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EXCITATORY INPUT FROM THE DISTAL COLON TO THE INFERIOR MESENTERIC GANGLION IN THE GUINEA-PIG

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SUMMARY

1. Intracellular recordings were obtained from ganglion cells in the guinea-pig inferior mesenteric ganglion (IMG) with a segment of the distal colon attached to the lumbar colonic nerves.

2. Continuous electrical activity consisting of excitatory synaptic potentials and action potentials was recorded from ganglion cells in all regions of the IMG.

3. The 'spontaneous' synaptic potentials were indistinguishable from those elicited by submaximal stimulation of any of the nerve trunks connected to the IMG.

4. The excitatory activity was irreversibly abolished when the lumbar colonic nerves were cut and reversibly abolished when tetrodotoxin (5×10^{-7} g/ml.) was added to the colon side of a two-compartment organ bath.

5. Addition of dihydro-β-erythroidine (5×10^{-6} g/ml.) to the ganglion side of the bath abolished the synaptic activity of colonic origin and the synaptic responses to stimulation of any of the nerve trunks connected to the IMG.

6. Addition of dihydro-β-erythroidine (1×10^{-5} g/ml.) to the colon side of the bath markedly depressed the synaptic input of colonic origin but had no effect on synaptic responses produced by preganglionic nerve stimulation.

7. Distension of the colonic segment and the application of 5-HT (1×10^{-5} g/ml.) to the mucosal surface of the colon increased the frequency of synaptic input.

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8. The synaptic input from the colon was transiently blocked following repetitive stimulation of any of the nerve trunks connected to the IMG. The discharge of miniature synaptic potentials was unaffected.

9. Addition of noradrenaline (1×10^{-7} to 1×10^{-6} g/ml.) to the colon side of the bath reduced, and in some cases completely abolished, the synaptic input to the IMG. Phentolamine (1×10^{-6} g/ml.), when added to the colon side of the bath, blocked the effect of noradrenaline and the transient inhibition following repetitive nerve stimulation.

10. Addition of noradrenaline (1×10^{-4} g/ml.) to the ganglion side of the bath reduced but never abolished the amplitude of the synaptic potentials of colonic origin.

11. It was concluded that in the guinea-pig, the IMG is involved in a peripheral reflex whose afferent limit of this reflex consists of the axons of cholinergic neurones within the wall of the colon. Many of these neurones are driven either directly or indirectly by cholinergic synapses. The efferent noradrenergic neurones of the IMG function as a group of inhibitory neurones which depress the activity of the excitatory neurones of the colon which are driving them.

INTRODUCTION

In the preceding paper it was shown that the lumbar colonic nerves of the guinea-pig contain fibres which excite the neurones of the inferior mesenteric ganglion (Crowcroft & Szurszewski, 1971). In view of this finding we have investigated the electrical activity of single neurones of this ganglion when it was left attached to a segment of distal colon via the lumbar colonic nerves. In contrast to the results obtained from the isolated ganglion (Crowcroft & Szurszewski, 1971), every neurone displayed 'spontaneous' activity consisting of excitatory synaptic potentials which often exceeded threshold for the initiation of action potentials. This paper describes the characteristics of this discharge and provides evidence that the neurones of the inferior mesenteric ganglion can be excited by presynaptic cholinergic fibres arising from neurones that are situated in the wall of the colon.

Some of the results have been communicated to the Physiological Society (Crowcroft, Holman & Szurszewski, 1970).

METHODS

Young guinea-pigs of either sex were stunned and bled. After the inferior mesenteric ganglion (IMG) was exposed, the distal colon was cut to give a length of 6–8 cm. The preparation was removed from the animal by cutting the mesocolon retaining the segment of colon, together with the IMG, the ascending mesenteric nerve and long lengths of the left and right hypogastric nerves. To obtain adequate lengths of the inferior splanchnic nerves, particularly when the IMG was surrounded by fat tissue, which made dissection of this region difficult, part of the aorta at the origin of the inferior mesenteric artery was also removed. The IMG itself and the lumbar colonic nerves which accompany the artery and the segment of colon (see Fig. 1)

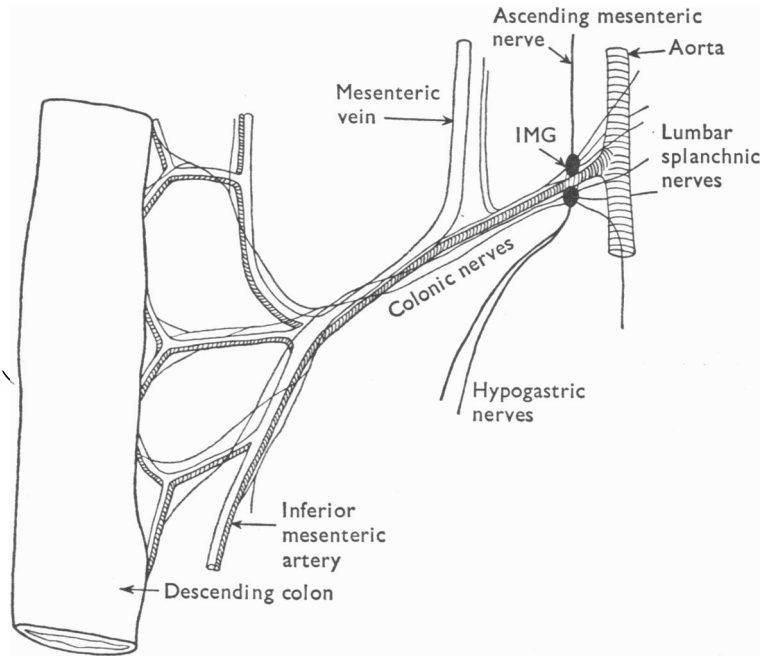

Fig. 1. Schematic drawing of the isolated preparation of the inferior mesenteric ganglion (IMG) and its associated nerve trunks.

were subjected to as little trauma as possible. In the first few experiments, faecal pellets were removed from the colonic segment; in later experiments, every precaution was taken to minimize handling of the colon and the pellets were left to be expelled by the contractions of the colon.

The preparation was placed in an organ bath consisting of two compartments separated by a wall made from silicone rubber. The IMG and associated nerve trunks were pinned down in one compartment and the colonic segment in the other. Only two pins, one at either end, were used to secure the colon without stretching it and to allow it freedom of movement. The mesocolon containing the inferior mesenteric artery and vein and the colonic nerves was draped over the centre wall and prevented from drying out by moistened strips of tissue paper. Both compartments were perfused at a rate of 3–5 ml./min with a modified Krebs solution equilibrated

with 95 % O_2 and 5 % CO_2, and warmed to 33–35° C as previously described (Crowcroft & Szurszewski, 1971). The methods used to stimulate nerve trunks, record intracellularly and to inject current intracellularly have been described (Crowcroft & Szurszewski, 1971). Since the voltage used for nerve stimulation depended on the size of the nerve trunk and on the amount of adherent connective tissue, the values of the stimulus strength used in each experiment will not be indicated. In all experiments, except for the one described in Fig. 3, a supramaximal strength was used and this was usually 10 V. The pulse duration was always 0·5 msec.

The following drugs were used: dihydro-β-erythroidine hydrogen bromide (DHβE), 5-hydroxytryptamine-creatinine-sulphate (5-HT), L-noradrenaline bitartrate (NA), phenoxybenzamine hydrochloride, phentolamine hydrochloride and tetrodotoxin. The drugs were injected into either compartment through separate side tubes after prewarming. 5-HT was added to the mucosal side of the colon by means of a polyethylene tube extending along the whole length of the segment. Small holes were cut along the length of the tubing so that the 5-HT reached all of the mucosa and the drug was injected slowly so that the colon was not distended.

To distend the colon, the distal end was tied off and a fine polyethylene tube which led to a syringe was tied into the proximal end. The segment was distended by injecting a small quantity of air for 5–10 sec after which the air was withdrawn.

RESULTS

General observations

Continuous electrical activity was recorded from more than 200 cells impaled during fifty experiments. This activity consisted of either excitatory synaptic potentials or of action potentials and excitatory synaptic potentials. Activity was recorded from cells found in all regions of the IMG. Although the frequency and amplitude of synaptic potentials varied from one preparation to another, the level of activity was fairly constant for all the cells studied in any one preparation.

Fig. 2 shows five examples of this activity. The record of Fig. 2*A* shows synaptic potentials occurring at fairly regular intervals. In Fig. 2*B*, synaptic potentials occurred in bursts and within each burst they frequently summed with each other. The most common pattern of activity recorded during these experiments is illustrated in Fig. 2*C*. In these ganglion cells the threshold for the initiation of an action potential was exceeded from time to time, either by an individual synaptic potential or as the result of summation. Fig. 2*D* and *E* shows records from cells where the synaptic activity was sufficiently intense to initiate many action potentials. In Fig. 2*E*, action potentials appeared to occur in bursts.

There was no obvious correlation between the pattern of the activity and contractions of the distal colon. In those cells where synaptic activity or action potentials were concentrated into bursts, there was no correlation between the frequency of these bursts and contractions of the colon. In some cells with particularly low levels of activity, synaptic potentials of

similar magnitude occurred at intervals ranging from 1 to 2 sec. It was noticed that excitatory activity was more pronounced in those preparations in which the faecal pellets had not been deliberately removed before the experiment.

The 'spontaneous' synaptic potentials were indistinguishable from those elicited by submaximal stimulation of any of the nerve trunks connected to the IMG. This is shown in Fig. 3 which consists of a series of traces in which the time course of synaptic responses to stimulation of a splanchnic nerve can be compared with the time course of the 'spontaneous' potentials.

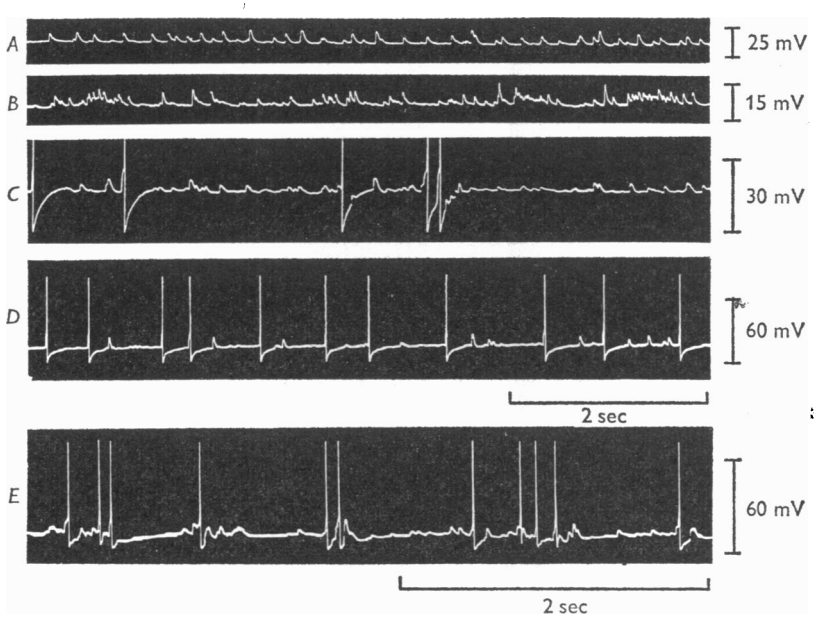

Fig. 2. Intracellular recordings of patterns of synaptic activity recorded from ganglion cells of the IMG in five preparations. Pattern in *C* most frequently observed.

When all the lumbar colonic nerves were cut, all 'spontaneous' synaptic activity was immediately and irreversibly abolished. Fig. 4 shows the burst of action potentials which were recorded when the nerves were transected. The activity was abolished reversibly when tetrodotoxin (10^{-6} g/ml.) was added to the colon side of the bath. During tetrodotoxin blockade, stimulation of any of the nerve trunks connected to the IMG still elicited synaptic and action potentials in the ganglion, showing that the toxin had not leaked from the colon bath to the ganglion bath. These findings demonstrated that the input to the IMG from the colon was mediated by nerve impulses initiated in the colon.

The excitatory input to each cell in the IMG appeared to come from all regions of the segment of distal colon removed in the dissection. In three experiments, 11–12 cm of colon was dissected and mounted in the organ bath. Ganglion cells in the IMG exhibited a massive synaptic activity such as that shown in Fig. 5*A*. When the colon itself was transected about 2 cm

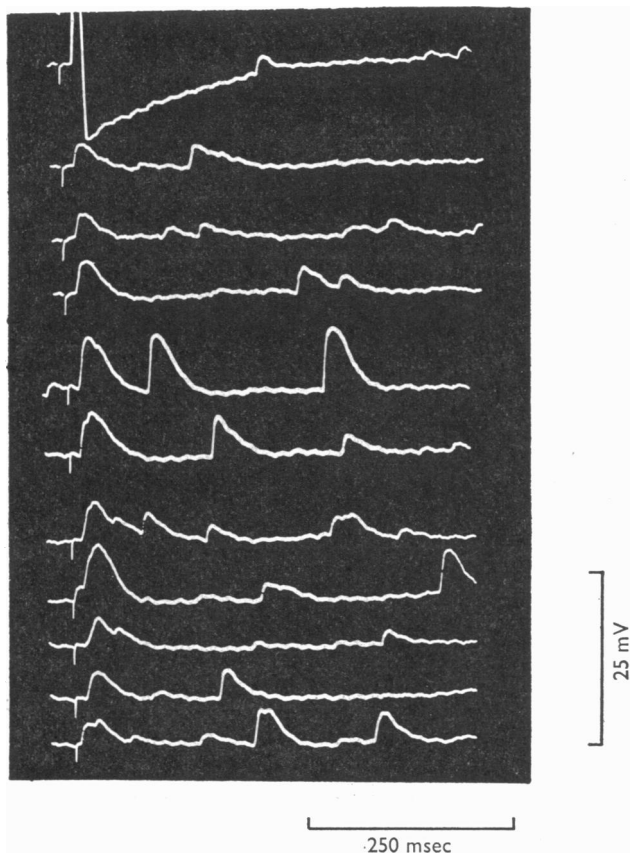

Fig. 3. Series of records taken from the same cell showing evoked synaptic potentials and 'spontaneous' synaptic potentials. Each trace begins with a synaptic response evoked by subthreshold stimulation of an inferior splanchnic nerve. The 'spontaneous' synaptic potentials which followed were due to input from the colon.

from the proximal end there was no change in the synaptic activity. However, when the nerve bundles and blood vessels connecting this isolated segment of colon to the main colonic nerve trunk were cut through, the synaptic activity was diminished (Fig. 5*B*). The records of Fig. 5*C*, *D* and *E* show the effect of a further series of transections through the arcades

of nerve fibres and blood vessels on the intensity of synaptic input to a single ganglion cell. A progressive decrease in activity occurred with each transection.

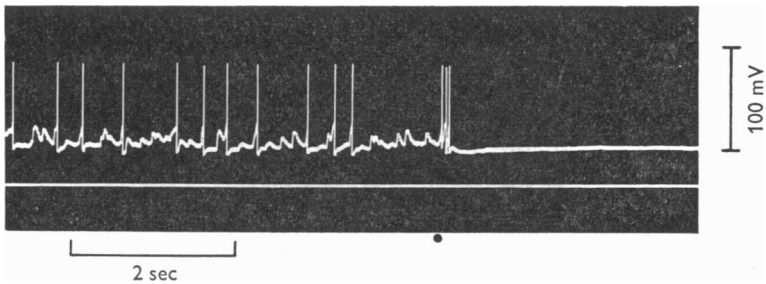

Fig. 4. Intracellular recording from a ganglion cell in the IMG before, during and after cutting the lumbar colonic nerves. The moment of cutting the nerve is indicated by a dot.

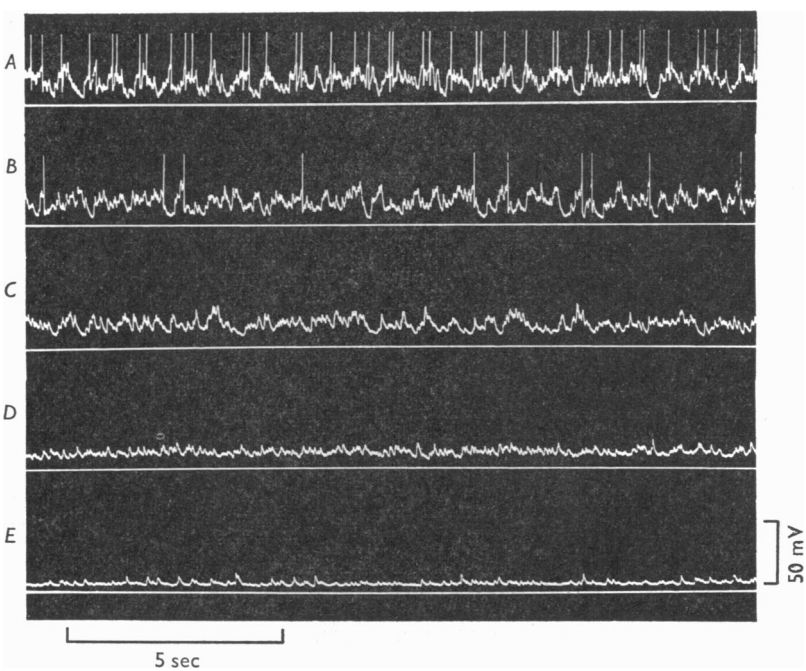

Fig. 5. Synaptic activity in a ganglion cell before and after a series of transections through the lumbar colonic nerves. In *A*, activity before cutting any nerve fibres. In *B* to *E*, number of intact fibres running between colon and IMG progressively reduced by transection so that in *E* only a few remained.

Effects of dihydro-β-erythroidine (DHβE)

Ganglion compartment. The synaptic activity of colonic origin was abolished when the IMG was exposed to DHβE at a concentration of 5×10^{-6} g/ml. (Fig. 6). This effect was reversible. Synaptic responses to stimulation of the lumbar colonic, ascending mesenteric, inferior splanchnic and hypogastric nerves were also blocked by this concentration. Depression

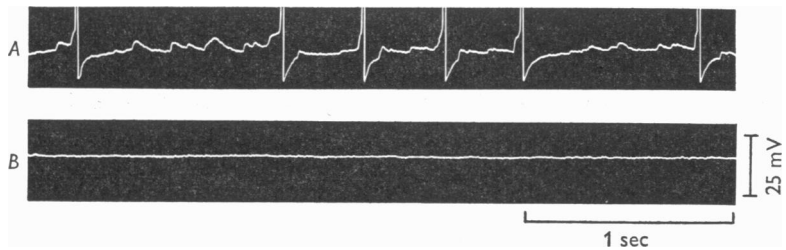

Fig. 6. Effect of DHβE (5×10^{-6} g/ml.) on the synaptic input to a ganglion cell in the IMG. *A*, control; *B*, DHβE present in solution bathing the ganglion.

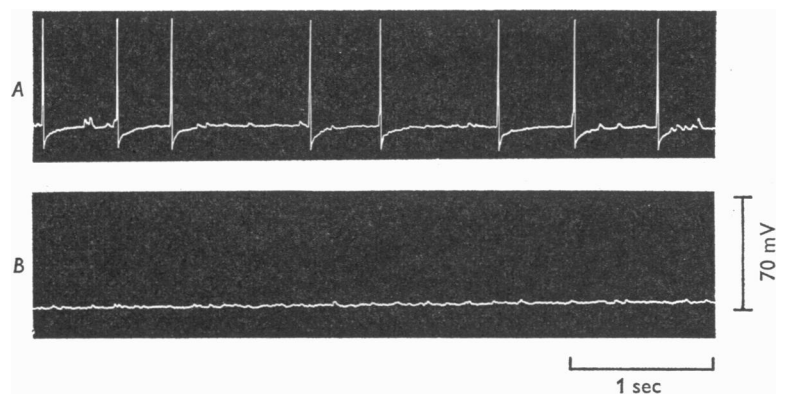

Fig. 7. Effect of DHβE (1×10^{-5} g/ml.) on the synaptic input to a ganglion cell in the IMG. *A*, control; *B*, DHβE present in the solution bathing the colon.

and blockade of synaptic activity of colonic origin and of evoked synaptic responses had a similar time course. Thus, action potentials which are initiated in the colon excite IMG cells by cholinergic synapses which are indistinguishable from those of the other preganglionic inputs.

Colon compartment. After the addition of DHβE (10^{-5} g/ml.) the synaptic activity of colonic origin was markedly depressed (Fig. 7). During the action of DHβE responses to stimulation of other nerve trunks connected to the IMG were unchanged indicating that the drug had not leaked to the ganglion side of the bath.

Effect of distension and 5-HT

In an attempt to learn more about the nerves which synapse in the IMG, we studied the effect of distension and 5-HT. Both types of stimuli are considered to excite enteric neurones.

Distension. When the colonic segment was distended by 1–3 ml. air, the frequency of the synaptic input to the IMG was increased. Two examples of this effect from different experiments are shown in Fig. 8. In

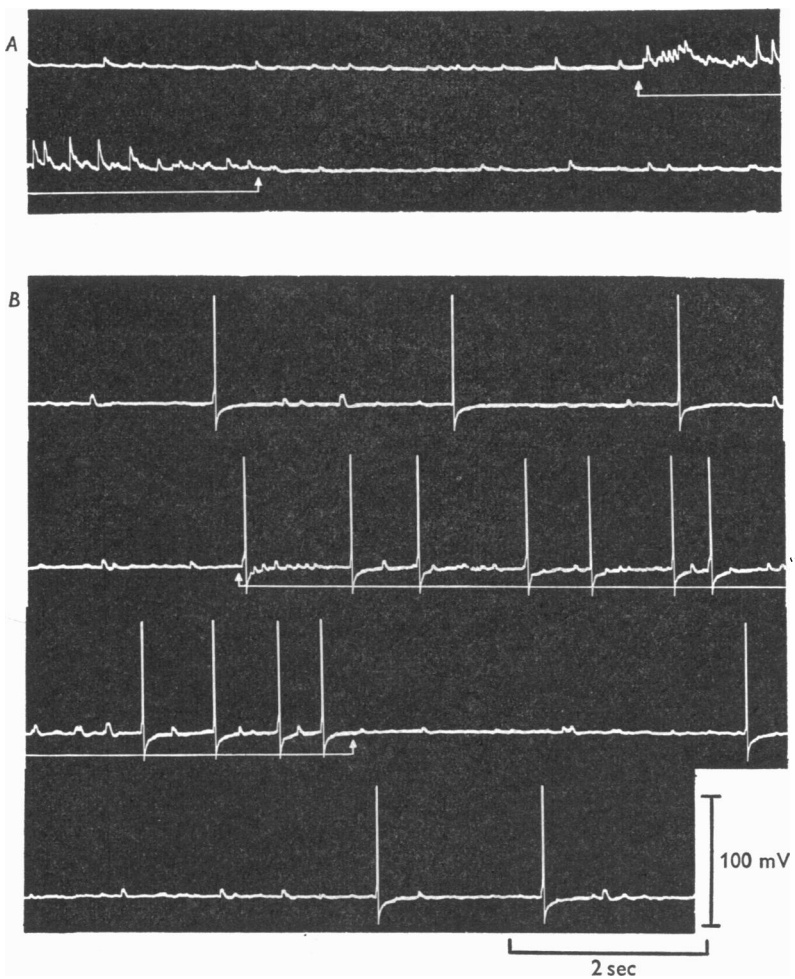

Fig. 8. Effect of distension of colon on synaptic input to two ganglion cells in the IMG in two preparations. In *A* and *B*, 1·0 ml. and 0·5 ml. air, respectively, injected into segment at first arrow, then withdrawn at second. Continuous recordings.

both instances after the air was withdrawn, there was a period during which the frequency of discharge was reduced and it usually took a few sec to return to the pre-distension level.

The application of DHβE (1×10^{-5} g/ml.) to the colon bath depressed not only the 'spontaneous' synaptic activity but also the effect of distension.

5-HT. Bülbring & Lin (1958) have shown that when 5-HT is introduced into the lumen of the small intestine the threshold for the initiation of the

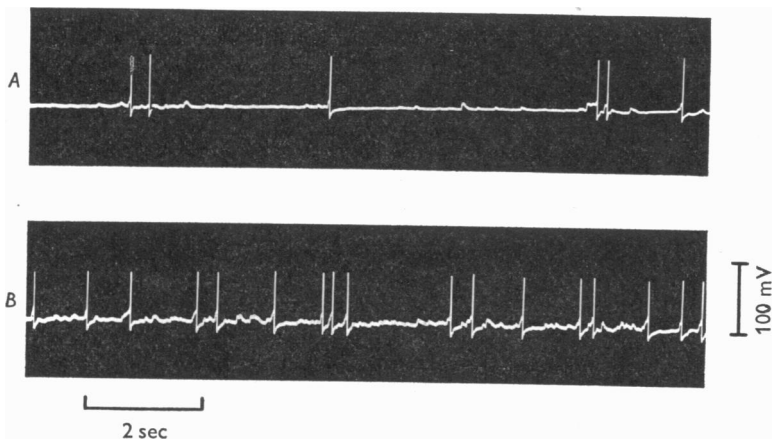

Fig. 9. Effect of 5-HT (1×10^{-5} g/ml.) on the synaptic input to ganglion cell in IMG. *A*, before and *B*, 2 min after introduction of 5-HT into the lumen of the colon.

peristaltic reflex is markedly reduced. We therefore decided to test the effect of 5-HT on the synaptic potentials recorded from IMG neurones, when this drug was introduced into the lumen of the colon. As shown in Fig. 9, 5-HT (1×10^{-5} g/ml.) caused a marked increase in the frequency of excitatory junction potentials and action potentials.

Effect of stimulating nerve trunks connected to the IMG

Repetitive stimulation of any of the nerve trunks connected to the IMG caused a transient inhibition of the synaptic input from the colon. In Fig. 10*A* the lumbar colonic nerves were stimulated at 20/sec for 1 sec. Synaptic input from the colon was inhibited for about 1·5 sec after the cessation of stimulation. In Fig. 10*B* the duration of the period of stimulation was increased to 2 sec and the period of inhibition increased to more than 3 sec. For all nerve trunks, the longer the duration and the higher the frequency of stimulation, the longer the period of inhibition. The degree of inhibition, and its time course also depended on which nerve trunk was stimulated. Stimulation of the lumbar colonic nerves was

generally most effective, though in some preparations an equally intense and prolonged inhibition followed stimulation of the ascending mesenteric nerve; stimulation of the hypogastric nerves was least effective but nevertheless this always caused a brief inhibition of the colonic input.

The burst of action potentials recorded from IMG neurones in response to repetitive preganglionic nerve stimulation was followed by a phase of after-hyperpolarization, in both the isolated ganglion (Crowcroft & Szurszewski, 1971) and in ganglia attached to the colon (Fig. 10*A* and *B*). To determine whether the after-hyperpolarization was responsible for the

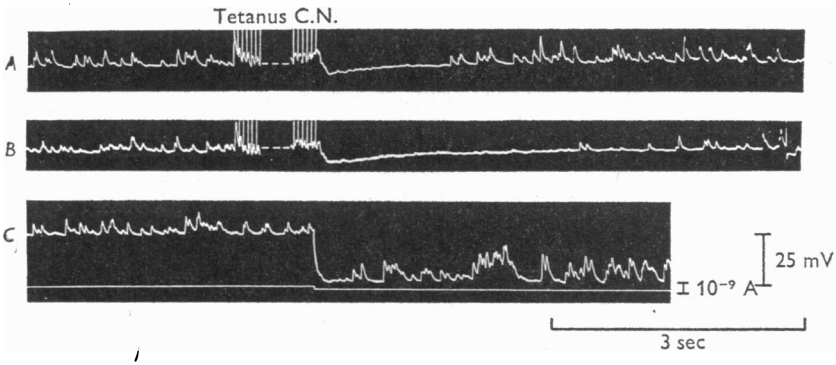

Fig. 10. Effect of repetitive stimulation of the lumbar colonic nerves at 20/sec for 1 sec (*A*) and for 2 sec (*B*) and effect of a hyperpolarizing pulse (1×10^{-9} A) applied through the micro-electrode (*C*) on the synaptic input from the colon. In *A* and *B*, tracing interrupted between first six and last six responses. In *C*, top record, membrane potential; bottom record, current monitor.

prolonged inhibition of the synaptic input, the effect of a hyperpolarizing current pulse on this input was studied. Fig. 10*C* shows the effect of hyperpolarizing the membrane of the same IMG neurone as that of Fig. 10*A* and *B* to a level greater than that observed following repetitive preganglionic nerve stimulation. The amplitude of the synaptic input from the colon was increased. Thus the inhibition of the colonic discharge following repetitive stimulation could not have been due to the after-hyperpolarization of the ganglion cell within the IMG.

In isolated mammalian ganglia, miniature (presumably quantal) synaptic potentials are rarely observed, but their frequency is markedly increased by repetitive stimulation (see Fig. 6 of the preceding paper). Fig. 11*A* shows the result of an experiment in which a splanchnic nerve was stimulated while the colon was attached to the IMG. The synaptic input was abolished after stimulation, but a much lower amplitude discharge of miniature synaptic potentials was recorded. Fig. 11*B* shows the effect of a similar period of stimulation, after the colonic nerves had been cut. The

discharge of miniature synaptic potentials was unaffected by this procedure. This finding also suggests that the inhibition of the synaptic activity coming from the colon, following the excitation of IMG neurones, was not due to a failure of transmission within the ganglion.

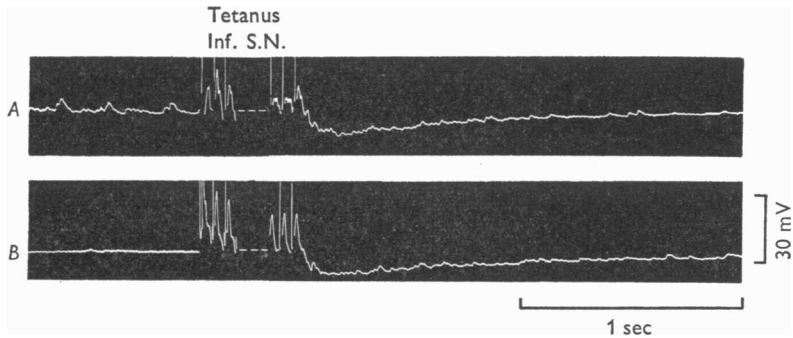

Fig. 11. Occurrence of miniature synaptic potentials following repetitive stimulation (15/sec for 2 sec) of an inferior splanchnic nerve before (*A*) and after (*B*) cutting the lumbar colonic nerves. In *A* and *B*, record interrupted between first three and last three responses. Records from same cell.

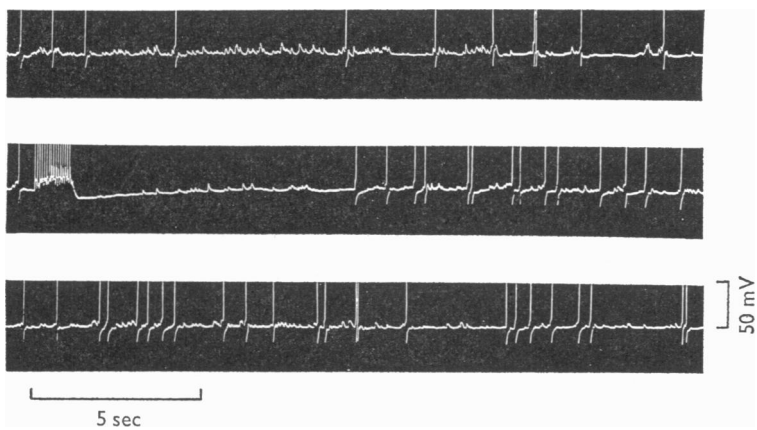

Fig. 12. Effect of repetitive stimulation of the lumbar colonic nerves (10/sec for 2 sec) on the 'spontaneous' activity in a ganglion cell in IMG. After the period of inhibition, more synaptic potentials reached threshold for initiation of action potentials.

In the preceding study on the IMG (Crowcroft & Szurszewski, 1971) it was noted that following repetitive preganglionic stimulation of ganglion cells, there followed a prolonged period of enhanced excitability which was unrelated to any change in membrane potential. Fig. 12 shows the effect of this period of enhanced excitability on the synaptic input from the colon. It can be seen that there were many more action potentials than

during the control period. This was not due to an increase in the frequency of synaptic potentials. Study of a small number of cells with relatively low levels of colonic input has shown that the amplitude of the synaptic potentials was increased. Thus this phase of increased excitability could be due to an increase in membrane resistance.

Effect of noradrenaline (NA) and α-adrenergic blocking drugs

Most, if not all the ganglion cells of the guinea-pig IMG are noradrenergic (A. Ostberg, personal communication) and excitation of these neurones will cause the release of NA from their terminal axons in the wall of the colon. It has been shown that the noradrenergic terminal axons of the gastrointestinal tract are concentrated in the vicinity of ganglion cells of the

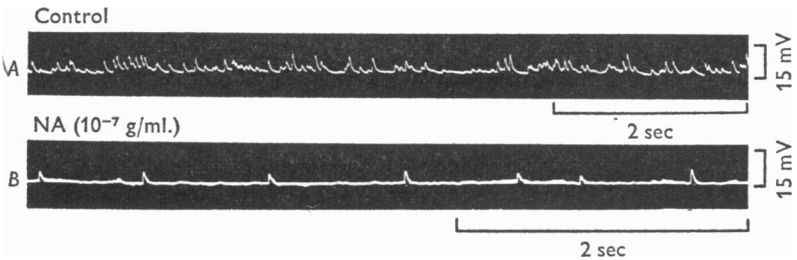

Fig. 13. Effect of adding noradrenaline (1×10^{-7} g/ml.) to the colon side of the bath on the synaptic input to a ganglion cell in the IMG. *A* before and *B* during the second minute of injection of noradrenaline.

enteric plexuses (Jacobowitz, 1965). We therefore decided to investigate the effect of exogenous NA on the synaptic activity recorded from IMG neurones in continuity with colon.

Addition of NA to the colon compartment. NA (10^{-7}–10^{-6} g/ml.) reduced and in some cases completely abolished the synaptic input to the IMG. In a typical experiment with NA (10^{-7} g/ml.), the frequency of synaptic and action potentials was reduced; after most of the synaptic potentials had disappeared it was possible to identify individual synaptic potentials occurring at rather regular intervals of 1–2 sec (Fig. 13). Finally, in many cells, all synaptic activity was abolished.

The effect of NA (10^{-9}–10^{-6} g/ml.) on the synaptic activity recorded from one cell is shown in Fig. 14. The height of each bar represents the total number of synaptic potentials in three consecutive 10-sec intervals. The open bars represent the last three 10-sec intervals before infusion of NA and the hatched bars represent the last three 10-sec intervals before ending the infusion. Identical volumes and rates of infusion were used for each concentration. Between experiments the NA was washed out and 30–45 min elapsed before giving the next dose. The percentage reduction

during the last 30 sec of infusion of NA, 10^{-9}, 10^{-8}, 10^{-7} and 10^{6} g/ml., was 29, 26, 43 and 99% respectively.

Exogenous NA therefore appears to mimic the inhibitory action of repetitive stimulation of the neurones of the IMG on the synaptic activity generated in the colon. Furthermore, both types of inhibition were blocked

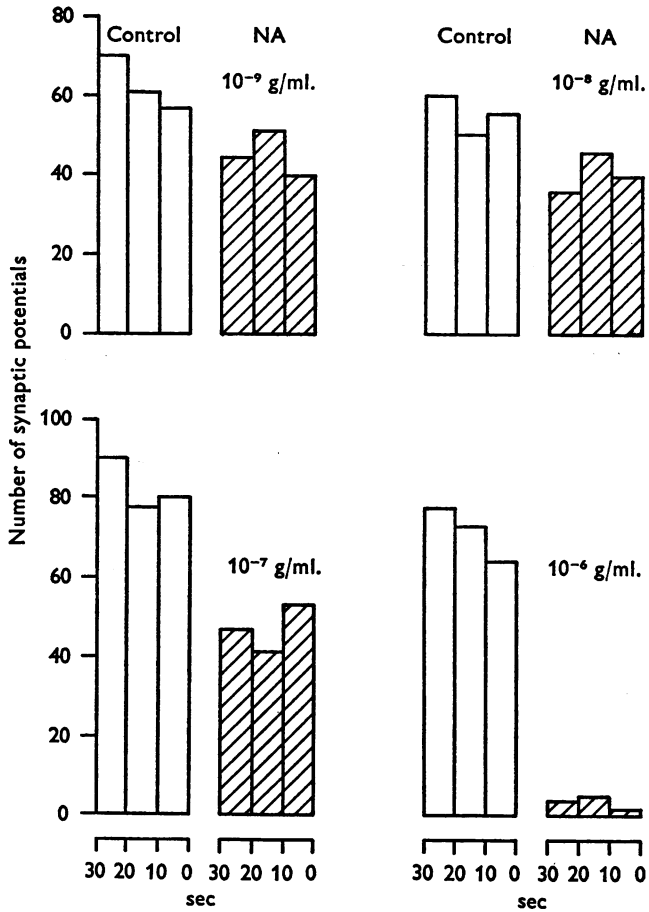

Fig. 14. Number of synaptic potentials (with and without action potentials) for the last three 10-sec intervals before (open columns) and for the last three 10-sec intervals during (hatched columns) injection of four different concentrations of noradrenaline (NA) to the colon side of the bath. 30–45 min elapsed between injections of noradrenaline.

by the α-receptor blocking drugs phentolamine (10^{-6} g/ml.) and phenoxybenzamine (1×10^{-6} g/ml.). An example of the effect of phentolamine is shown in Fig. 15. Inhibition of synaptic activity in response to stimulation of the ascending mesenteric nerve is shown in Fig. 15*A*. In Fig. 15*B*,

recorded in the presence of phentolamine (10^{-6} g/ml.) added to the bath containing the colon, this inhibition was almost abolished. The inhibitory effect of NA (10^{-5} g/ml.) was also blocked (Fig. 15*C*).

Addition of NA to the ganglion compartment. Although NA was most effective in abolishing the input when it was applied to the colon, only a

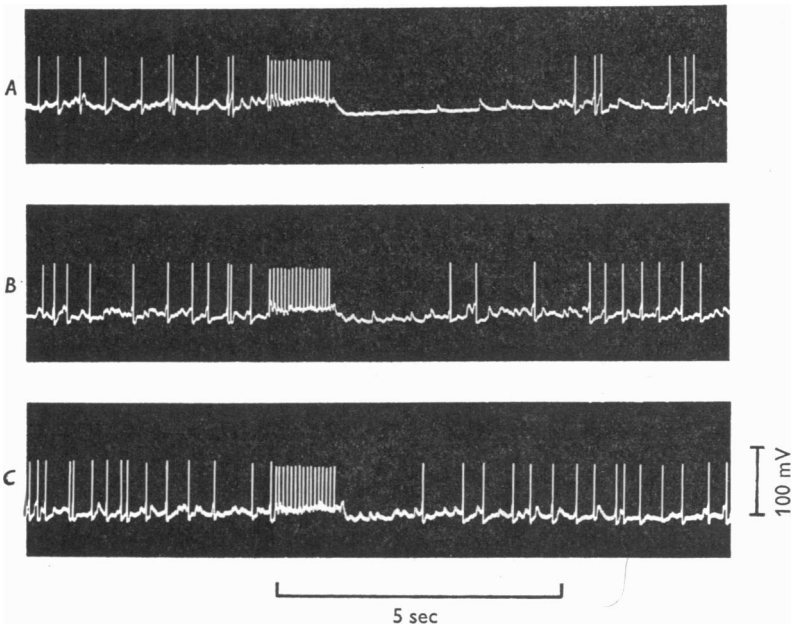

Fig. 15. Effect of phentolamine (1×10^{-6} g/ml.) on transient inhibition of input following repetitive stimulation of an inferior splanchnic nerve (15/sec for 1 sec) and on inhibitory action of noradrenaline (1×10^{-5} g/ml.). *A*, control; *B*, during fifth minute of perfusion of colon side of bath with Krebs solution containing phentolamine; *C*, during tenth minute of perfusion of phentolamine and fifth minute of injection of noradrenaline to colon side of bath. Records taken from same cell.

slight inhibitory effect was observed when NA was added to the bath containing the IMG. The amplitude of the synaptic potentials was reduced but never abolished by NA in concentration up to 10^{-4} g/ml.

DISCUSSION

The main findings of this study were: (1) neurones of the IMG receive excitatory synaptic input from axons coming from the colon and (2), noradrenergic neurones in the IMG which project to the colon can depress

this activity. It therefore seems that the IMG is involved in a peripheral reflex which may act to depress the activity of the colon.

The characteristics of the synaptic potentials due to the excitation of fibres of colonic origin were indistinguishable from those evoked by stimulation of any other presynaptic fibres to the IMG. Since all synaptic activity was blocked when the ganglion was exposed to DHβE we conclude that the colonic fibres must be cholinergic.

The addition of DHβE to the solution bathing the colon markedly depressed the input activity in nerve fibres projecting to the IMG. This suggests that many of these fibres must have come from neurones within the wall of the colon which are themselves activated by cholinergic synapses. Such synapses are known to occur within the enteric plexuses of the intestine. This would be in accordance with the hypothesis that many of the axons which project to the IMG arise from colonic neurones which are not 'spontaneously' active but are 'driven' by other enteric neurones. Using DHβE we have not been able to completely block the input activity coming from the colon. This may have been due to the limitations of the access of the drug to the plexuses. However, we cannot rule out the alternative explanations that (*a*) 'spontaneously' active enteric neurones exist which are cholinergic and project directly to the IMG, (*b*) enteric neurones which project to the IMG are 'driven' by non-cholinergic synapses from other enteric neurones, or (*c*) that some of these nerve fibres may be collaterals of primary afferent fibres synapsing in the IMG on their way to the central nervous system. This latter hypothesis implies that such fibres are cholinergic.

There have been a number of previous studies which suggest that enteric neurones may give rise to axons which synapse in the IMG (Garry, 1933; Lawson, 1934; Lawson & Holt, 1937; Kuntz, 1940; Kuntz & Saccomanno, 1944; Job & Lundberg, 1952; McLennan & Pascoe, 1954). Our electrophysiological observations at the single unit level strongly support this hypothesis.

Excitation of the IMG neurones by repetitive stimulation of any of the nerve trunks led to a transient hyperpolarization and inhibition of synaptic input from the colon. The inhibition was not dependent on the hyperpolarization because a hyperpolarizing pulse applied across the cell membrane increased the amplitude of the synaptic potentials. Thus, the inhibition occurred in the colon, probably due to the release of NA from the axon terminals of the IMG cells, and not within the IMG.

Inhibition of colonic input in response to repetitive stimulation of preganglionic fibres to the IMG was followed by a prolonged period (up to 2 min) during which transmission through the ganglion was facilitated. During this time there was no change in the frequency of the synaptic input from the colon but the amplitude of

synaptic potentials was increased. Synaptic potentials which, during the control period, were too small to initiate action potentials were now able to do so. A similar effect on evoked synaptic potentials and on brief depolarizations in response to direct stimulation was shown (Crowcroft & Szurszewski, 1971) and there it was concluded that this facilitation was due to a prolonged change in the properties of IMG neurones.

Fig. 16 summarizes our present hypothesis concerning the neural connexions between the IMG and the distal colon. We suggest that the noradrenergic neurones of the IMG function as a group of inhibitory neurones which depress the activity of the excitatory neurones of the colon which

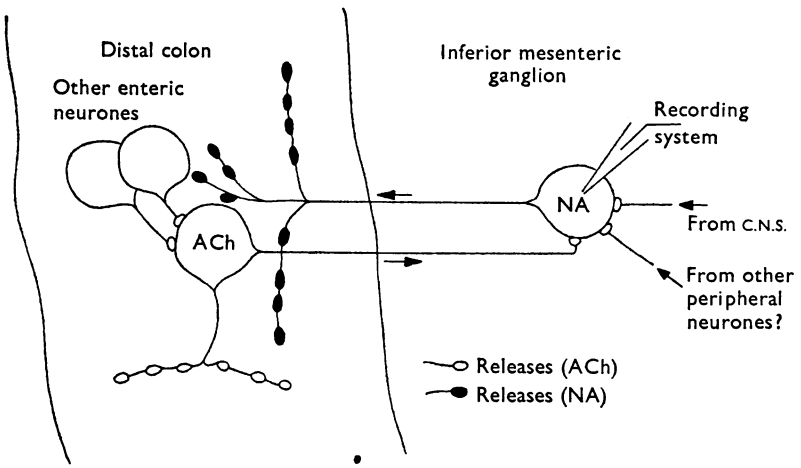

Fig. 16. Diagram of possible neural connexions between the IMG and the intrinsic plexus in the distal colon. For further description see text.

are driving them. Such a feed-back loop would be comparable in its action with the process of recurrent inhibition which has been observed in many parts of the central nervous system. To check this hypothesis, it would be a great advantage to be able to 'open the loop'. The results obtained so far, however, do enable us to draw some conclusions about the way in which this reflex might operate.

Our experiments indicate that many of the colonic neurones involved in this reflex were not spontaneously active but were driven, either directly or indirectly, by cholinergic synapses. Their activity might be altered by other enteric neurones such as those which responded to distension and 5-HT. It is possible that they may be the cholinergic motor neurones which supply the smooth muscle of the colon. In the diagram of Fig. 16, we have indicated that the NA released from axon terminals of neurones of the IMG inhibits the discharge from the colon by depressing transmission between enteric neurones. It was shown that the application of NA to the colon depressed

the colonic discharge. This action of NA and the action of NA released from colonic nerves was blocked by phentolamine and phenoxybenzamine, suggesting that the inhibitory action of NA was mediated through α-receptors. There is circumstantial evidence linking those inhibitory actions of NA on the gastrointestinal tract which are mediated by α-receptors, with a direct action of NA on transmission between enteric neurones. For example, Paton & Vizi (1969) and Kosterlitz, Lydon & Watt (1970) have shown that catecholamines depress the output of acetylcholine (ACh) from the small intestine and that this action is mediated by α-receptors. We would not rule out, however, the possibility that inhibition of the colonic discharge may be, in part, a secondary effect due to the direct inhibition of colonic motility.

In addition to their excitation by presynaptic fibres of colonic origin, the neurones of the IMG can also be excited by preganglionic fibres from the central nervous system. We have also indicated in the diagram of Fig. 16 that they may receive input from peripheral cholinergic neurones other than those within the wall of the colon. This possibility follows from the work of McLennan & Pascoe (1954) who found evidence in the rabbit that IMG cells could be excited by fibres which did not appear to be of central origin.

This concept of peripheral reflex is a departure from the generally accepted pattern of the organization of the autonomic nervous system. It remains to be determined whether or not peripheral cholinergic neurones other than those of the distal colon may also utilize the noradrenergic ganglion cells of the 'sympathetic' division of the autonomic nervous system in similar inhibitory feed-back loops.

It would be premature to attempt to assess the significance of the colon-IMG reflex in relation to the motility of the distal colon. The predominant effects of excitation of the lumbar colonic nerves are inhibition of motility and a decrease in responsiveness of the colon to excitation by both extrinsic and intrinsic nerves (see Hülten, 1969). In our experiments there was no obvious correlation between the pattern of the colonic input and the motility of the colon as observed under the dissection microscope. But this question needs further investigation with adequate means for the measurement of motility.

One useful result that has come from this study is the ability to monitor the activity of at least one kind of enteric neurone, by intracellular recording from the IMG. Thus these ganglion cells provide a 'window' into the enteric plexus of the distal colon under conditions in which the function of its musculature is undisturbed.

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