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Evidence for more than one type of 5-hydroxytryptamine receptor in the human colon

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The actions of methysergide, a 5-hydroxytryptamine (5-HT) antagonist, have been examined on muscle strips taken from the circular and longitudinal layers of the human colon. Relaxations of the longitudinal muscle to 5-HT were antagonized by concentrations of methysergide known to be selective. Relaxations of the circular muscle to 5-HT were unaffected by similar concentrations of methysergide. Responses of both types of muscle to 5-HT were partially reduced by tetrodotoxin. Furthermore there was evidence for 5-HT receptors in circular colonic muscle which were unaffected either by selective concentrations of methysergide or tetrodotoxin.

Peripheral 5-HT receptors have been classified by the use of 5-HT antagonists. There are M-receptors in neuronal tissue (ganglia or nerves) which are blocked by morphine, and D-receptors in smooth muscle which are blocked by phenoxybenzamine (dibenzyline) and lysergic acid diethylamide derivatives (Gaddum & Picarelli, 1957).

Relaxations of human longitudinal and circular colonic muscle strips by 5-HT were thought to be due to activation of D-receptors in smooth muscle (Bucknell & Whitney, 1964; Bennett & Whitney, 1966; Fishlock & Parks, 1966b; Misiewicz, Waller & Eisner, 1966; Wright & Shepherd, 1966; Crema, Del Tacca & others, 1968). This seems to be an oversimplification as the following work shows that not only do there appear to be neuronal receptors for 5-HT in both layers of human colonic muscle, but 5-HT also activates tryptamine receptors in the smooth muscle of the circular layer which are different from those in the longitudinal muscle layer.

MATERIALS AND METHODS

Circular and longitudinal (taeniae coli) muscle strips about 20 mm long and 2 mm wide were taken from specimens of human colon removed at operation. Muscle strips were prepared only from regions that appeared healthy and consisted of the full thickness of the bowel wall with the mucosa removed. The strips were suspended in a 10 ml organ bath in Krebs bicarbonate solution at 37° aerated with a mixture of 5% CO_2 in oxygen. Movements of the muscle were recorded either by a Devices recorder using an isotonic transducer or on a smoked Kymograph with an isotonic frontal writing lever (magnification ×8, load 1–2 g). The Krebs bicarbonate solution contained (mM): Na 140, K 5·9, Ca 2·5, Mg 1·2, Cl 122, HCO_3 25, HPO_4 1·2, SO_4 1·2, dextrose 11·5.

With circular colonic strips 5-HT was given cumulatively by increasing the concentration of the drug in the bath fluid, without washing out after each dose. For cumulative dosing an interval of at least 40 min was allowed between successive cumulative dose-response curves. The contact time for each dose was 3 or 4 min. As longitudinal muscle frequencly gave a biphasic response to 5-HT (relaxation followed by contraction) cumulative dosing was not used. For single dosing a cycle of 25 min or more was followed with a contact time of 2 or 3 min. It was found that under these conditions tachyphylaxis to 5-HT was minimal. Muscle strips from both layers were incubated with carbachol (6–16 ng ml^{-1}) which maintained the tone of the strips at a higher level and allowed constant responses to 5-HT.

The effects of premedication and anaesthesia were unknown. However, Fishlock & Parks (1966a) concluded that muscle strips from the human ileum and colon were unaffected by drugs used for premedication and anaesthesia. For instance they found that strips from patients who were given spinal anaesthesia but no atropine, responded in the same way as strips taken from patients who were given general anaesthesia and atropine.

The drugs used were carbachol chloride, 5-

hydroxytryptamine creatinine sulphate, noradrenaline bitartrate, methysergide bimaleate, tetrodotoxin, propranolol hydrochloride and phentolamine mesylate. Concentrations of drugs were expressed as the base.

RESULTS

As circular colonic muscle strips gradually lost tone during the experiment, tone was maintained by the addition of carbachol to the Krebs fluid. Without this procedure difficulties arise in estimating the antagonism of a compound like 5-HT which relaxes the muscle. With carbachol in the concentrations stated, constant responses to 5-HT were obtained.

The nature of the response and the sensitivity to 5-HT of longitudinal muscle strips varied from one experiment to another. Also such changes occurred during the course of an experiment. The range of concentrations of 5-HT giving submaximal responses was 17–870 ng ml^{-1} (58 strips). Methysergide effectively antagonized relaxations of longitudinal muscle to 5-HT (Fig. 1).

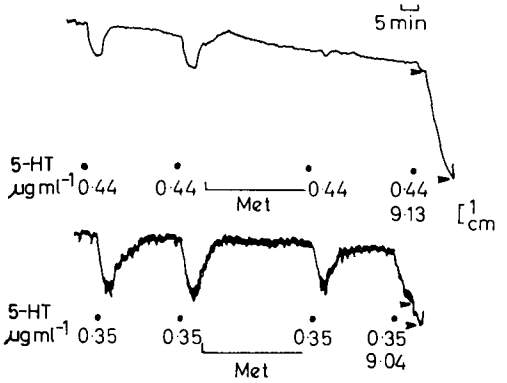

FIG. 1. Longitudinal sigmoid colon: inhibition of the 5-HT relaxation by methysergide (Met, 0·1 μg ml^{-1} upper tracing, 0·008 μg ml^{-1} lower tracing). The antagonist was left in contact with the muscle strips for 30 min. Carbachol (6 ng ml^{-1}) was present in both experiments. At the end of the experiment two 5-HT doses were given cumulatively; the arrows represent the maximum responses to each dose.

In three muscle strips methysergide (0·05 μg ml^{-1}) was tested against a biphasic response to 5-HT. The initial relaxation was abolished in all three strips, while the after contraction was abolished on one strip and partially reduced on two strips.

Tetrodotoxin (TTX, 0·5 μg ml^{-1}) when used to abolish all nerve activity, reduced submaximal responses to 5-HT significantly by 42·8 ± 11·8% (s.e.m.) on longitudinal muscle (Fig. 2, P <0·05 by paired t-test, 10 values from 10 strips).

Circular muscle strips always relaxed to 5-HT. The ED50 of 5-HT was 13 ± 2 ng ml^{-1}, the range of concentrations being 3–70 ng ml^{-1} (38 strips). Unlike longitudinal muscle, responses of circular muscle to 5-HT were not affected by methysergide except in very high concentrations. This was a non-selective effect as lower concentrations of the antagonist (10 μg ml^{-1}) reduced submaximal responses to noradrenaline by 54·5 ± 5% (13 values from 5 strips).

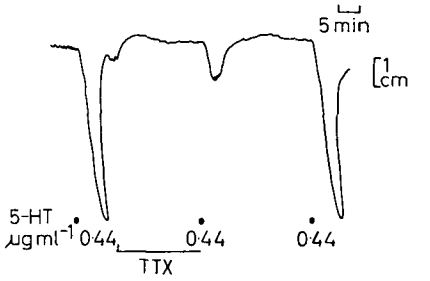

FIG. 2. Longitudinal sigmoid colon: reduction of 5-HT relaxation by tetrodotoxin (TTX, 0·5 μg ml^{-1} for 20 min). After removal of tetrodotoxin the 5-HT relaxation recovered within 30 min. Carbachol (16 ng ml^{-1}) was present throughout the experiment to maintain the tone of the muscle strips.

This difference in sensitivity is clearly seen in Fig. 3 where the concentration of methysergide required to give 50% inhibition of the 5-HT response is 1500 times greater in circular (27 μg ml^{-1}) than in longitudinal muscle (0·018 μg ml^{-1}).

Tetrodotoxin (0·5 μg ml^{-1} for 20 min) significantly reduced submaximal relaxations of circular sigmoid muscle to 5-HT by 46·8 ± 5·5% (P <0·001, 18 values from 9 strips).

Tetrodotoxin (0·5 μg ml^{-1}) had no significant effect on relaxations of the longitudinal colon to noradrenaline (relaxation to noradrenaline was increased on 3 strips and reduced on 3 strips, overall reduction of 2·7 ± 5%, n = 6, P >0·5). On circular colon relaxations to noradrenaline were increased after tetrodotoxin (38·4 ± 19·7%, 12 values from 4 strips). This potentiation was probably unrelated to tetrodotoxin as a similar increase (40·5 ± 32·5%, 6 values from 2 strips) was observed in control experiments where no tetrodotoxin was given. A combination of propranolol and phentolamine

($1\ \mu g\ ml^{-1}$ of each) did not significantly alter relaxations of circular colonic muscle to 5-HT (5-HT potentiated by 8·1 ± 5·9%, 15 values from 5 strips, $0{\cdot}05 > P > 0{\cdot}1$).

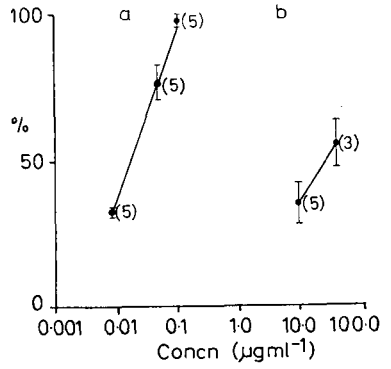

FIG. 3. 5-HT antagonism on (a) longitudinal and (b) circular colonic muscle strips. The effects of methysergide are shown at various concentrations ($\mu g\ ml^{-1}$) by the percentage inhibition (%) of submaximal relaxations to 5-HT. Vertical bars = standard error of the mean, figures in brackets = number of experiments.

DISCUSSION

Tetrodotoxin (TTX) has greatly aided the task of distinguishing direct from indirect (i.e. via nervous tissue) responses to drugs as it selectively abolishes all nerve conduction (Kao, 1966). TTX reduced the response of longitudinal colonic muscle to 5-HT but not noradrenaline, indicating that 5-HT was acting partly on neuronal receptors. It was surprising to find that the response of longitudinal muscle to 5-HT was reduced by TTX since the responses were completely blocked by selective concentrations of methysergide (Wright & Shepherd, 1966). These results confirm earlier observations that 5-HT had some indirect action on longitudinal colonic muscle. For instance Wright & Shepherd (1966) mentioned that the response of human longitudinal colonic muscle to 5-HT may be in part via nervous tissue, while Crema & others (1968) although concluding that 5-HT probably acts directly on the muscle, did observe some 5-HT antagonism by tetrodotoxin. Relaxation of muscle strips by 5-HT may have been partly due to an action on non-adrenergic inhibitory nerves which exist in the human colon (Bucknell, 1966; Crema & others, 1968; Bennett & Stockley, 1973). In contrast to the colon the response of human longitudinal ileal muscle to 5-HT was unaffected by TTX (Burleigh, 1976).

Relaxation of circular colonic muscle to 5-HT was also reduced by TTX indicating that 5-HT acts on neuronal (M) receptors in both muscle layers of the colon. Fishlock (1964) found lysergic acid diethylamide (LSD), a D-receptor antagonist was ineffective in preventing relaxations of human circular colonic muscle strips to 5-HT. However subsequent experiments showed that increasing the dose of LSD usually blocked 5-HT relaxations (Fishlock & Parks, 1966b); the selectivity of the larger doses of LSD was not investigated and this may be important as the compound has been shown to have post-synaptic α-receptor blocking properties unrelated to blocking of 5-HT receptors (Ambache, Killick & others, 1975). The results quoted above showed that the high concentrations of methysergide which partially reduced relaxations of circular colonic muscle to 5-HT were more effective in inhibiting relaxations to noradrenaline. The ineffectiveness of methysergide as a 5-HT antagonist on circular muscle was not due to insensitivity of this tissue to 5-HT. In fact circular muscle was more sensitive to 5-HT than longitudinal muscle. It was unlikely that the relaxation of circular muscle to 5-HT was by release of noradrenaline from adrenergic nerve endings as the response was unaffected by a combination of propranolol and phentolamine. The actions of 5-HT on the circular muscle layer of the colon appear to be mediated partly by M receptors and partly by a receptor which differs from the classical tryptamine receptor on smooth muscle, this receptor being unaffected by selective concentrations of a D-receptor antagonist.

Evidence for atypical tryptamine receptors on smooth muscle has also been found using sheep pulmonary vein (Eyre, 1975) and rat ovarian suspensory ligament (Davis, 1976). In both cases 5-HT caused relaxations, which were unaffected by methysergide, phenoxybenzamine (dibenzyline) and morphine (Eyre, 1975) or methysergide and tetrodotoxin (Davis, 1976).

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