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Investigation of the role of 5-HT_3 and 5-HT_4 receptors in ascending and descending reflexes to the circular muscle of guinea-pig small intestine

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1 The present study was undertaken to ascertain whether 5-hydroxytryptamine (5-HT) acting at either 5-HT_3 or 5-HT_4 receptors plays a significant role in motility reflexes in the guinea-pig small intestine.

2 An isolated segment of small intestine was opened along its mesenteric border and pinned, mucosa uppermost, in a three chambered organ bath so that the oral, middle and anal regions of a single preparation could be separately superfused.

3 Conventional intracellular recording methods were used to monitor the responses of the circular muscle in the oral or the anal end chambers when distension was applied in either of the other two chambers or the mucosal villi were compressed in the middle chamber. Drugs were added to the middle chamber.

4 5-HT_3 receptor antagonists (tropisetron, 0.1–10 μM; granisetron, 1 μM and BRL 46470, 1 μM) depressed the ascending excitatory reflex evoked by these stimuli but had no effect on the descending inhibitory reflex. The depression of the excitatory reflex was observed whether the reflex was evoked from the chamber containing the drug or was simply conducted, via interneurones, through this chamber.

5 The 5-HT_4 receptor antagonist, SDZ 205-557 (1 μM), had no significant effect on either the ascending or descending reflex pathways. However, 5-HT_4 receptors were present as cisapride (0.1 μM) significantly enhanced the ascending excitation without affecting the descending inhibition. This effect of cisapride was converted to a significant depression of the ascending reflex by SDZ 205-557.

6 The results suggest that 5-HT_3, but not 5-HT_4, receptors play an important role in the ascending excitatory reflex and that these receptors may be on interneurones in the reflex pathway.

Keywords: Enteric nervous system; peristalsis; enteric reflexes; 5-hydroxytryptamine (5-HT); 5-HT receptors; small intestine

Introduction

Since the discoveries that 5-hydroxytryptamine (5-HT) occurs in high concentration in the intestine and potently affects intestinal movements (Erspamer & Asero, 1952; Gaddum, 1953), the assumption has been that it is important for the regulation of intestinal movement (Bülbring, 1961; Gershon *et al.*, 1992). However, it has proved extremely difficult to ascertain the physiological role or roles of 5-HT in controlling motility (Tonini *et al.*, 1992; Kirschgessner *et al.*, 1992; Buchheit & Buhl, 1993). The reasons for the difficulty include the fact that two enteric stores of 5-HT exist, endocrine cells and neurones; 5-HT has multiple sites of action; and there are at least four different receptor types for 5-HT or its analogues through which motility can be influenced (Gunning & Humphrey, 1987; Costall & Naylor, 1990; Craig & Clarke, 1990; Tonini *et al.*, 1992). Sites of action began to be identified in the early 1950's. Amongst these were the endings of cholinergic nerve fibres, the cell bodies of excitatory neurones and the muscle (Rocha e Silva *et al.*, 1953; Gaddum & Picarelli, 1957). Further studies indicated that 5-HT stimulates enteric sensory neurones (Bülbring & Crema, 1958) and inhibitory motor neurones (Bülbring & Gershon, 1967). Surprisingly, manipulations to deplete enteric stores of 5-HT (Bülbring & Crema, 1959; Boullin, 1964) or drugs which block 5-HT receptors (Tonini *et al.*, 1992) have failed to identify the physiological roles of 5-HT in motility control. On the other hand, there has been considerable progress in determining the sites and classification of enteric receptors for 5-HT. For example, electrophysiological studies have identified at least two types of excitatory receptors on nerve cell bodies in the guinea-pig gastrointestinal tract, 5-HT_3 and putative 5-HT_{1P} receptors (e.g. Surprenant & Crist, 1988; Mawe *et al.*, 1989; Schemann, 1991). Hyperpolarization of nerve cells is mediated via 5-HT_{1A} receptors (Galligan *et al.*, 1988) and activation of 5-HT_4 receptors on nerve endings enhances transmitter release (Taniyama *et al.*, 1991; Kilbinger & Wolf, 1992).

In view of the number of receptors and multiple sites of action, we have sought to investigate the possible roles of 5-HT using a strategy of preferentially exposing parts of the enteric reflex pathways to drugs acting at receptors for 5-HT. We have done this by using an organ bath in which separate solutions bathe three regions, that from which reflexes are evoked, that in which reflexes are conducted along the intestine and that in which responses in the muscle are recorded. We have used specific receptor agonists and antagonists to evaluate the possible effects of endogenous 5-HT on 5-HT_3 and 5-HT_4 receptors on neurones in ascending and descending reflex pathways.

Methods

Guinea-pigs of either sex weighing between 200 and 400 g were used. The animals were killed by being stunned and bled via the carotid arteries. Segments of small intestine 4–6 cm in length were taken between 10–30 cm oral to the ileo-caecal junction and flushed to remove the content of intestine with physiological saline (composition in mM: Na^+ 151, K^+ 4.7, Ca^{2+} 2.8, Mg^{2+} 0.6, Cl^- 143.7, $H_2PO_4^-$ 1.3, SO_4^{2-} 0.6, HCO_3^- 16.3, glucose 7.7) containing 1 μM nicardipine. In the presence of nicardipine, movement of the muscle is substantially depressed so that cell impalements are more readily retained, but reflex responses can still be

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recorded electrically from the muscle (Smith & Furness, 1988; Yuan et al., 1991). Each segment was opened along its mesenteric border and pinned flat, mucosa uppermost, in a partitioned organ bath.

In order to analyze reflex nerve circuitry and 5-HT receptor locations on the different types of neurones in the pathway, the organ bath was divided into three chambers of different sizes by two plastic partitions (the bottom edge resting against mucosa; 1 mm in thickness). The chambers included one recording chamber (volume 3 ml) and two stimulation chambers (middle; 1 ml and far; 3 ml) each of which had a distending balloon (diameter 5 mm) set into its base. The centres of the distending balloons were separated by 11 mm (Figure 1). Vacuum grease was used to seal the partition area around the preparation and a lower level of superfusion solution in the middle chamber was also used to prevent the drugs in the middle chamber leaking into adjacent chambers. Preliminary experiments were done by applying phenol red (concentration 0.02%) into each of three chambers separately to check the leakage of dye. No leakage was observed. During the experiment, the preparation in the three chambers was superfused individually and continuously by a gravity feed system for the recording and far chambers and by a peristaltic pump for the middle chamber, both flow rates were kept at 0.7 ml min^{-1} except during the rapid application and washout of drugs (2.8 ml min^{-1}) in the middle chamber. The physiological saline was maintained at 37°C and bubbled with carbogen (95% O_2/5% CO_2) on entering each chamber. Drugs were administered into the middle chamber by the peristaltic pump from a reservoir containing the final concentration of drugs in the superfusate.

Reflexes were evoked either by distension (stimulating stretch receptors; 0.1 ml distending volume, duration 9 s) in the middle or far chambers, or by distortion of the mucosa by compression with a compressor (stimulating mucosal mechanoreceptors; distance moving down 1 mm, duration 9 s) in the middle chamber, which was centred over the distending balloon. The distance between the recording electrode in the recording chamber and the centres of both distending balloons and compressor in the middle chamber was 11 mm. The compressor consisted of three parts: a plastic holder on the top, a sponge rubber block in the middle and a sylgard block at the bottom. The sylgard was intended to prevent the lower part of the compressor from absorbing the drugs and to be cleaned easily after each stimulus (Figure 1). The compressor had a compressibility of 0.08 kPa mm^{-1} and the surface of the sylgard block that touched the mucosa was flat and measured 5 mm in the longitudinal axis of the intestine and 11 mm in its circumferential axis. The compressor was mounted in a rigid support that was advanced and withdrawn vertically by hand using a calibrated rackwork bosshead.

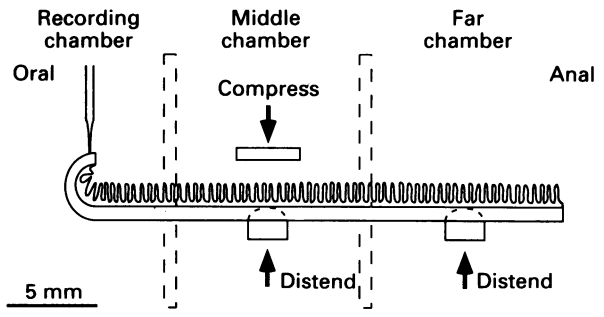

Figure 1 Diagram showing the partitioned organ bath used to evoke the intestinal reflex by distension with a balloon against the serosal surface or mucosal compression with a piece of stiff sponge rubber and to record the reflex responses from the circular muscle. The antagonists or agonists at 5-hydroxytryptamine (5-HT) receptors were added to the middle chamber. The dashed rectangles show the location of the partitions. Note, although the length of the preparation is drawn to scale, the thickness of the preparation is not to scale.

During the experiments, control responses were recorded prior to addition of the drugs to the middle chamber 15 min before a series of test stimuli. Stimuli applied in each of the stimulation chambers tested whether different neuronal components of the reflex circuitry were affected by the drugs. For example, balloon distension in the far chamber was used to test the effect of the drug on the interneurones in the middle chamber which receive inputs from interneurones excited by stretch-sensitive sensory neurones in the far chamber. Balloon distension or mucosal compression in the middle chamber was used to test the effect of drugs on the middle chamber stretch or mucosal distortion-sensitive sensory neurones and some interneurones in the same chamber (Figure 7). Previous studies have shown that responses evoked by mechanical stimuli similar to those used in this present study are completely abolished by tetrodotoxin (Smith & Furness, 1988; Smith et al., 1990) and hence are neurally mediated. The responses of the circular muscle in the recording chamber were recorded by conventional intracellular recording techniques (Yuan et al., 1991). Ascending reflexes were studied by impaling circular muscle cells at the oral end of an opened segment. Descending reflexes were studied in separate preparations whose orientation was reversed so that the anal end was in the recording chamber.

As compression of the mucosal villi can evoke both ascending and descending reflexes, it might have been expected that the partitions dividing the organ bath would also excite such reflexes. However, a previous study (Yuan et al., 1992) has shown that maintaining a constant stimulus causes the reflex responses to decline in amplitude and ultimately to disappear, but it does not suppress reflexes evoked at other sites. Such reflexes can still propagate past the point of a sustained, but ineffective, stimulus.

In several experiments, the mucosa was removed from the wall of the intestine in the middle and far chambers to eliminate the influence of 5-HT released from mucosal enterochromaffin cells. The submucosa and the muscle layers were left intact.

Drugs

The drugs used in this study were: tropisetron (ICS 205-930), granisetron (BRL 43694) and BRL 46470 (endo-N-(8-methyl-8-azabicyclo[3,2,1]oct-3-yl)-2,3-dihydro-3,3-dimethyl-indol-1-carboxamide, hydrochloride), kindly supplied by SmithKline Beecham Pharmaceuticals; SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino)ethyl ester; gift from Sandoz, Basle, Switzerland). All the drugs above were dissolved in physiological saline. Cisapride (Janssen pharmaceutica, Belgium) was firstly dissolved in DMSO (dimethyl sulphoxide) to make a stock solution (10 mM) and then diluted with saline for the experiment. Vehicle solution was identical except for cisapride. Nicardipine (Sigma) was dissolved in warm distilled water at a concentration of 10 mM, stored in frozen aliquots and dilutions for experiments were freshly prepared every day.

Statistical procedures

Amplitudes of responses were measured at their peaks and statistical comparisons were made using Student's paired t test. All data are expressed as mean ± s.e.mean.

Results

Although consistent responses can be achieved by repeated stimulation when the stimulation intervals are greater than 2 min in conventional organ baths (Yuan et al., 1991), when the partitioned organ bath was used, the amplitudes of res-

ponses to the same amount of stimulation were observed to vary if the time interval between the beginning of the stimulation and the end of the whole experiment was longer than 1 h. Thus, it was necessary to determine the time interval over which consistent responses could be obtained.

Time control experiments

In these experiments, either distension in the far and middle chambers or compression in the middle chamber, were separated by intervals of 2 min (see Yuan *et al.*, 1992). In the middle chamber stimulation by either distension or compression caused consistent compound e.j.ps during the first 30 min of the experiment and the amplitudes of responses were reduced slightly, but not significantly, when the time was extended to 45 min. The amplitudes of e.j.ps evoked by distension in the far chamber were also consistent and reproducible during the first 15 min and then the amplitudes of e.j.ps became depressed with time (Figure 2a). These results indicated that the responses in these time control experiments were consistent and repeatable during the first 30 min. For the comparison of responses with or without drug treatments, the best time interval between control stimuli and stimuli in the presence of drugs was 15 min. Unlike the ascending reflex response, the descending reflex responses evoked by the stimuli either in the middle or in the far chamber were quite consistent and reproducible during the first 30 min of the experiment and, even when the time of experiment was extended to 45 min, the amplitudes of inhibitory junction potentials (i.j.ps) were still not significantly changed (Figure 2b).

Descending inhibitory reflexes

Low concentrations of the 5-HT$_3$ receptor antagonist ICS 205-930 (0.1–1 μM) in the middle chamber had no significant effect on the compound i.j.ps elicited by either distension or compression in both stimulation chambers, only the highest concentration of ICS 205-930 (10 μM) significantly depressed the amplitude of i.j.ps (a fall of 40–50% in four preparations from different animals). This effect was probably not due to a specific action of ICS 205-930 on the descending inhibitory reflexes, because the concentration needed to produce it was 10 times that needed to block either 5-HT$_3$ or 5-HT$_4$ receptors. The amplitudes of the i.j.ps were not significantly affected by the 5-HT$_3$ receptor antagonists, granisetron (1 μM) and BRL 46470 (1 μM), by the 5-HT$_4$ receptor antagonist, SDZ 205557 (0.1 μM), or the mixed 5-HT$_3$ antagonist and 5-HT$_4$ agonist, cisapride (0.1 μM).

Ascending excitatory reflexes

Effect of 5-HT$_3$ receptor antagonists ICS 205-930 in the middle chamber substantially depressed the compound e.j.ps evoked either by distension or by mucosal compression in both stimulation chambers. The effects of ICS 205-930 were dose-dependent. ICS 205-930 0.1 μM reduced the e.j.ps to

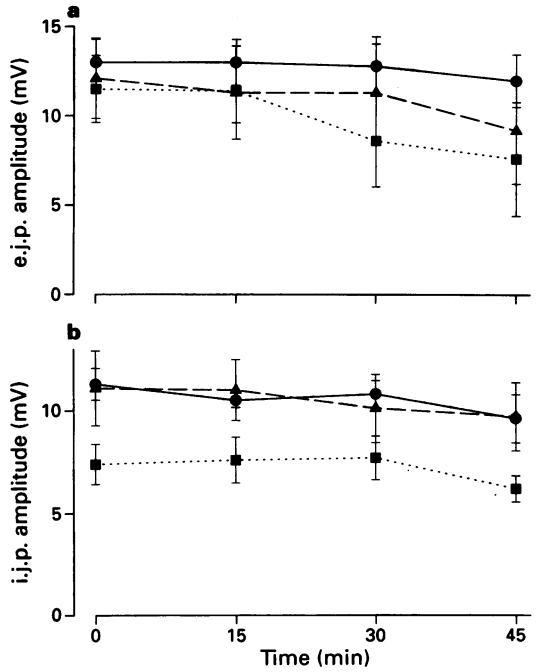

Figure 2 Time control experiments illustrating the consistency of responses evoked by stimuli in different chambers over a 45 min period. (a) Amplitudes of excitatory junction potentials (e.j.ps) recorded from circular muscle oral to the stimuli. (b) Amplitudes of inhibitory junction potentials (i.j.ps) recorded from circular muscle anal to the stimuli: (●) mean responses evoked by distension in the central chamber; (▲) responses evoked by compression of the mucosa in the central chamber; (■) mean responses evoked by distension in the far chamber; s.e.mean are shown. $n = 5$ preparations from different animals for each curve.

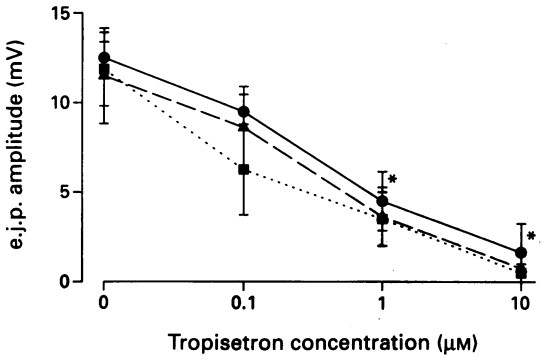

Figure 3 Effect of tropisetron (ICS 205-930) at different concentrations on the ascending excitatory reflex evoked either by distension in the middle or far chamber or by mucosal compression in the middle chamber. Tropisetron at 1 μM and 10 μM markedly reduced the e.j.ps oral to stimuli applied in both stimulation chambers ($*P < 0.05$): (●) distension in central chamber; (▲) mucosal compression in the central chamber; (■) distension in the far chamber. $n = 4$ preparations from different animals.

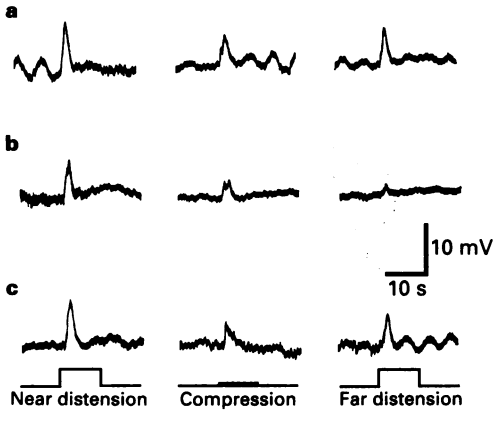

Figure 4 Effects of granisetron on the ascending excitatory reflexes evoked in a single circular smooth muscle cell of the guinea-pig small intestine. (a) Shows compound e.j.ps evoked by near distension (left panel), compression (middle panel) and far distension (right panel), each separated by 2 min. (b) Shows responses evoked by identical stimuli after 1 μM granisetron had been in the central chamber for 15 min. (c) Shows responses evoked by identical stimuli 15 min after beginning to wash out granisetron. The periods during which the stimuli were applied are shown in the bottom panels. Granisetron produced a marked depression of the reflex responses, which reversed after washout.

60–80% of control levels. ICS 205-930, 1 μM further reduced the e.j.ps to 20–30% of control ($P<0.05$) (Figure 3).

Other, more specific, 5-HT_3 receptor antagonists, granisetron (1 μM) (Figure 4) and BRL 46470 (1 μM) also depressed the e.j.ps elicited either by distension or by compression in both stimulation chambers when added to the middle chamber. Each compound reduced the e.j.ps to 50–70% of control levels ($P<0.05$ in the middle chamber; $P<0.01$ in far chamber) (Figure 5a), ($P<0.02$ in the middle chamber; $P<0.01$ in far chamber) (Figure 5b). These observations suggest that endogenously released 5-HT acting on 5-HT_3 receptors plays a role in the ascending excitatory reflex.

To test whether the endogenous 5-HT was released from the enterochromaffin cells of the mucosa or from the neurones in the myenteric plexus, the experiments were repeated after removing the mucosa. Granisetron (1 μM) significantly depressed the e.j.ps evoked by distensions in the middle chambers even when the mucosa was absent ($P<0.05$). After removing the mucosa, the responses to stimulation by compression in the middle chamber were abolished and responses to distension in the far chamber were markedly depressed (Figure 5c).

Effect of a 5-HT_4 receptor antagonist As 1 μM ICS 205-930 might be expected to block 5-HT_4 as well as 5-HT_3 receptors, the effects of a more selective 5-HT_4 receptor antagonist, SDZ 205-557 (0.1 μM) (Buchheit *et al.*, 1991), were examined. However, this compound, when applied in the middle chamber, had no significant effect on the excitatory responses to distension or compression in either stimulation chamber (not illustrated), although it was able to block the effects of the 5-HT_4 receptor agonist, cisapride (see below).

Effect of a 5-HT_4 receptor agonist Cisapride (0.1 μM), a 5-HT_4 receptor agonist, applied in the middle chamber substantially enhanced the responses elicited either by distension or by compression in both stimulation chambers. The amp-

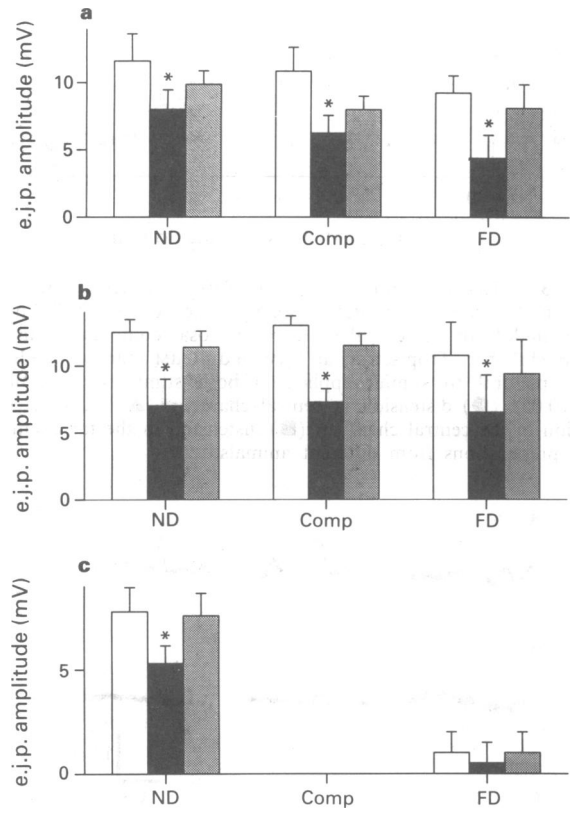

Figure 5 Effect of 5-HT_3 receptor antagonists on ascending excitatory reflexes. (a) Granisetron significantly reduced the amplitudes of e.j.ps evoked by stimuli applied in both stimulation chambers (*$P<0.05$ in middle chamber; $P<0.01$ in far chamber) ($n=4$). (b) BRL 46470 markedly depressed the amplitudes of e.j.ps evoked by stimuli applied in both stimulation chambers (*$P<0.02$ in middle chamber; $P<0.01$ in far chamber) ($n=4$). (c) The depression of e.j.ps amplitudes by granisetron persisted for distensions applied in the near distension chamber after removal of mucosa to prevent the release of 5-HT from enterochromaffin cells (*$P<0.05$) ($n=5$). The reflexes evoked by compression were abolished. Open columns, responses in control solution; solid columns, responses in the presence of the drugs; cross-hatched columns, responses after 15 min washout. ND, distension in central chamber (near distension): Comp, mucosal compression in the central chamber; FD, distension in the far chamber.

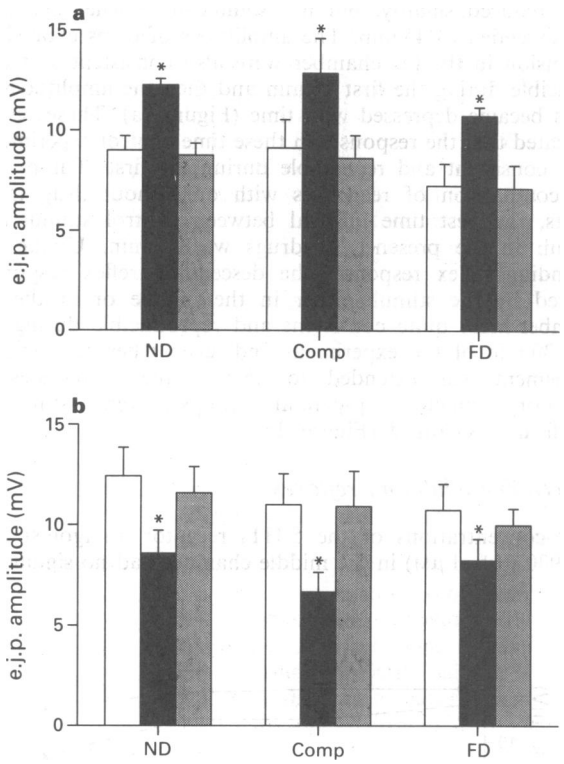

Figure 6 Effect of a 5-HT_4 receptor agonist and an antagonist on ascending excitatory reflexes. (a) Cisapride significantly enhanced the amplitudes of e.j.ps evoked by the stimuli applied in either stimulation chamber (*$P<0.05$ for distension; $P<0.01$ for compression) ($n=4$). (b) In the presence of SDZ 205-557, the effect of cisapride was converted to a significant depression (*$P<0.05$ for distension; $P<0.01$ for compression) ($n=6$). Open columns, responses in control solution; solid columns, responses in the presence of the drugs; cross-hatched columns, responses after 15 min washout. ND, distension in central chamber (near distension); Comp, mucosal compression in the central chamber; FD, distension in the far chamber.

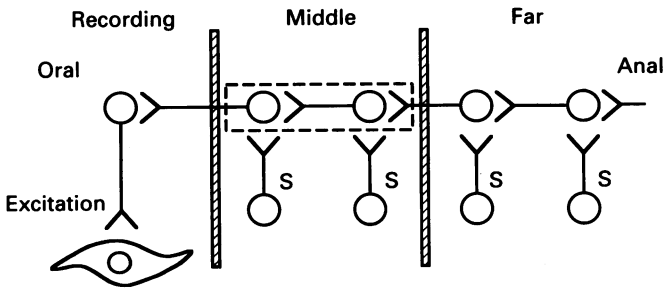

Figure 7 Diagram of the neuronal circuit underlying the ascending excitatory reflex in guinea-pig ileum (Furness & Bornstein, 1993). Sensory neurones (S) provide inputs to ascending interneurones that form chains with outputs to excitatory motor neurones that innervate the circular muscle. The hatched bars show the locations of the partitions that separated the independently perfused compartments. The possible sites of action of the drugs are shown by dashed lines.

litudes of the excitatory responses to the stimuli in both stimulation chambers increased by about 30–50% of control level ($P<0.05$ for distension stimulus; $P<0.01$ for compression stimulus) (Figure 6a).

This effect of cisapride was due to its agonist action on 5-HT$_4$ receptors because it was reversed when these receptors were blocked by SDZ 205-557. Control responses were obtained in the presence of SDZ 205-557 (0.1 μM) to block 5-HT$_4$ receptors in the middle chamber. After cisapride (0.1 μM) was administered in the same chamber for 15 min the responses evoked, either by distensions in middle and far chambers or by compression in middle chamber, were no longer enhanced, but were significantly depressed by cisapride presumably acting via 5-HT$_3$ receptors. Cisapride, in the presence of SDZ 205-557, reduced the excitatory responses to 60–77% of control level ($P<0.05$ for distension stimulus; $P<0.01$ for compression stimulus) (Figure 6b).

Vehicle solution without cisapride was used to repeat the experiments on ascending and descending reflexes. DMSO alone had no effect on i.j.ps or e.j.ps elicited by the stimuli tested. The 5-HT receptor agonist and antagonists used in these experiments did not significantly change the resting membrane potential recorded from the circular muscle or evoke reflex responses.

Discussion

In previous studies, it was difficult to deduce at which points in reflex pathways drugs that affect 5-HT receptors were acting. In contrast, the divided organ bath system used in this work has made it possible for drugs to be applied to separate compartments in such a way that whether a drug acts on sensory neurones, interneurones or motor neurones can be deduced.

The present work revealed consistent effects of antagonists of 5-HT$_3$ receptors on enteric reflexes, despite the fact that previous pharmacological attempts to implicate 5-HT as a transmitter in these reflexes had been inconclusive. Early experiments had tried to uncover a role for 5-HT by depleting its stores in the intestine. 5-HT was depleted by treatment with reserpine (Bülbring & Crema, 1959) or by a tryptophan-free diet (Boullin, 1964). Nevertheless, no effects on intestinal reflexes were found. Although 5-HT receptor antagonists were tested in the years following the first serious suggestion that 5-HT is an enteric transmitter (Gershon *et al.*, 1965; Gershon & Ross, 1966; Bülbring & Gershon, 1967), no convincing pharmacological evidence of a transmitter role of 5-HT in motility reflexes was forthcoming (Furness & Costa, 1982; Furness *et al.*, 1987; Tonini *et al.*, 1991). More recently, immunohistochemical studies have shown that within the small intestine of the guinea-pig, neurones containing 5-HT are descending interneurones which form ultrastructurally identifiable synapses on the cell bodies and dendrites of myenteric neurones (Furness & Costa, 1982; Erde *et al.*, 1985; Young *et al.*, 1993), but no 5-HT terminals were found in the muscle layers. These results indicated that the physiological site of action of neuronal 5-HT was the neurone. Pharmacological evidence suggested that the most likely role of 5-HT would be as an excitatory transmitter, possibly mediating a slow excitatory postsynaptic potential (e.p.s.p) in myenteric neurones (Wood & Mayer, 1978; 1979; Galligan *et al.*, 1988; Mawe *et al.*, 1989). It would thus be anticipated that receptor antagonists for 5-HT would inhibit descending reflexes, because the interneurones that contain 5-HT run anally. We were thus surprised to discover that the inhibitory effects of antagonists for 5-HT$_3$ receptors were selective for orally directed reflex pathways. This suggests that the effects of the antagonists were not a consequence of blocking the actions of 5-HT released from enteric neurones. Indeed, this study provides no support for the idea that 5-HT released from enteric neurones plays a role in motility reflexes.

The lack of correlation of pharmacological observations with the projections of 5-HT neurones suggest that the 5-HT$_3$ receptor antagonists used in our experiments blocked the action of an unknown endogenous ligand released as the synapses in ascending reflex pathways. Three 5-HT$_3$ receptor antagonists all significantly depressed ascending reflex responses but not descending reflexes evoked by either distension or mucosal compression. The extent of the depression was similar whether the drugs were in the middle chamber from which the reflex was initiated or the tissue was exposed to antagonists in the middle chamber and the reflex was evoked from the far chamber (Figure 5a and b). This suggests that the site of the antagonism within the reflex pathway is at neuro-neuronal connections, probably between ascending interneurones (Figure 7). In addition, our results show that the depression of the response to distension by 5-HT$_3$ receptor antagonists remained even after removal of the mucosa, which suggested that 5-HT$_3$ receptors on the nerve terminals in the mucosa did not play a key role in initiating the excitation of the reflex pathways activated by distension. Removal of the mucosa did, however, appear to depress the reflexes evoked by distension in the far stimulation chamber. This observation contrasts with an earlier observation that removal of the mucosa does not affect either initiation of distension reflexes or propagation of reflexes past the region from which the mucosa had been removed (Smith & Furness, 1988; Smith *et al.*, 1990). The major difference between these experiments is in the use of the partitions that divided the organ bath in the present study. It may be that the mucosa normally cushions the partitions, preventing them compressing nerve trunks running in the outer muscle coat, and that when the mucosa is removed the nerve trunks are compressed, thus interrupting the reflex pathways. Although 5-HT$_3$ receptors in the mucosa do not appear to have a role in initiation of the reflexes, our data do not exclude the possibility that putative 5-HT$_{1p}$ receptors on nerve terminals in the mucosa may influence the reflex responses to distension (Kirschgessner *et al.*, 1992). The hypothesized ligand could be an excitatory cotransmitter or a modulator with excitatory presynaptic action.

An earlier study also suggested that a 5-HT-like transmitter might be involved in transmission along the ascending excitatory reflex pathway in the guinea-pig colon (Costa & Furness, 1976). In this region, immunohistochemical studies also show the 5-HT neurones to be in descending pathways (Wardell *et al.*, 1993), suggesting the endogenous transmitter of the ascending pathway in the colon is not 5-HT. If the endogenous substance that acts on 5-HT$_3$ receptors in the ascending pathway is not 5-HT, the failure of depletion of 5-HT stores (Bülbring & Crema, 1959; Boullin, 1964) to affect the peristaltic reflex might be explained.

The 5-HT$_4$ receptor antagonist, SDZ 205-557 (Buchheit *et al.*, 1991) had no action by itself on the peristaltic reflex, clearly implying that endogenous 5-HT acting via 5-HT$_4$ receptors does not play an important role in the peristaltic reflex. However, these receptors can be activated by adding exogenous 5-HT or its agonists (Craig & Clarke, 1991). Cisapride, for example, in the present experiments facilitated the ascending excitatory reflex, consistent with its prokinetic actions (Schuurkes *et al.*, 1988; Tonini *et al.*, 1989). This effect of cisapride can be converted to a significant depression of the ascending excitatory reflexes by SDZ 205-557. The depression is probably via an antagonist action of cisapride at 5-HT$_3$ receptors. Thus, cisapride, but not endogenous 5-HT, had a facilitating action on the peristaltic reflex via 5-HT$_4$ receptors, which probably were located on ascending interneurones.

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