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A pharmacological analysis of the peristaltic reflex in the isolated colon of the guinea-pig or cat

A. CREMA, G. M. FRIGO AND S. LECCHINI

Department of Pharmacology, University of Pisa, Italy

Summary

1. The peristaltic reflex in the colon was elicited by a localized intraluminal stimulus. The contractile response of the longitudinal coat, which consists of two phases, begins before the start of propulsion. Although the contractions of the longitudinal and circular musculature are usually associated, they may be independent of each other. In particular, the longitudinal contraction does not seem to be necessary for propulsion.

2. Both the longitudinal reflex contraction and the segmental responses of the circular muscle to distension, namely a contraction above and a relaxation below the bolus, are abolished by tetrodotoxin and ganglion blocking agents.

3. In the guinea-pig, longitudinal and circular reflex contractions are usually resistant to antimuscarine, antihistamine and antitryptamine drugs but in the cat they are abolished by antimuscarine drugs. In both species, however, atropine and hyoscine can impair propulsion by blocking selectively the descending inhibition. In the cat, it is possible to find doses which abolish the descending inhibition without affecting the contractile responses of the longitudinal and circular muscle.

4. Sympathetic denervation and pretreatment with reserpine do not affect the propulsive activity. The maintenance of the descending inhibition in denervated organs suggests that the inhibitory neurones to the circular muscle are not adrenergic.

5. On the basis of the effects of drugs, the possible nervous mechanism subserving the polarity of propulsion has been examined. Such a mechanism seems to require an inhibitory pathway involving muscarinic receptors at some point.

6. Pelvic nerve stimulation facilitates propulsive activity. The effect of transmural stimulation is different at low and at high frequencies of stimulation. The inhibitory effect of sympathetic stimulation on the reflex responses seems to be due mainly to an action on intrinsic nervous structures.

Introduction

The peristaltic reflex in the isolated colon has so far obtained relatively little attention. It has been studied by Langley & Magnus (1905), Currie & Henderson (1926), Lembeck (1958), Lee (1960), Hukuhara, Nakayama & Nanba (1961), Hukuhara & Neya (1968), and MacKenna & McKirdy (1969).

Many important problems have been poorly investigated and are still unsolved, among them the role of the longitudinal musculature and the functional relationship between the two muscle coats, the mechanisms subserving the response of the muscle layers and the polarity of propulsion, and the effects of stimulation of the intrinsic and extrinsic nervous system. In particular, the inhibitory phenomena involved in the peristaltic reflex and the actions of drugs have been rarely investigated and the published results are often in conflict with each other.

One reason for the relatively few publications dealing with the colon and the discrepancies of the results could be the lack of a proper method for the investigation of the peristaltic reflex in the colon. In the present paper, a localized intraluminal stimulus was used to evoke a mode of propulsion which simulates the physiological propulsion in the large intestine ; this method made it possible to investigate the effects of drugs and of nervous stimulation.

Methods

The experiments were carried out in the guinea-pig and in the cat by the method described in the preceding paper (Frigo & Lecchini, 1970).

Both in the cat and in the guinea-pig, the periarterial and pelvic nerves were stimulated with rectangular pulses of supramaximal strength and of 0·2 ms duration. The frequency of stimulation ranged from 1 to 20 Hz. Supramaximal transmural stimulation of the guinea-pig colon was carried out by means of two S5 Grass stimulators connected in parallel ; the pulse duration was 0·5 ms and the frequency 1–20 Hz.

In the guinea-pig, sympathetic denervation of the colon was performed by removal of the inferior mesenteric ganglion and freezing of the periarterial plexus of the inferior mesenteric artery 3–5 days before the experiment (Del Tacca, Lecchini, Frigo, Crema & Benzi, 1968). In the cat, the procedure was the same, except that, in addition, the superior mesenteric ganglion was removed and the periarterial plexus of the superior mesenteric artery frozen. The efficacy of denervation was checked functionally by the lack of response to periarterial stimulation and morphologically by the histochemical fluorescence method of Falck & Owman (1965). A few guinea-pigs were pretreated with an intraperitoneal injection of reserpine (2·5 mg/kg) 40 and 16 h before killing.

The following drugs were used: acetylcholine chloride, atropine sulphate, hyoscine hydrobromide, hexamethonium bromide, pentolinium tartrate, cyproheptadine hydrochloride, methysergide maleate, bretylium tosylate, piperoxan hydrochoride, propranolol hydrochloride, tetrodotoxin (Sankyo), reserpine (Serpasil 2·5 mg/ml, Ciba). In the experiments on the guinea-pig colon, the drugs were added to the bath fluid, except for a few preparations in which drugs were also injected into the cannulated inferior mesenteric artery. In the cat, on the other hand, the drugs were always added to the fluid perfusing the arteries. The concentrations and the doses of drugs refer to the salts.

Results

Relationship between the responses of the two muscular coats

Guinea-pig

The threshold volumes at which the longitudinal and circular musculature contracted were very similar, so that any distension sufficient to elicit a contraction

of the longitudinal coat also initiated a response of the circular muscle. However, the two muscular coats often contracted independently of each other. For instance, in 8% of the experiments there was no preliminary shortening of the colon before propulsion although during propulsion there was a maintained contraction of the longitudinal muscle coat. Moreover, in 4·6% of the preparations propulsion occurred without any longitudinal contraction.

The time lapse between the beginning of the longitudinal response and the start of propulsion decreased with increasing distension. At a distension causing maximal velocity of propulsion the average duration of the lag was $6{\cdot}02 \pm 0{\cdot}24$ s ($\pm$ S.E.M. ; $n=71$). No relationship was found between the degree of distension and the amplitude of longitudinal contraction.

Cat

In the cat, contractions of the two muscular coats seem to be strictly correlated, so that propulsion takes place by means of a typical peristaltic wave and occurs only rarely without a longitudinal contraction and then only over a short distance. The mean duration of the lag period between the beginning of longitudinal response and the start of propulsion was $18{\cdot}28 \pm 1{\cdot}77$ s (thirty-six experiments) when the distension caused maximal velocity. In 10·4% of the preparations there was no distinct preliminary contraction of the longitudinal muscle before propulsion because the longitudinal contraction and the propulsion started simultaneously.

Effects of drugs on the peristaltic reflex

Tetrodotoxin, added to the bath containing the guinea-pig colon (0·25–0·5 μg/ml, four experiments) or infused in the arteries supplying the cat colon (0·5–1·5 μg in 5 min, three experiments) inhibited all the components of the peristaltic reflex although the spontaneous motility of the preparations was only slightly affected.

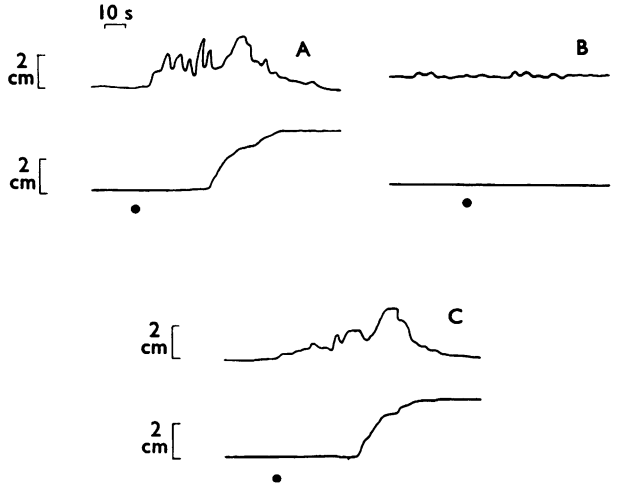

FIG. 1. Effect of hexamethonium on the peristaltic reflex in the guinea-pig isolated distal colon. In each panel: upper tracing, longitudinal movements, shortening upwards; lower tracing, displacement of the bolus, aboral movements upwards. The mark (●) indicates distension of the intraluminal balloon, causing maximal velocity of propulsion. A, Typical peristaltic reflex response; B, in the presence of hexamethonium (50 μg/ml) added to the bath 10 min before distension of the balloon; C, after washing.

In the guinea-pig preparations that showed an initial relaxation of the longitudinal musculature in response to distension, bretylium (25 μg/ml) or piperoxan and propranolol (both 1 μg/ml) did not abolish the inhibitory response. In the guinea-pig (six experiments), hexamethonium (10–50 μg/ml) abolished all the components of the peristaltic reflex (Fig. 1), except in two preparations in which about 20% of the longitudinal contraction was still present. A similar action was exhibited by pentolinium (10–50 μg/ml) in three experiments. That ganglion blocking drugs inhibit propulsion by impairment not only of the ascending contraction but also the descending inhibition, together with an inhibition of the longitudinal response, was clearly observed in four cats after intra-arterial infusion of 100–300 μg of hexamethonium in 10 min (Fig. 2).

In the guinea-pig, atropine (thirty-one experiments) and hyoscine (five experiments), in concentrations from 0·01 to 5 μg/ml, blocked propulsion. In only four experiments, atropine, at all concentrations tested, caused an inhibition of the contractile responses of both muscular coats, but in the majority of the experiments atropine (twenty-seven experiments) and hyoscine (five experiments) blocked propulsion without inhibition of the contractile responses. In fact, from a measurement of the colonic diameters, the block of propulsion seemed to be due to a simultaneous contraction of the circular musculature above and below the bolus (Fig. 3). Moreover, the block of propulsion due to aboral spasm was associated with a contraction of the longitudinal coat of even greater magnitude than that which occurred in the absence of atropine or hyoscine. The same effect was observed after an intra-arterial infusion of 0·1–0·5 μg of atropine in 10 min at a rate of 0·5 ml/min (three experiments).

The contractile responses of the longitudinal muscle coat and of the circular musculature above and below the bolus were maintained even when atropine was

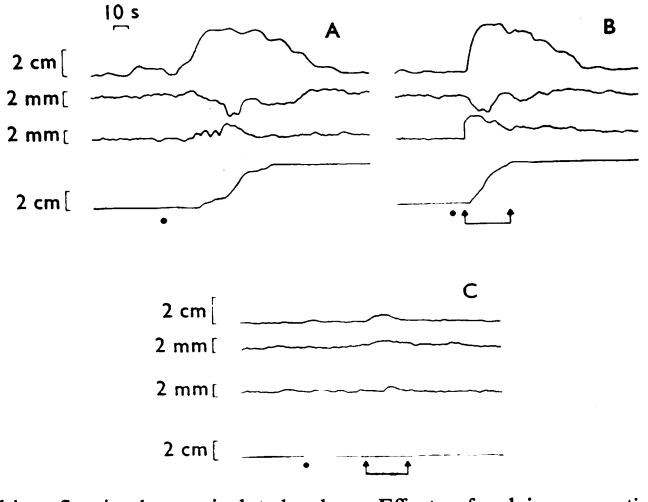

FIG. 2. Peristaltic reflex in the cat isolated colon. Effects of pelvic nerve stimulation and of hexamethonium. In each panel, from top to bottom, records of: longitudinal movements, movements of the circular musculature below the bolus, movements of the circular musculature above the bolus, oral-aboral displacement of the bolus. The mark (●) indicates distension of the intraluminal balloon, causing maximal velocity of propulsion. Between arrows, stimulation of pelvic nerve (0·2 ms, 6 Hz, supramaximal strength). Between B and C, intra-arterial infusion of hexamethonium (300 μg/10 min).

present in a concentration of 5 μg/ml, except in three experiments in which all contractile responses disappeared at concentrations larger than 0·1 μg/ml. The contraction of the longitudinal and circular musculature produced by the bolus in the presence of atropine or hyoscine was not affected by cyproheptadine and methysergide (both 1 μg/ml), but was abolished by hexamethonium (10 μg/ml) (Fig. 3).

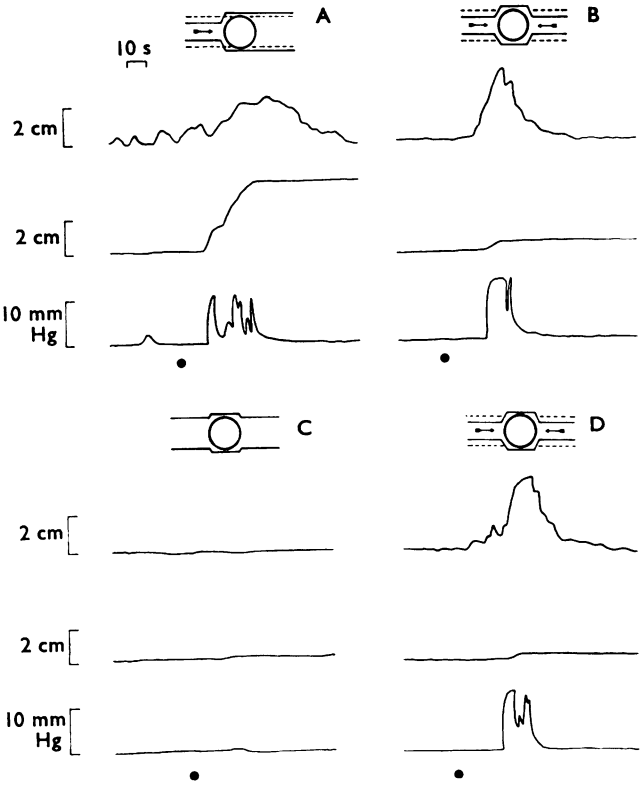

FIG. 3. Effects of atropine and hexamethonium on the peristaltic reflex in the guinea-pig isolated distal colon. In each panel from top to bottom: sketch of the colonic profile as deduced from photographs taken during the reflex, record of longitudinal musculature, record of oral-aboral displacement of the bolus, record of the pressure (1 mmHg≡1·333 mbar) inside the balloon. A, Normal pattern of the reflex; B, in the presence of atropine (0·05 μg/ml); C, in the presence of atropine (0·05 μg/ml) and hexamethonium (50 μg/ml); D, after washing, atropine (1 μg/ml) was added again.

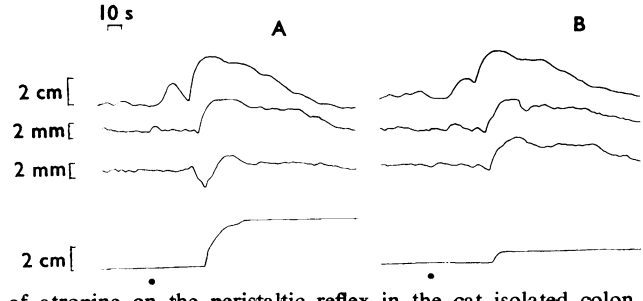

FIG. 4. Effect of atropine on the peristaltic reflex in the cat isolated colon. Records as in Fig. 2. The mark (●) indicates distension of the intraluminal balloon, causing maximal velocity of propulsion. Between A and B, 1 μg of atropine was infused into the arteries over a period of 10 min.

The fact that antimuscarinic drugs inhibited propulsion by impairing the descending inhibition was clearly demonstrated in the cat. In ten out of twelve experiments, intra-arterial infusion of atropine (0·2–1·5 μg in 10 min), did not affect the contractions of the longitudinal coat or of the circular muscle above the bolus, but abolished the descending inhibition or converted it into a contraction (Fig. 4). These contractile responses of both muscular coats were abolished by intra-arterial infusion of hexamethonium (100–300 μg in 10 min). Contrary to what was observed in the guinea-pig, the contractile responses of the longitudinal and circular musculature to distension were abolished when the rate of intra-arterial infusion of atropine was raised to 10–30 μg in 10 min.

Effects of stimulation of the nervous supply to the colon

In both the guinea-pig and the cat, stimulation of the pelvic nerves for 10–40 s before, during or after filling of the balloon decreased the threshold of distension, shortened the latent period and enhanced the velocity of propulsion. It is noteworthy that pelvic nerve stimulation increased not only the velocity of propulsion evoked by submaximal distension but also enhanced the maximal velocity of propulsion (Fig. 5). In both the guinea-pig and the cat the maximum enhancing effect was reached at a frequency of 6 Hz. Moreover, pelvic nerve stimulation was able to initiate propulsion in four preparations of cat colon in which distension alone did not evoke it.

In neither the guinea-pig nor the cat did any of the frequencies of stimulation used (1–20 Hz) prevent propulsion of the bolus by causing a simultaneous contraction of the circular musculature above and below the bolus. In fact, in the cat colon pelvic nerve stimulation increased the contraction above the bolus, while the degree of the descending inhibition was only slightly affected (Fig. 2B). Hexamethonium added to the bath (10–50 μg/ml) or infused into the arteries (100–300 μg in 10 min) inhibited all the responses to pelvic nerve stimulation, except for a small residual contraction of the longitudinal musculature.

In the guinea-pig colon, atropine inhibited the contractile effects of pelvic nerve stimulation. In most experiments (twenty-two out of twenty-seven) complete inhibition of pelvic nerve stimulation occurred at the lowest concentration of atropine

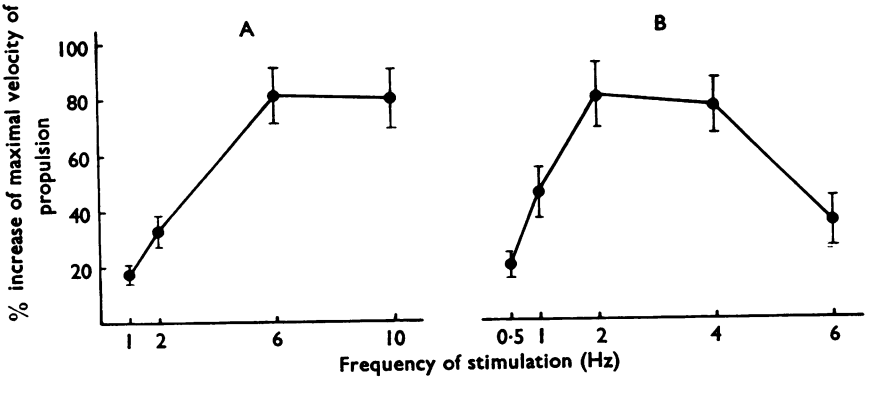

FIG. 5. Effect of pelvic nerve (A) and transmural (B) stimulation on propulsive activity in the guinea-pig isolated distal colon. The percentage increase of maximal velocity of propulsion is plotted against frequency of stimulation. Each point represents the mean of nine experiments in A and of six experiments in B ; vertical lines indicate S.E.M.

which impaired propulsion. On the other hand, when in the cat (nine out of ten experiments), doses of atropine had been infused that impaired propulsion by converting the descending inhibition into a contraction, pelvic nerve stimulation still caused contractions of both the longitudinal and circular muscular coats but did not initiate propulsion.

The effect of transmural stimulation of the guinea-pig colon for 10–30 s depended on the pulse frequency used. At a low frequency (1–6 Hz), the velocity of propulsion was enhanced. This enhancing effect was greatest at 2 Hz (Fig. 5B). At higher frequencies (10–20 Hz) propulsion was impaired, mainly due to a contraction of the circular musculature above and also below the bolus (Fig. 6).

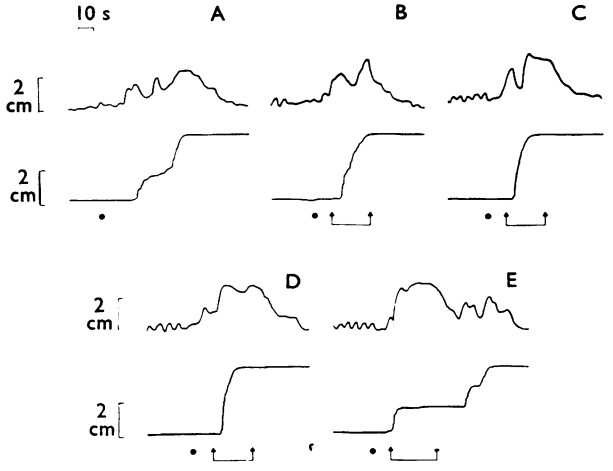

FIG. 6. Effect of pelvic nerve and transmural stimulation on propulsive activity in the guinea-pig isolated distal colon. Records as in Fig. 1. The mark (●) indicates distension of the intraluminal balloon, causing maximal velocity of propulsion. Between arrows, supramaximal pelvic nerve stimulation at a frequency of 2 Hz (B) and 20 Hz (C) and supramaximal transmural stimulation at 2 Hz (D) and 20 Hz (E).

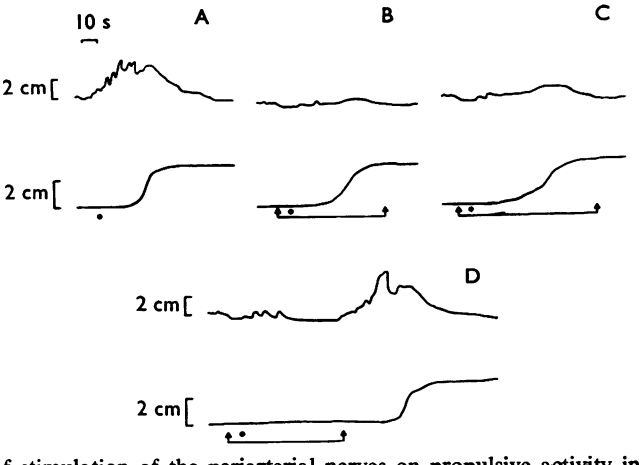

FIG. 7. Effect of stimulation of the periarterial nerves on propulsive activity in the guinea-pig isolated distal colon. Records as in Fig. 1. The mark (●) indicates distension of the intraluminal balloon, causing maximal velocity of propulsion. Between arrows, supramaximal stimulation of periarterial plexus at 2 Hz (B), 4 Hz (C) and 8 Hz (D).

When acetylcholine was infused into the arteries in the guinea-pig or the cat colon, low doses (0·1–0·3 μg in the guinea-pig and 0·5–2 μg in the cat, injected in 10 s) increased propulsive activity, whereas with doses higher than 2 μg in the guinea-pig and higher than 10 μg in the cat, the circular muscle contracted below the bolus and thus prevented propulsion.

In both the guinea-pig and the cat, stimulation of the periarterial plexus for 20–90 s impaired the reflex responses and the propulsive activity to an extent which was dependent on the frequency of stimulation (Fig. 7). In twenty-one experiments on the guinea-pig colon, propulsion due to any degree of distension was blocked at a frequency of 2 Hz in one, of 4 Hz in eight, of 6 Hz in eight and 8 Hz in four experiments. When propulsion was not blocked, the bolus was propelled at slower speed and during propulsion the longitudinal contraction was reduced or absent. At 2 Hz the inhibition of maximal velocity was $34{\cdot}42 \pm 3{\cdot}22\%$. In the cat (five experiments), the reflex responses of both the longitudinal and circular muscle layers were blocked in three experiments at a frequency of 2 Hz and in two experiments at a frequency of 4 Hz.

Both in the guinea-pig and in the cat, stimulation of the periarterial plexus, at a frequency sufficient to inhibit the peristaltic reflex, did not impair the responses of the longitudinal and circular musculature to intra-arterial injection of acetylcholine in a dose that produced a contraction similar in amplitude to that elicited by distension (Fig. 8). On the other hand, the responses of the longitudinal and circular muscle to pelvic nerve stimulation (1–20 Hz) were greatly reduced by stimulation of the periarterial plexus.

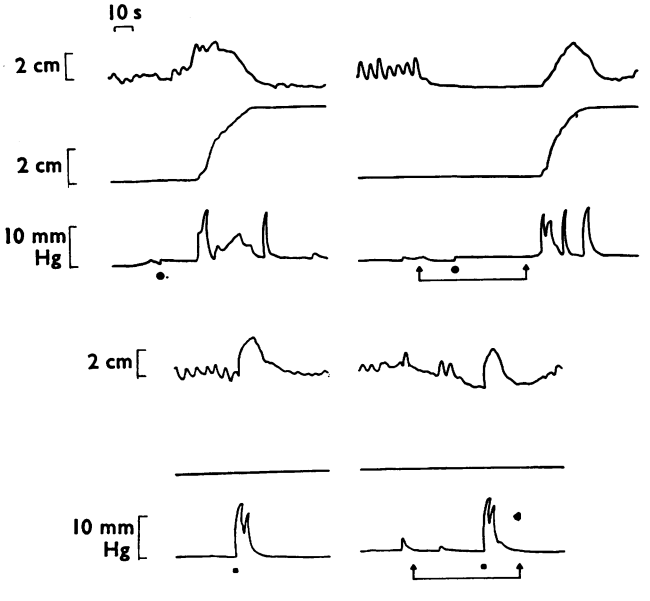

FIG. 8. Guinea-pig isolated distal colon. Effect of stimulation of the periarterial nerves on the reflex responses to distension and on the responses to intra-arterial injection of acetylcholine. Records as in Fig. 1. The mark (●) indicates distension of the intraluminal balloon, causing maximal velocity of propulsion. Between arrows, supramaximal stimulation of periarterial plexus (0·2 ms pulse duration, 6 Hz). At the mark (■) 0·2 μg of acetylcholine was injected into the artery over a period of 5 s.

Sympathetic denervation, carried out in twelve guinea-pigs and six cats, and pretreatment with reserpine (four guinea-pigs) did not affect the propulsive activity of the colon. The average velocity of propulsion in the denervated organs was $0{\cdot}54 \pm 0{\cdot}063$ mm/s in the guinea-pig (eight experiments) and $0{\cdot}34 \pm 0{\cdot}089$ mm/s in the cat (four experiments) at threshold distension; the maximal velocity was $1{\cdot}38 \pm 0{\cdot}11$ mm/s in the guinea-pig (twelve experiments) and $0{\cdot}97 \pm 0{\cdot}21$ mm/s in the cat (six experiments). The mean velocities evoked by threshold or maximal distension of the denervated preparations were not significantly different from those of the normal colons. Even the degree of distension at which threshold or maximal effects were obtained did not differ in the normal and denervated preparations. In four out of twelve guinea-pig denervated colons, the quick relaxing phase of the longitudinal response preceding the contraction was present and, in the cat, the descending inhibition was not impaired (Fig. 9).

Discussion

The peristaltic reflex in the colon, elicited by the localized stimulus of a solid bolus, shows some differences in the responses to drugs and to nervous stimulation from the reflex elicited by raising the hydrostatic pressure in the lumen both in the ileum and in the colon. We shall consider separately the responses of the longitudinal and circular musculature.

By analysing the pattern of the reflex in the isolated colon (Frigo & Lecchini, 1970), we can distinguish in the response of the longitudinal muscle coat a phasic contraction, which develops before the start of propulsion, and a tonic contraction, which increases in magnitude as propulsion progresses. Since both phases are blocked by tetrodotoxin and ganglion-blocking drugs, the nerve fibres and the ganglion cells of the intramural plexus are involved. The final transmitter involved in the excitation of the longitudinal muscle layer appears to be acetylcholine in the cat colon and a substance which is not blocked by antimuscarine, antihistamine and antitryptamine drugs in the guinea-pig colon. The presence of non-cholinergic excitatory neurones to the longitudinal muscle has been postulated for the guinea-pig

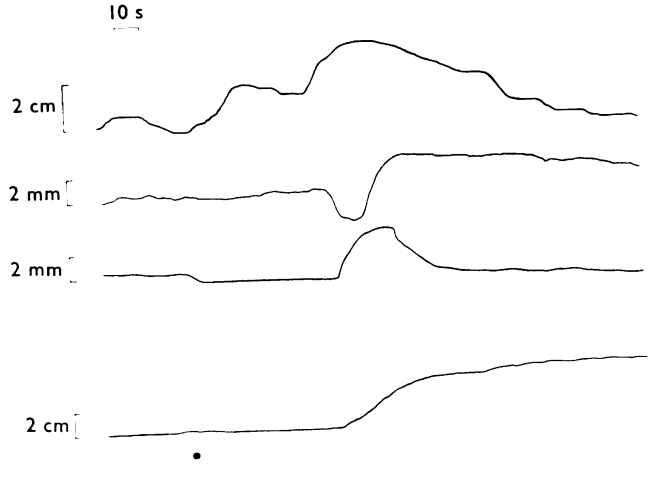

FIG. 9. Peristaltic reflex in the cat isolated colon after sympathetic denervation. Records as in Fig. 2. The mark (●) indicates distension of the intraluminal balloon.

ileum by Ambache & Freeman (1968) and Goldenberg (1969) and for the guinea-pig colon by Bennett & Fleshler (1969).

It would appear that a solid bolus is propelled when a contraction of the circular coat above the bolus occurs simultaneously with a relaxation below the bolus. Both in the guinea-pig and the cat the responses of the circular muscle are nervous in origin and involve a ganglionic site. There is general agreement that in the guinea-pig ileum ganglion-blocking drugs abolish the emptying phase of the peristaltic reflex (Schaumann, 1955 ; Kosterlitz & Lees, 1964). In the colon the contraction above the bolus and the descending inhibition are suppressed by hexamethonium. Our results agree with most authors (Raiford & Mulinos, 1934 ; Schaumann, 1955 ; Kosterlitz & Robinson, 1957 ; Lee, 1960 ; Thouvenot & Harichaux, 1961 ; Kosterlitz, 1967 ; Hukuhara & Neya, 1968) in that atropine blocks the emptying phase of the peristaltic reflex not only in the ileum but also in the colon. However, when a solid bolus is the stimulus, it is found that, both in the guinea-pig and the cat, atropine is able to block propulsion by preventing selectively the relaxation below the bolus. The contraction above the bolus is abolished in the cat by large doses of atropine, which are without effect in the guinea-pig, a finding similar to that obtained by Kottegoda (1969) for the ileum. There are therefore in the guinea-pig colon atropine-resistant excitatory neurones in the longitudinal and circular muscle layers, which are activated by intrinsic stimulation and essential for propulsion ; this is of particular interest because the response to stimulation of the pelvic nerves is inhibited by atropine.

In our opinion the descending inhibition, which involves muscarinic receptors, is the basis for an explanation of the polarity of propulsion. This polarity has been claimed to be of myogenic or neurogenic nature (Raiford & Mulinos, 1934 ; Bozler, 1949a, b ; Hukuhara, Yamagami & Nakayama, 1958 ; Nishio & Saito, 1965). Our experiments indicate that, in the presence of atropine, the distension of the balloon causes contractions above and below the bolus ; we may therefore infer that the polarity is not inherent in the excitatory pathways. The simplest explanation is to assume the existence of a caudally directed inhibitory pathway triggered by the same stimulus which also excites the excitatory neurones. The effects of ganglion-blocking drugs indicate that both the descending inhibition and the excitatory responses involve nicotinic receptors. Therefore, the excitatory and inhibitory reflex arcs could have a common first limb and the inhibitory pathway originate distally to the nicotinic synapse also common to both arcs. Since atropine prevents the descending inhibition, the inhibitory neurones are probably excited through muscarinic receptors, as has been found in other synapses (Volle, 1966 ; Trendelenburg, 1967 ; Koketsu, 1969). In the cat colon, in which the excitatory pathway is cholinergic, the inhibitory muscarinic receptors are more sensitive to antimuscarine drugs than are the excitatory muscarine receptors, so that low concentrations of these drugs can block the descending inhibition without affecting the contractions of the longitudinal and circular muscle layers. The mode in which the inhibitory neurones act, either by modulation of the excitatory pathway or by a direct action on the smooth muscle, is open to speculation. However, an adrenergic origin of the descending inhibition can be excluded because it is not abolished by sympathetic denervation. On the other hand, the presence in the intestinal wall of non-adrenergic inhibitory neurones has been postulated by many authors (see Crema, Del Tacca, Frigo & Lecchini, 1968).

Our experiments present evidence that in the colon a solid bolus is propelled by the associated contractions of the longitudinal and circular muscle coats; there is no relaxation of the longitudinal muscle layer during propulsion. However, contraction of the longitudinal muscle layer seems not essential for propulsion, which in the guinea-pig colon takes place when there is no spontaneous contraction or the contraction is inhibited by stimulation of the periarterial nerves.

The peristaltic reflex in the colon is very sensitive to stimulation of the extrinsic nervous supply. Since sympathetic stimulation blocks propulsion without affecting the responses of the muscle to drugs stimulating it directly, it may be assumed that the sympathetic fibres make contact with the neurones involved in the peristaltic reflex; a similar mechanism was suggested for the ileum by Lee (1970). Pelvic nerve stimulation facilitates propulsion at low and high frequencies of stimulation whereas transmural stimulation increases the rate of propulsion at low frequencies and blocks it at high frequencies. During pelvic nerve stimulation the descending inhibition is not increased, and so the facilitation of propulsion is probably due to increased force of contraction above the bolus. One possible explanation of the differences in the responses to pelvic and transmural stimulation could be the transmural excitation of nervous tissue which is not activated by stimulation of the parasympathetic nerves, as suggested by Paton & Vane (1963) for the guinea-pig stomach. This observation may explain why transmural stimulation, but not stimulation of the pelvic nerves, is able to overcome the descending inhibition.

Whether the differences in the results obtained in the colon and the ileum reflect true organ differences or are simply dependent on the type of intraluminal stimulus used, cannot be decided on the evidence available at present.

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REFERENCES

AMBACHE, N. & FREEMAN, M. A. (1968). Atropine-resistant longitudinal muscle spasm due to excitation of non-cholinergic neurones in Auerbach's plexus. *J. Physiol., Lond.*, **199**, 705–727.

BENNETT, A. & FLESHLER, B. (1969). A hyoscine-resistant excitatory nerve pathway in guinea-pig colon. *J. Physiol., Lond.*, **203**, 62–63*P*.

BOZLER, E. (1949a). Myenteric reflex. *Am. J. Physiol.*, **157**, 329–337.

BOZLER, E. (1949b). Reflex peristalsis of the intestine. *Am. J. Physiol.*, **157**, 338–342.

CREMA, A., DEL TACCA, M., FRIGO, G. M. & LECCHINI, S. (1968). Presence of non-adrenergic inhibitory system in the human colon. *Gut.* **9**, 633–637.

CURRIE, G. C. & HENDERSON, V. E. (1926). A study of the movements of the large intestine in the guinea-pig. *Am. J. Physiol.*, **78**, 287–298.

DEL TACCA, M., LECCHINI, S., FRIGO, G. M., CREMA, A. & BENZI, G. (1968). Antagonism of atropine towards endogenous and exogenous acetylcholine before and after sympathetic system blockade in the isolated distal guinea-pig colon. *Eur. J. Pharmac.*, **4**, 188–197.

FALCK, B. & OWMAN, CH. (1965). A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. *Acta Univ. Lundens.*, Sect II, **7**, 1–23.

FRIGO, G. M. & LECCHINI, S. (1970). An improved method for studying the peristaltic reflex in the isolated colon. *Br. J. Pharmac.*, **39**, 346–356.

GOLDENBERG, M. M. (1969). Effect of atropine and DMAE (a hemicholinium derivative) on contractile responses of the guinea-pig ileum. *Can. J. Physiol. Pharmac.*, **47**, 185–192.

HUKUHARA, T., NAKAYAMA, S. & NANBA, R. (1961). The role of the intrinsic mucosal reflex in the fluid transport through the denervated colonic loop. *Jap. J. Physiol.*, **11**, 71–79.

HUKUHARA, T. & NEYA, T. (1968). The movements of the colon of rats and guinea-pigs. *Jap. J. Physiol.*, **18**, 551–562.

HUKUHARA, T., YAMAGAMI, M. & NAKAYAMA, S. (1958). On the intestinal intrinsic reflexes. *Jap. J. Physiol.*, **8**, 9–20.

KOKETSU, K. (1969). Cholinergic synaptic potentials and the underlying ionic mechanisms. *Fedn Proc.*, **28**, 101–112.

KOSTERLITZ, H. W. (1967). Intrinsic intestinal reflexes. *Am. J. dig. Dis.*, **12**, 245–254.

KOSTERLITZ, H. W. & LEES, G. M. (1964). Pharmacological analysis of intrinsic intestinal reflexes. *Pharmac. Rev.*, **16**, 301–339.

KOSTERLITZ, H. W. & ROBINSON, J. A. (1957). Inhibition of the peristaltic reflex in the isolated guinea-pig ileum. *J. Physiol., Lond.*, **136**, 249–262.

KOTTEGODA, S. R. (1969). An analysis of possible nervous mechanisms involved in the peristaltic reflex. *J. Physiol., Lond.*, **200**, 687–712.

LANGLEY, J. N. & MAGNUS, R. (1905). Some observations of the movements of the intestine before and after degenerative section of the mesenteric nerves. *J. Physiol., Lond.*, **33**, 34–51.

LEE, C. Y. (1960). The effect of stimulation of extrinsic nerves on peristalsis and on the release of 5-hydroxytryptamine in the large intestine of the guinea-pig and of the rabbit. *J. Physiol., Lond.*, **152**, 405–418.

LEE, C. Y. (1970). Adrenergic receptors in the intestine. In *Smooth Muscle*, ed. Bülbring, E. London: Edward Arnold.

LEMBECK, F. (1958). Die Beeinflussung der Darmmotilität durch Hydroxytryptamin. *Pflügers Arch. ges. Physiol.*, **265**, 567–574.

MACKENNA, B. R. & MCKIRDY, H. C. (1969). The role of nervous structures in propulsive activity of the rabbit distal colon. *J. Physiol., Lond.*, **202**, 99–100*P*.

NISHIO, T. & SAITO, K. (1965). Muscular architecture of large intestine. *Proc. 2nd int. Congress for the Study of Diseases of Colon and Rectum*, pp. 151–155. Tokyo: Hedrologicum Conlegium.

PATON, W. D. M. & VANE, J. R. (1963). An analysis of the responses of the isolated stomach to electrical stimulation and to drugs. *J. Physiol., Lond.*, **165**, 10–46.

RAIFORD, T. & MULINOS, M. G. (1934). The myenteric reflex as exhibited by the exteriorized colon of the dog. *Am. J. Physiol.*, **110**, 129–136.

SCHAUMANN, W. (1955). The paralysing action of morphine on the guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **10**, 456–461.

THOUVENOT, J. & HARICHAUX, P. (1961). Sur le péristaltisme intestinal du cobay. Essais pharmacologiques. *C.R. Séanc. Soc. Biol., Paris*, **155**, 1986–1989.

TRENDELENBURG, U. (1967). Some aspects of the pharmacology of autonomic ganglion cells. *Ergebn. Physiol.*, **59**, 2–85.

VOLLE, R. L. (1966). Modification by drugs of synaptic mechanisms in autonomic ganglia. *Pharmac. Rev.*, **18**, 839–869.

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