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VAGAL CONTROL OF COLONIC MOTILITY IN THE ANAESTHETIZED FERRET: EVIDENCE FOR A NON-CHOLINERGIC EXCITATORY INNERVATION

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SUMMARY

1. Spontaneous colonic motility in the urethane-anaesthetized ferret consists of two distinct types of contraction which correspond to the patterns recorded myoelectrically in conscious animals.

2. This motility was abolished or greatly reduced when nervous conduction was prevented in the cervical vagi by cooling to below 4 °C. On rewarming the nerves the colonic motility returned, after a short latency, to the pre-cool level.

3. Atropine transiently abolished colonic motility. On its return the motility was significantly reduced but still sensitive to vagal integrity. Thus the atropine-resistant colonic motility was also abolished or markedly reduced by cooling the cervical vagi to below 4 °C. On rewarming there was a longer latency for the return of motility compared to that before atropinization.

4. Electrical vagal stimulation produced, after a short latency, large-amplitude colonic contractions. Following atropine, the short-latency response to electrical vagal stimulation was replaced in the majority of animals by a long-latency response whose characteristics were quite different from those of the cholinergic response.

5. These results are consistent with the vagus containing two functional motor pathways to the colon, one to cholinergic post-ganglionic neurones and the other operating via a non-cholinergic mechanism.

INTRODUCTION

The intestines of the ferret appear as a continuous tube with no gross anatomical division between small and large intestine, although the terminal 10 cm is dilated and has a morphologically distinct mucosal surface recognized as being typical of the colon in other species (Podder & Murgatroyd, 1976). Myoelectrical activity recorded from intestinal smooth muscle of the conscious fasted ferret reveals a difference in motor function at about the same point. The intestine orad to this region shows migrating myoelectric complexes characteristic of the small intestine whilst more distal regions are devoid of this activity and are therefore considered to be large intestine (Bueno, Fioramonti & More, 1981). The motility in this more distant region is characterized

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by two types of electrical activity, described as either long spike bursts which are propagated and therefore propulsive, or short spike bursts which are localized and therefore taken to represent segmentary-type contractions (Bueno *et al.* 1981). This distinction has also been made in records of colonic motility from other animals and in man (Bueno & Fioramonti, 1981).

The anaesthetized ferret shows well developed gastrointestinal motility and in the colon has components which correlate with the long and short bursts described in the conscious animal. The present paper describes the colonic motor responses both to removal of the vagal supply by cooling and to electrical vagal stimulation, and provides evidence for a vagal non-cholinergic excitatory innervation of the colon. A preliminary account of this work has been published (Collman, Grundy & Scratcherd, 1982).

METHODS

The experiments were performed on male and female ferrets anaesthetized with a single intraperitoneal injection of urethane (1·5 g/kg body weight). They were fed on a standard carnivore diet with free access to water, but were deprived of food for 18 h before experimentation. The preparation was essentially the same as described previously (Collman, Grundy & Scratcherd, 1983) with the only difference being the site at which intestinal intraluminal pressure was recorded. In the present study, motility in the terminal intestine (here designated colon from morphological considerations (Podder & Murgatroyd, 1976)) was recorded from a saline-filled cannula, attached to a pressure transducer (Elcomatic EM 760 system) inserted in an oral direction at a point 5 cm above the anus. A loop of intestine was formed by ligating the intestine 10 cm orad to the cannula. The pressure transducer was calibrated periodically by reference to a water manometer and the output was displayed on a flat-bed chart recorder (Bryans 2800).

Vagal influences on colonic motility were investigated by either acute cervical vagotomy and subsequent stimulation of the peripheral end of the severed vagal trunks or by temporary interruption of the vagal supply by cooling the cervical vagi to below 4 °C (Linden, Mary & Weatherill, 1981). Vagal cooling was accomplished by circulating a freezing mixture of alcohol and water through copper tubes positioned around the cervical vagi, the rate of flow through the tubes being adjusted to control the temperature of the nerves monitored by thermocouples. The temperature of the nerves was maintained at 37 °C between periods of cooling. Thin slivers of polystyrene were inserted between the nerves and underlying tissue to insulate against heat loss. Body temperature was maintained throughout by means of a Palmer Homeothermic blanket.

The animals were left for 30 min after completion of the surgery before any motility was recorded. Data on the spontaneous patterns of motility were obtained from a 10 min period prior to any experimental intervention. In the results peak pressure is taken as the maximum pressure generated above the base line while 'tone' refers to the amplitude of the base line above atmospheric pressure. All results are expressed as the mean ($\pm$S.E. of mean), with the number of animals in parentheses. A paired-sample t test was used to assess statistical significance with the control for each test being taken from the 10 min period immediately before the experimental procedure.

RESULTS

The anaesthetized ferret showed well developed spontaneous colonic motility which had two components. The first type of contraction occurred at a maximum frequency of 11/min and had a duration of $5{\cdot}12 \pm 0{\cdot}05$ s (range 4–8 s). The contractions were of low amplitude ($0{\cdot}53 \pm 0{\cdot}02$ kPa, $n = 870$ contractions in twenty-four animals) when compared with the second type of long-duration contraction ($1{\cdot}9 \pm 0{\cdot}09$ kPa (range 0·1–9·9 kPa), $n = 248$ contractions in twenty-four animals). This second type of contraction had a mean duration of $14{\cdot}4 \pm 0{\cdot}3$ s (range 8–36 s) with a maximum frequency of <4/min and often had the faster, smaller-amplitude contractions

superimposed (Fig. 1*A*). Tonal increases were also seen in four animals and were generally associated with a burst of large-amplitude contractions giving the appearance of a cyclical pattern of motility (Fig. 1*B*).

Atropine (0·1–5 mg/kg) did not abolish all the spontaneous colonic motility. In animals given atropine (1 mg/kg) motility was affected to some degree. In three animals motility was completely abolished, whereas in the remaining nine only a transient inhibition occurred with motility returning after $9 \pm 1{\cdot}6$ min. The motility present after the injection of atropine was always greatly reduced, with a mean peak amplitude of colonic contractions of $0{\cdot}39 \pm 0{\cdot}07$ kPa compared with $2{\cdot}9 \pm 0{\cdot}57$ kPa before atropine ($P < 0{\cdot}01$, $n = 12$), but it still showed the two types of contraction seen before atropine (see Fig. 5).

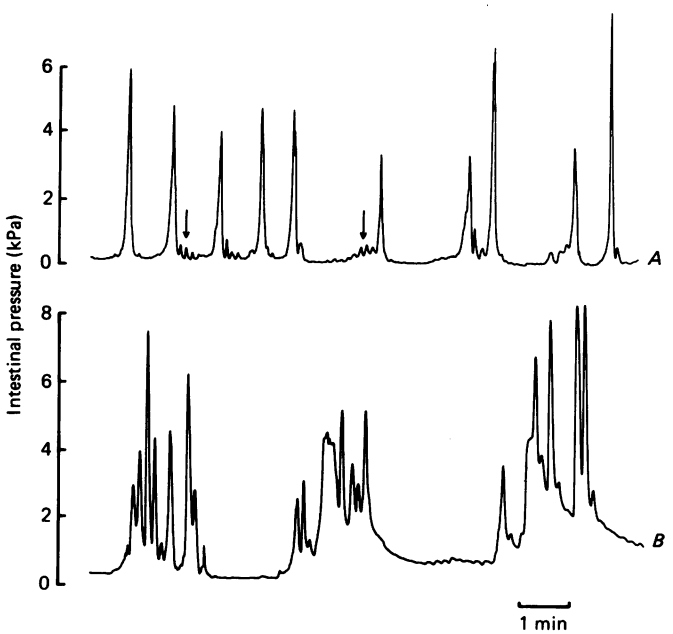

Fig. 1. Spontaneous colonic motility recorded in two separate experiments. In *A* both short and long duration contractions can be distinguished whilst *B* also shows tonal increases with associated contractions giving a cyclical pattern of activity. Arrows point to the low-amplitude, short-duration contractions.

Effect of vagal stimulation

After vagotomy, colonic motility was markedly attenuated (see next section) but could be initiated or enhanced, after a short latency (2–4 s), by electrical stimulation of the peripheral end of the cut cervical vagi (20 V, 0·5 ms, 1–20 Hz for 1 min). Stimulation at 1 Hz resulted in only small tonus changes, while stimulation frequencies of 2 Hz and above gave rise to large-amplitude contractions at approximately 4/min which developed throughout the period of stimulation and occurred on top of the tonal increase (Fig. 2*A*). Peak contractions were reached at 10 Hz stimulation when the peak contraction amplitude was $4{\cdot}35 \pm 1{\cdot}2$ kPa, $n = 12$. The lower-amplitude,

higher-frequency contractions were superimposed on the large-amplitude contractions but tended to be obscured by the latter, especially at higher stimulation frequencies, so that they appeared only as a ripple on the large waves. The contractions ceased abruptly on removal of the stimulus.

Vagal stimulation after atropine gave more variable responses. In four animals vagal stimulation had no effect, whilst in two others there was a transient fall in colonic tone, indicating an inhibitory innervation. In the latter cases and in the

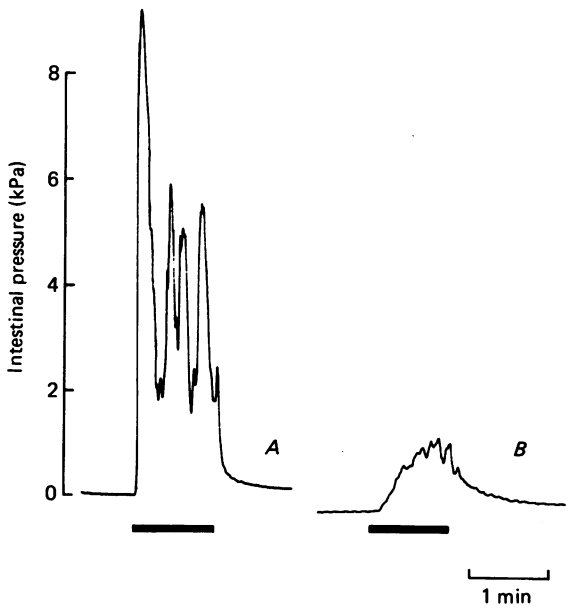

Fig. 2. Colonic responses to vagal stimulation (20 V, 0·5 ms, 10 Hz in *A* and 20 Hz in *B*). Bar denotes stimulus period. In *A* the response is in the absence and in *B* in the presence of atropine (1 mg/kg). Note the difference in response following atropinization.

remaining six animals vagal stimulation after atropine revealed a contractile response, the characteristics of which were quite different from that seen in the absence of atropine (Figs. 2*B* and 3). The latency was longer (4–54 s, mean 20·6 ± 5·0 s, $n = 8$), the threshold for stimulation was higher (5 Hz rarely elicited a response after atropine), the amplitude lower (2·4 ± 1 kPa at 20 Hz compared to 3·97 ± 1·5 kPa at the same frequency before 1 mg/kg atropine ($P < 0·05$, $n = 8$)), and the response persisted for some time (10–300 s) after removal of the stimulus. The responses before ($n = 11$) and after ($n = 9$) atropine were not abolished by pre-treatment with phentolamine (2 mg/kg). Indeed phentolamine caused a marked increase in both amplitude of the response and in its persistence following removal of the stimulus (Fig. 3).

Effect of vagal cooling

The influence of tonic vagal activity on spontaneous colonic motility was investigated in sixteen animals by reversible vagal blockade by cooling the cervical vagal trunks to below 4 °C. This not only allowed repeated observations to be made on the

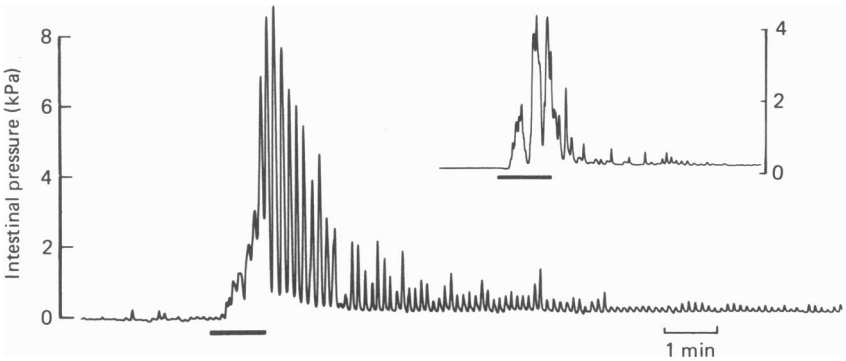

Fig. 3. Vagal stimulation in the presence of atropine (2 mg/kg) and phentolamine (2 mg/kg). Inset shows the response in the presence of atropine alone. Both stimulations were at 20 Hz for 1 min (denoted by bar). Note the latency of the response and the persistence of activity after the stimulation had ceased.

Fig. 4. The effect of vagal cooling on spontaneous colonic motility. The bar indicates the period in which the nerves were below 4 °C.

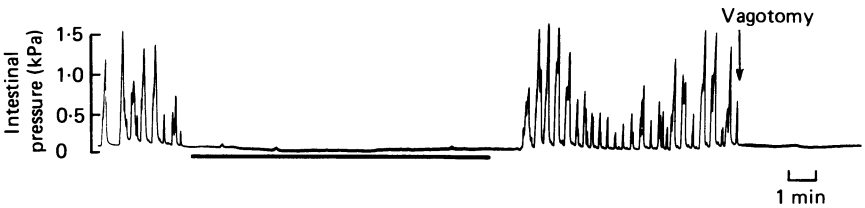

Fig. 5. The effect of vagal cooling on spontaneous colonic motility after atropine (1 mg/kg). Bar indicates period when nerves were cooled below 4 °C. Same animal as Fig. 4. Note from the pressure calibration the reduced amplitude of the atropine-resistant contractions. Note also that bilateral cervical vagotomy had the same effect as vagal cooling.

same animal but allowed the effect of removal of vagal activity to be investigated before and after atropinization. Before atropine, vagal cooling abolished (fifteen out of twenty-nine trials) or reduced (fourteen out of twenty-nine trials) spontaneous colonic motility. Where a reduction was seen peak contraction amplitude was reduced from $3{\cdot}06 \pm 0{\cdot}6$ kPa to $0{\cdot}21 \pm 0{\cdot}05$ kPa ($P < 0{\cdot}001$) by vagal cooling. On rewarming the nerves motility returned, after a short latency ($15{\cdot}3 \pm 3{\cdot}4$ s), to pre-cool levels (Fig. 4).

The effect of vagal cooling on the motility resistant to atropine (1 mg/kg) was investigated in eight animals. Under these conditions vagal cooling abolished motility in eight out of fourteen trials and in the remainder reduced the peak contraction amplitude from $1{\cdot}5 \pm 0{\cdot}3$ kPa to $0{\cdot}3 \pm 0{\cdot}1$ kPa ($P < 0{\cdot}001$) (Fig. 5). On rewarming,

motility returned to pre-cool levels but after a significantly longer latency (89·2 ± 25 s) than before atropinization (10·4 ± 2·9) ($P < 0{\cdot}01$, $n = 8$). These effects persisted after pre-treatment with phentolamine and propranolol (2 mg/kg) in three animals and after spinal section at L1 in two others.

DISCUSSION

The spontaneous pattern of colonic motility recorded in the anaesthetized ferret shows components that correspond to myoelectric events recorded in conscious animals including man (Taylor, Duthie, Smallwood & Linkens, 1975; Fioramonti, Bueno & Ruckebusch, 1980; Bueno *et al.* 1981). The short-duration, low-amplitude contractions correspond to the short spike bursts recorded myoelectrically from the conscious ferret and occurred at the same frequency as the slow waves (Bueno *et al.* 1981). The larger-amplitude, long-duration contractions correspond to the long spike bursts described in the proximal colon of the conscious ferret which had a mean duration of 16 s (Bueno *et al.* 1981). These short and long spike bursts correspond to type I and type II waves described originally by Templeton & Lawson (1931). Tonal changes corresponding to Templeton & Lawson's type III waves were also seen in the present study.

This colonic motility depended upon an intact vagal supply since cooling the cervical vagi to <4 °C caused a marked reduction and in many cases complete abolition of all contractile activity. Since the vagus contains both afferent and efferent fibres the effect of vagal cooling could be due to removal of afferent activity which reflexly reduces the pelvic supply to the colon, or to the direct removal of tonic vagal efferent supply to the colon. Evidence in favour of a direct vagal effect can be derived from the persistence of the inhibition after spinal section. A vagal efferent supply exists since electrical stimulation of the peripheral stump of the cervical vagal trunk results in the initiation of large-amplitude contractile activity. Indirect effects via reflex increases in sympathetic activity during vagal cooling were eliminated by combined α- and β-adrenergic blockade. Other indirect effects via changes in temperature, acid/base status or blood flow have been discounted in the efficacy of vagal cooling on other regions of the gastrointestinal tract (Grundy, Hutson & Scratcherd, 1983; Collman *et al.* 1983). Thus a background of vagal activity is required for full spontaneous contractile activity in the colon. This is in contrast to other studies in which acute vagal section had relatively little effect (Hulten, 1969; Rostad, 1973). This may represent a species difference in vagal dependence or, equally possible, the short term effects of removal of vagal activity may be overcome by plasticity between the dual parasympathetic innervation of this region of intestine, the pelvic supply taking over control following removal of the vagal influence (Gray, Hendershot, Whitrock & Seevers, 1955).

Evidence for plasticity between cholinergic and non-cholinergic influences may be inferred from the only transitory abolition of colonic motility by muscarinic blockers seen in the present study and in previous studies on other species (Gray *et al.* 1955; Stoddard, Bickerstaffe & Johnson, 1982). Both cholinergic and non-cholinergic excitatory pathways to the colon have been demonstrated by electrical stimulation of the pelvic supply in cats (Hulten, 1969; Rostad, 1973; Fasth, Hulten & Nordgren,

1980; Andersson & Jarhult, 1981; Andersson, Bloom & Jarhult, 1983) and dogs (Wells, Mercer, Gray & Ivy, 1942; Gray *et al.* 1955; Goldenburg & Burns, 1968), but previously the vagal excitatory pathway has been considered to be atropine sensitive (see Costa & Furness, 1982). In the present study vagal stimulation in the presence of atropine (up to 5 mg/kg) has revealed an excitatory response whose characteristics are quite distinct from the response prior to atropinization but are similar to those of the response to stimulation of the non-cholinergic pelvic pathway described above in the cat and dog. Thus the response has a long latency and persists for several seconds after removal of the stimulus. The arguments for this representing a non-cholinergic excitatory response have been discussed in detail previously in connexion with a similar innervation to the ferret jejunum (Collman *et al.* 1983). Thus, it would appear that the vagal supply to the colon, like the pelvic innervation, contains both cholinergic and non-cholinergic components.

More convincing evidence for the long-latency atropine-resistant response representing a preganglionic input to a non-cholinergic pathway and not some other indirect effect is that the spontaneous atropine-resistant contractions are sensitive to vagal integrity. Thus vagal cooling resulted in marked attenuation of the spontaneous atropine-resistant colonic contractions. This observation suggests that tonic vagal activity is responsible, via the intramural ganglion, for release of a substance which operates via a non-cholinergic mechanism. Whether this substance is released directly onto the smooth muscle cells or has its effect indirectly by a humoral mechanism cannot be determined in the present study. Certainly, direct excitatory effects, via the release of an unknown transmitter, have been demonstrated *in vitro* by transmural electrical stimulation of segments of colon (Bennett & Fleshler, 1969; Costa & Furness, 1972; Furness & Costa, 1973; Stockley & Bennett, 1974). However, the long latency of the response to electrical vagal stimulation and the long latency for the return of colonic motility on rewarming after a period of vagal cooling may suggest some humoral involvement. Irrespective of the mechanism, however, the observation that tonic vagal activity is sufficient to initiate or enhance atropine-resistant colonic motility would suggest that such a pathway is of functional significance.

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