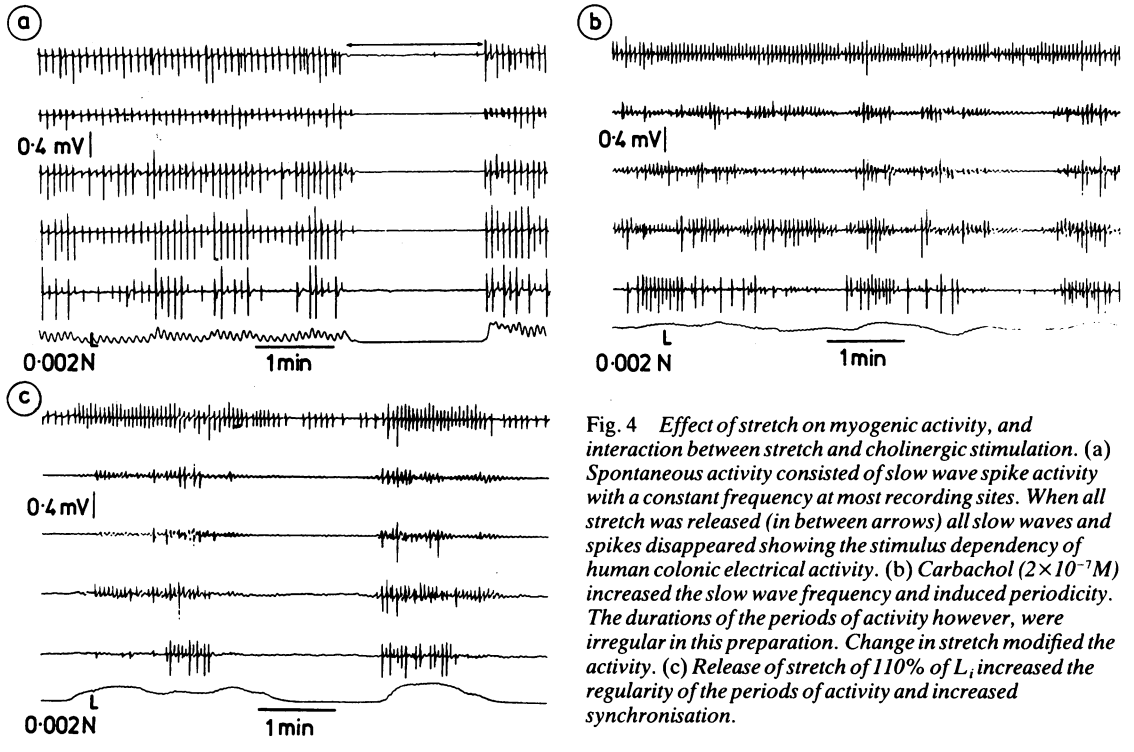

Fig. 4 *Effect of stretch on myogenic activity, and interaction between stretch and cholinergic stimulation.* (a) *Spontaneous activity consisted of slow wave spike activity with a constant frequency at most recording sites. When all stretch was released (in between arrows) all slow waves and spikes disappeared showing the stimulus dependency of human colonic electrical activity.* (b) *Carbachol ($2\times10^{-7}M$) increased the slow wave frequency and induced periodicity. The durations of the periods of activity however, were irregular in this preparation. Change in stretch modified the activity.* (c) *Release of stretch of 110% of L_i increased the regularity of the periods of activity and increased synchronisation.*

activities at different recording sites over a distance of 2–3 cm, in most preparations studied. The electrical activities are different with respect to slow wave frequency and amplitude, and amount of spiking activity. This results in an irregular pattern of contractile activity.

Cholinergic stimulation induces regular periodic slow wave activity with superimposed spiking activity which is synchronised over the length of the segment studied. This synchronised electrical activity results in regular phasic contractions at the frequency of the periods of electrical activity (~1/min). The response to carbachol is mediated by muscarinic receptors since it is blocked by atropine.

The periodic activity in the continuous presence of carbachol is not because of periodic input of neural activity as it occurs in the presence of TTX. Intrinsic properties of the muscle cells are responsible for the carbachol induced pattern of activity and discussion about the mechanism by which carbachol changes the electrical activity remains speculative. Carbachol might increase calcium conductance, thereby increasing amplitude of slow waves and increasing spiking activity. Both slow waves and spikes are sensitive to calcium influx blockers.[16] Calcium, accumulating intracellularly during the carbachol stimulation might turn on a potassium conductance which would block inward current and result in the period of relative electrical quiescence. A similar mechanism has been proposed for neurones showing bursting type action potentials.[17] Concurrent with changes in ionic conductance, carbachol might increase cell to cell coupling. Such coupling was shown to be sensitive to a variety of agents.[18]

Periodic synchronised activity, as induced by carbachol, occasionally occurs 'spontaneously', that is without addition of excitatory substances into the organ bath. This synchronised activity is not mediated by intrinsic cholinergic nerves as neither TTX nor atropine abolishes it. It is dependent on stretch, however, and thus, the specific patterns of activity, associated with synchronised circumferential contractions are only observed when the tissue is appropriately stimulated, with stretch and cholinergic substances as possible stimuli.

The periods of slow waves of increased amplitude described in this study have a duration of 10–45 sec and the slow wave frequency is 20–40 cpm. Such periodic activity has been observed *in vivo* in the human colon.[19,20] There is a strong correlation between this type of slow wave activity and spiking activity not only *in vitro* (this study) but also *in vivo*[21]

(see also Fig. 6 in reference 22). In *in vivo* recordings where slow wave events are filtered out, long bursts of spikes between 10 and 45 sec are observed,[5 8] and these bursts are frequently seen to propagate after stimulation.[4–7 21] Furthermore, such propagating spike bursts are associated with propulsive motor activity.[8] The present study suggests that the propagating long spike burst observed *in vivo* is controlled by periodic high frequency slow wave activity.

The present study presents evidence that the electrical correlate of circumferential contractions is different in man compared with the most commonly studied animal models.[11 23–26] Synchronised circumferential contraction of circular muscle of the human colon only occurs after development of a specific stimulus induced pattern of slow wave-spike activity which occurs concomitantly with increased synchronisation of the electrical activity.

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