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The effects of drugs on circular muscle strips from the isolated ileum of the rabbit

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Circular muscle strip preparations were contracted by high concentrations of acetylcholine, methacholine or carbachol, but not by other smooth muscle stimulants. The responses to acetylcholine were antagonized by muscarinic blocking agents but not by ganglion-blocking drugs or local anaesthetics. Anticholinesterases induced violent and prolonged activity which was unaffected by repeated washing, by atropine or by local anaesthetics. The insensitivity to acetylcholine and the anomalous responses to anticholinesterases and to the stimulant drugs are discussed. It is suggested that there is some basic difference between the pharmacological responses of the two layers of the mammalian small intestine.

THERE have been many accounts of the effects of drugs on the longitudinal smooth muscle of the mammalian small intestine (see, for example, Kosterlitz & Lees, 1964). By comparison, the pharmacology of the circular muscle layer of the small intestine has attracted little attention. Isolated preparations from only three species have so far been investigated. These are the cat (Gasser, 1926; Evans & Schild, 1953), the guinea-pig (Harry, 1963; Brownlee & Harry, 1963) and man (Fishlock & Parks, 1963; Fishlock, 1964). In addition, there have been some experiments on circular muscle strips from the large intestine. In the present work, the pharmacological investigation of the intestinal circular muscle has been extended to a fourth species, the rabbit.

Experimental

METHODS

Rabbits of either sex weighing between 1·25 and 3·0 kg were killed by a blow on the neck and bled. The abdomen was opened and a marking thread was sewn into the wall of the small bowel close to the ileo-caecal junction. The gut was transected distal to this thread and then freed from its mesenteric attachments. Approximately one third of the distal small bowel was so mobilized, removed from the animal and placed in Krebs solution chilled to 10°.

Thirty min later, when the gut was fully relaxed, it was measured and any length in excess of 95 cm from the ileo-caecal junction was discarded. Circular muscle strips were made from the proximal 10 cm of the remaining length of small gut, i.e., from 85–95 cm proximal to the ileo-caecal junction.

A small segment 2·5 cm long was cut from the chosen portion of the small bowel (Fig. 1a), freed from mesenteric debris and opened by a cut along the mesenteric border (Fig. 1b). The resulting sheet of intestinal wall was pinned out, lightly stretched, under chilled Krebs solution with the mucosal surface uppermost. Threads were tied into the margins of the sheet of intestinal wall, opposite one another and in the line of the circular muscle fibres (Fig. 1c). A strip 4 mm wide was cut between the

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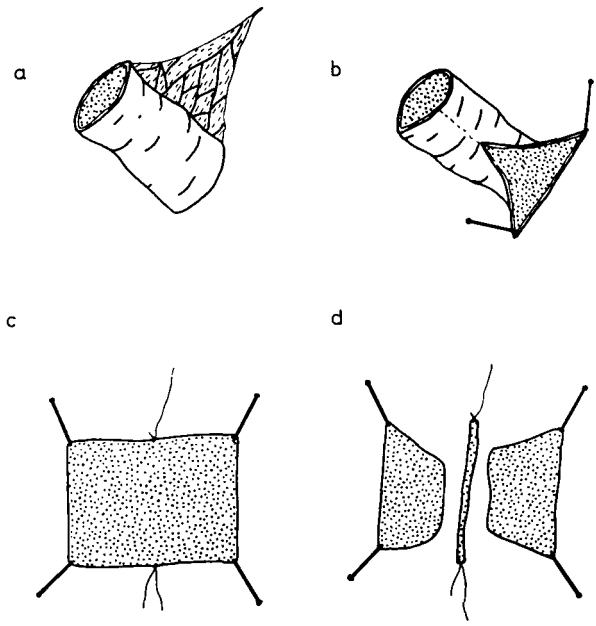

FIG. 1. The preparation of a circular muscle strip. (a) A segment 2·5 cm long is removed from the ileum about 90 cm proximal to the ileo-caecal junction (see text). (b) Cleaned of mesenteric attachments, the segment is opened along the mesenteric border. (c) The sheet of intestinal wall is pinned out, mucosa uppermost, under chilled Krebs solution. Threads are tied into the margins of the sheet in the direction of the circular muscle fibres. (d) The strip, 4 mm wide, is cut between the threads.

threads (Fig. 1d) using two parallel scalpel blades attached to a handle. Usually two such strips were cut from each 2·5 cm segment of ileum.

Longitudinal muscle strips were prepared in a similar manner except that the threads were tied and the cuts were made at right-angles to the line of the circular muscle fibres.

Each circular muscle strip prepared by the method described was anchored at one end to a glass tissue holder and mounted vertically in 15 ml of Krebs solution maintained at 37° (±0·5°) and gassed with 5% carbon dioxide in oxygen. The upper end of the strip was attached to a light, isotonic, balsa-wood lever fitted with a glass side-writing point (Foster, 1963) recording on a lightly smoked drum. The movements of the tissue were magnified 10 times and the load on the tissue was 300 mg. All preparations were left for 1 hr before the experiments were begun.

Immediately before the addition of a drug to the bath fluid, 1 ml of bath fluid was withdrawn. Each dose of a drug was added to the bath fluid in this volume (1 ml) of Krebs solution. A drug was left in contact with the preparation for 60 sec in a 10 min cycle. The bath fluid was changed at least three times at ½ min intervals after each dose of a drug. A resting period of 45–60 min was required between each series of six 10 min cycles if the preparation was to survive.

When the dose response relations were being investigated the first dose of each series was repeated because the sensitivity of the preparations increased transiently after each rest period. Successive doses were quadrupled to reduce the number of cycles required to cover a wide range of drug concentrations.

Antagonist drugs were made up to the required concentration in the Krebs solution used to replace the bath fluid. All antagonists were in contact with the tissue for the duration of a rest period (i.e., 45–60 min) before responses to the agonist were again tested.

Each experiment was made with at least four preparations.

DRUGS AND SOLUTIONS

Drugs used were: acetylcholine chloride, acetyl-β-methylcholine chloride, carbachol, atropine sulphate, hyoscine hydrobromide, hexamethonium bromide, dimethylphenylpiperazinium iodide, cocaine hydrochloride, procaine hydrochloride, neostigmine methylsulphate, eserine sulphate, di-isopropyl-phosphodiamidic fluoride (Mipafox), histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, nicotine acid tartrate, crude substance P, potassium chloride, barium chloride, angiotensin II and sucrose. All concentrations are in mg/ml or μg/ml of base.

The Krebs solution had the following composition (in g/litre of distilled water): NaCl 6·92; KCl 0·354; $CaCl_2$ 0·282; $NaHCO_3$ 2·10; NaH_2PO_4 0·162; $MgSO_4.7H_2O$ 0·294; and glucose 2·00.

Results

Circular muscle strips from the rabbit ileum were never spontaneously active. Most preparations survived for about 5 hr after the start of the experiment. This was sufficient to allow three dose response series, each consisting of six cycles, to be completed.

ESTERS OF CHOLINE

Typical responses of a circular muscle strip to increasing concentrations of acetylcholine, methacholine and carbachol are shown in Fig. 2. The responses to acetylcholine or to methacholine increased with increasing concentrations of the agonist over a wide range (50 μg/ml–12·8 mg/ml for acetylcholine; 2·5–640 μg/ml for methacholine). Most preparations were more sensitive to carbachol than to the other two choline esters. Usually two phases were seen; the first an increase in response with increasing concentrations followed by the second, a subsequent decrease with increasing concentrations. In some preparations, the response to the second and subsequent concentrations of carbachol was delayed for a variable latent period. Because of these inconsistencies, the responses of circular muscle strips to carbachol were not further investigated.

ATROPINE AND HYOSCINE

Fig. 3 shows the results of four experiments in which circular muscle strips were treated with atropine (0·01 μg/ml) before the repetition of the

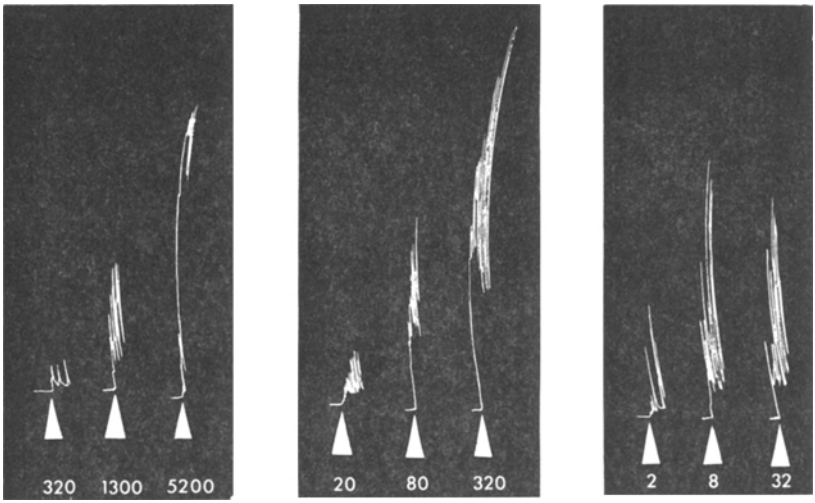

FIG. 2. The responses of a circular muscle strip from the rabbit ileum to acetylcholine, methacholine and carbachol. The first panel shows the responses to increasing concentrations of acetylcholine and the second and third panel to methacholine and carbachol. All concentrations are in μg/ml. Methaboline was 20 times more active than acetylcholine, but 10 times less active than carbachol, The responses to carbachol did not increase with increasing concentrations of the agonist.

dose response curve to methacholine. Atropine (0·01 μg/ml) or hyoscine (0·005 μg/ml) always depressed the responses of preparations stimulated by either acetylcholine or methacholine.

HEXAMETHONIUM

Four preparations were treated with the competitive ganglion-blocking agent hexamethonium (100 μg/ml) after a control dose response curve to acetylcholine had been completed. The responses of two preparations to acetylcholine were unaffected by hexamethonium, and two additional preparations showed a slight potentiation of the responses to the lower concentrations of acetylcholine.

Three out of four preparations stimulated by methacholine were unaffected by hexamethonium (100 μg/ml) and one preparation showed potentiation of the responses to the lower concentrations of methacholine.

DIMETHYLPHENYLPIPERAZINIUM

The compound, 1,1-dimethyl-4-phenylpiperazinium (DMPP) has been shown to cause ganglion blockade by depolarization (Leach, 1957; Ling, 1959). Brownlee & Johnson (1963) presented evidence that depolarizing blockade by DMPP differed from that produced by nicotine in not reverting to a competitive type of blockade after a short time. The dose response curves to acetylcholine and methacholine of circular muscle strips from the rabbit ileum were unaffected by the presence of DMPP (100 μg/ml).

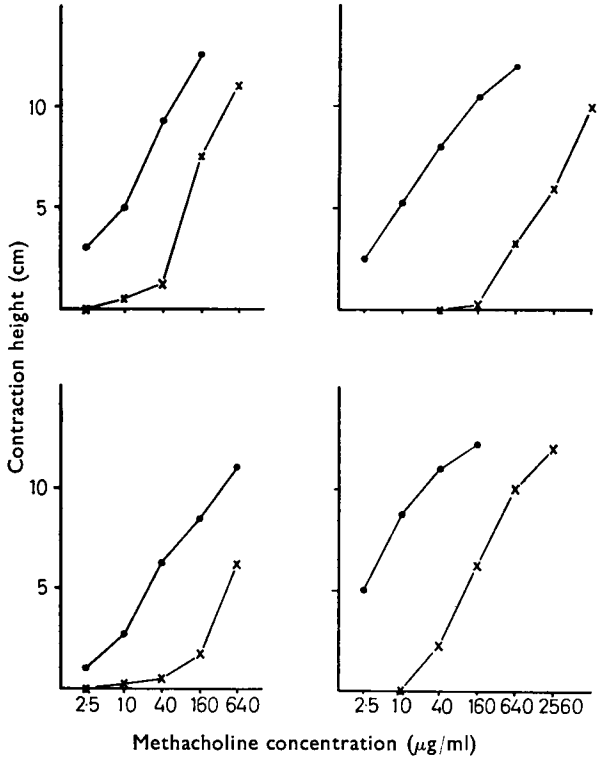

FIG. 3. The depressant effect of atropine on the responses to methacholine. The dose response curves from four circular muscle strip preparations are shown. The contractions of each preparation (cm) are plotted against the concentration of methacholine (μg/ml). The closed circles represent the control responses; the crosses represent the responses obtained 1 hr later in the presence of atropine (0·01 μg/ml). Atropine antagonized the responses to methacholine, moving the dose response curve to the right.

COCAINE AND PROCAINE

The responses of two acetylcholine-treated circular muscle strips were unchanged in the presence of cocaine (50 μg/ml). Two other preparations showed a slight potentiation of the responses to acetylcholine after cocaine.

Three preparations stimulated by methacholine showed potentiation of the responses after cocaine (50 μg/ml) and another was unaffected. A further methacholine-treated preparation went into spasm on the addition of cocaine (50 μg/ml) to the bath fluid and this experiment was abandoned.

Procaine (100 μg/ml) failed to modify the responses of circular muscle strips stimulated by acetylcholine or methacholine.

ANTICHOLINESTERASES

Neostigmine (1·0–2·6 μg/ml) always stimulated circular muscle strips from the rabbit ileum. Three types of activity were recorded. Firstly,

large, irregular contractions which continued for 60–90 min and which were followed by spasmodic activity for a further 60–90 min. During this latter period, bursts of activity were triggered by any interference with the preparation (e.g., changing the bath fluid). Following the period of intermittent activity, the preparation became quiescent and unresponsive to electrical, mechanical or pharmacological stimuli. Secondly, a slow, well-maintained contracture which persisted for several hours, and thirdly, occasional preparations contracted intensely and then relaxed slowly. Such contractions occurred singly, or in groups of two or three, at irregular intervals for many hours. Eserine (1·0–5·0 μg/ml) produced similar effects on the preparations, as did the organophosphorus anti-cholinesterase di-isopropylphosphodiamidic fluoride (Mipafox, 50 μg/ml).

The activity produced by these anticholinesterases persisted in spite of repeated washings. In other experiments, the anticholinesterase agent was added to the bath fluid together with atropine (0·01 μg/ml), cocaine (50 μg/ml) or procaine (100 μg/ml). None of these drugs prevented the anticholinesterase-induced activity.

OTHER SMOOTH MUSCLE STIMULANTS

Circular muscle strips from the rabbit ileum failed to respond to histamine, 5-hydroxytryptamine (5-HT), nicotine, DMPP, substance P, barium ions or potassium ions (Fig. 4). Each of these substances was added to the bath fluid in a concentration of 1 mg/ml, the metallic ions

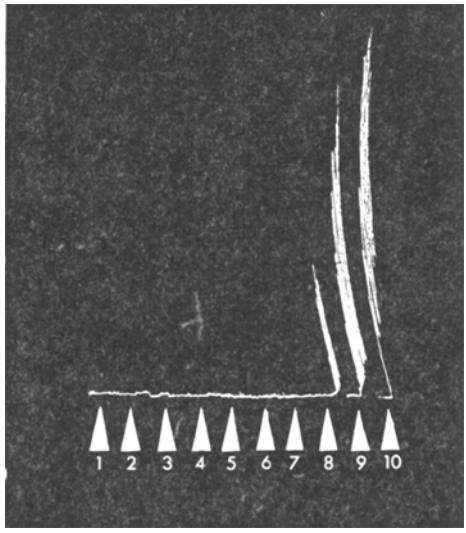

FIG. 4. Spasmogenic drugs on the circular muscle strip preparation. The record shows the effect of 1, 5-hydroxytryptamine, 2, barium chloride, 3, crude substance P, 4, nicotine, 5, histamine, 6, potassium chloride and 7, dimethylphenylpiperazinium. Each of these substances was applied to the preparation in a concentration of 1 mg/ml. The preparation did not respond to any of these drugs but graded responses were obtained to methacholine 8, 10 μg/ml, 9, 40 μg/ml and 10, 160 μg/ml.

being added as the chloride. The four circular muscle strips used for these experiments responded normally to methacholine (see Fig. 4). No

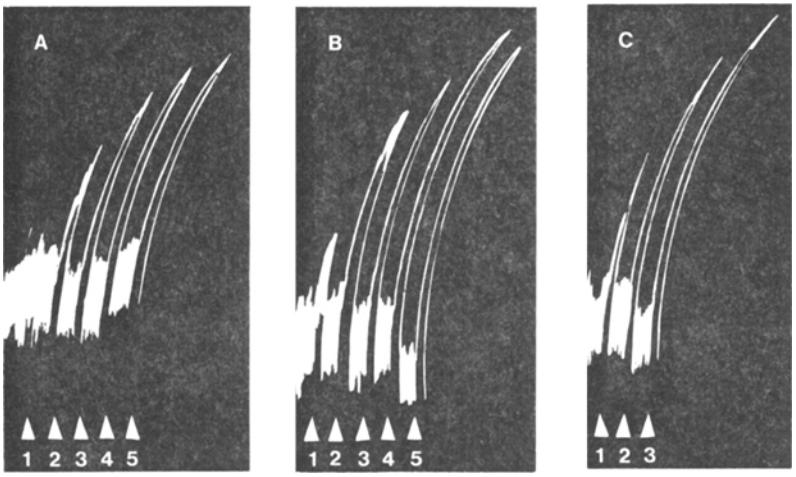

FIG. 5. The effect of acetylcholine, methacholine and carbachol on a longitudinal muscle strip from the rabbit ileum. The first panel shows the responses of the preparation to increasing concentrations of acetylcholine. The other two panels show, respectively, the responses to methacholine and carbachol. All concentrations are in μg/ml. The longitudinal muscle strip is spontaneously active and very sensitive to the stimulant drugs (compare with Fig. 2). The responses to all three agonists increase with increasing concentrations. A. Acetylcholine; 1, 0·015; 2, 0·06; 3, 0·25; 4, 1·0; 5, 4·0. B. Methacholine; 1, 0·015; 2, 0·06; 3, 0·25; 4, 1·0; 5, 4·0. C. Carbachol; 1, 0·004; 2, 0·016; 3, 0·064.

response was obtained from two preparations treated with angiotensin II (50 μg/ml).

SUCROSE

Sucrose in a concentration (330 mg/ml) osmotically equivalent to the highest concentration of acetylcholine used in these experiments produced only negligible activity compared with the large and rapid response to acetylcholine.

LONGITUDINAL MUSCLE STRIPS

Unlike circular muscle strips, preparations cut in the direction of the longitudinal muscle coat were spontaneously active. Further, a high level of inherent tone was demonstrable by the use of relaxant drugs. Longitudinal muscle strips responded to choline esters, histamine, nicotine or substance P in concentrations similar to those required on the Magnus preparation of the rabbit ileum. The responses of one longitudinal muscle strip to acetylcholine, methacholine and carbachol are shown in Fig. 5.

Discussion

Circular muscle strips from the rabbit ileum responded with concentration-dependent contractures when treated with acetylcholine or methacholine. Preparations were more sensitive to carbachol than to the other two esters of choline, but the responses to carbachol showed a mixed action and a variable latency. This pattern of responses to drugs differs from that previously reported (Tweeddale, 1963), the difference being attributable to the use of different recording systems on the two occasions. The reasons for these differences are fully discussed elsewhere (Tweeddale, 1965).

The circular muscle strip of the rabbit ileum is remarkable for its insensitivity to drugs. Very high concentrations of acetylcholine (up to 12·8 mg/ml) were needed to produce adequate contractions, and concentrations in excess of this did not produce a maximal response. The need for such high concentrations of acetylcholine leads one first to consider indirect mechanisms. The responses to acetylcholine or to methacholine were not reduced by the competitive ganglion-blocking agent hexamethonium, nor by its depolarizing counterpart, dimethylphenylpiperazinium. The possibility of a site of action of acetylcholine distal to the ganglion-cell body was excluded by the failure of local anaesthetic agents to reduce the responses. In fact, both hexamethonium and cocaine increased some of the responses to the choline esters. For hexamethonium this observation is attributed to the weak anticholinesterase activity of this drug in the concentration used of 100 μg/ml of base (Paton & Zaimis, 1949) and for cocaine to a stimulant action of the type reported by Feldberg & Lin (1949a). Because drugs which abolish neural activity failed to modify the responses to acetylcholine, or to methacholine, the responses to these agonists must occur wholly by activation of receptors on the smooth muscle cells.

The muscarinic blocking agents atropine and hyoscine in low concentrations antagonized the actions of acetylcholine or methacholine. This antagonism seemed to be competitive since the dose response curves in the presence of the antagonist were parallel to and to the right of the control curves. The effects of acetylcholine and methacholine on the circular muscle of the rabbit ileum must therefore be attributed to the activation of muscarinic receptors.

It remains to consider why such high concentrations of choline esters were required to activate the circular muscle of the rabbit ileum. The muscarinic receptors of the circular muscle were 10,000 times less sensitive to acetylcholine than those of the longitudinal muscle. A number of possibilities for this insensitivity must be considered. Firstly, that the trauma of preparation adversely affected the responses of the circular muscle strips. However, longitudinal muscle strips were subjected to similar procedures and yet retained a high sensitivity to acetylcholine. Secondly, a non-specific barrier to the diffusion of drugs might diminish the responses of the circular muscle to drugs. This is unlikely, since atropine and hyoscine were active in their usual low concentrations.

Finally, it is possible that the apparent insensitivity of rabbit intestinal circular muscle to acetylcholine is due to the receptors being protected by a high concentration of cholinesterase. The experiments with the anticholinesterase agents, which might have clarified this issue, did not allow assessment of the effect of acetylcholine in the presence of cholinesterase inhibition.

Considering now the effects of anticholinesterases on intestinal circular muscle, Gasser (1926) reported that eserine was not only unreliable in potentiating responses to acetylcholine, but was itself capable of stimulating circular muscle strips from the ileum of the cat which were unresponsive to acetylcholine. This latter effect of eserine was blocked by atropine. Evans & Schild (1953) also using cat intestine, but with lower concentrations of eserine than those used by Gasser (1926), found no evidence of stimulation and always observed potentiation of the responses to acetylcholine. The effect of eserine and neostigmine on circular muscle strips from the guinea-pig ileum has not been reported, but Harry (1963) observed a slowly developing irregular activity of his preparations after incubation with Mipafox. This effect was probably due to the accumulation of acetylcholine (cf. Johnson, 1963), as it was not a prominent feature of any other of his experiments. Feldberg & Lin (1949b) reported that eserine caused uncoordinated, intermittent spasms of the circular muscle of the rabbit ileum Trendelenburg preparation. These workers also reported a large output of acetylcholine from the eserinized rabbit ileum which they concluded came from non-nervous sources in the gut wall.

In the present experiments, eserine, neostigmine and even Mipafox induced vigorous activity of rabbit intestinal circular muscle strips and it was not possible to assess whether responses to acetylcholine were potentiated. The anticholinesterase-induced activity was not due to the accumulation of acetylcholine from nervous sources within the gut wall (Johnson, 1963), since it was not reduced by repeated washing, by atropine or by local anaesthetic drugs. Even if acetylcholine were arising from non-nervous sites (Feldberg & Lin, 1949b), its effects should be blocked by atropine although Cuthbert (1962) has described an atropine-resistant stimulant action of anticholinesterases upon the chick amnion. This latter effect was limited to the tertiary anticholinesterases whereas neostigmine and Mipafox were as effective as eserine in the present experiments. This is the first time that Mipafox has been reported to show significant spasmogenic activity (Harry, 1962, 1963; Cuthbert, 1962; Brownlee & Johnson, 1963; Carlyle, 1963; Johnson, 1963). It may be that the stimulant effects of the anticholinesterases in the present experiments represent a direct excitatory action independent of both cholinesterase inhibition and of the muscarinic receptor.

In the absence of satisfactory results with the anticholinesterases it is difficult to assess the failure of nicotine, DMPP, histamine and 5-HT to stimulate circular muscle strips from the rabbit ileum. Evans & Schild (1953) found that nicotine readily stimulated whole-wall circular muscle strips from the cat intestine. On the other hand, Harry (1963) and

Brownlee & Harry (1963) showed that responses of guinea-pig circular muscle strips to nicotine, 5-HT or histamine appeared only after cholinesterase inhibition by Mipafox. The failure of potassium and barium to stimulate circular muscle strips from the rabbit ileum in concentrations which were more than adequate to activate the Magnus preparation of the rabbit ileum may be explicable in terms of the membrane potential level of the two types of smooth muscle cell. The longitudinal muscle is spontaneously active and should therefore possess an unstable membrane potential (Burnstock, Holman & Prosser, 1963), which is possibly susceptible to the stimulant effect of potassium and barium. The circular muscle is quiescent and atonic and should possess a stable membrane potential which is resistant to depolarization by acetylcholine as well as by potassium or barium.

There are relatively few reports in the literature on the pharmacology of isolated intestinal circular muscle, and only one direct comparison between the longitudinal and circular muscle layers (Brownlee & Harry, 1963). However, these reports and the present work suggest that there is some basic difference between cells of the two muscle layers. Even should this prove to be untrue, it is apparent that conclusions drawn from experiments made with one of the muscle layers may not be indiscriminately applied to the other.

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