Br. J. Pharmac. Chemother. (1966), **28**, 164–171.

THE EFFECT OF 5-HYDROXYTRYPTAMINE ON THE HUMAN ILEUM AND COLON *IN VITRO*

BY

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(*Received May* 16, 1966)

It is well known that 5-hydroxytryptamine (5-HT) causes alimentary smooth muscle to contract. Most regions of the gut show this response although the sensitivity varies. The rat stomach (Vane, 1957), duodenum (Erspamer, 1940) and colon (Gaddum, 1953) all contract in the presence of small concentrations of this amine, and the guinea-pig duodenum, jejunum and ileum also show the same response (Gaddum & Hameed, 1954).

The human lower bowel, however, appears to be different. The taenia coli preparation (Bucknell & Whitney, 1964) and the circular muscle of the sigmoid colon (Fishlock & Parks, 1963; Fishlock, 1964) relax in the presence of 5-HT although other regions of the human alimentary tract have been shown to contract under identical experimental conditions (Fishlock, Parks, & Dewell, 1965). This paper describes the effect of 5-HT on both the longitudinal and circular muscle layers of the human terminal ileum and colon and includes evidence as to the site of action of this amine.

METHOD

Longitudinal and circular muscle strips from 12–30 mm long and 1–2 mm wide have been taken at operation from the ileum and colon and immersed in Krebs solution in an isolated organ bath and placed under a tension of usually 1.5 g. The solution has been gassed with 95% O_2 and 5% CO_2 and maintained at 37° C. Two methods of obtaining muscle strips have been described previously (Fishlock & Parks, 1966). Both were used in this investigation but differences in response and sensitivity of the strips could not be related to the method. All strips consisted of full thickness bowel wall with the mucosa removed. It should be noted that the longitudinal strips of colon were taken through the taenia coli and that the circular muscle strips were taken from between the taeniae where there is a thin investing layer of longitudinal muscle.

The preoperative medication and anaesthesia of the patients from whom the strips were taken varied as described previously (Fishlock & Parks, 1966).

The following drugs were used: acetylcholine chloride, atropine sulphate, hexamethonium bromide, 5-hydroxytryptamine creatinine sulphate, lysergic acid diethylamide (LSD), mepyramine maleate, procaine hydrochloride and pronethalol hydrochloride. Concentrations refer to the salts. All solutions were made with physiological saline.

RESULTS

Ileum

Forty-eight circular muscle strips and 15 longitudinal ones were taken from 42 patients. All came from the terminal part of the ileum (last 0.6 m).

All the circular muscle strips contracted in the presence of 5-HT and acetylcholine (0.01–0.1 μg/ml.). The smallest concentration of 5-HT required to evoke a response varied between 0.5–10 ng/ml. in the majority of experiments. Five strips were not as sensitive as this and required as much as 1 μg/ml. The majority gave maximal responses with 0.05–0.1 μg/ml. Tachyphylaxis was found to be variable. Some strips failed to respond to further doses of 5-HT after as little as 5 ng/ml.; other strips became less sensitive with repeated small doses. Most could be rendered insensitive to 5-HT with a concentration of 0.1–0.5 μg/ml. and this blockade would last for about 30 min. The variability of this phenomenon made investigation of other antagonists very difficult and this fact had not been appreciated when a preliminary report was made (Fishlock, 1964). To overcome the difficulty of tachyphylaxis it was necessary to give small concentrations of 5-HT (sufficient to provoke a 50% response) at 10-min intervals. Atropine (10 μg/ml. 10 min) completely antagonized acetylcholine but did not affect the response to 5-HT. Procaine, 30 μg/ml. for 30 min, which is sufficient to block the effect of nicotine on the human ileum (Fishlock & Parks, 1966) did not affect either 5-HT or acetylcholine. LSD (0.1 μg/ml. 5 min) completely antagonized 5-HT but did not affect the response to acetylcholine. The partial blockade with hexamethonium reported earlier (Fishlock, 1964) was not substantiated. When 5-HT was given at 10-min intervals as described above, hexamethonium (50 μg/ml. 5 min) did not affect the response of the tissue to 5-HT or acetylcholine.

All the longitudinal muscle strips contracted in the presence of 5-HT and acetylcholine, the sensitivity to both substances being similar to that of the circular muscle. Tachy-

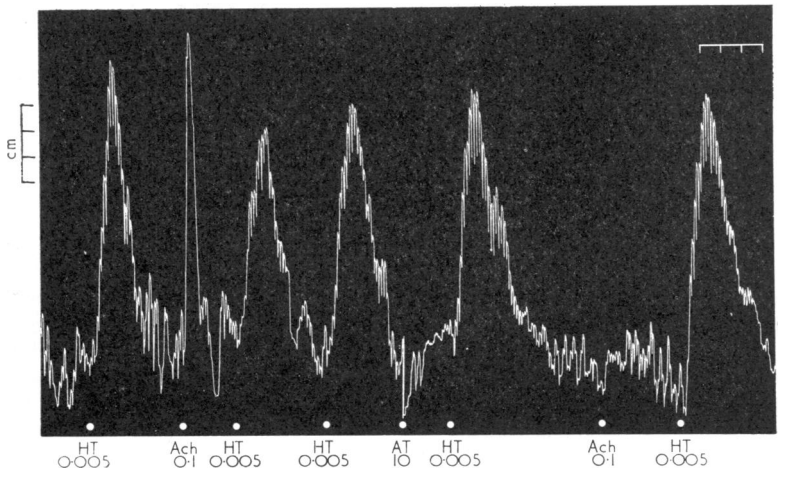

Fig. 1. Longitudinal muscle terminal ileum preparation: 5-hydroxytryptamine caused contraction which could not be antagonized by atropine. HT=5-hydroxytryptamine (exposure 1 min). Ach=acetylcholine (exposures: first dose 20 sec, second dose 1 min). AT=atropine (exposure 10 min); concentrations in μg/ml.; strip length 30 mm; lever magnification ×7; tension 1.5 g; times marks 1 min. In the ileum tachyphylaxis to 5-HT varied from strip to strip. This particular record was chosen as an illustration because the preparation did not show that phenomenon at low concentrations of 5-HT and therefore allowed the completion of the experiment in a short time.

phylaxis to 5-HT occurred and was as variable as in the circular preparation. Atropine antagonized acetylcholine but not 5-HT (Fig. 1). LSD blocked 5-HT without affecting the responses to acetylcholine but procaine and hexamethonium failed to antagonize either. The concentration of antagonists was the same as that used in the circular muscle investigation.

Sigmoid colon

From this region 53 circular muscle strips and 15 taenia preparations were investigated. Specimens were taken from 51 patients.

All of the 53 circular muscle strips contracted in the presence of acetylcholine (0.1 μg/ml. and above) and all but two of them relaxed to 5-HT. The smallest concentration required to cause relaxation varied between 0.01–0.1 μg/ml. in the majority of experiments. A few strips were not as sensitive as this and required up to 2 μg/ml. Tachyphylaxis occurred only after higher concentrations—i.e., after 5 μg/ml. (Fig. 2). The

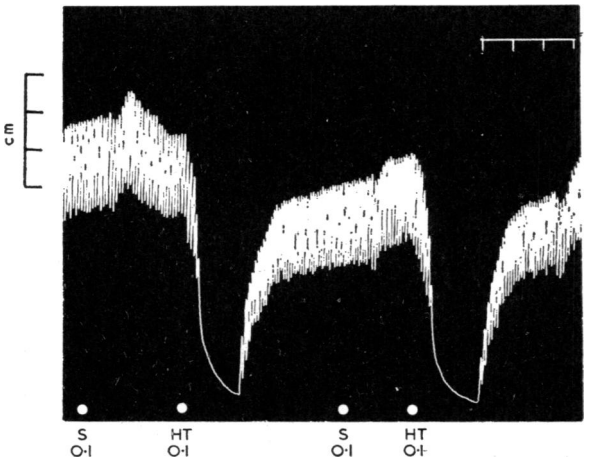

Fig. 2. Circular muscle sigmoid colon preparation: 5-hydroxytryptamine caused relaxation. S = physiological saline (in ml.), HT = 5-hydroxytryptamine (in μg/ml.). All exposures 60 sec; strip length 24 mm; lever magnification $\times 7$; tension 2 g; time marks 1 min. This preparation does not always show such regular spontaneous activity. Although the responses to 5-HT are maximal tachyphylaxis did not occur. A higher concentration of 5-HT is required to demonstrate this phenomenon in the sigmoid colon.

relaxation caused by 5-HT could not be antagonized by procaine (30 μg/ml. 30 min), hexamethonium (50 μg/ml. 5 min), pronethalol (30 μg/ml. 5 min) or mepyramine (50 μg/ml. 5 min). It was previously reported that LSD (0.5–1 μg/ml.) did not abolish the effect of 5-HT (Fishlock, 1964). When, however, the exposure time and concentration were increased LSD (2 μg/ml. for 15 min) usually caused complete blockade. Two of the 53 strips gave small contractions to 5-HT (0.1 μg/ml.). Higher concentrations did not increase the response. Because of its rarity this could not be investigated further.

All of the taenia preparations contracted to acetylcholine and relaxed to 5-HT ; the sensitivity being the same as the circular muscle. The effect of 5-HT was abolished by LSD (0.1–0.5 μg/ml.) (Fig. 3). A more detailed investigation of the antagonism of these responses has been recorded by Bucknell & Whitney (1964).

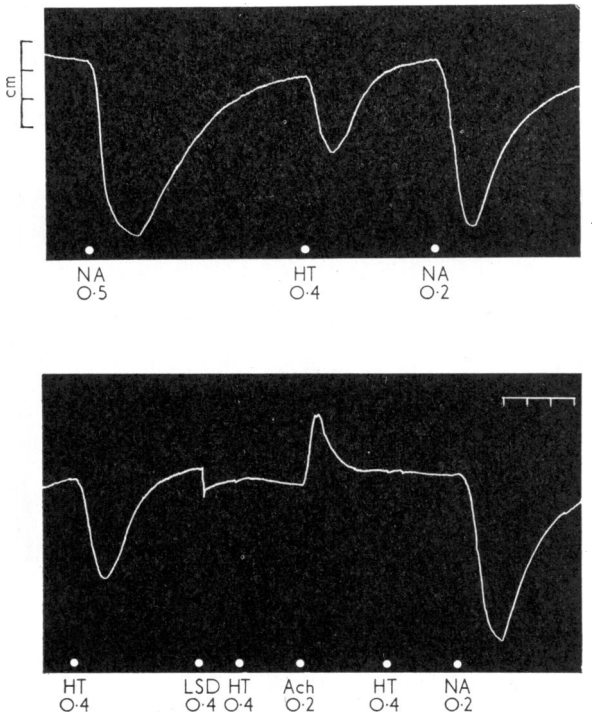

Fig. 3. Taenia preparation from sigmoid colon. 5-hydroxytryptamine caused relaxation which could be antagonized by lysergic acid diethylamide. NA=noradrenaline ; HT=5-hydroxytryptamine ; LSD=lysergic acid diethylamide ; Ach=acetylcholine ; concentrations in μg/ml. Exposures: LSD 5 min, all other 40 sec. Strip length 12 mm ; tension 5 g ; lever magnification $\times 7$; time marks 1 min. The taenia preparation frequently did not exhibit the spontaneous relaxation and contraction seen in the circular muscle and it tended to maintain a higher tone.

The fact that the human terminal ileum contracted in the presence of 5-HT and that the sigmoid colon relaxed raised the problem as to the response of the ascending colon. It is well known that right and left sides of the human colon have a different embryological origin and there is some evidence that they behave differently in response to drugs *in vivo* (Fink & Friedman, 1960). Accordingly it was decided to investigate strips of muscle from the ileocaecal region to the hepatic flexure. Unfortunately it is not so easy to obtain healthy muscle from this region.

Ascending colon

Strips from 12 patients were used. Neither the longitudinal nor the circular muscle preparations showed the rapid spontaneous activity that is seen in strips from the ileum

and the sigmoid colon. A high state of tone was maintained but usually the responses to acetylcholine and 5-HT were slow in onset and the recovery on washout was gradual. Eleven circular muscle strips were investigated and all of them contracted to acetylcholine (0.1–1 μg/ml.) but the response to 5-HT was variable. Four strips relaxed (0.2–2 μg/ml.), two failed to respond (0.2–5 μg/ml.) and the remainder gave small contractions usually above 5 μg/ml. (although one of the strips contracted at 0.02 μg/ml.). Tachyphylaxis was easily induced. There did not appear to be a relationship between the exact anatomical origin of the strip and its responsiveness. Two strips taken 2 cm distal to the ileocaecal valve both relaxed to 5-HT (Fig. 4). Ten longitudinal muscle strips were

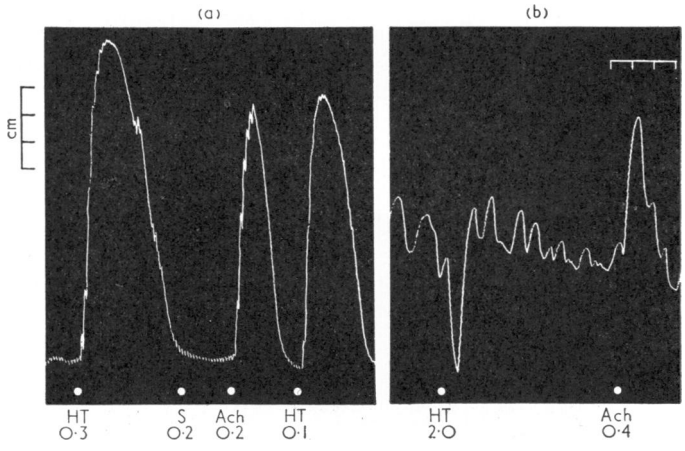

Fig. 4. Ileocaecal region (a) circular muscle from ileum 2 cm proximal to ileocaecal valve, (b) caecal circular muscle 2 cm. distal to valve. Both preparations contracted to acetylcholine but showed different responses to 5-hydroxytryptamine. HT=5-hydroxytryptamine, S=physiological saline (in ml.); Ach=acetylcholine; concentrations in μg/ml. All exposures 40 sec; lever magnification $\times 7$; time marks 1 min. (a) strip length 15 mm, tension 1.5 g; (b) strip length 20 mm, tension 1.5 g. These strips were taken from the same patient.

investigated and although they all contracted to acetylcholine the response to 5-HT was variable. Five strips relaxed (0.1–5 μg/ml.) and two failed to respond (0.01–10 μg/ml.). The remainder gave small contractions at 0.1 μg/ml. but relaxed at concentrations over 1 μg/ml. Tachyphylaxis could be induced very easily.

Because of the variability of response and the difficulty in obtaining specimens the effect of antagonists was not investigated.

DISCUSSION

The site of action of 5-HT has been investigated by many workers. This amine can act directly on the muscle cells or indirectly by stimulating some part of the nervous network in the bowel wall. Paton & Vane (1963) concluded that 5-HT acted directly on the muscle cells of the mouse, rat and kitten stomach preparations because the response was not antagonized by ganglion blocking agents or by hyoscine. Similar evidence of a direct action of 5-HT has been described in the human stomach and jejunum (Fishlock *et al.*, 1965; Whitney, 1965). There have been several reports of

the indirect effect of 5-HT on the guinea-pig ileum (Rocha e Silva, Valle & Picarelli, 1953; Robertson, 1953; Gaddum & Hameed, 1954) and evidence for two separate 5-HT receptors in this preparation was produced by Gaddum & Picarelli (1957). D receptors were sited on smooth muscle cells and could be blocked by dibenzyline. M receptors were considered to be in nervous tissue and could be blocked by morphine. However, other investigators have shown that in the guinea-pig ileum the predominant site of action of this amine is on receptors in nervous tissue (Day & Vane, 1963) probably situated on autonomic ganglion cells in the intramural plexus (Brownlee & Johnson, 1963), the nervous pathway being cholinergic. The longitudinal muscle strip of the guinea-pig ileum is sensitive to 5-HT but the circular muscle does not respond unless it is incubated with an anticholinesterase (Brownlee & Harry, 1963). The circular and longitudinal muscle preparations of the human terminal ileum both contract to 5-HT and are equally sensitive. The effect of 5-HT on both layers can be antagonized by LSD without affecting the responsiveness to acetylcholine. Atropine blocks acetylcholine without altering the response to 5-HT. Hexamethonium and procaine in concentrations sufficient to antagonize nicotine (Fishlock & Parks, 1966) do not alter the response obtained with either 5-HT or acetylcholine. It is concluded from these results that 5-HT acts directly on the smooth muscle of the human ileum and we can offer no evidence for an indirect action. Recently Bennett (1965) has published the results of a preliminary investigation of the human isolated ileum in which he came to the same conclusion.

The rat distal colon contracts in the presence of 5-HT and a recent pharmacological analysis has shown that this amine has both a direct and an indirect action in that preparation (Ulrich, 1965). In contrast, the human distal colon shows the remarkable inhibitory effect of 5-HT which proved to be so difficult to antagonize in the circular muscle preparation. We have reported previously that pronethalol (25 μg/ml. 5 min) antagonizes adrenaline and noradrenaline in this tissue (Fishlock & Parks, 1966) but it was found to have no effect on the responses caused by 5-HT. Similarly procaine and hexamethonium were also found to antagonize the relaxation caused by nicotine but to be ineffective in blocking 5-HT. Mepyramine antagonizes the inhibitory effect of histamine on the human colon (Fishlock & Parks, 1963; Bucknell & Whitney, 1964) but it did not affect the responses to 5-HT. LSD, however, was effective, although a higher concentration was required than in the ileum. From these results we conclude that it is unlikely that 5-HT produces the relaxation of circular muscle by acting on the nervous elements or on the noradrenaline tissue stores but acts on the smooth muscle itself. The taenia exhibits the same inhibitory response which has been investigated by Bucknell & Whitney (1964) who came to the same conclusion.

It can be misleading to use results from work *in vitro* and apply them to the normal physiological situation of the whole animal but there are certain reports, in this case, which support such an interpretation of the findings described. It is well known that 5-HT increases the motility of the human small intestine (Hendrix, Atkinson, Clifton & Ingelfinger, 1957; Haverback & Davidson, 1958; Debray & Besançon, 1961) and Daniel, Honour & Bogoch (1960) concluded from an investigation *in vivo* that 5-HT directly stimulated the smooth muscle cells of the human ileum. Also, there are several reports of the inhibitory effect of 5-HT on the motility of the human distal colon (Fink & Friedman, 1960; Debray & Besançon, 1961). The ascending colon is difficult to investi-

gate *in vivo* but Fink & Friedman believed that their results showed a stimulatory effect of 5-HT on this region. It does appear, therefore, that the results described by us in this paper bear some relationship to the responses *in vivo* of the human alimentary tract.

If our conclusions as to the site of action of 5-HT are correct then it is probable that the smooth muscle cells of the distal half of the colon are physiologically different from the muscle cells of the human ileum. The distal half of the colon is embryologically developed from the hind-gut. The derivatives of the mid-gut—that is, the distal part of the duodenum, jejunum and ileum—all respond similarly to 5-HT (Fishlock *et al.*, 1965) except for that region distal to the ileocaecal valve, the proximal part of the colon. This region appears to be relatively insensitive to 5-HT and responds variably. In a previous investigation we found that the stomach (except for the longitudinal muscle of the body) and the first part of the duodenum were insensitive to 5-HT. These are all fore-gut derivatives. In all the regions of the human gastrointestinal tract so far investigated where 5-HT has an effect it appears to be the result of a direct action on the muscle cells and there are several confirmatory reports of this conclusion (Whitney, 1965 ; Bennett, 1965). It is likely, therefore, that there are physiological differences in the muscle cells of the fore-, mid- and hind-gut regions.

SUMMARY

1. Longitudinal and circular muscle strips were taken at operation from the human terminal ileum, ascending colon and sigmoid colon. They were immersed in Krebs solution in an isolated organ bath at 37° C.

2. The response of these strips to acetylcholine and 5-hydroxytryptamine (5-HT) was recorded.

3. Both longitudinal and circular muscle layers of the ileum contracted in the presence of 5-HT and this response could not be antagonized by atropine, procaine or hexamethonium, but was abolished by low concentrations of lysergic acid diethylamide (LSD).

4. Both longitudinal and circular strips from the sigmoid colon relaxed in the presence of 5-HT. The response of the circular muscle could not be prevented by procaine, hexamethonium, pronethalol or mepyramine. LSD antagonized the effect of 5-HT on both the taenia and the circular muscle.

5. The ascending colon was comparatively insensitive to 5-HT and its response was variable.

6. It is concluded that 5-HT acts directly on the muscle cells of the terminal ileum and on the circular layer of the sigmoid colon. No evidence was found of an effect mediated by the intrinsic nerves.

7. It is suggested that the regional differences in response to 5-HT found in the human alimentary tract may reflect physiological differences in the muscle cells and appear to be related to the embryological origin of the region.

This work has been made possible by a grant from the Medical Research Council.

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