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TWO DESCENDING NERVE PATHWAYS ACTIVATED BY DISTENSION OF GUINEA-PIG SMALL INTESTINE

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SUMMARY

1. Intracellular recordings were made from myenteric neurones of the guinea-pig small intestine in preparations which had a synaptic input from an orally situated segment of intestine.

2. Excitatory synaptic potentials could be evoked in most neurones by distension of the attached intestinal segment.

3. It was possible to distinguish two distinct firing patterns of synaptic potentials in response to distension. A transient short latency discharge was recorded from some neurones. From the others, a persistent synaptic discharge was recorded only after a long latency (2–11 sec).

4. Distension of intestinal segments evoked short latency transient inhibitory junction potentials in the circular muscle layer followed by excitatory junction potentials in both the circular and longitudinal muscle layers.

5. It is suggested that distension may cause both descending inhibition and, after a delay, descending excitation of the guinea-pig small intestine.

INTRODUCTION

In a previous report we described experiments where intracellular recordings were made from myenteric neurones of guinea-pig small intestine using a preparation in which these neurones received synaptic input from an adjacent segment of intestine (Hirst & McKirdy, 1974). Most long synaptic pathways in the myenteric plexus were found to be anally directed. Distension of the intestine produced impulse traffic in these pathways. It was apparent that distension also produced inhibition of circular muscle only on the anal side of the distended region (descending

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inhibition) and it was suggested that inhibition resulted from impulse traffic in some of the anally directed pathways of the myenteric plexus.

Clearly there is an excitatory component in the peristaltic reflex since peristalsis is characterized by a wave of contraction which moves in an anal direction; since the wave of contraction is abolished by atropine (Trendelenburg, 1917) it is probably mediated by the release of acetylcholine from excitatory neurones. From the experiments of Kuriyama, Osa & Toida (1967) it would appear that excitatory neurones are present in the myenteric plexus; unless we failed to record from these neurones they must also be influenced by anally directed pathways (Hirst & McKirdy, 1974). Since our experiments were carried out in the presence of atropine, we failed to detect changes in the membrane potential of either smooth muscle layer which would result from activation of cholinergic excitatory neurones.

In the experiments described in this paper, we have examined the discharge of excitatory synaptic potentials produced in myenteric neurones by distension of an orally situated segment of intestine. We have identified two distinct nerve pathways; one is characterized by a short latency transient discharge of synaptic potentials in response to distension whilst the other is characterized by a persistent discharge of synaptic potentials only after a long latency. When recording from the two smooth muscle layers, we have found that in the absence of atropine distension produces a short latency transient inhibition of circular muscle which is followed by excitation of both circular and longitidunal muscle. It is suggested that one nerve pathway mediates descending inhibition and that the other mediates descending excitation.

METHODS

Intracellular recordings were made from myenteric neurones lying in a flap of plexus attached to the aboral end of a segment of mid-small intestine as described previously (Hirst & McKirdy, 1974). A metal tube with a small balloon attached to one end was filled with water and placed inside the gut lumen. The other end of the metal tube was connected via a T piece to a syringe and a pressure transducer. The intestinal segment was distended by injecting small volumes of water (0·1–0·6 ml.) into the balloon; the output from the pressure transducer was used to monitor the onset and offset of distension.

When recordings were made from smooth muscle cells, an intact segment of intestine was placed in the tissue bath and intracellular recordings were made from cells in the longitudinal or circular muscle layer some 3–4 cm aboral to the balloon. In some experiments these preparations were stimulated transmurally as described previously (Hirst & McKirdy, 1974). Both preparations were bathed in modified Krebs solution (Hirst & McKirdy, 1974); unless stated otherwise the solution contained atropine sulphate (2×10^{-7} g/ml.) (Bull Ltd) and the bath temperature was 24–26° C. Other drugs used were tubocurarine chloride (Burroughs Wellcome Ltd) and tetrodotoxin (Sankyo).

RESULTS

General observations. Intracellular recordings were made from the two different types of neurones (S cells and AH cells) previously described in the myenteric plexus (Hirst, Holman & Spence, 1974). Of sixty-six neurones eight were classified as AH cells since action potentials, initiated in their somas, were followed by a prolonged afterhyperpolarization. The other fifty-eight neurones were characterized as S cells on the basis that action potentials could be repeatedly initiated in the cell soma by passing outward current through the recording electrode (Hirst *et al.* 1974; Hirst & McKirdy, 1974). Many of the S cells showed evidence of a low frequency spontaneous synaptic input similar to that described previously (Hirst & McKirdy, 1974). The soma of one S cell was invaded repeatedly by antidromic action potentials which persisted in the presence of curare (5×10^{-5} g/ml.); they were therefore unlikely to have resulted from the release of acetylcholine at an axo-axonal synapse (cf. Dennis, Harris & Kuffler, 1971). The cause of this behaviour is obscure. The synaptic responses evoked by distending the attached intestinal segment were examined in the remaining fifty-seven S cells.

Synaptic responses recorded from myenteric neurones following distension of the adjacent intestinal segment. In each experiment, the plexus flap was attached to the anal end of an intestinal segment; 0·2 ml. fluid was injected into the intraluminal balloon and, after 10–20 sec, the balloon was deflated. If no synaptic response was detected, this was repeated, the distending volume being increased in 0·1 ml. steps up to a maximum volume of 0·6 ml. In fifty-four of the fifty-seven cells a synaptic response was detected in response to such stimulation. Two of the cells which did not respond to distension showed evidence of a spontaneous synaptic input whilst no synaptic potentials were detected from the third. It has previously been shown that nearly all myenteric neurones may be influenced by descending polysynaptic pathways when an oral gut segment attached to the plexus flap was stimulated transmurally (Hirst & McKirdy, 1974). It is surprising that such a large proportion of these pathways may be activated by distension since there is evidence that nervous activity may be provoked in the intestine by a variety of stimuli (Hukuhara, Yamagami & Nakayama, 1958). Evidently if these other stimuli activate specific sensory receptors there must be a great deal of convergence in the pathways which lead to changes in muscle excitability. No activity has been recorded from AH cells in response to distension; this is not surprising since the closest that recordings could be made to the distended region is 2 cm.

The pattern of discharge of synaptic potentials observed after distension

varied from neurone to neurone. However it was apparent that two different discharge patterns could be observed. An example of one type of response is illustrated in Fig. 1. The first excitatory synaptic potential (e.s.p.) was recorded 0·6 sec after the balloon had been distended. It can be seen that synaptic potentials occurred during the following 0·6 sec but even though the distension was maintained for a further 5 sec, no synaptic responses were subsequently detected. Twenty-seven of the fifty-four neurones

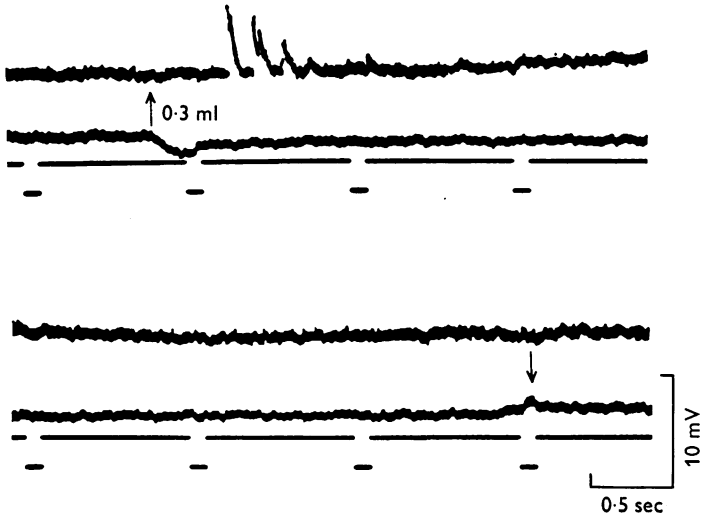

Fig. 1. Discharge of synaptic potentials in a myenteric neurone, evoked by distension of segment of intestine attached to the oral end of a flap of plexus. The upper traces monitor the membrane potential of the myenteric neurone, the middle traces show the pressure changes produced by the inflation of the intraluminal balloon (distending volume 0·3 ml.). The lower trace is one sec time marker. The onset and offset of distension are indicated by upward and downward arrows. Note that the discharge of e.s.p.s is transient even though distension is maintained for 6·2 sec. For convenience the pressure record and time marker are not shown in subsequent Figures; the onset and offset of distension will again be indicated by two arrows.

activated by distension showed a similar response; after a short latency (0·2–1·2 sec) one or more synaptic potentials were recorded; synaptic activity never persisted for more than 1·2 sec after the first synaptic potential even though the stimulus was usually applied for several seconds.

In contrast, a persistent discharge of synaptic potentials was recorded from the remaining neurones in response to persistent distension of the attached intestinal segment. This is illustrated in Fig. 2. It can be seen that after a delay of 3·5 sec e.s.p.s were recorded intermittently until the balloon was deflated.

Thus the two firing patterns could be distinguished by two criteria: the

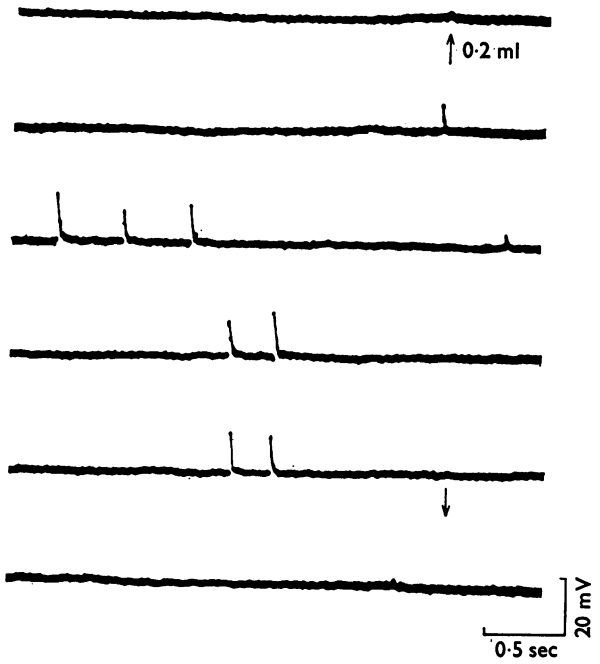

Fig. 2. Synaptic potentials in a myenteric neurone in a flap attached to the anal side of an intestinal segment; e.s.p.s were evoked by maintained distension (0·2 ml.) of the intestinal segment; onset and offset of stimulus indicated by upward and downward arrows respectively. Note that e.s.p.s occur intermittently throughout the distension but only after a latency of 3·5 sec.

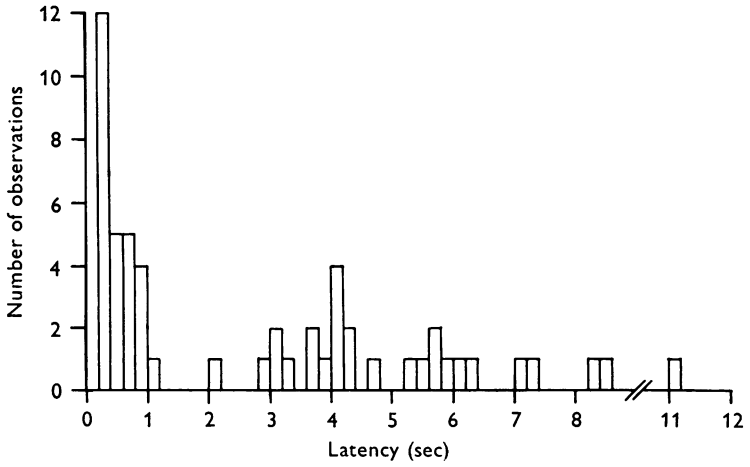

Fig. 3. Histogram of latency between onset of distension and onset of synaptic discharge recorded from fifty-four myenteric neurones. Each value is the mean of three to six determinations for each neurone.

latency of onset of e.s.p.s and their persistence or otherwise throughout the period of distension. The differences in latencies between the two groups is illustrated in Fig. 3; all neurones having a latency of greater than 1·2 sec (see Fig. 3) gave a persistent discharge of e.s.p.s. Both discharge patterns could be recorded from any one preparation and on three occasions the two distinct firing patterns were recorded from different neurones in the same node of plexus. Although the latency to onset of discharge varied somewhat for successive stimuli (see Figs. 4 and 5), no neurone once impaled gave different firing patterns during the repeated application of distension.

In some experiments, it was possible to record from myenteric neurones even though the attached gut segment was spontaneously active. During this activity, increases in intraluminal pressure were frequently detected on the pressure record. Even large increases in intraluminal pressure did not cause an obvious discharge of e.s.p.s. This would be in accord with the suggestion (Ginzel, 1959) that distension rather than an increase in intraluminal pressure was the adequate stimulus for nervous activity in the enteric plexuses.

Short latency descending pathways. The effect of varying the amount of distension was studied in cells in which synaptic discharge had latencies of up to 1·2 sec. In most experiments, the number of e.s.p.s evoked by distension could be varied by varying the volume of liquid injected into the intraluminal balloon. It can be seen from Fig. 4, that as the stimulus was increased in a stepwise manner, one, two and three synaptic potentials were recorded. This could be explained if larger stimuli could cause repetitive firing of one descending pathway or if each pathway could only initiate one synaptic potential but more pathways converging on the one neurone could be recruited. In a few experiments, we were only able to initiate one synaptic potential with a number of different stimuli. One of these experiments is illustrated in Fig. 5. Inflation of the balloon with 0·2 ml. (or with 0·3 ml.) of water, failed to evoke an e.s.p. However, distending volumes of 0·4, 0·5 (and 0·6 ml.) each evoked only one e.s.p. It can also be seen that a persistent stimulus failed to initiate more than one synaptic potential. This finding would suggest that each descending pathway initiates only one synaptic potential (in this experiment other pathways may have been damaged during dissection). It is also apparent that successive e.s.p.s fluctuate in amplitude; a similar variation in the amplitudes of successive e.s.p.s has been observed when they were evoked by close transmural stimulation of a flap of plexus (Hirst *et al.* 1974; Nishi & North, 1973). The variation in latency also observed may well reflect the precision with which the stimulus (radial stretch of the intestinal segment) could be applied, though it is possible that transmission delays

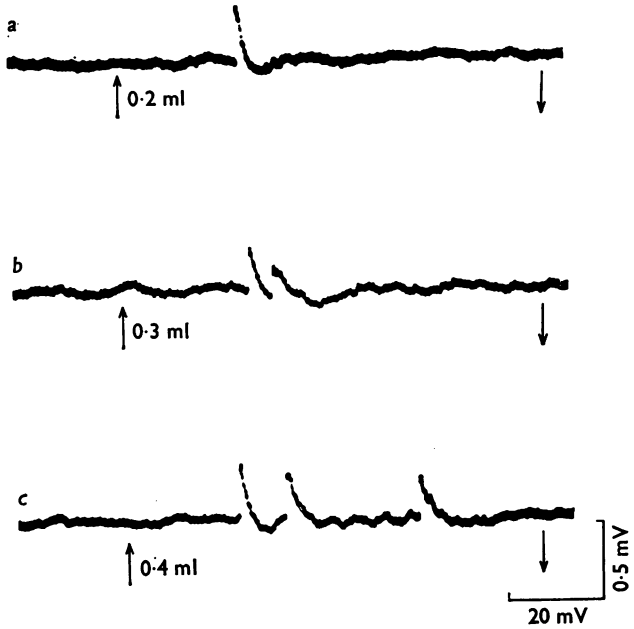

Fig. 4. *a*, *b*, *c*. Effect of different distending volumes upon the synaptic discharge recorded from a myenteric neurone. The distending volume was increased from 0·2 ml. (Fig. 4*a*) to 0·3 ml. (Fig. 4*b*) and finally to 0·4 ml. (Fig. 4*c*); a period of 10 sec elapsed between the end of one stimulus and beginning of the subsequent stimulus. It can be seen that these stimuli initiated, one, two and three synaptic potentials respectively. In this experiment the recording system was a.c. coupled.

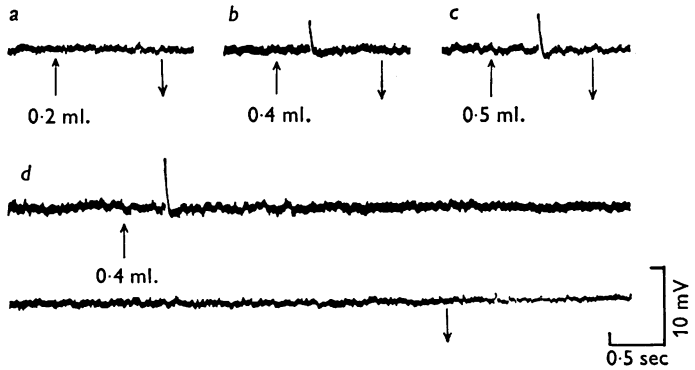

Fig. 5. *a*, *b*, *c*, *d*. Effect of distension on a myenteric neurone. It can be seen that in this cell an e.s.p. is evoked only after a distending volume greater than 0·3 ml. has been used as a stimulus. Larger or more prolonged stimuli failed to cause a repetitive discharge of synaptic potentials.

at synapses on the descending pathway could vary from stimulus to stimulus. Each pathway may only initiate one synaptic potential because transmission through some synapses may not occur at high frequency or because the sensory input is 'gated' in some way.

In four experiments where a variety of amplitudes of stimuli only initiated one synaptic potential, the responses evoked by repeated brief stimuli were examined. The preparation was distended for 1 sec, every 2·5 sec for 15 sec. Synaptic potentials failed to occur in response to the 3rd, 4th or 5th stimulus; it was found that several sec (3–7 sec) had to elapse before a synaptic potential could again be evoked. The possible significance of these observations will be discussed later.

Long latency descending pathways. A discharge of synaptic potentials was recorded from some neurones only after a delay of 2–11 sec (see Fig. 3) from the onset of maintained distension of the aboral intestinal segment. In each of these experiments e.s.p.s were recorded intermittently throughout the duration of the stimulus (see Fig. 2); occasionally the discharge persisted after the balloon had been deflated (see Fig. 7, Hirst & McKirdy, 1974). It was previously reported that the rate of synaptic discharge could be further increased by increasing the volume of fluid injected into the balloon (Hirst & McKirdy, 1974); this was reconfirmed in a few of these experiments.

The mean time between onset of distension and onset of synaptic discharge varied from neurone to neurone (see Fig. 3); it also varied for successive stimuli applied during each experiment. On occasions the variation was quite large, for example, in one experiment the preparation was distended for 10 sec, once every 50 sec, synaptic potentials were first recorded after 4·3, 3·3, 4·2, 3·6 and 3·7 sec. No clear explanation for the long delay or for its variability can be offered. One possibility was considered, namely that nervous activity was evoked in these descending pathways after several hypothetical nervous circuits had been made to fire repetitively. It would be expected that a persistent stimulus would be required to maintain repetitive firing in such circuits. However, in several experiments it was found that a brief stimulus (1 sec) could trigger the long latency discharge of e.s.p.s. One of these experiments is illustrated in Fig. 6. Initially the gut segment was distended for 20 sec; it can be seen that a discharge of e.s.p.s occurred after 6 sec (Fig. 6*a*); e.s.p.s were recorded intermittently until the balloon had been deflated (not shown). When the gut segment was distended for only 1 sec (see Fig. 6*b*) a discharge of e.s.p.s still occurred some 5·6 sec after the onset of distension (that is 4·6 sec after the stimulus had been removed). Thus synaptic activity may be provoked in these long latency pathways either by a persistent or a transient distension of the adjacent aboral segment of intestine.

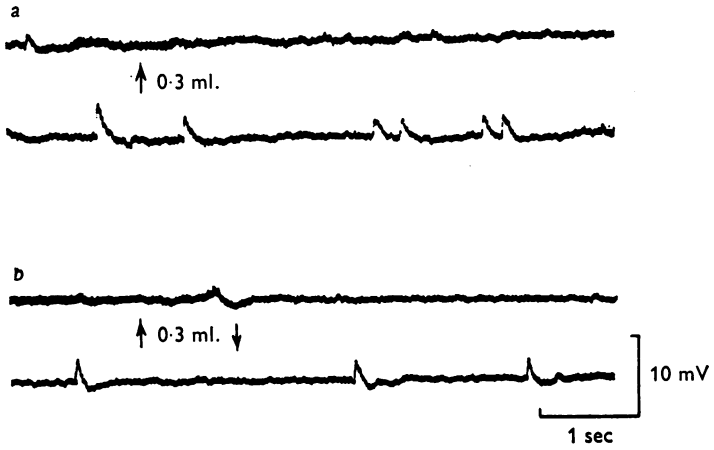

Fig. 6. Effect of stimuli of varying duration (distending volume 0·3 ml.) on discharge of e.s.p.s. In Fig. 6*a* the stimulus was maintained for 20 sec, only the first 10 sec of this is shown. It can be seen that e.s.p.s occur intermittently after a latency of 4·8 sec. In Fig. 6*b* the stimulus was applied for approximately 1 sec but it can be seen that e.s.p.s are still evoked after a latency of 4·5 sec. This record was also made using an a.c. amplifier.

Inhibitory responses recorded from circular muscle in response to distension of an aborally situated segment of intestine. In these experiments, the effect of acetylcholine (ACh) released from excitatory neurones was prevented by atropine (2×10^{-7} g/ml.). Distension of the intestinal segment by inflation of the intraluminal balloon evoked an inhibitory junction potential (i.j.p.) after a latency of about 1 sec (see Table 1) only in the circular muscle layer. Their amplitude could be varied by changing the intensity of the stimulus; as the distending volume was increased from 0·2 to 0·6 ml., larger and larger i.j.p.s were initiated up to a maximum of some 20 mV. The duration of these i.j.p.s was not much affected by intensity of the stimulus nor was it affected by the length of time that the intestine was distended. There was no difference in the 1/2 duration of i.j.p.s, which ranged from 0·6 to 1·3 sec, evoked by stimuli of 1 sec or of 10 sec duration. Descending inhibition produced by the localized distension of a segment of intestine is therefore essentially a transient response. It may be of interest also to note that i.j.p.s evoked by distension or by a transmural stimulus from electrodes close to the balloon had very similar time courses (see also Figs. 6 and 7, Hirst & McKirdy, 1974).

In many experiments recordings were made from the outer layer of smooth muscle cells (longitudinal muscle layer). In spite of the fact that the circular and longitudinal muscle had not been separated in these experiments (cf. Hirst & McKirdy, 1974) inflation of the intraluminal balloon with volumes as large as 0·6 ml. failed to initiate a detectable

change in muscle membrane potential. If the two muscle layers are coupled together electrically as has been suggested to occur in the cat and the dog (Prosser & Bortoff, 1968) the coupling must be such that a change in membrane potential of the order of 15–20 mV in the circular muscle layer produces an undetectable change in the membrane potential of longitudinal muscle cells. Moreover since we were unable to detect i.j.p.s from this layer it would appear that it does not have a functional inhibitory innervation (cf. Kuriyama, Osa & Toida, 1967).

This point was examined in more detail in several experiments using a preparation identical to that described by Hirst *et al.* (1974). Inhibitory junction potentials of long duration could be detected from the longitudinal muscle layer in response to trains of transmural stimuli (10 pulses at frequencies of 5–20 Hz) but a single stimulus never initiated a response. Recordings from myenteric neurones indicated that single stimuli of identical amplitude were sufficient to initiate action potentials in S cells. Since these slow i.j.p.s persisted in the presence of guanethidine (5×10^{-5} g/ml.) for up to 2 hr they do not result from release of noradrenaline. These experiments suggest that inhibition of this layer by intrinsic inhibitory neurones may occur, but probably only as a result of overflow of inhibitory transmitter from neurones whose processes terminate in the circular muscle layer.

When the intestinal segment was distended repeatedly with brief stimuli (distending volume 0·2 ml. for 1 sec, every 2·5 sec for 15 sec), successive i.j.p.s, recorded from the circular muscle, decreased in amplitude until the sixth stimulus failed to initiate a response. In contrast, when the intestinal segment was stimulated transmurally with electrodes positioned close to the distending balloon, although the first three i.j.p.s decreased slightly in amplitude, each stimulus initiated a clearly detectable response. These experiments indicate that the nervous pathway causing release of inhibitory transmitter has many properties similar to the pathways which cause a short latency transient discharge of e.s.p.s in some myenteric neurones. Furthermore it is unlikely that the pathways causing a long-latency persistent discharge of e.s.p.s in other neurones are involved in the release of inhibitory transmitter since i.j.p.s only have a duration of one to two seconds.

Excitatory responses recorded from the longitudinal or circular muscle in response to distension of an aborally situated segment of intestine. Intracellular recordings were made either from longitudinal muscle cells or from circular muscle cells of preparations bathed in normal solution containing no atropine. In each experiment, the intraluminal balloon was first distended for 10 sec (distending volume 0·2–0·4 ml.), an i.j.p. was detected from the circular muscle layer but usually the preparation contracted shortly after this and dislodged the micro-electrode. When the preparation was observed visually, it could be seen that, a few seconds after the balloon had been inflated, a ring of contraction would originate at the distended region and

then travel along the intestine in an aboral direction. Occasionally the contraction was observed to travel both in an oral and an aboral direction.

In the experiments when the micro-electrode was not dislodged, an i.j.p. (in the circular muscle layer) was followed some 2–7 sec later by an excitatory junction potential (e.j.p.). If the e.j.p. reached threshold a muscle action potential was initiated. On occasions the e.j.p. was followed a few sec later by a second e.j.p. The latencies between the onset of distension and the initiation of i.j.p.s and e.j.p.s from ten different preparations are given in Table 1. When the experiments were repeated using only a brief stimulus (1 sec distension of intraluminal balloon) the same sequence of events was recorded except that only one e.j.p. was initiated.

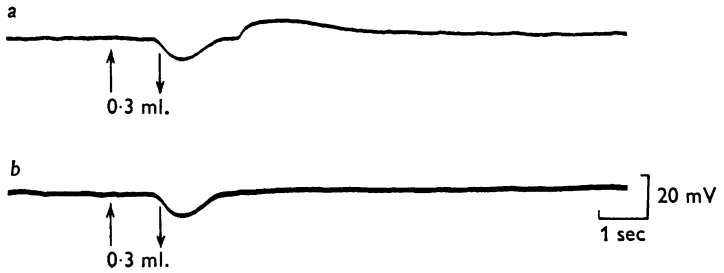

Fig. 7. Intracellular recording from circular layer of smooth muscle showing the sequence of changes in membrane potential evoked by a brief distension (1 sec duration, distending volume 0·3 ml.). In Fig. 7*a*, the record was made using drug free solution. An i.j.p. followed by an e.j.p. was recorded. Fig. 7*b* was made 8 min after changing to atropine solution (2×10^{-7} g/ml.), it can be seen that the e.j.p. has been abolished.

A record from such an experiment is shown in Fig. 7*a*. It can be seen that there is distinct time lag between the end of the i.j.p. and the onset of the e.j.p. Moreover the e.j.p. was selectively abolished by atropine (2×10^{-7} g/ml.) (Fig. 7*b*). These findings rule out the possibility that the delayed depolarization results not from the release of ACh but from rebound excitation of the smooth muscle. Excitatory junction potentials were abolished by curare (5×10^{-5} g/ml.) or tetrodotoxin (1×10^{-6} g/ml.). It has been reported previously that i.j.p.s evoked by distension were also blocked by curare or tetrodotoxin (Hirst & McKirdy, 1974). We would suggest therefore that distension may evoke both descending inhibition and descending excitation of the circular muscle layer. Both are nervously mediated and involved transmission through cholinergic synapses. It seems likely that neurones which only show a discharge of e.s.p.s after a long latency may be excitatory neurones or interneurones lying along the excitatory pathway, since e.j.p.s only occurred after a similar long latency.

It should be stressed that all previous experiments were carried out at

24–26° C. In five further experiments the bath temperature was 35° C. The latencies between onset of inhibition and of excitation are shown in Table 1. It would appear likely that although transmission in the excitatory pathway was slightly faster, these pathways have very similar properties at either temperature.

TABLE 1. Latency (in sec) to onset of i.j.p.s and e.j.p.s in circular muscle of guinea-pig small intestine with bath temperatures of 35 and 25° C. Each figure is mean of five observations from each preparation

	35° C			25° C	
Preparation	i.j.p.	e.j.p.	Preparation	i.j.p.	e.j.p.
1	1·2	4·0	6	1	4·8
2	0·9	3·9	7	0·9	2·4
3	0·9	2·0	8	1	7·3
4	1·0	3·3	9	1·1	4·0
5	1·1	3·8	10	1	3·1
	Mean 1·02	Mean 3·4		Mean 1·0	Mean 4·32

As previously noted no i.j.p.s have yet been detected in the longitudinal muscle layer. However, e.j.p.s could be detected from this layer, and again after a latency of 2–7 sec (experimental temperature 24–26° C). Such e.j.p.s were abolished by atropine (2×10^{-7} g/ml.) but it is of interest to note that in lower concentrations of atropine ($1–10 \times 10^{-9}$ g/ml.) it was occasionally possible to detect an e.j.p. from the longitudinal layer but not from the circular muscle layer. This is surprising since it appears that longitudinal muscle does not have an intimate innervation (Gabella, 1972) whereas axon profiles containing clear vesicles have been observed in circular muscle (R. Hamilton, personal communication). We would have expected atropine to prevent excitation of the outer layer more readily. However these experiments again suggest that if the two muscles are electrically coupled, the coupling must only permit the transmission of larger voltage changes, for example an action potential.

DISCUSSION

Experiments on myenteric neurones demonstrated that two distinct descending polysynaptic pathways could be activated by distension of the intestine. Following distension an e.s.p. or a cluster of e.s.p.s is recorded from neurones lying on one pathway; this discharge occurs after a latency of about 0·5 sec and is transient. When records are made from neurones lying on the other pathway e.s.p.s are recorded intermittently during maintained distension (10–20 sec) but the latency to onset of synaptic discharge is several seconds (2–11 seconds).

When intracellular recordings were made from circular muscle cells on the anal side of the intraluminal balloon, distension evoked a transient hyperpolarization (i.j.p.) about 1 sec after the balloon had been inflated. In drug free solution, this was followed by a more prolonged depolarization which began some 3–7 sec after the onset of distension. Both these responses are neurally mediated and involve transmission through cholinergic synapses. The latency of the onset of transient discharges of e.j.p.s corresponded with the latency of the i.j.p. We suggest that this is the 'descending inhibitory pathway'. The latency to onset of depolarization corresponds with the latency to onset of synaptic discharge in the other pathway. We suggest that this is a 'descending excitatory pathway'.

Both pathways could be activated by brief (1 sec) or prolonged (10–20 sec) distension of the adjacent intestinal segment. A discharge of synaptic potentials was still recorded from neurones on the excitatory pathway several sec after a brief stimulus. Similarly an e.j.p. was still recorded from circular (and longitudinal) muscle several seconds after the intraluminal balloon had been deflated (see Fig. 7). It is not clear why there is such a long latency before the onset of a synaptic discharge in neurones on the excitatory pathways nor why the discharge of e.s.p.s persists even though the stimulus has long been removed (see Fig. 6). It is unlikely that such a delay could result simply from summed transmissional delays of a number of neurones along the excitatory pathway. Even if one assumed that there was a delay of some 20 msec between the arrival of an action potential in the presynaptic terminals and the initiation of an action potential in the neurone they innervated, several hundred such synapses would have to be connected in series to give an overall delay of 2–3 sec. Moreover, when preparations were stimulated transmurally using a pair of stimulating electrodes positioned close to the intraluminal balloon, most cells gave a short latency discharge of e.s.p.s (Hirst & McKirdy, 1974). We interpret this observation to mean that the delay mechanism may be bypassed and that nervous activity once in the myenteric plexus may be rapidly conducted in an anal direction. In a few experiments it was found that removal of the submucosa appeared to prevent descending excitation of the circular muscle layer but did not prevent descending inhibition of this layer (G. D. S. Hirst & H. C. McKirdy, unpublished observations). It may be that some of the neuronal connexions responsible for descending excitation lie in the submucous plexus.

The descending inhibitory pathway appears to be 'gated' in some way. Single transmural electrical stimuli, brief and persistent distension of the intestinal segment each evoke very similar i.j.p.s in the circular muscle. After this pathway had been repeatedly discharged, it became refractory for several seconds. As a working hypothesis we suggest that AH cells provide

afferent input to this pathway. The firing of the AH cells is self limiting; after one or a brief burst of action potentials the cell cannot be excited for several seconds; these cells do not appear to have a synaptic input (Hirst *et al.* 1974; Nishi & North, 1973). This hypothesis is supported by the observation that descending inhibition may still be evoked after removal of the submucous plexus; it is known that AH cells are present in the myenteric plexus (Hirst *et al.* 1974; Nishi & North, 1973).

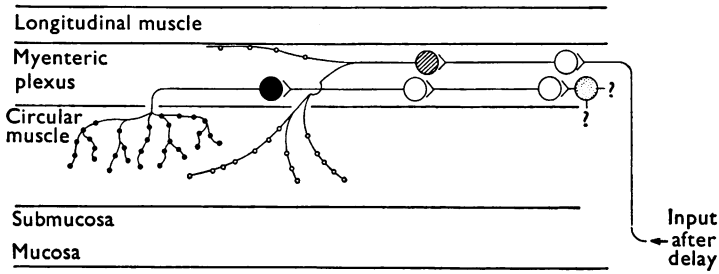

Fig. 8. Schematic representation of the two descending nerve pathways. The lower pathway represents the descending inhibitory pathway. An AH cell (cell body stippled) is in some way activated by distension; its efferent process impinges on a chain of cholinergic interneurones (open circles) which in turn activate an inhibitory neurone (large filled circle). Inhibitory fibres, running in an anal direction, terminate in the circular muscle; inhibitory transmitter is assumed to be released from varicosities (small filled circles). The upper pathway represents the descending excitatory pathway. Input to this pathway only occurs after a delay. Both interneurones (open circles) and excitatory neurones (hatched circles) are activated. Excitatory transmitter (ACh) is then assumed to be released from varicosities (small open circles) within the circular muscle layer or at the surface of the longitudinal muscle layer.

The two descending pathways are illustrated schematically in Fig. 8. Distension presumably causes activation of sensory neurones of both the inhibitory and the excitatory pathway. It may be that a process of the AH cell detects radial stretch of the intestinal segment; we suggest that efferent processes of this cell impinge on an interneurone on the inhibitory pathway. Activation of such an interneurone leads to the subsequent discharge of an inhibitory neurone and causes hyperpolarization of the circular muscle layer. The firing of e.s.p.s along this pathway is limited by the properties of the AH cell. In contrast, input to the excitatory pathway always occurs after more than 2 sec delay; interneurones and excitatory neurones influencing both the circular and longitudinal muscle are then discharged and a wave of excitation sweeps in an aboral direction along the intestine. We would suggest that if the stimulus (e.g. a bolus) were allowed to move that these sequences would recur along the intestine until the bolus had in some way been dissipated. It is also apparent from

our experiments that if the bolus is prevented from moving, as indeed it is when the pathways are activated by distension of the intraluminal balloon, the wave of excitation will still pass in an aboral direction.

We have previously suggested that the aborally directed wave of inhibition corresponds to the descending inhibition described by Bayliss & Starling (1899). It is not clear why Bayliss & Starling did not elaborate on the descending wave of excitation which they detected in their experimental records using an enterograph; the wave passed analwards from a stationary bolus to be recorded some distance below. Visual observation of our preparations indicated that distension could also cause an ascending wave of excitation; since there are only a few orally directed nerve pathways in the myenteric plexus of guinea pigs (Hirst & McKirdy, 1974) we would suggest that ascending excitation arises from the myogenic spread of muscle action potentials initiated at the bolus. It is reassuring to note that as in our experiments, Bayliss & Starling (1899) reported that excitation of the intestine only occurred after a long delay (3–30 sec). The mechanism for such a long delay remains unknown.

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