Interactions of Neurogenic Responses of Longitudinal and Circular Muscle in the Guinea-Pig Ileum

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Abstract. The relationship between neurogenic responses of longitudinal and circular muscle was studied by measuring contractions and EMG or non-adrenergic, non-cholinergic (NANC) relaxations and NANC inhibitory junction potentials in different preparations of the guinea-pig ileum. NANC relaxation of longitudinal muscle was observed also without any preceding or concomitant circular muscle contraction ruling out the possibility that the latter might be the cause of the NANC relaxation. Circular muscle twitches or powerful contractions were absent if there was no preceding neurogenic or myogenic excitation of longitudinal muscle; in preparations with myenteric plexus-longitudinal muscle layers removed only small residual responses were seen although still under neurogenic influences. Thus excitation of longitudinal muscle seemed a prerequisite for synchronized and powerful contractions of circular muscle to occur. Cholinergic contraction and NANC relaxation of longitudinal muscle evoked by field stimulation were partly inhibited if the submucous plexus was also present suggesting the involvement of a more complex neuronal circuitry in these responses.

Key words: Guinea-pig ileum — Neurogenic responses — Longitudinal and circular smooth muscle

Introduction

The relationship between activities of longitudinal and circular muscle of the mammalian small intestine has not been entirely understood as yet. It has been suggested that relaxation along the longitudinal axis of the cat jejunal segments might be a sheer mechanical consequence of circular muscle contractions rather than of nervous inhibition of the longitudinal muscle (Wood and Perkins 1970). Also it has been proposed that synchronized contractions of longitudinal and

circular muscle layers are either due to a high degree of electrical coupling between the two muscle layers (Bortoff and Sachs 1970; Chow and Huzinga 1987) or to non-neutral interstitial cells of Cajal (ICC) located between the longitudinal and circular muscle layers which might be essential for the generation of synchronizing electrical slow waves (Hara et al. 1986; Suzuki et al. 1986). On the other hand, the neurogenic control of a coordinated muscle activity in the guinea-pig ileum rather than a simple synchrony between the two muscle layers was stressed during reflex activity (Yokoyama and North 1983). Further, Bywater and Taylor (1986) in discord with Bauer and Kuriyama (1982a,b) and Sanders and Smith (1986) suggested that non-cholinergic excitatory and inhibitory nerve fibres primarily supply the circular rather than longitudinal muscle layer in the guinea-pig ileum.

A number of questions concerning mechanical, myogenic and neurogenic levels of interaciton between longitudinal and circular muscle layers have thus been left open; at least one reason for this were different techniques of dissection of guinea-pig ileum preparations applied by different investigators. The present study was therefore undertaken to investigate whether longitudinal muscle relaxation is caused by circular muscle contraction, or whether there are synchronous and quantitatively related contractions of both layers; and finally, how the activity of one muscular layer is affected by the presence or absence of the other one and of the myenteric and submucous plexi.

Materials and Methods

The methods, solutions and electrophysiological recording techniques used in this study were similar to those described previously (Bauer and Kuriyama 1982a,b: Kadlec and Horáček 1980; Kadlec et al. 1982; 1984a,b; 1985; 1986; 1987a,b). Briefly, male short-hair guinea-pigs weighing 300—600 g were used, a piece of the middle part of the ileum was dissected and used to make different preparations.

Preparations

For some experiments the longitudinal muscle layer or the myenteric plexus-longitudinal muscle (MPLM) layers were dissected from the surface of ileum segments as described by Paton et al. (1971). The integrity of the myenteric plexus attached either to the longitudinal or circular muscle layers was checked by histological examination (Fig. 1). Adequate vital staining of whole mounts was accomplished within 10—30 min by injecting 0.3 ml saturated solution of methylene blue in 20 ml of aerated Krebs solution (Erwin et al. 1978). MPLM strips were also prepared (Paton et al. 1971). In some cases, circular muscle with the submucous plexus were left to adhere to the strips over a certain length.

The ileal segment with or without MPLM layers was reversed inside out and the mucosa was always gently stripped off; in some instances the submucosa with the plexus were even removed from the underlying circular muscle (Bornstein et al. 1986). The presence or absence of the submucous plexus was also histologically checked.

Helically cut circular muscle strips were prepared from ileal segments of different wall thickness and composition (with MPLM layers present or absent).

The preparations were placed in separate baths containing Krebs solution (37 °C) of the following composition (mmol/l): NaCl 120, KCl 5.9, $CaCl_2$ 2.5, $NaHCO_3$ 15.4, $MgCl_2$ 1.2, NaH_2PO_4 1.2, glucose 11.5, and gassed with a constant stream of 95 % O_2 and 5 % CO_2.

Peristalsis and contractions

The peristaltic reflex activity was studied in isolated segments of the guinea-pig ileum by means of a modification of the Trendelenburg method (Van Nueten et al. 1973; Kadlec and Horáček 1980). Longitudinal muscle contractions and intramural pressure were recorded; or, contractions of a ring of circular muscle were measured between the two opposite sites of the ring circumference by means of a thread attached under the serosa (Perkins 1971).

Contractions of whole segments with different wall thickness and composition or of different strips were recorded. Platinum wire electrodes were used for electrical stimulation (Kadlec et al. 1982). The electrodes were either placed at both the top and the bottom of the organ bath (field stimulation) or formed 2 rings (4 mm i.d.) embedded 2 mm apart in acryl (Rand electrode) with the segment drawn through. Single pulses at 0.1 Hz or trains of different duration at a frequency of 30 Hz were applied; to obtain powerful twitches the pulse width was set at 0.3 ms. The voltage of stimulation was 6—10 V with the Rand electrode and 20—200 V with the field stimulation; in the latter case only stimulation with voltages below 50 V was considered neurogenic as responses to single pulses were completely blocked by TTX (Kadlec et al. 1985).

Triple-bath experiments

Oriented MPLM strips with or without the underlying tissue were drawn through narrow orifices in 2 rubber membranes dividing the bath into 3 compartments. Two rubber membranes set 10 mm apart anchored the preparation giving the possibility to record contractions of each peripheral part separately (Kadlec et al. 1985; 1987a,b). A pair of platinum wire electrodes (7 mm apart) was placed in each peripheral compartment. In this study we report only the neurogenic contractions of the aboral part; they were evoked either by local stimulation with electrodes sited in the aboral compartment or by distant stimulation with electrodes sited contralaterally in the oral compartment. Neurogenic field stimulation by 0.2 ms pulses of 20—35 V amplitude at a frequency of 30 Hz for 5 s was used.

EMG

Electromyograms of the muscle layers were picked up by glass suction electrodes touching the external surface of different segment preparations (Kadlec et al. 1984a,b; 1986). Platinum wire point electrodes were placed on the opposite sides of the gut wall so that focal stimulation was achieved. Trains of 10—100 pulses (0.2 ms; 10—50 V) were applied at a frequency of 100 Hz. The high frequency used was chosen so that the stimulation artifact evoked by trains be terminated before the EMG responses appeared to preclude interferences.

Intracellular microelectrodes

In some experiments membrane potential changes were recorded using conventional glass capillary microelectrodes filled with 3 mmol/l KCl. The longitudinal muscle cells were impaled from the

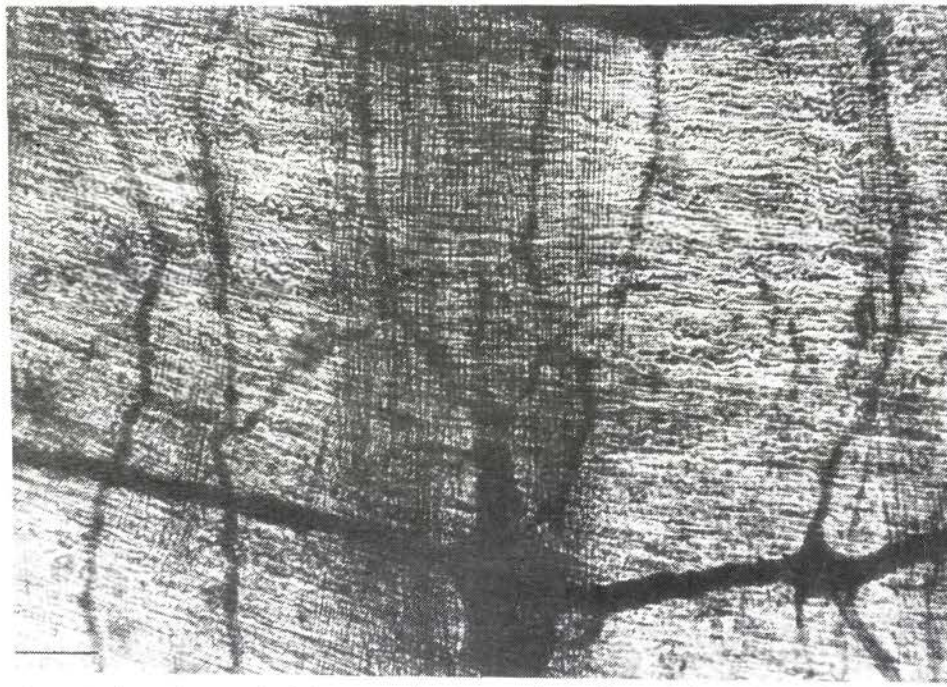

Fig. 1. A photomicrograph of the myenteric plexus stained with methylene blue on the background of the circular muscle layer; the middle part is overlaid by longitudinal muscle fibres. The presence or absence of a muscle layer or the plexus, or respective positioning of an EMG electrode could be specified. Bar: 100 μm.

serosal and the circular muscle cells from the mucosal surface. Ag/AgCl stimulatory electrodes, one placed on the tissue 1—2 mm from the impaled microelectrode and the other at a distance of 10—15 mm, were used. Trains of 40 or 200 pulses (0.15 ms; 50 V) were delivered at 20 Hz for 2 or 10 s. These parameters were found optimal to evoke NANC inhibitory junction potentials followed by rebound changes in membrane potential (Bauer and Kuriyama 1982a); atropine and guanethidine were present in the bath solution throughout these experiments.

Drugs used were: adenosine 5′ triphosphoric acid disodium salt (ATP, Serva); atropine sulphate (Spofa; 1 μmol/l); guanethidine sulphate (CIBA; 10μmol/l) histamine dichloride (Spofa; 1 μmol/l); and tetrodotoxin (TTX, Sigma; 1 μmol/l).

The results were expressed as means $\pm$ standard error of the mean, with the number of experiments given in parentheses. The significance of differences was assessed with Student's two-tailed *t*-test for paired or unpaired data as appropriate. In some cases a typical experiment has been shown in a figure and the total number of experiments is given.

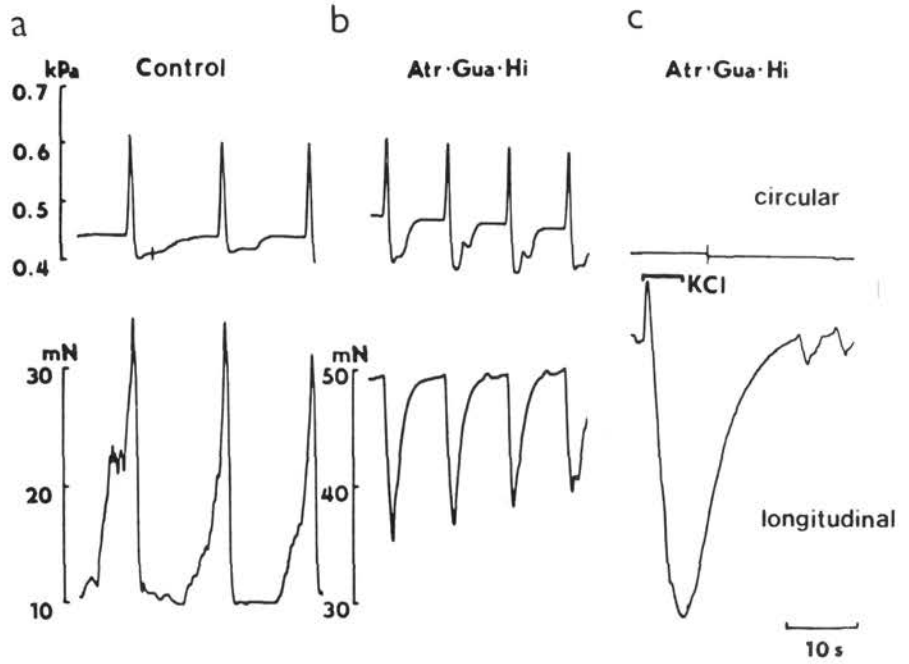

Fig. 2. Peristaltic movements of the ileal segment. Intraluminal pressure (circular) and tension in the longitudinal axis were recorded under control conditions (panel *a*) and in the presence of atropine (Atr), guanethidine (Gua) and histamine (Hi) (panel *b*). Potassium chloride (KCl) concentration was raised by 30 mmol/l (panel *c*).

Results

Whole segments: Longitudinal muscle tension and intraluminal pressure

In whole ileal segments intraluminal pressure was elevated to 0.4 kPa and longitudinal muscle tension to 10 mN eliciting in 6 experiments rhythmic peristaltic activity with synchronous contractions of longitudinal and circular muscle layers (Fig. 2*a*). The addition of histamine in the presence of atropine and guanethidine increased the longitudinal muscle tension to about 50 mN. Under these conditions the elevation of the intraluminal pressure to 0.4 kPa in 5 preparations tested regularly evoked rhythmic reflex activity again; however, now the circular muscle contractions were synchronous with longitudinal muscle relaxations (Fig. 2*b*). On the other hand, the elevation of potassium chloride concentration in the bathing solution by 30 mmol/l caused in all 5 experiments a large relaxation of the longitudinal muscle without any preceding or concomitant contraction of the circular muscle (Fig. 2*c*).

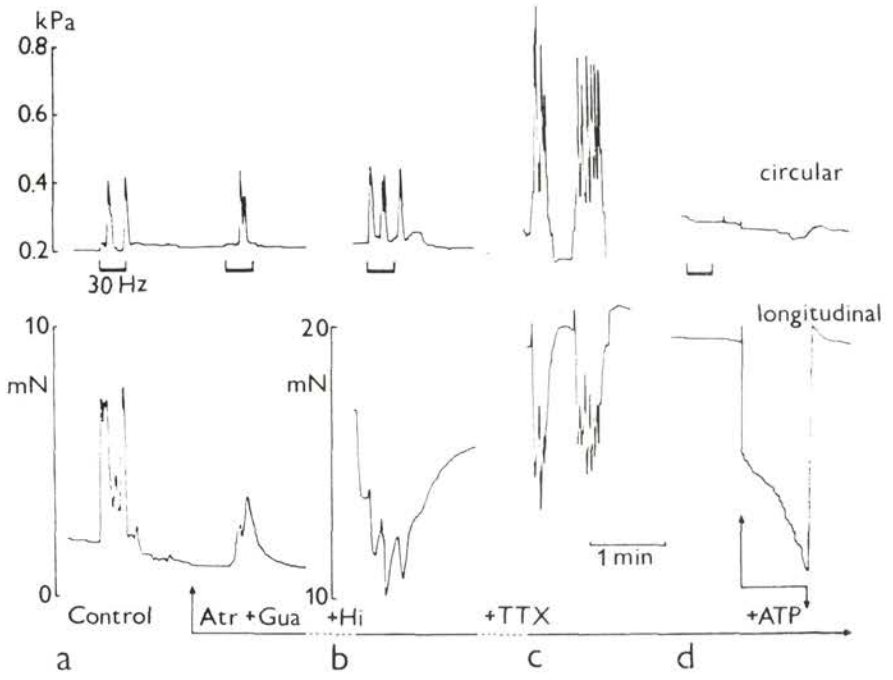

Fig. 3. Movements of muscle layers of the ileal segment evoked by neurogenic stimulation (pulses of 0.3 ms and 20 V at a frequency of 30 Hz for 20 s; field stimulation; panels *a*,*b* and *d*), by increased intraluminal pressure (elevation to 0.4 kPa; panel *c*) or by the addition of ATP (2.5 mmol/l; panel *d*). Intraluminal pressure changes (circular) and tension in the longitudinal axis were recorded under control conditions or in the presence of atropine (Atr), guanethidine (Gua), histamine (Hi) and TTX.

Neurogenic field stimulation in the absence of an increased intraluminal pressure evoked concomitant contractions of both muscle layers in 31 experiments and only longitudinal muscle contractions were depressed in the presence of atropine plus guanethidine (Fig. 3*a*). In the presence of atropine, guanethidine and histamine this stimulation evoked contractions of the circular muscle and relaxations of the longitudinal muscle which were but partly synchronous (Fig. 3*b*; 31 experiments). The addition of ATP (0.05—1 mmol/l) caused an irregular relaxation of the longitudinal muscle without any concomitant contraction of the circular muscle. At the highest concentration of ATP tested (2.5 mmol/l) the relaxation was always distinct (Fig. 3*d*) both in the absence (4 experiments) or in the presence (6 experiments) of TTX. The addition of TTX

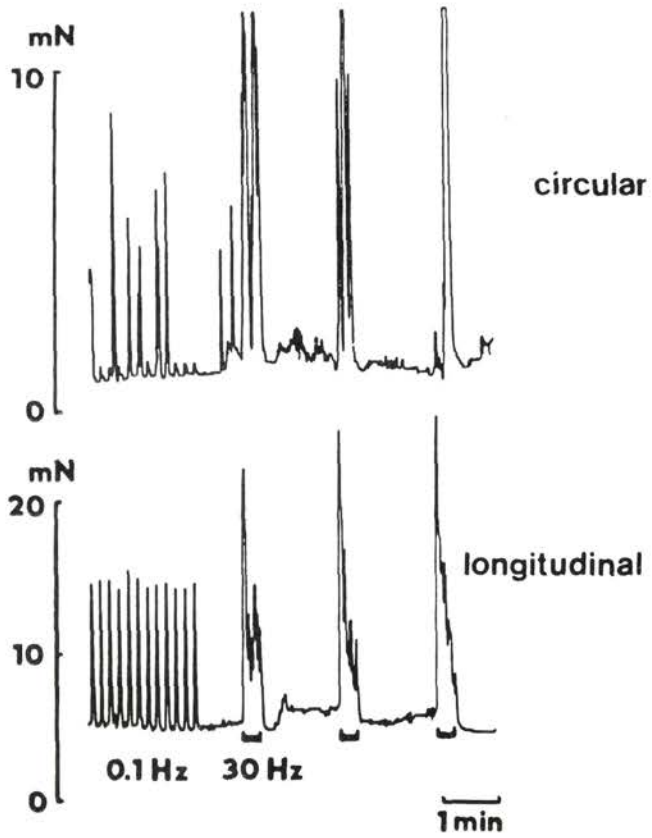

Fig. 4. Contractions of an ileal segment were recorded in the longitudinal axis and from a ring of circular muscle. Neurogenic stimulation by the Rand electrode (pulses of 0.3 ms and 6 V) was delivered at 0.1 Hz (single pulses) or at 30 Hz (20 s trains).

blocked the neurogenic responses to electric stimulation (Fig. 3*d*). If the voltage of field stimulation was raised from 50 to 200 V, small contractions of the circular muscle were always observed in the presence of TTX (5 experiments, not shown). In 2 cases out of the 5 experiments however, rhythmic activity of both layers was induced by an elevation of intraluminal pressure: circular muscle contractions were synchronous with longitudinal muscle relaxations (Fig. 3*c*). This rhythmic activity in the presence of atropine, guanethidine, histamine and TTX resembled by its frequency (15—20 c/min) pendular movements rather than peristalsis (3—6 c/min). In the other 3 preparations no rhythmic activity could be evoked.

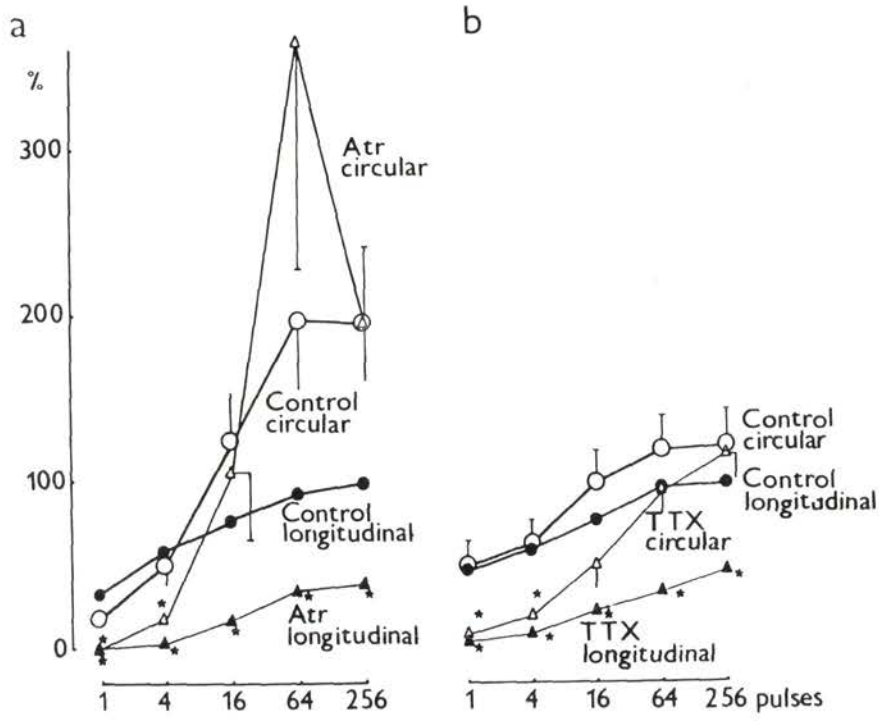

Fig. 5. The effect of atropine (panel *a*) and TTX (panel *b*) on contractions of ileal segment in the longitudinal axis and of a circular muscle ring; contractions were evoked by single pulses (0.3 ms, 40 V, field stimulation) or by trains of 4—256 pulses delivered at a frequency of 30 Hz. The values are percentages of maximum contraction of the longitudinal muscle in the respective control group. Means from 13 and 22 experiments are given in panels a and b, respectively. Vertical lines show S.E.M.; where not shown, S.E.M. was less than the size of the symbol. The asterisks indicate significant decreases in contraction height in the presence of a drug compared to the respective control value ($P < 0.05$).

Whole segments: Longitudinal and circular muscle tension

Contractions along the longitudinal axis of whole ileum segments and of a circular muscle ring of the same segments evoked by neurogenic stimulation were recorded. Stimulation by the Rand electrode sited at the oral end of 8 segments evoked contractions of both layers. Longitudinal contractions evoked by 0.1 Hz stimulation were of regular height whereas circular contractions were different; either regular small contractions synchronous with longitudinal contraction were seen, or larger irregular contractions superceding on the small ones were present. The larger contractions occurred also spontaneously. During

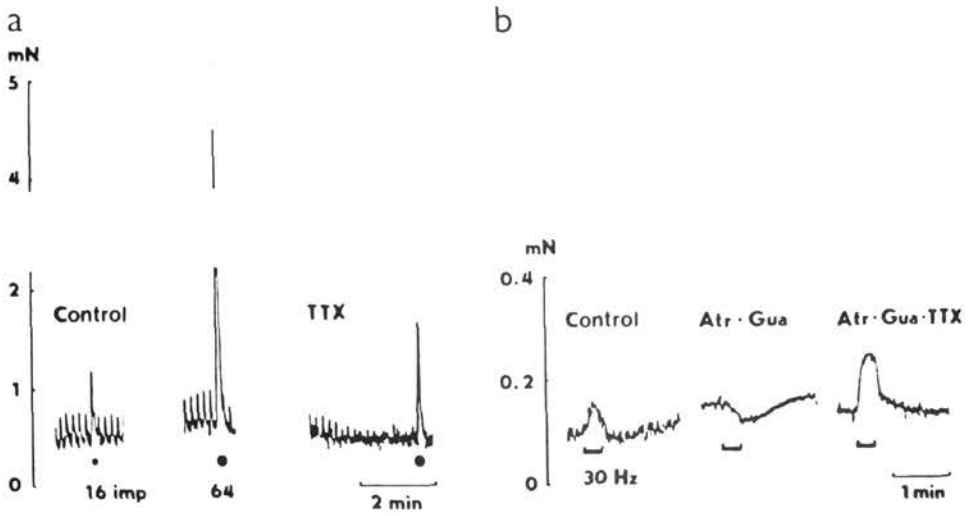

Fig. 6. Contractions of helical strips cut from ileal segments either with the attached MPLM layers (panel *a*) or after their removal (panel *b*). In panel *a* responses were evoked by single pulses (0.3 ms, 30 V, 0.1 Hz; field stimulation) or by trains of 16 or 64 pulses at a frequency of 30 Hz under control conditions or in the presence of TTX; in panel *b*, 20 s trains (30 Hz) of 600 pulses of high voltage (100 V) were applied and the effects of atropine (Atr), guanethidine (Gua) and TTX were tested.

train stimulation, again, circular contractions were irregular and pendular movements of the ring were also observed (Fig. 4).

With field stimulation, circular muscle contractions evoked by single pulses at 0.1 Hz, or by trains of 4—256 pulses at a frequency of 30 Hz, were more regular than those evoked by stimulation with the Rand electrode. The tension of longitudinal muscle reached by the longest train was taken for 100 % and the values measured for other longitudinal and circular responses were related to this reference value (Fig. 5). In the presence of atropine (13 experiments) or TTX (22 experiments), longitudinal muscle contractions evoked by single pulses were practically abolished, and contractions evoked by the longest trains were below 50 % of the control responses. Circular muscle contractions elicited by single pulses were also abolished but contractions evoked by longer trains were not lower than those under control conditions.

Helically cut circular muscle strips

Either intact ileal segments or segments without MPLM layers were utilized. In the former case longitudinal muscle and myenteric plexus were present and

Table 1. The effect of underlying tissue on the responses of the aboral part of guinea-pig ileal strip preparations to electrical train stimulation at 30 Hz for 5 s. Neurogenic excitation was evoked in the aboral and oral parts (local and distant stimulation, respectively). Contractions were recorded under control conditions and NANC relaxations (signed —) in the presence of atropine, guanethidine and histamine. The preparations contained MPLM layers either without the underlying tissue (—) or with circular muscle (cm) and the submucosa with the submucous plexus (sp) (+) at some or all parts. Means ± S.E.M. are given and the number of experiments (N) is indicated. The responses were expressed in terms of tension changes in mN. $^{*}P < 0.05$, $^{**}P < 0.005$ compared to the respective value of the 1st row; $^{+}P < 0.05$ compared to the respective value of the preceding (4th) row.

Preparation parts				Site of stimulation			
Layers			N	Aboral	Oral	Aboral	Oral
				Contractions		Relaxations	
cm	−	−	32	9.3 ± 0.7	3.2 ± 0.3	−3.0 ± 0.3	−1.1 ± 0.2
sp	−	−					
cm	−	+	8	12.8 ± 2.0*	2.9 ± 0.8	−4.3 ± 0.4	−0.4 ± 0.2
sp	−	+					
cm	+	−	10	6.0 ± 1.5*	4.3 ± 1.1	−0.3 ± 0.1**	−0.4 ± 0.2
sp	+	−					
cm	+	+	10	2.7 ± 0.4**	0.1 ± 0.04**	−0.2 ± 0.05**	0**
sp	+	+					
cm	+	+	9	6.4 ± 1.0*+	1.3 ± 0.3*+	−1.4 ± 0.3*+	0**
cp	−	−					

neurogenic contractions evoked by single pulses or by trains at 30 Hz were recorded; in the presence of TTX, trains of pulses still evoked smaller contractions (Fig. 6*a*). In 9 preparations without MPLM layers, only contractions by 1 to 2 orders smaller were evoked by long trains of strong pulses; although small, these contractions were still modified by neurotropic drugs. In the presence of atropine and guanethidine contraction were lowered or reversed into a relaxation, and the addition of TTX restored the contractions (Fig. 6*b*). The same results were obtained with 8 ring preparations of the circular muscle from ileal segments after removal of MPLM layers.

MPLM strips and underlying tissue

Preparations without underlying tissue or with circular muscle and with submucosa containing the submucous plexus were used. The underlying tissue was present either over the entire length of the preparation (strips from the ileal segments were cut longitudinally) or covered only a portion of the preparation and the rest consisted of MPLM strip prepared as described in Methods. Neurogenic stimulation was applied either in control Krebs solution or in the

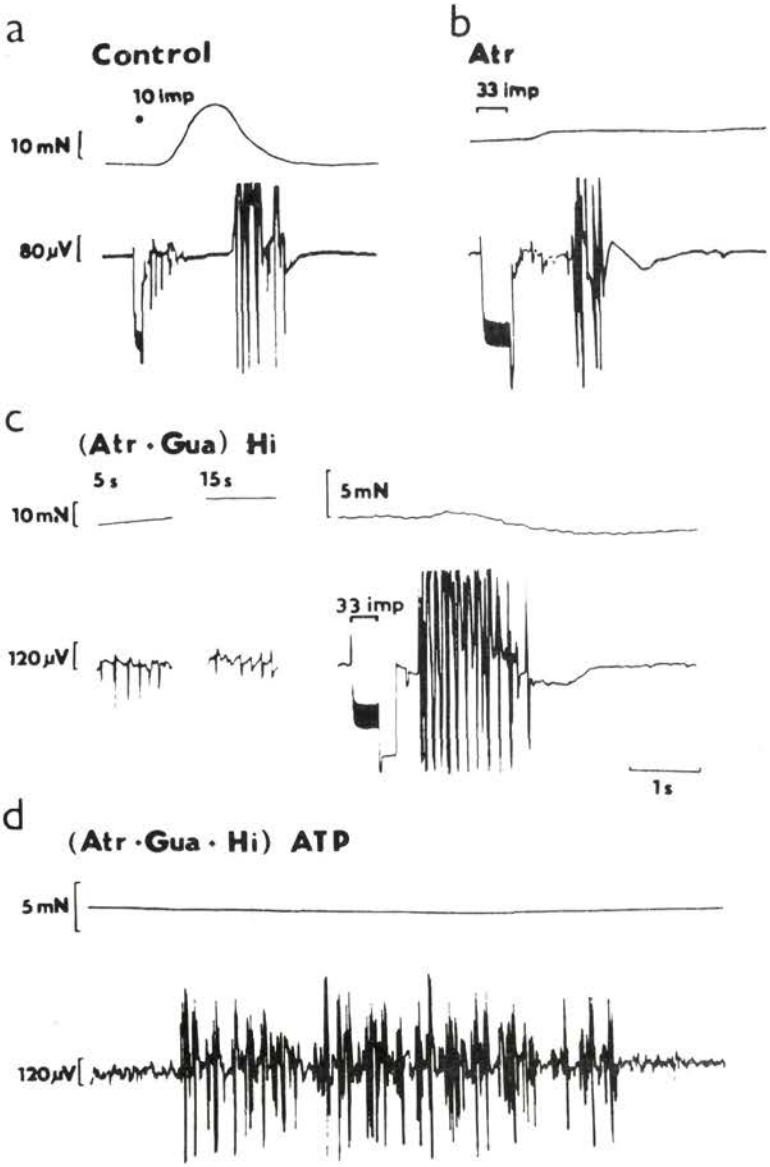

Fig. 7. An EMG record of the activity of longitudinal and circular muscle layers by neurogenic stimulation and drugs. Panels *a-c* show responses to trains of pulses (10 or 33 pulses of 0.2 ms and 30 V at 100 Hz) under control conditions or in the presence of atropine (Atr), guanethidine (Gua), 5 and 15 s after the addition or in the presence of histamine (Hi). Panel *d* shows the response to the addition of ATP (100 µmol/l). The upper traces are contractions (force in mN) in the longitudinal axis; the lower traces were recorded with an electrode placed 4 mm aborally from the site of focal stimulation.

presence of atropine, guanethidine and histamine evoking contractions or relaxations (Table 1). The presence of underlying tissue aborally significantly reduced the aboral responses evoked from the aboral site only (local stimulation). In preparations with the underlying tissue over the entire length, both local and distant stimulation (from aboral and oral sites, respectively) evoked smaller aboral responses. However, the removal of submucosa with the submucous plexus caused a partial but significant restoration of contractions evoked both locally and by distant stimulation as well as locally evoked relaxations.

EMG recording

The typical sequence of responses to focal electrical stimulation in a whole ileum segment is shown in Fig. 7*a*, representative of 30 experiments. In control situation stimulation artifacts were followed by EMG spikes and longitudinal muscle contractions which were atropine sensitive, whereas the excitation that appeared 0.5—2 s later was atropine resistant (Fig. 7*b*) and corresponded to the circular muscle contraction (not shown). Addition to the bathing fluid of histamine in the presence of atropine and guanethidine (Fig. 7*c*) produced spikes and longitudinal muscle contraction for approximately 30 s. Three min later electrical stimulation evoked NANC relaxations of the longitudinal muscle (Fig. 7*c*); spikes of the circular muscle regularly preceded this relaxation in these 30 experiments. If instead of electrical stimulation ATP (0.1 mmol/l) was added to preparations in the bath, spikes of the circular muscle preceded relaxations of the longitudinal muscle (Fig. 7*d*) in 6 experiments; using tenfold higher concentration of ATP (1 mmol/l) spikes of the circular muscle were evoked in additional 5 experiments; in the other 20 out of 31 cases the addition of ATP evoked just relaxation with no preceding spikes. The fact that circular muscle spikes were not recorded in all experiments could have been due to the fact that in some instances circular muscle responses did not spread over the whole segment and EMG electrodes reflected only local excitation.

From a part of ileal segment circumference a strip of longitudinal muscle plus the attached myenteric plexus was dissected as shown in Fig. 8*a*. In 8 such experiments EMG responses to focal neurogenic stimulation from this site and from the intact surface of the longitudinal muscle showed the typical features of circular and longitudinal muscle activity, respectively. In the next step, the longitudinal muscle layer was dissected from the whole surface of 10 segments with only some longitudinal fibres left (Fig. 1) and with the myenteric plexus remaining largely intact. EMG responses of the longitudinal muscle were absent and contractions were largely suppressed; EMG responses of the circular muscle were seen only upon stimulation by trains of longer duration (Fig. 8*b*). If

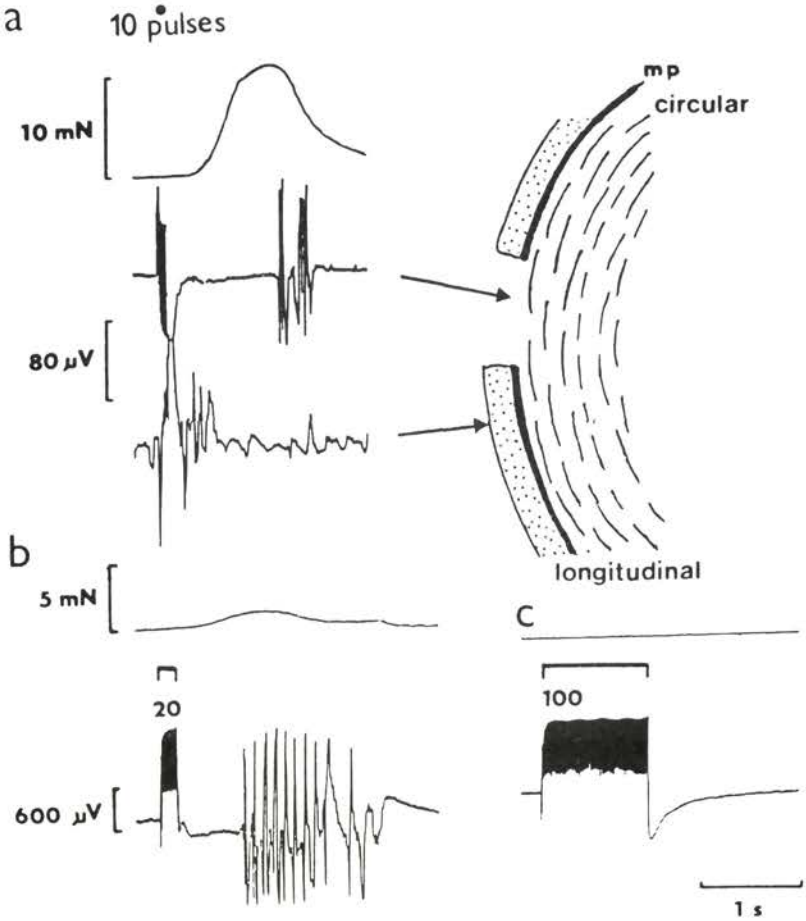

Fig. 8. An EMG record from the surface of either an intact ileal segment or from the sites of a segment where myenteric plexus (mp) and/or longitudinal muscle were dissected. Panel *a* shows the responses from the intact surface of the longitudinal muscle and from the denudated surface of the circular muscle; neurogenic stimulation (10 pulses of 0.2 ms, 20 V at 100 Hz; focal stimulation). Panels *b* and *c* show, respectively, the effect of stimulation in preparations with the longitudinal muscle layer partially removed (20 pulses) and with MPLM layers completely removed (100 pulses).

however, in 6 segments the longitudinal muscle layer was dissected completely together with the myenteric plexus, no EMG responses of the circular muscle were recorded even with trains (100 Hz) of 100 pulses (Fig. 8*c*).

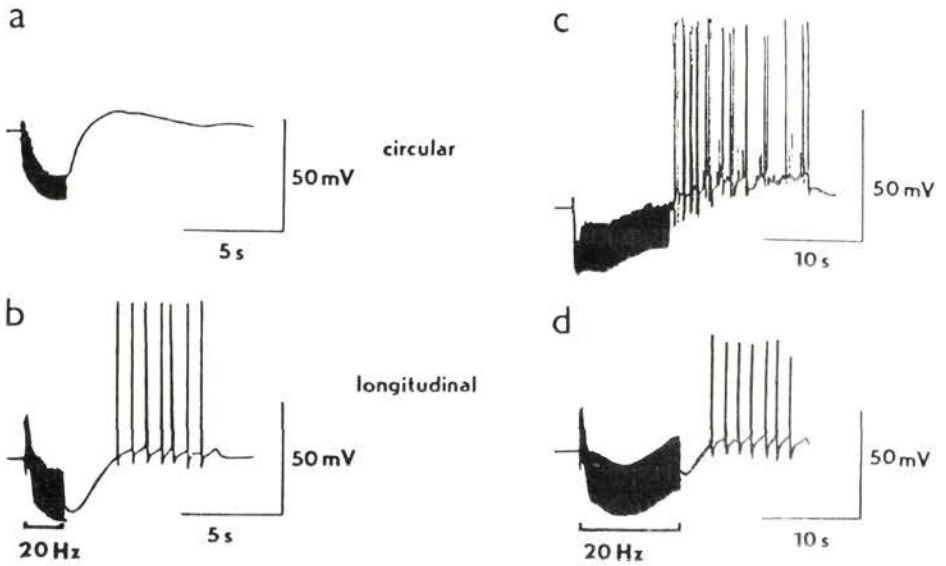

Fig. 9. NANC inhibitory junction potentials elicited by field stimulation; 40 (*a, b*) or 200 (*c, d*) pulses (0.15 ms, 50 V) were delivered at 20 Hz. The responses of circular and longitudinal muscle cells were recorded.

Intracellular recording

In 25 circular muscle cells NANC inhibitory junction potentials were elicited by trains of pulses. The stimulation-evoked hyperpolarization lasted only during the stimulation and turned into rebound depolarization and spike discharge immediately after the train stimulation was discontinued (Fig. 9*a,c*). NANC inhibitory junction potentials were also elicited in another 30 longitudinal muscle cells. As soon as train stimulation was discontinued, the membrane was further hyperpolarized; in comparison to the circular muscle rebound excitation developed only after a delay of more than 2 s (Fig. 9*b,d*).

Discussion

Is there a causal relationship between circular muscle contractions and longitudinal muscle relaxations?

Many investigators studying ileal peristalsis by the naked eye noticed that

circular muscle contractions might be a factor important for relaxation in the longitudinal axis (Hirst 1979; Costa and Furness 1976; Yokoyama nad North 1983; Elden and Bortoff 1984) and even suggested that relaxation might be a passive movement (Wood and Perkins 1970). Mechanical recording of the longitudinal and circular activities may be however difficult to interpret quantitatively because these methods have limited temporal resolution. Electrographic recording from a segment with precontracted longitudinal muscle with more precise temporal resolution suggested that circular muscle contractions in some experiments really preceded NANC relaxations (Fig. 7). However, intracellular recording revealed that rebound depolarization and spike discharge of circular muscle cells occurred immediately after stimulation was discontinued, whereas longitudinal muscle cells were further hyperpolarized (Fig. 9). This could be hardly reconciled with the suggestion of Bywater and Taylor (1986) that NANC inhibitory responses were primarily evoked in the circular muscle layer. Further, if longitudinal muscle cells were also subject to NANC inhibitory influences in a specific way, as actually observed, then longitudinal muscle relaxation would not be just a passive consequence of circular muscle contraction. The coincidence of circular muscle contractions with longitudinal muscle NANC relaxations following neurogenic stimulation as observed in EMG studies (Fig. 7*c*) could thus be well explained by different time courses of stimulus bound hyperpolarization and rebound depolarization. Such kind of relatively independent movements of both layers was confirmed by mechanical recording. During peristalsis in the relaxed segment circular and longitudinal muscle contractions were synchronous (Fig. 2*a*) whereas circular muscle contractions were synchronous with NANC relaxations in segments with precontracted longitudinal muscle (Fig. 2*b*). Large relaxations of the longitudinal muscle evoked either by elevated potassium concentration, which might release NANC transmitters, or by ATP acting postsynaptically (Burnstock 1981; 1986; Den Hertog et al., 1985) were also seen without any preceding contractions of the circular muscle (Fig. 2*c*).

After the addition of TTX to preparations in the presence of atropine, guanethidine and histamine, the preparations were unresponsive to electrical neurogenic stimulation and incapable of peristaltic reflex activity (Fig. 3*d*); moreover, myogenic electrical stimulation evoked synchronous contractions of both muscle layers. Raising the intraluminal pressure could cause pendular movements of both layers in the opposite sense, circular muscle contractions synchronous with longitudinal muscle relaxations (Fig. 3*c*). Thus, circular muscle contraction might causally precede longitudinal muscle relaxation at the myogenic and/or mechanical level (cf. Wood and Pekins 1970); however, it should be strictly differentiated from more complex neurogenic activities where no such causal relationship has been detected.

Can circular muscle contractions be evoked without preceding longitudinal muscle contractions ?

Circular muscle twitches (0.1 Hz) were not a consequence of mechanical interactions between the axes of tension measurement as they were present in the whole segments as well as in helically cut circular strips taken from the whole wall of an ileal segment (Fig. 6*a*). However, the twitches were of irregular height especially during stimulation by the Rand electrode (Fig. 4). Twitches of the circular muscle were completely absent if neurogenic contractions of longitudinal muscle were prevented by atropine or TTX (Fig. 5) or by the removal of MPLM layers (Fig. 6*b*). Field stimulation by trains of pulses induced limited contractions of the longitudinal muscle by non-cholinergic and myogenic mechanism but circular muscle responses were unimpaired (Fig. 5) unless MPLM layers were removed. In the latter case (Fig. 6*b*), only very small responses were seen and even those were subject to neurogenic influences. Also, EMG responses of the circular layer were usually of higher voltage and longer duration than the responses of the longitudinal muscle (Fig. 7); the EMG responses were completely absent in preparations without MPLM layers (Fig. 8). Several previous studies revealed a striking unresponsivness of the circular muscle to substances which had powerful spasmogenic effects on the intestinal longitudinal muscle, and the absence of the myenteric plexus was believed to be responsible for the insensitivity (for review see Holzer et al. 1980). However, authors of these studies worked with preparations lacking longitudinal muscle, too.

It could therefore be hypothesized that previous activation of longitudinal muscle is a prerequisite for both twitches and powerful circular muscle contractions to occur. This hypothesis was based mainly on evidence that a) circular muscle twitches were abolished by atropine or TTX which primarily blocked longitudinal muscle twitches; b) as soon as non-cholinergic or myogenic excitation evoked a longitudinal muscle response, circular muscle contractions fully reappeared; c) in the absence of MPLM layers; neurogenic influences were still present but circular muscle contractions were very weak. Alternatively to this tentative role of the longitudinal muscle in circular muscle activation (cf. Connor et al. 1977), a key role in the synchronized excitation and powerful contractions of the circular muscle was proposed also for non-neural ICC present at the outer aspect of the circular muscle layer that may have been damaged during dissection when MPLM layers were severed (Hara et al. 1986; Suzuki et al. 1986); some role was ascribed to the motoneurones of the submucous plexus, too (Sanders and Smith 1986).

Can the underlying tissue influence longitudinal muscle responses?

Cholinergic contractions and NANC relaxations in the longitudinal axis of the aboral part of MPLM preparations were also influenced by the presence or absence of the underlying tissue including the circular muscle and submucous plexus (Table 1). The inhibitory influence of the underlying tissue was probably not due to mere mechanical hindrances on both contractions and relaxations as the removal of the submucosa with submucous plexus alone (which obviously was not as important from the mechanical point of view as the circular muscle) significantly reduced this inhibition. The inhibitory influence was more related to the site of stimulation (aboral or local vs. contralateral, oral or distant) than to the site of neuromuscular transmission (aboral part only). The inhibitory influence of the underlying tissue was more expressed with relaxations and, as they were in general of lower amplitude, clear differentiation of their changes with respect to the site of stimulation or neuromuscular transmission was precluded.

These results suggested that the observed neurogenic responses might include more than just action potential initiation at the axonal hillock of the respective set of nerves; at least during electrical field stimulation exciting additionally more neural elements, the responses could thus manifest the participation of a more complex neuronal circuitry, especially with NANC relaxation (North 1982; Sanders and Smith 1986).

Acknowledgements. We wish to thank Mrs. A. Hloušková and Mr. V. Růžička for excellent technical assistance.

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Final version accepted February 2, 1989