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# A PRELIMINARY INVESTIGATION OF THE PHARMACOLOGY OF THE HUMAN ISOLATED TAENIA COLI PREPARATION

BY

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The effects of drugs on smooth muscle strips of human taenia coli, obtained from operation specimens, were studied *in vitro*. Both nicotinic and muscarinic sites of action of acetylcholine were demonstrated, the nicotinic effect being a relaxation. The sympathomimetic amines, adrenaline, noradrenaline, and isoprenaline produced a relaxation of the tissue by an action on adrenaline $\alpha$- and $\beta$-receptors. The presence of both types of receptor was demonstrated by selective adrenergic blockade with pronethalol or Hydergine. Pronethalol in high concentrations gave a nonselective adrenergic blockade. The ganglion-stimulating agents nicotine and dimethylphenylpiperazinium produced a relaxation of the tissue in all concentrations. This relaxation was inhibited by pronethalol or physostigmine but no contractile component to ganglion stimulation was revealed when these two drugs were present together. These results indicate the presence of either sympathetic ganglia in the intrinsic nerve plexuses, or adrenergic stores in the bowel wall. There is no pharmacological evidence for parasympathetic ganglia in human sigmoid colon. Histamine produced relaxant, contractile or biphasic responses. The type of response was independent of the "tone" of the preparation. The responses were not modified by procaine, hyoscine or pronethalol, which result indicates that both the contractile and relaxant responses to histamine were due to a direct action of the drug on smooth muscle. 5-Hydroxytryptamine produced either a contraction or a relaxation of the tissue. The relaxation was due to a direct effect of the drug, since hexamethonium, procaine or pronethalol did not affect the response. No conclusions have been drawn regarding the mechanism of the contractile response to 5-hydroxytryptamine. The nature of the responses of the tissue to drugs was independent of the disease for which the specimen of colon was removed.

The effects of drugs on the human intestine have been investigated chiefly in experiments on the motility of the bowel in patients (Fink, 1959 ; Fink & Friedman, 1960 ; Chaudhary & Truelove, 1961 ; Ritchie, Ardran & Truelove, 1962 ; Painter & Truelove, 1963). The interpretation of these results is complicated by both intrinsic and extrinsic nervous pathways which are involved in the normal propulsive activity of the bowel *in vivo*.

Some experiments have been carried out on muscle isolated from the human alimentary tract. Ellis, Kauntze, Nightingale & Trounce (1960) studied the effect of drugs on normal and diseased oesophageal muscle. Similar investigations were

made on colonic muscle in experiments on the pathology of Hirschsprung's disease (Trounce & Nightingale, 1960). Pharmacological observations on circular muscle from normal human colon have been reported recently (Fishlock & Parks, 1963).

## METHODS

Material, obtained from resections of bowel for carcinoma, diverticulitis or ulcerative colitis, was cut from the specimen immediately after resection and transported to the laboratory in cooled Krebs solution. The composition of Krebs solution, expressed in g/l. was NaCl 6.9, KCl 0.35, $CaCl_2.6H_2O$ 0.55, $KH_2PO_4$ 0.16, $MgSO_4.7H_2O$ 0.29, (+)-glucose 1, and $NaHCO_3$ 2.1. The tissue was removed from a site, at least 6 cm from the pathological region, where the muscle appeared macroscopically normal and inflammation was absent, except in ulcerative colitis specimens where the disease, in each case, involved the whole of the sigmoid colon. In diverticulitis, the taeniae appeared thickened. Tissue which had been obviously distended by obstruction was excluded.

Taeniae coli, in human colon, run as three well-defined bands down to the region of the rectum where they become less distinct. Longitudinal strips, 2 to 4 mm wide, and approximately 20 mm in length, were cut from the taeniae of the sigmoid region, and it was usually possible to cut two or three preparations from each taenia. The mucosa was removed and the muscle strip set up in a 10 ml. organ-bath containing Krebs solution at 37° C and gassed with 5% carbon dioxide in oxygen. In some cases of ulcerative colitis it was impossible to remove the mucosa on account of fibrosis. Recordings of the tissue responses were made on a smoked drum using a frontal-writing isotonic lever. The load on the tissue was 2 g. The responses were magnified eight-times. Tissue was often stored overnight or for 48 hr in Krebs solution at 4° C.

*Drugs.* The drugs used were: acetylcholine perchlorate, (−)-noradrenaline bitartrate, (±)-isoprenaline sulphate, (−)-adrenaline bitartrate, 5-hydroxytryptamine creatinine sulphate (5HT), histamine acid phosphate, nicotine acid tartrate, dimethylphenylpiperazinium iodide (DMPP), physostigmine sulphate, (−)-hyoscine hydrobromide, mepyramine maleate, procaine hydrochloride, hexamethonium bromide, pronethalol, Hydergine (a mixture of equal parts of dihydroergocornine, dihydroergocryptine and dihydroergocristine), and bromolysergic acid diethylamide. Drug concentrations are expressed as μg/ml. of the final bath concentration of the base, with the exception of DMPP, procaine, pronethalol, Hydergine and bromolysergic acid diethylamide which are expressed as the salt.

## RESULTS

### *Spontaneous activity*

Most preparations exhibited spontaneous activity which occasionally was so great as to render the tissue unsuitable for accurate pharmacological investigations. Any one preparation could exhibit several types of spontaneous activity, abruptly changing from one type to another during the course of the experiment (Fig. 1). The activity varied in both frequency and amplitude in preparations which had been cut from the same taenia.

### *Actions of acetylcholine*

The tissue contracted to acetylcholine in a concentration range of 0.1 to 2 μg/ml. Physostigmine (1 μg/ml.) left in contact with the tissue for 10 min potentiated the effect of acetylcholine, shifting the log dose/response line to the left. The contractile response to acetylcholine was not modified by hexamethonium (10 μg/ml.), which completely blocked the effect of DMPP. Exposure of the tissue to hyoscine (0.01

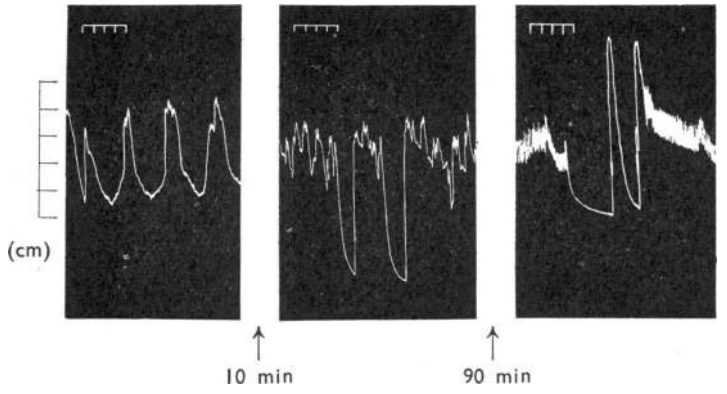


Fig. 1. The kymograph record shows different types of spontaneous activity exhibited by one preparation. Time marks, 30 sec.

$\mu$g/ml.) for 3 min completely inhibited the effect of acetylcholine. A relaxation was obtained with acetylcholine (8 $\mu$g/ml.) in the presence of hyoscine (1 $\mu$g/ml.).

*Actions of noradrenaline, adrenaline and isoprenaline*

The sympathomimetic amines, noradrenaline (0.1 $\mu$g/ml.), adrenaline (0.1 $\mu$g/ml.) and isoprenaline (0.1 $\mu$g/ml.), relaxed the tissue and inhibited spontaneous activity. In the few experiments in which the amines failed to produce a relaxation the usual response was obtained after raising the " tone " with either barium chloride (100 $\mu$g/ml.) or acetylcholine (1 $\mu$g/ml.). The $\beta$-receptor blocking agent pronethalol (10 $\mu$g/ml.) when present for 3 min produced almost complete inhibition of submaximal responses to noradrenaline, adrenaline or isoprenaline. At a concentration of 2 $\mu$g/ml., pronethalol inhibited the relaxation caused by isoprenaline leaving the response to noradrenaline unaffected.

The $\alpha$-receptor blocking agent Hydergine (5 $\mu$g/ml.) abolished the response to noradrenaline, leaving the response to isoprenaline unaffected.

*Actions of nicotine and DMPP*

Nicotine or DMPP (1 $\mu$g/ml.) caused a relaxation of the tissue. A 10 min cycle for nicotine and a 5 min cycle for DMPP were required to give constant responses. Minimal responses were obtained with a concentration of 0.5 $\mu$g/ml. and maximal with 1 $\mu$g/ml. No contractile component was revealed either by increasing the concentration to 40 $\mu$g/ml. or by decreasing it to 0.1 $\mu$g/ml. Hexamethonium, in a concentration of 5 $\mu$g/ml., reduced the response and 10 $\mu$g/ml. caused complete inhibition. Incubation with physostigmine (1 $\mu$g/ml.) for 10 min reduced or abolished the relaxation to nicotine or DMPP but did not reveal a contraction (Fig. 2). Procaine (100 $\mu$g/ml.) in contact with the tissue for 10 min abolished the responses to DMPP or nicotine. The effect of procaine was maintained by keeping the drug in the bath between doses of agonist. The response to DMPP was blocked by pronethalol (10 $\mu$g/ml.) (Fig. 3). Incubation of the tissue with physostigmine (1 $\mu$g/ml.) for 10 min and pronethalol (10 $\mu$g/ml.) for 3 min abolished the response

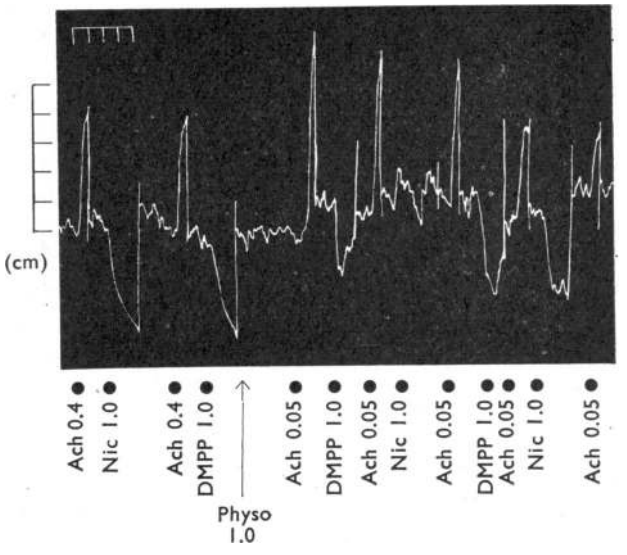


Fig. 2. The effect of physostigmine (Physo) on the responses of the longitudinal muscle strip to acetylcholine (Ach), nicotine (Nic) and dimethylphenylpiperazinium (DMPP). Submaximal responses to these drugs are shown before and after exposure of the tissue to physostigmine (1 $\mu$g/ml.) for 10 min. Numbers in this and subsequent figures refer to drug concentrations, expressed as $\mu$g/ml. of bath fluid. Time marks, 30 sec.

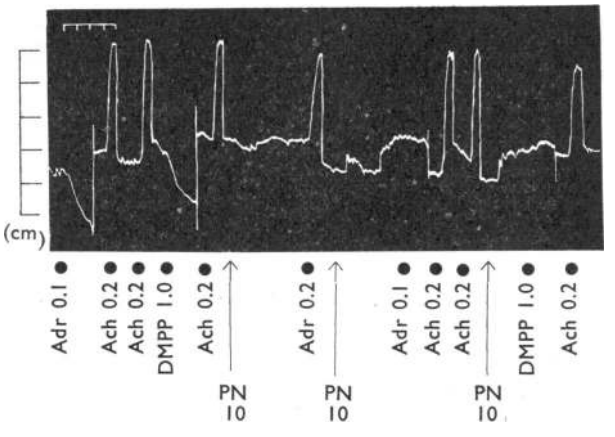


Fig. 3. The effect of pronethalol (PN) on the responses of the taenia coli to adrenaline (Adr), acetylcholine (Ach) and dimethylphenylpiperazinium (DMPP). Submaximal responses to these drugs are shown before, and in the presence of pronethalol (10 $\mu$g/ml.) added to the bath 2 min before the test drug in each instance. Time marks 30 sec.

to DMPP. Preparations which had been stored for up to 4 days in Krebs solution at 4° C still responded to nicotine and DMPP.

*Actions of histamine*

The tissue responded to histamine (0.1 to 0.5 μg/ml.). In twenty-three experiments, six preparations gave only a contractile response, eleven preparations a biphasic response and six gave only a relaxation. Storage of the tisue at 4° C appeared to influence the nature of the response; the contractions to histamine were usually obtained on fresh preparations whereas stored tissue more frequently produced a biphasic or relaxant response. Tissue giving only a contractile reponse to histamine was shown to be capable of a relaxation with noradrenaline.

Mepyramine (0.01 μg/ml.) in contact with the tissue for 10 min inhibited both the contractile and relaxant responses to histamine, leaving the response to acetylcholine unaffected.

Neither hexamethonium (10 μg/ml.), physostigmine (1 μg/ml.) nor hyoscine (0.01 μg/ml.) modified the response to histamine. Procaine, in a concentration (100 μg/ml.) which abolished submaximal responses to DMPP, reduced the sensitivity of the tisue to acetylcholine or histamine (Fig. 4). A twofold increase

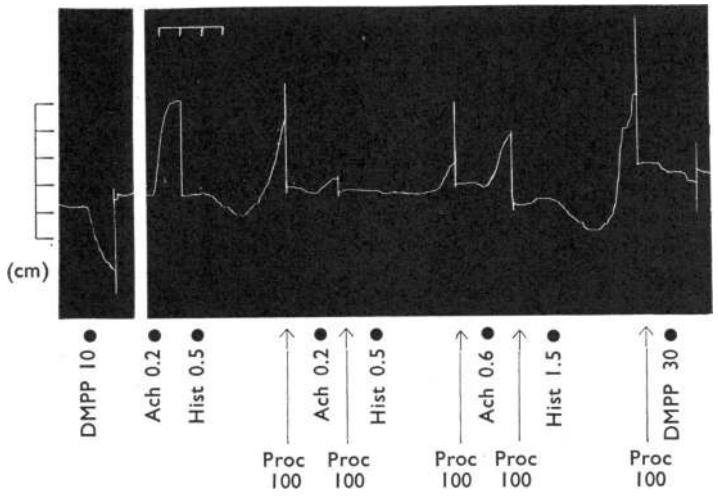


Fig. 4. The effect of procaine (Proc) on the biphasic response of the taenia coli to histamine (Hist.) Submaximal responses to dimethylphenylpiperazinium (DMPP), acetylcholine (Ach) and histamine are shown before, and in the presence of procaine (100 μg/ml.) added to the bath 10 min before the test drug in the first instance, and then 3 min before each successive test drug. Time marks 30 sec.

in the concentration of the agonists restored the responses to acetylcholine and histamine to their original levels, whereas the response to DMPP was still inhibited even with a tenfold increase in concentration. Exposure of the tissue to pronethalol, in a concentration (10 μg./ml.) which inhibited the response to noradrenaline, did not alter the response to histamine (Fig. 5).

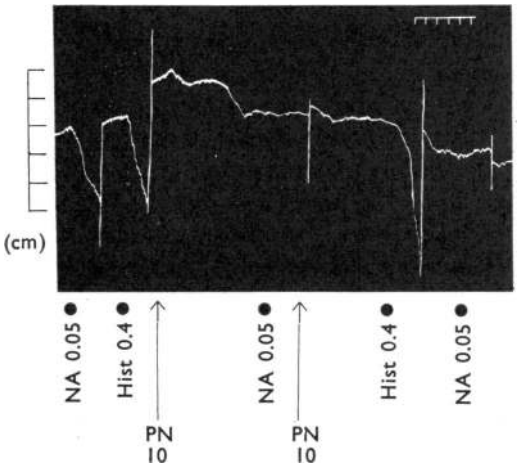


**Fig. 5.** The effect of pronethalol (PN) on the relaxant response of the taenia coli to histamine (Hist). Submaximal responses to noradrenaline (NA) and histamine are shown before and in the presence of pronethalol (10 $\mu$g/ml.) added to the bath 3 min before each test drug. Time marks 30 sec.

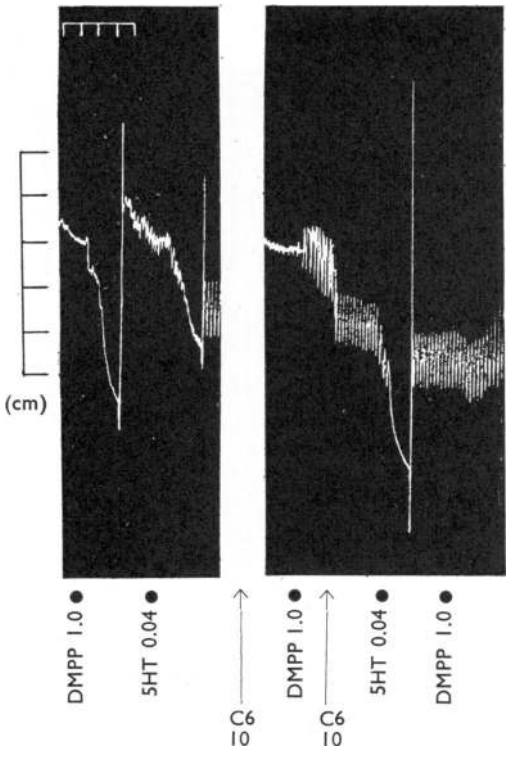


**Fig. 6.** The effect of hexamethonium (C6) on the responses of the tissue to dimethylphenylpiperazinium (DMPP) and 5-hydroxytryptamine (5HT). The left-hand panel shows submaximal responses of the taenia coli to these drugs and the right-hand panel the responses after each drug addition has been preceded (by 3 min) by hexamethonium (10 $\mu$g/ml.). Time marks 30 sec

*Actions of 5-hydroxytryptamine*

The tissue responded to 5HT in a concentration range of 0.1 to 1 $\mu$g/ml. with either a contraction or a relaxation. In twenty-six experiments, eighteen preparations gave a relaxation and eight a contraction to 5HT. The relaxant responses were sometimes followed by a prolonged contractile phase on washing the drug out, but a biphasic effect could not be demonstrated, even by leaving the drug in contact with the tissue for 5 min. The nature of the response was independent of the concentration of 5HT used, and of the " tone " of the preparation. In two preparations the contractile response changed to a relaxation during the course of the experiment. Time cycles of 40 min were usually required to prevent tachyphylaxis to 5HT. During the desensitized period, acetylcholine, noradrenaline and DMPP still produced their characteristic responses.

Concentrations of hexamethonium (10 $\mu$g /ml.) which blocked the response to DMPP, had no effect on the relaxation to 5HT (Fig. 6). Procaine, in a concentration which abolished submaximal responses to DMPP (100 $\mu$g/ml.) reduced the relaxations produced by both adrenaline and 5HT. Doubling the concentration of agonists restored the responses to adrenaline and 5HT to their previous levels whereas the response to DMPP was still inhibited even with a tenfold increase in concentration (Fig. 7). Pronethalol (10 $\mu$g/ml.), which produced an inhibition of the responses

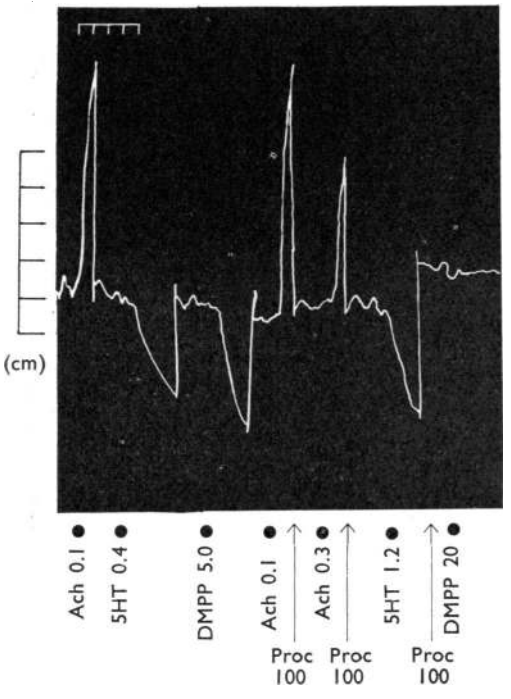


Fig. 7. The effect of procaine (Proc) on the responses of the taenia coli to 5-hydroxytryptamine (5HT), dimethylphenylpiperazinium (DMPP) and acetylcholine (Ach). The responses are shown before and in the presence of procaine (100 $\mu$g/ml.) added to the bath 10 min before the test drug in the first instance, and 3 min before in successive doses. Time marks 30 sec.

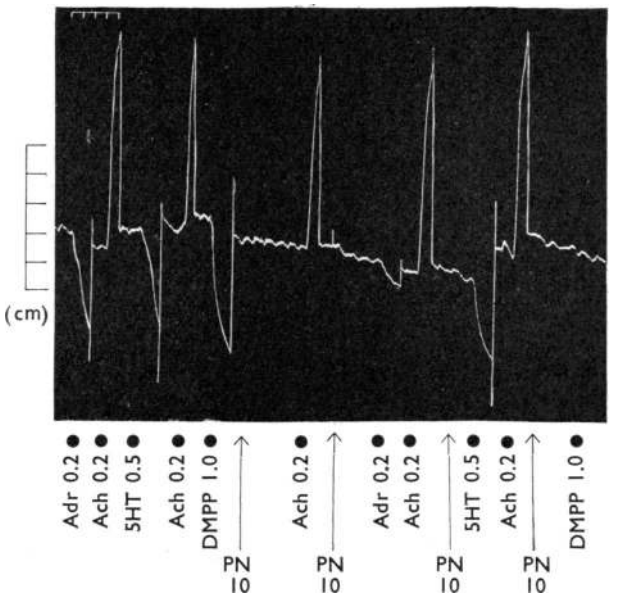


Fig. 8. The effect of pronethalol (PN) on the responses of the taenia coli to 5-hydroxytryptamine (5-HT), dimethylphenylpiperazinium (DMPP), acetylcholine (Ach) and adrenaline (Adr). The responses are shown before, and then after each drug addition has been preceded (by 3 min) by pronethalol (10 $\mu$g/ml.). Time marks, 30 sec.

to adrenaline or DMPP, had no effect on the relaxation produced by 5HT (Fig. 8). Bromolysergic acid diethylamide (1 $\mu$g/ml.) completely inhibited the relaxation produced by 5HT.

## DISCUSSION

These results probably represent the pharmacology of normal colonic muscle since the responses of the tissue to drugs were not influenced by the disease for which the operation was performed.

The investigations have been carried out on mucosa-free preparations, as it was possible that the redundant folds of mucosa offered a mechanical barrier to the diffusion of drugs, and Walder (1953) has shown that the isolated mucosa of the human stomach secretes a substance which inhibits drug action. In the present experiments removal of the mucosa damaged the submucous nerve plexus, and thus no conclusions should be drawn about the possible action of drugs on either sensory receptors in the mucosa or their associated reflex arcs.

Some of the experiments were performed on tissue which had been stored for 24 or 48 hr in Krebs solution at 4° C. This did not seem to impair either myogenic or neurogenic responses to drugs, except that the contractile phase of the response to histamine did not occur so frequently in the stored preparations.

Acetylcholine produced typical muscarinic effects on human colonic muscle, the contractile response being blocked by hyoscine, potentiated by physostigmine and

unaffected by hexamethonium. Elimination of the contractile response to acetylcholine with a high concentration of hyoscine revealed a relaxant response when the concentration of acetylcholine was increased twenty-times, which demonstrated the nicotinic effect of acetylcholine.

According to Ahlquist & Levy (1959) catechol amine receptors in the bowel are of two types, $\alpha$ and $\beta$. It is now accepted that noradrenaline acts mainly on $\alpha$-receptors, isoprenaline mainly on $\beta$-receptors and that adrenaline acts on both. The action of these sympathomimetic amines on different receptors can be demonstrated using selective adrenergic blocking agents. Pronethalol and Hydergine were chosen for this purpose since they have been shown to be selective antagonists for $\beta$- and $\alpha$-receptors respectively (Black & Stephenson, 1962).

At a concentration of 2 $\mu$g/ml. pronethalol inhibited the relaxation caused by isoprenaline, but not the response to noradrenaline. However, when the concentration of pronethalol was increased to 10 $\mu$g/ml. almost complete inhibition of the responses both to noradrenaline and to adrenaline could be demonstrated. In rabbit colon only partial inhibition of the response to isoprenaline was observed with a concentration of pronethalol of 1 $\mu$g/ml. (Vanov, 1963). Hydergine (1 $\mu$g/ml.) completely blocked the response to noradrenaline leaving the relaxation to isoprenaline unaffected indicating a selective $\alpha$-receptor blockade. On this basis, therefore, both $\alpha$- and $\beta$-receptors are present in human taenia coli, and stimulation of either produced a relaxation.

Ganglion stimulation by nicotine or DMPP produced a relaxation of the tissue which was blocked at neuronal sites by hexamethonium and procaine, and peripherally by pronethalol. Gillespie & Mackenna (1960) showed that the response of the rabbit colon to nicotine could be converted from a relaxation to a contraction by increasing the concentration. No such variation in the response to either nicotine or DMPP could be demonstrated on human colon.

A contractile response to ganglion stimulation was not revealed by adrenergic blockade with pronethalol. Physostigmine, however, abolished the relaxation produced by DMPP or nicotine but no contractile phase occurred, even when the concentration of agonist was increased five times. If this effect of physostigmine represented potentiation of a cholinergic mechanism it should have been possible to demonstrate a contractile response when physostigmine and pronethalol were present together. However, no contractile response was obtained under these conditions.

There was in these experiments no evidence, on a pharmacological basis, for the presence of cholinergic ganglia in human sigmoid colon, despite the classical teaching that preganglionic parasympathetic fibres synapse in the intrinsic plexuses of the bowel wall. On the other hand we demonstrated the presence of an adrenergic mechanism which could operate either by stimulation of postganglionic adrenergic nerve terminals or by stimulation of sympathetic ganglia in the bowel wall.

Histamine was capable of relaxing or contracting human taenia coli. It seems possible that the nature of the response is influenced by storage, since the contractile phase was observed more frequently in fresh preparations. The dual action of histamine suggested the possibility of two mechanisms, one being neurogenic.

Hexamethonium, in a ganglion-blocking concentration, did not affect the response to histamine. This does not, however, exclude the possibility of a ganglionic site of action of histamine, as it has been shown that histamine can stimulate autonomic ganglia at a receptor site not affected by hexamethonium (Trendelenburg, 1957). Procaine, which has been shown to depress neuronal activity in the isolated gut (Feldberg & Lin, 1949) also depresed myogenic activity in the concentrations used (100 $\mu$g/ml.). This depression was overcome by increasing the concentration of acetylcholine, adrenaline or histamine threefold, but a tenfold increase in the concentration of DMPP did not overcome the inhibition. This result implied that histamine does not have a neurogenic effect. The evidence for a myogenic (direct) site of action of histamine is supported by the fact that hyoscine, in a concentration which abolished the responses of the tissue to acetylcholine, had no effect on the contraction produced by histamine, and that the adrenergic blockade produced by pronethalol did not depress the relaxant phase to histamine. Mepyramine abolished all types of response to histamine but this does not exclude a neurogenic site of action of histamine.

A myogenic site of action of histamine has been demonstrated in guinea-pig ileum (Innes, Kosterlitz & Robinson, 1957 ; Kosterlitz & Robinson, 1958 ; Day & Vane, 1963). Two sites of action of histamine were demonstrated by Ambache (1946), but Emmelin & Feldberg (1947) were unable to confirm these observations. Ambache & Lessin (1955) have since suggested that histamine has a direct action on guinea-pig ileum but a dual site of action on rabbit intestine, the predominant effect being neuronal in the latter. Further evidence of a neurogenic action of histamine has been put forward by Harry (1963) and Paton & Vane (1963).

5HT produced either a contraction or a relaxation of human taenia coli. The relaxation of this tissue to 5HT appears to be a direct effect on the muscle, since this response was unaffected by pronethalol or by hexamethonium. Procaine (100 $\mu$g/ml.) depressed not only the neuronal activity of the tissue, but also the responses to direct-acting drugs. The depression of the responses to adrenaline and 5HT was overcome by doubling the concentration of these drugs, whereas the response to DMPP was still inhibited when the concentration was increased ten-times, thus confirming the direct site of action of 5HT. No conclusions have yet been drawn on the nature of the contractile response to 5HT since constant responses could not be obtained.

A physiological role of 5HT in the colon has been suggested by Lee (1960), who showed that intraluminal application of 5HT in guinea-pig colon converted non-propulsive contractions into co-ordinated waves. Motility studies on human intestine showed that 5HT stimulates activity in the proximal colon and depresses it in the distal part (Fink & Friedman, 1960). Bülbring & Lin (1958) analysed the action of 5HT on the peristaltic reflex in guinea-pig ileum and showed that it sensitized pressure receptors in the mucosa. 5HT is also known to act directly on smooth muscle (Paton & Vane, 1963), and the possibility of two sites of action was suggested by Gaddum & Picarelli (1957). Brownlee & Johnson (1963) have shown a neuronal site of action of 5-HT in guinea-pig ileum. The present experiments on human colon indicate a direct site of action of 5HT producing a relaxation.

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