In vitro electrical activity in canine colon

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SUMMARY  In vitro slow wave activity was studied in strips of right and left canine colon with silver/silver chloride electrodes. Using visual and computer analysis, slow wave frequency and coupling was assessed between different recording sites and the effect of a cholinergic agonist on coupling and frequency was determined. A regular slow wave was always found to be present. Frequency in the left colon was slightly higher than in the right with a slight decline noted with time. Spike activity was rarely seen in unstimulated specimens. Administration of a cholinergic agonist produced a decrease in frequency with no improvement in coupling. Coupling was usually better in a circular than in a longitudinal direction. It was concluded that if electrical activity is important in the control of colon contractions, it is more likely to be involved in the control of segmentation than in propagated contractions.

Electrical activity in the colon has been studied in vivo and in vitro in different experimental animals and in humans (Couturier et al., 1969; Weinbeck et al., 1972; Taylor et al., 1975; Snape et al., 1976). In most of these studies electrical slow waves have been recognised only intermittently at two or more frequencies. However, Christensen et al. (1969) found electrical slow wave activity in the in vitro cat colon to be omnipresent at 0·05 Hz (3 c/m). We have studied the in vitro electrical activity of muscle strips from canine colon. Coupling between different recording sites was assessed to determine whether electrical activity could be responsible for propagated contractions.

Methods

Strips of colon (1·5 × 8 cm) were removed from healthy dogs anaesthetised with chloralose. The mucosa was removed by sharp dissection and the tissue placed in one of two tissue chambers (1·5 × 5 × 13 cm). A Krebs-Ringer solution kept at 37-0–37-5° and aerated with a gas mixture of 95% O2 and 5% CO2 was allowed to flow through the tissue chambers and over the tissue at a flow rate of 5–6 ml/min. Eight glass insulated silver/silver-chloride electrodes (1 mm diameter) were gently pressed on to the circular muscle tissue surface until a satisfactory record was obtained. The electrodes were separated by a distance of 1·0 cm. A heavy silver wire was placed in the tissue chamber to serve as a reference electrode. This apparatus is similar to that previously described by Christensen et al. (1969). Recordings of electrical activity were made on a Beckman Dynograph R 411 polygraph (Beckman Instruments Inc., Shiller Park, Illinois, USA) with filters set to produce a pass band from 0·2 (12 cpm) to 30 Hz. The signals were stored on an SR 300 Ampex tape recorder (Ampex, Redwood City, Calif., USA). The low cut-off frequency is the result of the time constant which is introduced when the recorder is used in the a.c. coupled mode. This mode of coupling was required because of the large d.c. offsets which tend to fluctuate. On the equipment available the a.c. coupling introduced a time constant of one second—that is, a corner frequency of 0·16 Hz. The roll off towards frequencies less than 0·16 Hz is not more than 6 dB/octave. Signals with a period of 0·05 Hz, for example, are attenuated to about one-third of their original value.

A total of 54 strips of colon were studied. Thirty-four strips were analysed in detail; 11 were cut in a circular direction (five right colon; six left colon) and 23 in a longitudinal direction (14 right colon; nine left colon).

A 30 minute basal record was obtained from each specimen. The effect of neostigmine methylsulphate (0·3 μm/ml) in the perfusion fluid was then determined. In four paired colon specimens (right and

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Fig. 1 Typical frequency power spectra of electrical activity recorded in vitro from three sites 1 cm apart (○ = Channel 1; □ = Channel 2; ⊙ = Channel 3). Horizontal axis: 10.9 cycles/min. Vertical axis: arbitrary units with the maximum values normalised to 1000 units. If the values in two or more sites are identical, only the symbol of the first channel having that value is printed.

left colon from four dogs) basal recordings were continued for six hours. Electrical slow wave frequency was determined manually by counting three non-consecutive five minute segments of record from each electrode. A five minute interval was chosen because during periods of this length the frequencies did not appear to vary significantly and because the interval was large enough for beat to beat variations to be averaged out.

The records were also analysed on a digital computer. Before processing the data, the taped analogue signals from the instrumentation recorder were low-pass filtered (0.5 Hz cut-off frequency), digitised (sampling rate of one per second) and stored on magnetic tape. The digitised data were then processed on the digital computer to obtain the frequency power spectra (Jenkins and Watts, 1968). Dominant frequencies were obtained from these frequency power spectra. A typical frequency spectrum is shown in Fig. 1.

Coupling between different recording sites was assessed by comparing frequencies and phase and determining coherence and cross-correlation. The first two assessments were made visually; the latter two by computer.

1. Frequencies obtained from different recording electrodes on the same specimen were compared. The results are expressed as the number of specimens in which the same frequency was present in the first three recording electrodes (frequency comparison). The distance between the two outer electrodes was 2 cm.

2. The number of cycles during which the phase shift between two recording electrodes 2 cm apart remained within 360°.

3. Coherence between signals from pairs of electrodes, 2 cm apart was calculated for narrow frequency bands (0.033 Hz) on the computer. Coherence is a measure of the linear dependence of one signal on another. If one signal is completely dependent on the other, coherence is unity. Otherwise the coherence may have values between zero and unity. The mathematical definition of coherence (which holds for stationary processes only) is given by:

$$\gamma_{xy}(f) = \frac{|G_{xy}(f)|}{G_{xx}(f) G_{yy}(f)}$$

Where:

$G_{xx}(f)$ and $G_{yy}(f)$ = one-sided measured power spectrum of the signals.

$G_{xy}(f)$ = one-sided measured cross-power spectrum of the two signals.

Coherence was determined in the frequency bands in
which the power spectra of both signals were maximal.

The cross spectral density function $G_{xy}(f)$ is a complex function and can be expressed as:

$$G_{xy}(f) = C_{xy}(f) + j\theta_{xy}(f)$$

Where:

$$j = \sqrt{-1}$$

$C_{xy}(f) =$ co-spectral density function.

$\theta_{xy}(f) =$ quadrature spectral density function.

One may also write:

$$G_{xy}(f) = G_{xy}(f) e^{j\theta_{xy}(f)}$$

Where:

$$G_{xy}(f) = [C_{xy}(f) + j\theta_{xy}(f)]$$

and:

$$\theta_{xy}(f) = \text{arc tan} \frac{\theta_{xy}(f)}{C_{xy}(f)}$$

In the case under consideration, $\theta_{xy}(f)$ represents the phase angle as a function of frequency between the two signals (Bendat and Piersol, 1966).

4. Cross-correlation of slow wave activity measured at electrodes spaced 2 cm apart was calculated on the digital computer. Cross-correlation as a function of the time shift with $\tau$ was displayed on a large screen cathode ray tube and then photographed. Observations of the cross-correlation graph on the CRT enable classifying the results into one of three categories of coupling: good, fair, poor. An example is shown in Fig. 2. To facilitate comparison of

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**Fig. 2** Cross-correlation between pairs of channels. Horizontal axis: timeshift, 1 per division. Vertical axis: Arbitrary units. 1: good correlation. 2: fair correlation.

**Fig. 3** Electrical slow wave activity of the type most commonly observed in recordings from canine colon. Electrodes are arranged in a longitudinal orientation on the colon strips (Ch. 1–4 = left colon; Ch. 5–7 = right colon).
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Results, a numerical grading system was used: (good = 100%; fair = 50%; poor = 0%). The period (and thus frequency) and the phase shift between channels could also be obtained from the graphs. The cross-correlation function is defined by:

\[ \varphi(\tau) = \lim_{T \to \infty} \frac{1}{2T} \int_{-T}^{+T} f_1(t) f_2((t-\tau)) d\tau \]

Where \( f_1(t) \) and \( f_2(t) \) are the signals to be correlated.

5. Phase shift between different recording sites was determined visually by using the relation \( \varphi = t \times 360^\circ \), where \( t \) is the delay between the onset of slow wave depolarisation at different recording sites and \( T \) is the period of a complete wave cycle. The phase angle was calculated only when coupling was present. Phase shift was calculated on the computer in those specimens in which the coherence value exceeded 0.5.

Results

A regular electrical slow wave was always found in strips of canine colon muscle studied in vitro (Fig. 3). Frequency in the left colon was only slightly higher than that in the right colon in both paired (4.90 ± 0.63 and 4.13 ± 0.30 c/m) and unpaired (5.02 ± 0.35 and 4.81 ± 0.38 c/m) experiments. The differences were not significant (\( p > 0.1 \)). These frequencies are only slightly lower than those noted in the chronic in vivo dog colon (Bowes et al., 1978). The in vitro frequency was well maintained in the tissue chamber. Only a slight decline was noted with time (Fig. 4). Spike activity was rarely seen in unstimulated specimens.

Although slow waves were always recognisable at single regular frequencies, there were variations in wave form. The most commonly observed signal was biphasic and its wave form resembled the first time

Fig. 4 Change in electrical slow wave frequency with time in perfusion chamber.

Fig. 5 Variations in slow wave form. Electrodes are arranged in a longitudinal orientation on the colon strips (Ch. 1-4 = left colon; Ch. 5-7 = right colon).
Table 1  Slow wave frequency in canine colon (mean±SEM)

<table>
<thead>
<tr>
<th></th>
<th>Basal Left colon</th>
<th>Basal Right colon</th>
<th>After neostigmine-methylsulphate Left colon</th>
<th>After neostigmine-methylsulphate Right colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpaired experiments</td>
<td>4·81±0·38</td>
<td>5·02±0·35</td>
<td>3·71±0·37</td>
<td>3·53±0·16</td>
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<tr>
<td>Paired experiments</td>
<td>4·13±0·30</td>
<td>4·90±0·63</td>
<td>3·37±0·12</td>
<td>3·41±0·25</td>
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</table>

Table 2  Various methods used to assess coupling

<table>
<thead>
<tr>
<th></th>
<th>Phase comparison*</th>
<th>Frequency comparison†</th>
<th>Coherence value‡</th>
<th>Cross-correlation§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L colon c-o</td>
<td>47·67± 2·33</td>
<td>50</td>
<td>0·37±0·10</td>
<td>40%±19%</td>
</tr>
<tr>
<td>L colon L-o</td>
<td>39·44± 3·96</td>
<td>38</td>
<td>0·16±0·03</td>
<td>84%±9%</td>
</tr>
<tr>
<td>R colon c-o</td>
<td>47·40± 2·60</td>
<td>80</td>
<td>0·63±0·10</td>
<td>60%±19%</td>
</tr>
<tr>
<td>R colon L-o</td>
<td>41·00± 3·80</td>
<td>29</td>
<td>0·33±0·06</td>
<td>28%±12%</td>
</tr>
<tr>
<td>After neostigmine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L colon c-o</td>
<td>19·67±19·67</td>
<td>67</td>
<td>0·43±0·05</td>
<td>10%±10%</td>
</tr>
<tr>
<td>L colon L-o</td>
<td>25·60±6·56</td>
<td>7</td>
<td>0·20±0·05</td>
<td>28%±12%</td>
</tr>
<tr>
<td>R colon c-o</td>
<td>43·40±6·60</td>
<td>50</td>
<td>0·58±0·12</td>
<td>20%±12%</td>
</tr>
<tr>
<td>R colon L-o</td>
<td>23·90±4·22</td>
<td>13</td>
<td>0·26±0·05</td>
<td>20%±20%</td>
</tr>
</tbody>
</table>

*Phase comparison: the number of cycles during which electrical slow waves recorded from two electrodes 2 cm apart remained in phase by 360°.
†Frequency comparison: percentage of specimens in which the same frequency was present in the first three recording electrodes (a distance of 2 cm).
‡Mean±SEM; good = 100%, fair = 50%, poor = 0%.
§Mean±SEM.
**Number of counts.

Table 3  Phase shift between coupled recording sites*

<table>
<thead>
<tr>
<th></th>
<th>Visual analysis</th>
<th>Computer analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase</td>
<td>Phase lag</td>
<td>Coherency</td>
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<tr>
<td>Basal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L colon c-o</td>
<td>94·00±17·222</td>
<td>51·00±27·64</td>
</tr>
<tr>
<td>L colon L-o</td>
<td>56·88±7·40</td>
<td>81·43±19·53</td>
</tr>
<tr>
<td>R colon c-o</td>
<td>46·91±8·12</td>
<td>40·50±12·23</td>
</tr>
<tr>
<td>R colon L-o</td>
<td>75·51±7·21</td>
<td>76·00±0·00</td>
</tr>
<tr>
<td>After neostigmine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L colon c-o</td>
<td>95·00±22·55</td>
<td>42·00±19·24</td>
</tr>
<tr>
<td>L colon L-o</td>
<td>76·00±15·46</td>
<td>62·00±15·18</td>
</tr>
<tr>
<td>R colon c-o</td>
<td>54·75±6·57</td>
<td>28·67±13·44</td>
</tr>
<tr>
<td>R colon L-o</td>
<td>114·16±31·29</td>
<td>23·00±0·00</td>
</tr>
</tbody>
</table>

*Mean±SEM.
†Number of measurements.
‡Results are expressed in degrees.

Fig. 6  Electrical spike activity observed in left colon after 0·3 μmol neostigmine methylsulphate. The tissue strip was orientated in a circular fashion.

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derivative of the monophasic wave recorded from intracellular microelectrodes (Bulbring et al., 1958) (Fig. 1). Variations from this wave form were also observed (Fig. 5). Occasionally, the common wave form would gradually split into two signals of lesser amplitude. This gave the appearance of a doubling in frequency.

Neostigmine methylsulphate resulted in a fall of slow wave frequency in both the right and left colon (Table 1). The record frequently became less clear, presumably because of superimposed mechanical artefacts. Electrical spike activity appeared in all studies. When the slow wave had a more irregular form, spiking appeared at any time during the wave cycle. Spike activity was present near the peak depolarisation when the slow wave had the most typical form (Fig. 6).

In most cases it appeared that coupling was better in the circular than in the longitudinal direction (Table 2). The visual methods of assessment suggested better coupling than did the computer methods. There were no significant differences between the different parts of the colon. No improvement in coupling was seen after neostigmine.

Coupling was often poor, and therefore only a limited number of specimens could be assessed for phase shift. Beat to beat variations in wave form made phase angle difficult to measure. There was
considerable variation in phase angle in measurements from different parts of the colon using computer or visual methods of analysis. Similarly, there was little agreement between visual and computer methods in the same place.

Discussion

We have found electrical activity to be always present at essentially only one frequency in specimens of the canine colon. These findings are in striking contrast with earlier in vitro canine studies (Vanasin et al., 1974). We have previously suggested that noisy records in which a regular slow wave cannot be recognised and the presence of different frequencies could both be due to the recording of multiple oscillating regions by a single recording electrode. Our use of smaller silver surface recording electrodes minimises this possibility and may be the reason that we were always able to recognise a single slow wave frequency. This concept is supported by two observations made in this study. First, when slow waves had the most typical form, spike activity occurred at the peak; when the form was less typical, activity occurred at any time in the slow wave cycle. Second, when higher slow wave frequencies were seen they appeared to be the result of splitting of a larger wave into a wave of lesser amplitude at double the original frequency. The appearance suggests two oscillating regions initially coupled in a mode without significant phase shift, changing to a coupled mode with approximately 180° phase shift.

In the stomach—and to a lesser extent in the small bowel—myogenic electrical activity is the major factor controlling frequency and propagation of contractions (Sarna et al., 1972, 1976). This control function depends upon the coupling of activity between different electrically active areas. For this reason we were particularly interested in assessing coupling in our specimens. This is usually done by noting slight fluctuations in the intervals between slow waves and noting the propagation of this interval to other sites. However, the presence of small, apparently random fluctuation in colon slow wave periods made recognition of the propagation of irregular intervals impossible. We, therefore, assessed it by several indirect methods.

Unfortunately, all of the methods of assessing coupling used in this experiment have inherent errors. The first two methods are largely based on comparison of frequencies over a 2 cm segment of colon. It should be remembered that the frequency difference between isolated specimens of the right and left colon is small—that is, less than one cycle per minute. It is probable that the frequency change is a gradual one and over a 2 cm distance the intrinsic frequency difference is probably extremely small. Thus, even in the absence of coupling, only slight frequency differences would be expected over the distances studied. Without coupling two oscillators with respective frequencies of 5 and 5.1 would take 50 cycles to become out of phase by 360°. Similarly, comparing frequencies based on five minute counts and averaging the counts could also give a false impression of identical frequencies and thus coupling. Both methods will tend to err on the side of recognising coupling when none is present. It is unlikely that significant coupling would be missed.

The computer methods of evaluation (coherence and cross-correlation) on the other hand are rather conservative. When using a visual method of analysis, for example, one can delete parts of recordings which are obviously corrupted by excessive motion artefacts.

On the computer, however, complete recordings were evaluated without deleting bad sections. When the signals are noisy and vary from beat to beat, longer periods of observation are necessary to average out the noise and variation. However, when the period averaged becomes longer, since the statistical properties of the signals are not stationary, a loss of detail (smearing out) occurs.

The experimental results indicate that the coupling in the circular direction is usually better than that in the longitudinal direction. This would tend to support the concept that stationary circular contractions could be under myogenic control. It is unlikely that oscillators which are coupled suddenly become coupled at the time of need for a propagated contraction. Certainly we have found no evidence for improved coupling after prostigmine.

On the basis of these findings, it seems likely that colonic myogenic electrical slow wave activity could control segmentation contractions. Propagated contractions are, however, probably controlled by some other mechanism.

References


