Effects of drugs and of electrical stimulation on the muscularis mucosae of rabbit large intestine

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Summary

1. The muscularis mucosae of the rabbit large intestine shows spontaneous rhythmic contractions with an average frequency of about 11/minute.

2. The muscularis mucosae responds to acetylcholine, adrenaline and nor-adrenaline in a manner similar to that of whole segments of terminal large intestine.

3. The muscularis mucosae would seem to have a cholinergic motor innervation.

Introduction

The muscularis mucosae may be considered the ' Cinderella ' of the muscle layers of the gut since it has received much less attention than the muscularis externa. Hughes (1955) reviewed the relevant literature. Recently Hooper & Schneider (1970) have examined the effect of various drugs on the movement of villi in pigeon intestine; villous muscle orginates in the muscularis mucosae. Onori, Friedmann, Frigo & Tonini (1971) studied the effect of drugs on the muscularis mucosae of cat colon. We have now examined the drug responses of the muscularis mucosae of the rabbit distal colon and rectum. These regions of rabbit bowel have been used for *in vitro* preparations (see Mackenna & McKirdy, 1972) where the possible contribution of the muscularis mucosae has been largely disregarded.

Methods

Thirty-four Dutch rabbits of either sex, weighing 1–2 kg, were used. They were killed by stunning and bleeding or were anaesthetized by intravenous injection of pentobarbitone sodium and exsanguinated. Segments of distal colon and rectum were opened along the ventral aspect and the mucosa with its muscularis mucosae was removed. Strips of muscle, with overlying glandularis mucosae, 1–2 cm long and 0·25–0·5 cm broad, were excised with a scalpel in the direction of either longitudinal muscle or circular muscle. In some experiments the mucosa with its muscularis was maintained as an intact tube by dissecting off the muscularis externa.

The muscle strip was suspended in an isolated organ bath and was connected to a Grass FT 03C tension transducer, the output from which was displayed on a Grass Model 7 Polygraph. In some experiments, auxotonic recordings were made by inserting a fine calibrated spring between the tension transducer and muscle strip. Isometric and auxotonic recordings were made from the Magnus preparation of intact tube of mucosa.

In some experiments muscle strips were pulled into an electrode of the type described by Burn & Rand (1960) and were stimulated electrically with rectangular pulses from an American Electronic Laboratories stimulator. The pulse duration was 0·1 ms and the strength supramaximal.

Frequencies of 1–100 Hz were employed, either for fixed periods of 15 and 30 s or for variable periods to ensure equal numbers of pulses at the various frequencies. Intact tubes of mucosa were stimulated coaxially by the method of Paton (1955).

Preparations were bathed in Krebs solution of the following composition (mM): NaCl 119 ; KCl 4·7 ; $CaCl_2$ 2·5 ; KH_2PO_4 1·2 ; $MgSO_4$ 1·2 ; $NaHCO_3$ 25 ; glucose 11·7. The solution was maintained at 38–39° C and a gas mixture of 95% O_2 and 5% CO_2 was delivered through a sintered glass bubbler.

The drugs used were pentobarbitone sodium (Abbott), acetylcholine chloride (Roche), atropine sulphate (Macfarlan Smith), physostigmine sulphate (Macfarlan Smith), (—)-adrenaline bitartrate (Sigma), (—)-noradrenaline bitartrate (Koch-Light), (±)-isoprenaline (Aldrich), histamine acid phosphate (B.D.H.), 5-hydroxytryptamine creatinine sulphate (Koch-Light) and tetrodotoxin (Sankyo). Concentrations of adrenaline, noradrenaline and isoprenaline are expressed in μg/ml of the active base and concentrations of all other drugs in μg/ml of the salt.

Transverse and longitudinal sections and spread preparations of mucosa and of whole bowel were stained with haematoxylin and eosin or methylene blue and examined histologically.

Results

Histology

The muscularis mucosae is composed of two layers, an inner circular and outer longitudinal, both of which are arranged in ill-defined bundles. Strips of muscle from the inner circular layer running into the lamina propria were occasionally observed. Nerve fibres were seen in the muscularis mucosae. Ganglia of the submucous plexus were occasionally seen in spread preparations from which the muscularis externa had been removed ; in such preparations no trace of muscularis externa could be detected on microscopic examination.

Spontaneous activity

The muscularis mucosae exhibited spontaneous rhythmic activity (Figs. 1 and 2). This activity varied from preparation to preparation and also with time in any one preparation. The frequency of spontaneous contractions varied between 4 and 18 contractions/min with an average of about 11/minute.

Effects of drugs

Acetylcholine (0·1–10 μg/ml) or histamine (1–10 μg/ml) caused contraction of the longitudinal and circular muscle strips, with a variable effect on the frequency and amplitude of rhythmic contractions (Fig. 1A and B). Atropine 0·1 μg/ml blocked the effect of acetylcholine 10 μg/ml. Physostigmine 10 μg/ml also caused contraction.
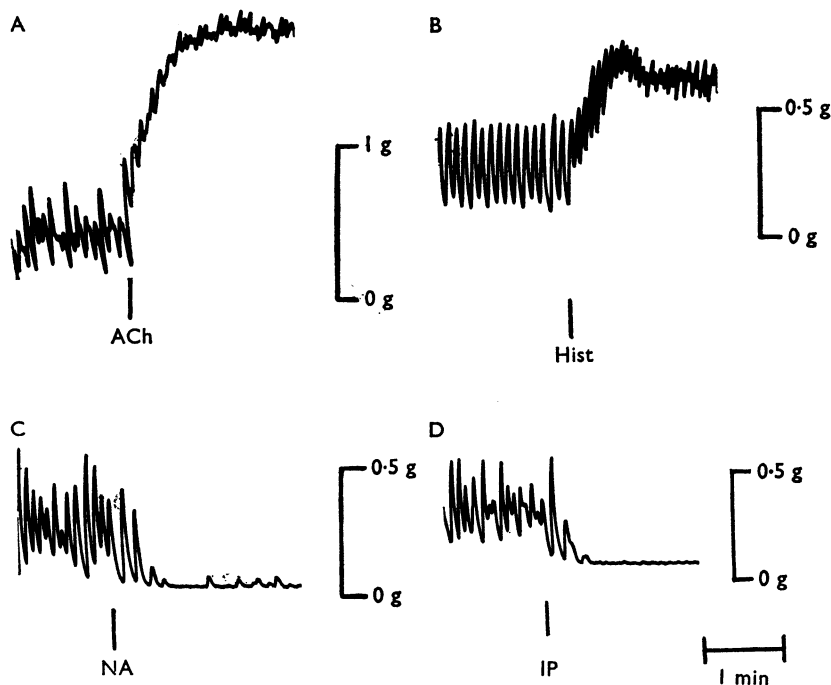
FIG. 1. The effect of (A) acetylcholine (ACh, 10 μg/ml), (B) histamine (Hist, 1 μg/ml), (C) noradrenaline (NA, 1 μg/ml) and (D) isoprenaline (IP, 1 μg/ml) on the muscularis mucosae of the rabbit large intestine. (A), (B), (C) and (D) are from different preparations. All strips were cut in direction of longitudinal muscle and recorded isometrically.

Adrenaline, noradrenaline or isoprenaline (Fig. 1C and D) (0·1–10 μg/ml) always produced relaxation with decrease in frequency and amplitude of spontaneous contractions.

5-Hydroxytryptamine (1–10 μg/ml) caused contraction in thirteen out of eighteen experiments ; the response diminished on repeated exposure.

Tetrodotoxin (0·1 μg/ml) produced small changes in amplitude and frequency of spontaneous activity. The responses to acetylcholine, histamine and catechol-amines were qualitatively unaltered. This concentration of tetrodotoxin is more than sufficient to block the response to stimulation of extrinsic nerves of the colon (Mackenna & McKirdy, 1972).

Effects of electrical stimulation

Only about one-half of preparations responded to electrical stimulation, although histological examination revealed that nervous structures were present in some of those preparations which did not respond. The response was apparent at 10 Hz and increased when the frequency was increased up to 25–100 Hz but higher frequencies were not more effective. The response was usually biphasic in character with excitation followed by inhibition (Fig. 2) although pure excitation or inhibition was occasionally seen. The excitatory response was reduced by atropine (0·1 μg/ml) and the whole response abolished by tetrodotoxin (0·01 μg/ml).
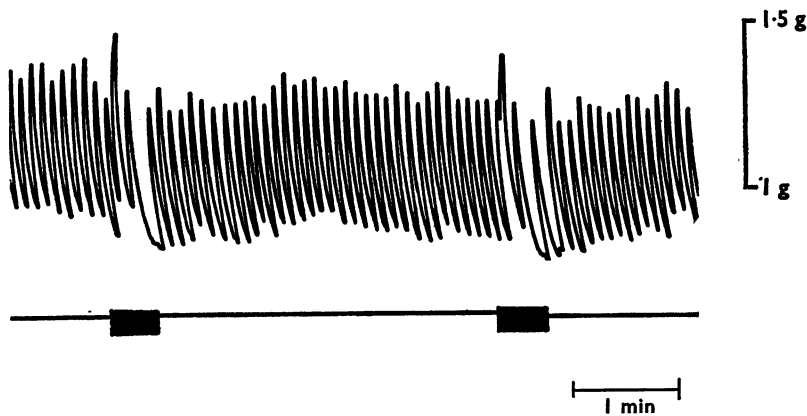
FIG. 2. The response of the muscularis mucosae of the rabbit large intestine to electrical stimulation (Burn & Rand, 1960), with pulses of 0·1 ms duration at 100 Hz and supramaximal voltage. The period of stimulation is shown by the black bar. Slight excitation is followed by slight inhibition. Strip cut in direction of longitudinal muscle. Isometric recording.

Discussion

The function of the muscularis mucosae is obscure at present. It may play a part in absorption by changing the surface contact between the epithelial layer and intraluminal contents and by promoting propulsion in mucosal veins and lymph vessels by means of its rhythmic contractions, or it may be involved in secretion by causing ejection of material from cells and glands when it contracts. It is possible that the smooth muscle layers may have a sensory as well as a motor function and may in some way be involved in detecting distension of the viscus. Finally, it may act as a barrier to the diffusion of bio-active substances (McKirdy, Jones & Ballard, 1972).

The control of the muscularis mucosae is similarly obscure. Acetylcholine and histamine produce a contraction of the muscularis mucosae which is independent of nerve impulses since the response is still observed in the presence of tetrodotoxin. We cannot exclude, however, the possibility that these substances act by releasing other substances from the glandularis mucosae or from nerve endings. Excitation in the presence of physostigmine suggests that a choline ester is released in the mucosa, but we have no information on the source, whether neural or non-neural, of this choline ester.

Isoprenaline, adrenaline and noradrenaline relax the muscularis mucosae independently of nerve impulses. The muscularis mucosae of the rabbit large bowel thus responds to acetylcholine, adrenaline and noradrenaline in a manner similar to that of whole segments of the rabbit large intestine in the presence of tetrodotoxin (Mackenna & McKirdy, 1972). Some of the above findings are quite different from those of Onori et al. (1971) on the muscularis mucosae of cat colon, where adrenaline or noradrenaline causes contraction and acetylcholine relaxation.

The biphasic response to electrical stimulation would seem to be neurally mediated since the response is blocked by tetrodotoxin. The question arises why only about one-half of the preparations responded to electrical stimulation, particularly since nerve fibres were found in some of the preparations which did not respond.

The nerve damage which occurred during separation of the mucosa from the bowel would seem unlikely to be the whole answer. Perhaps a large proportion of the nerve fibres in the muscularis mucosae is sensory in function.

The excitatory component of the response to nerve stimulation would appear to be due to stimulation of cholinergic nerves since this component is reduced by atropine. This finding is in keeping with the observation of McKirdy *et al.* (1972) that nerve fibres which stain intensely for acetylcholinesterase are present in the muscularis mucosae, but is in direct contrast to the finding of Onori *et al.* (1971) that there is no cholinergic innervation of the muscularis mucosae of cat colon. The inhibitory component of the response to nerve stimulation may be due to stimulation of adrenergic nerves. A sparse adrenergic innervation has been detected in the muscularis mucosae of the colon of the guinea-pig (Furness, 1970) and the rabbit (H. C. McKirdy, unpublished observation). Since we have not used adrenergic blocking agents, we have no information on the possibility of innervation by non-adrenergic inhibitory fibres.

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