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Evidence for a 5-HT_3 receptor involvement in the facilitation of peristalsis on mucosal application of 5-HT in the guinea pig isolated ileum

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1 The 5-HT receptor involved in the effect of mucosal application of 5-HT to facilitate peristalsis was investigated in the isolated guinea pig ileum.

2 An application of 5-HT (3–100 μM) to the mucosal surface (by inclusion of 5-HT in the Krebs-Henseleit solution passing through the lumen of the ileum) caused a concentration related facilitation of peristalsis characterized by a reduction in the peristaltic threshold.

3 Peristalsis was not modified by methiothepine (0.1 μM), ritanserin (0.1 μM), ondansetron (5 μM), granisetron (1 μM) or SB 204070 (0.1 μM) administered alone to the mucosal surface.

4 The concentration–response curve to mucosally applied 5-HT was not altered by the mucosally applied 5-$HT_{1/2}$ receptor antagonist methiothepine (0.1 μM), the 5-HT_2 receptor antagonist ritanserin (0.1 μM) or the 5-HT_4 receptor antagonist SB 204070 (0.1 μM). However, the mucosally applied 5-HT_3 receptor antagonists ondansetron (5 μM) and granisetron (1 μM) shifted the response curves to mucosally applied 5-HT to the right in a parallel and surmountable manner. The pD_2 values in the absence and presence of ondansetron were 5.42 ± 0.07 and 4.12 ± 0.10, respectively, ($n=6$) and that of granisetron were 5.45 ± 0.12 and 4.50 ± 0.10 respectively, ($n=5$).

5 Serosally applied ondansetron (5 μM) or granisetron (1 μM) had no effect on the concentration–response curve to mucosally applied 5-HT. However, the serosally applied ondansetron and granisetron antagonised the facilitatory effect of serosally applied 5-HT (10 μM) when administered in the presence of serosally applied SB 204070 (0.1 μM).

6 It is concluded that the facilitatory effect of mucosally applied 5-HT to reduce the peristaltic threshold in the guinea pig ileum is mediated via a 5-HT_3 receptor located on the mucosal and not the serosal side of the ileum.

Keywords: 5-Hydroxytryptamine; 5-HT_3 receptor; mucosal application; guinea pig ileum; peristalsis; ondansetron; granisetron

Introduction

5-Hydroxytryptamine (5-HT) is known to produce both a facilitation and inhibition of peristalsis in the guinea pig ileum (Kosterlitz & Robinson, 1957; Bülbring & Crema, 1958). 5-HT applied to the mucosal side is known to cause only a facilitation of peristalsis (Bülbring & Crema, 1958) whereas the effect of serosal application of 5-HT is more complex. At submicromolar concentrations, the serosal application of 5-HT causes only facilitation of peristalsis (Craig & Clarke, 1991; Costall *et al*., 1993). However, it causes a short lasting facilitation which is followed by an inhibitory effect at higher concentrations (Bülbring & Crema, 1958; Costall *et al*., 1993; Tuladhar *et al*., 1995).

There is good evidence to suggest that serosal and mucosal applications of 5-HT act at different sites in the ileum and that a tissue barrier prevents the 5-HT applied to one side from reaching the other (Bülbring & Crema, 1958; Gershon & Tamir, 1981). Thus, peristalsis which is blocked by a serosal application of 5-HT could be reversed by a mucosal application of 5-HT (Bülbring & Crema, 1958). Although no anatomical structure acting as the barrier has been identified, Gershon & Tamir (1981) considered such a barrier to lie either between the mucosa and submucosa or within the mucosa itself. This was based on their findings that ^{3}[H]5-HT added to the serosal side was taken up by myenteric and submucosal plexus neurons but not by the enterochromaffin cells, whereas the ^{3}[H]5-HT was taken up by only enterochromaffin cells and not by the enteric neurons following a mucosal addition.

The facilitatory effect of a serosal application of submicromolar concentrations (0.1–1 μM) of 5-HT in the guinea pig ileum is mediated by a 5-HT_4 receptor (Craig & Clarke, 1991; Costall *et al*., 1993). 5-HT_3 receptors are also recruited to its facilitatory effect when higher concentrations ($\geqslant 3$ μM) of 5-HT are applied to the serosal side (Tuladhar *et al*., 1995). At such higher concentrations of 5-HT, the facilitatory effect of serosally applied 5-HT lasts only for a short time probably because of an additional inhibitory effect of 5-HT which follows the facilitatory effect (Costall *et al*., 1993; Tuladhar *et al*., 1995). The receptor mediating the inhibitory effect of 5-HT is not known. Unlike the serosal application, mucosal application is reported to produce only a facilitation of peristalsis (Bülbring & Crema, 1958). The aim of the present study is to investigate the 5-HT receptor(s) mediating the facilitatory effect of mucosally applied 5-HT in the guinea pig ileum.

Methods

Measurement of peristalsis

Up to four 5 cm lengths of ileum taken 5–30 cm from the ileocaecal junction were obtained from guinea pigs (Dunkin Hartley Bradford strain) of either sex weighing 500–1000 g. The tissues were mounted horizontally as described previously (Costall *et al*., 1993). Briefly each segment was perfused from an oral inlet tube and the aboral outflow end was cannulated via a tube; one wide bore arm of the tube had a tap to restrict outflow when required and the other arm was of narrow bore and permanently open. The apparatus differed in two respects to that previously described. The wide bore aboral

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outflow arm was repositioned to be level with the bath fluid. Second, as soon as peristalsis was induced the inflow was stopped for 10 s to avoid pumping fluid during contraction of circular muscle and to facilitate relaxation between each peristaltic contraction. Krebs-Henseleit solution was perfused into the lumen of the ileum from the oral side at a constant rate. Perfusion rates were 1–1.5 ml min^{-1} to obtain comparable levels of peristalsis. Peristalsis could be induced reversibly and rapidly by restricting outflow through the wide bore arm of the tube.

Non-cumulative concentration–response curves to 5-HT were constructed approximately 1 h after the mounting of the tissues. Equilibration with antagonists was achieved by adding the relevant drug to the perfusion solution once regular peristalsis had been initiated. The effect on peristalsis was observed for 30 min and then peristalsis was stopped by opening the aboral outflow tube for a further equilibration period of 30 min.

The concentration–response curves to 5-HT were established as follows. Peristalsis was elicited in cycles of 10–12 min duration with resting periods of same duration. 5-HT was applied to the mucosal side in an ascending order 5–6 min after the initiation of peristalsis in each cycle and with a contact time of about 2–3 min which was found to be optimal in the preliminary experiments. The concentration–response curves to 5-HT were expressed as a percentage decrease in threshold below the minimum threshold measured in the 2 min period before the addition of 5-HT (Figure 2b). When examining the effects of antagonists on the 5-HT response, a control response curve to 5-HT was always constructed using a tissue taken from the same animal.

In some experiments, a single concentration of 5-HT was applied to one side of the ileum 5–6 min after the initiation of peristalsis; without removing this treatment with 5-HT, another single concentration of 5-HT was added to the other side of the ileum 10–15 min after the first application and the effect observed for a further 5 min. In the experiments where mucosal application was followed by serosal application, some of the tissues were also equilibrated with a 5-HT receptor antagonist added to the serosal side for 1 h before initiation of peristalsis.

Analysis of results

The ability of 5-HT to reduce the peristaltic threshold was expressed in terms of pD_2 values. pD_2 values were determined graphically. All results are expressed as the arithmetic mean ± s.e.mean. Apparent pK_B values for antagonists were calculated from:

$$\text{apparent } pK_B = \log_{10}(\text{concentration ratio} - 1) - \log_{10}(\text{antagonist concentration})$$

The concentration ratio was calculated at 50% of the 5-HT maximum response. The significance of differences between the values was determined at $P < 0.05$ using either a Student's t test (unpaired two-tail) or ANOVA followed by a Bonferroni–Dunnett's t test.

Drugs

5-Hydroxytryptamine maleate (Sigma), ondansetron hydrochloride dihydrate (Glaxo), SB 204070 (8-Amino-7-chloro-(*N*-butyl-4-piperidyl)methylbenzo-1,4-dioxan-5-carboxylate hydrochloride), granisetron hydrochloride (SmithKline Beecham) and methiothepine mesylate (RBI) were dissolved in distilled water and diluted in Krebs-Henseleit solution. Ritanserin (RBI) was dissolved in methanol to give 1 mM solution and the required volume of this solution was added to Krebs-Henseleit solution to give a final concentration of 0.1 μM. When drugs were added to the serosal side the volume added did not exceed 1% of the bath volume.

Results

General observations

The peristalsis obtained was regular, stable and remained so for many hours. The threshold for peristalsis varied only slightly between the tissues obtained both from the same animal and also from those of different animals. The threshold for peristalsis changed slightly in different cycles during the construction of the concentration–response curve to 5-HT. However, the changes were random and were not related to the time or the previous applications of 5-HT (Figure 1).

Effect of 5-HT

The addition of 5-HT at concentrations equal to or greater than 3 μM produced a concentration-related facilitation of peristalsis; a pD_2 value of 5.61 ± 0.21 ($n = 5$) was calculated. The facilitation of peristalsis was characterized by a reduction in the threshold for peristalsis with a consequent increase in the frequency of peristalsis. A typical reduction in the threshold for peristalsis with 3 μM 5-HT and the concentration–response curves to four tissues taken 5–30 cm from the ileocaecal junctions are shown in Figure 2. The concentration–response curves in such tissues were indistinguishable and such tissues were used in the subsequent experiments to determine the effect of 5-HT receptor antagonists.

Effect of ondansetron or granisetron

Ondansetron (5 μM) or granisetron (1 μM) alone had no effect on peristalsis whether they were applied to the mucosal side ($n = 6$ and $n = 5$, respectively) or to the serosal side ($n = 4$ both; data not shown). Mucosally applied ondansetron and granisetron shifted the concentration–response curve to mucosally applied 5-HT to the right in a parallel and surmountable manner with apparent pK_B values of 6.58 ± 0.12 ($n = 6$) and 6.89 ± 0.11 ($n = 5$), respectively (Figure 3, Table 1). However, neither ondansetron (5 μM) nor granisetron (1 μM) had any effect on the concentration–response curve to mucosally applied 5-HT when they were added serosally ($n = 4$ both; data not shown).

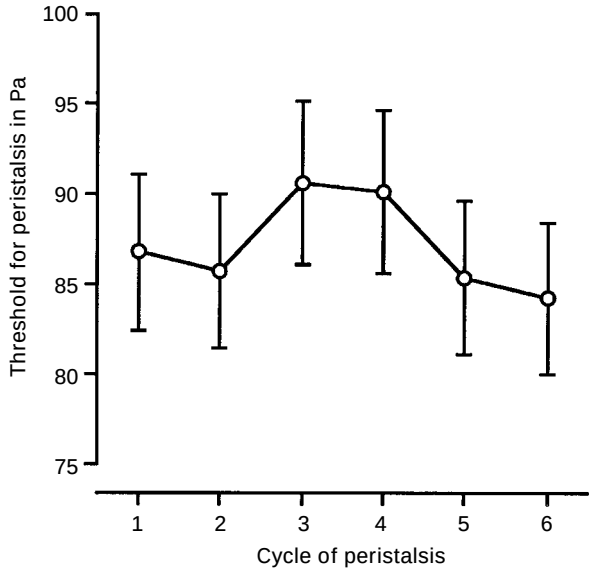

Figure 1 Change in threshold for peristalsis (see example data in Figure 2) in different cycles during construction of concentration–response curves to 5-HT. The thresholds shown are the thresholds for peristalsis in tissues before 5-HT is applied to the mucosal surface in each cycle. Values shown are the means from 22–28 animals with s.e.mean shown by vertical bars.

Effect of methiothepine, ritanserin and SB 204070

Mucosally applied methiothepine (0.1 μM), ritanserin (0.1 μM) and SB 204070 (0.1 μM) had no effect on their own to modify peristalsis and had no effect on the concentration–response curve to mucosally applied 5-HT (Figure 4, Table 1).

Effect of applications of 5-HT to both the serosal and mucosal surface of the ileum

When 5-HT (10 μM) was applied first to the mucosal side the reduction in the threshold for peristalsis lasted for 2–3 min after which the threshold returned nearly to the original value. In these tissues, a further addition of a 10 μM concentration of 5-HT to the serosal side caused again a reduction in the threshold for peristalsis in the next peristaltic stroke. The latter reduction was, however, followed by an increase in the threshold that in many tissues led to a complete loss of peristalsis. The reduction in the threshold due to the mucosally applied 5-HT in the first part of the experiment was not altered significantly in tissues which were subjected to serosally applied SB 204070 (0.1 μM) alone or in combination with ondansetron (5 μM) or granisetron (1 μM) (Figure 5a). However, the reduction in threshold due to the serosally applied 5-HT in the second part of the experiment was completely abolished in tissues which were subjected to the combination of serosally applied ondansetron (5 μM) or granisetron (1 μM) with SB 204070 (0.1 μM) but not SB 204070 alone (Figure 5b). The inhibitory effect (ie. the increase in threshold for peristalsis) of the serosally applied 5-HT which followed the reduction in threshold was neither affected by serosally applied SB 204070 nor its combination with either ondansetron or granisetron (data not shown).

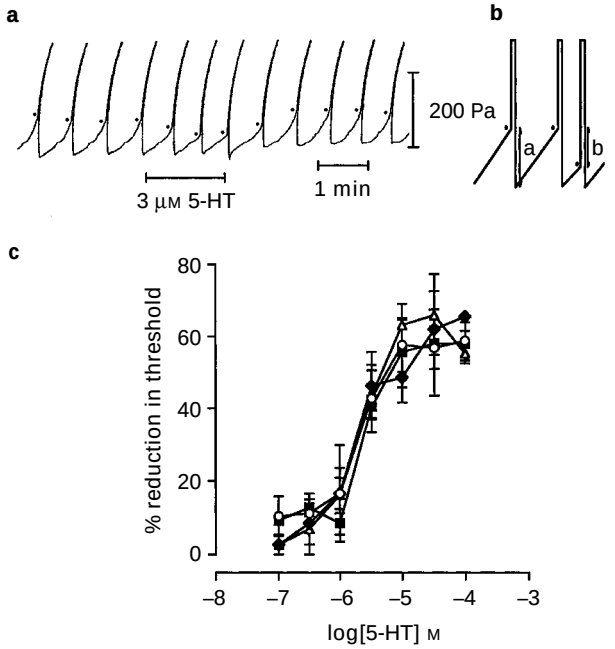

Figure 2 (a) Intraluminal pressure recordings of the peristalsis in the guinea pig ileum and the reduction of threshold caused by mucosal addition of 3 μM 5-HT. Dots indicate the threshold of peristalsis. (b) Schematic diagram of the tracing showing the variables measured. The percentage reduction in threshold was calculated as [100b/a]. (c) Concentration–response curves to mucosally applied 5-HT in four tissues obtained 5–30 cm from the ileocaecal junction: (○) nearest to the ileocaecal junction, (△) adjacent to the first tissue, (■) adjacent to the second tissue and (◆) adjacent to the third tissue. The reduction in the threshold is expressed as the reduction from the minimum threshold in the 2 min period before the addition of 5-HT. Values are the means from four animals with s.e.mean shown by vertical bars.

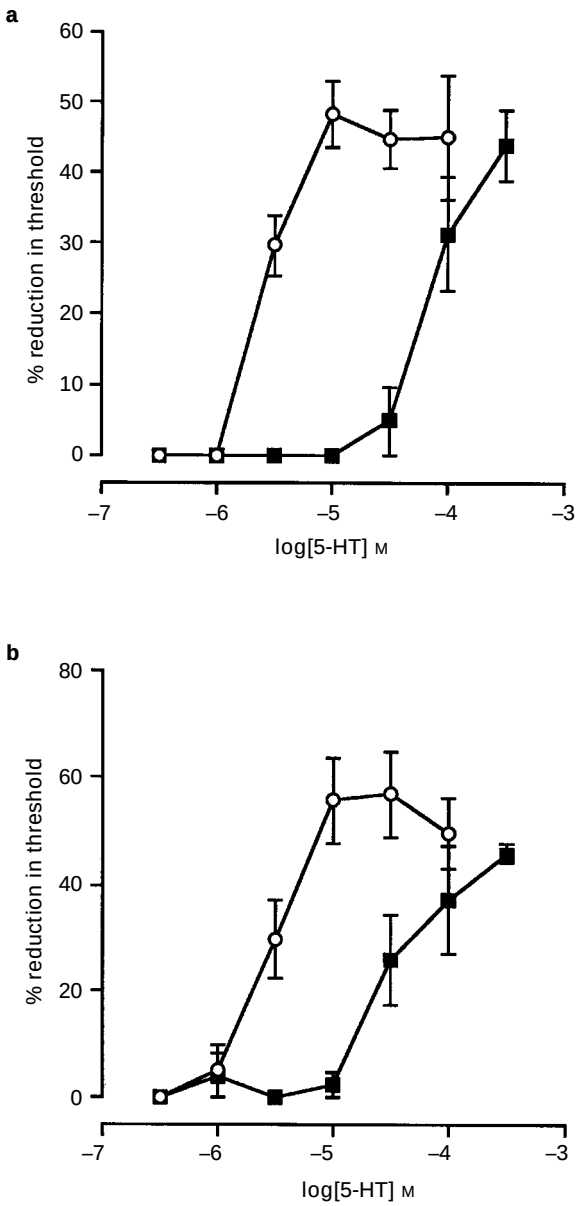

Figure 3 Antagonism of the reduction in the threshold for peristalsis caused by the mucosal addition of 5-HT by mucosally applied ondansetron and granisetron. Concentration–response curves to mucosally applied 5-HT (a) in the absence (○, $n=6$) and presence of 5 μM ondansetron (■, $n=6$) (b) in the absence (○, $n=5$) and presence of 1 μM granisetron (■, $n=5$). The reduction in threshold is expressed as the reduction from the minimum threshold in the 2 min period before the addition of 5-HT. Values shown are the means with s.e.mean from a number of animals indicated by n.

Table 1 Potency of mucosally applied 5-HT to reduce the threshold for peristalsis in the guinea-pig ileum in the absence and presence of mucosally applied 5-HT receptor antagonists

		Reduction in peristaltic threshold $pD_2 \pm$ *s.e.mean*		
Antagonists	*Concentration tested* (μM)	*Without antagonist*	*With antagonist*	*n*
Ondansetron	5	5.42±0.07	4.12±0.10*	6
Granisetron	1	5.45±0.12	4.50±0.10*	5
Methiothepine	0.1	5.46±0.04	5.34±0.12	4
Ritanserin	0.1	5.40±0.01	5.26±0.09	5
SB 204070	0.1	5.49±0.09	5.51±0.12	5

*Difference statistically significant $P<0.001$, Student's t test, unpaired two-tailed.

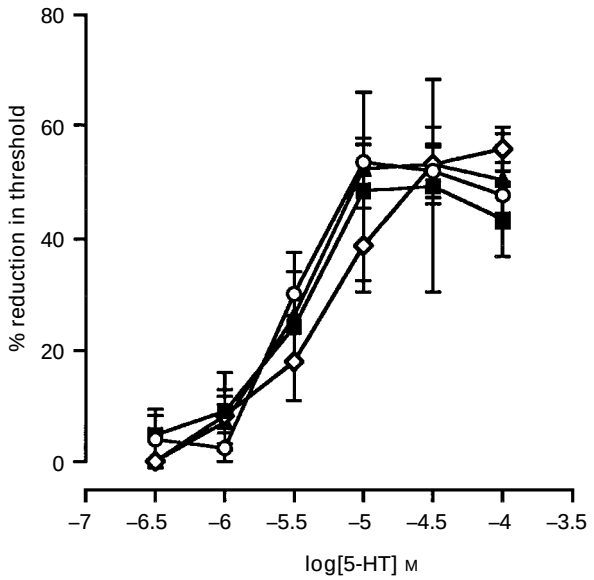

Figure 4 Inability of mucosally applied methiothepine, ritanserin or SB 204070 to antagonise the reduction in the peristaltic threshold by mucosally applied 5-HT. Concentration–response curves to mucosally-applied 5-HT (a) in the absence (○, $n=10$) and presence of 0.1 μM methiothepine (■, $n=4$) or 0.1 μM ritanserin (◇, $n=5$) or 0.1 μM SB 204070 (▲, $n=5$). The reduction in the threshold is expressed as the reduction from the minimum threshold in the 2 min period before the addition of 5-HT. Values shown are the means with s.e.mean from a number of animals indicated by n.

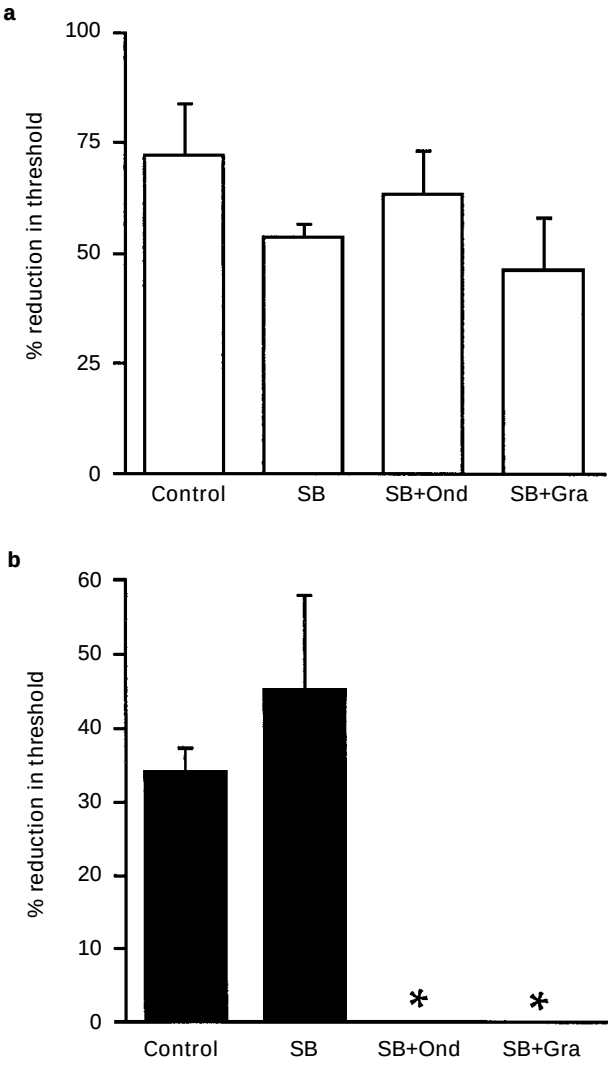

Figure 5 Effect of a mucosal followed by a serosal addition of 10 μM 5-HT in the absence and presence of serosally applied SB 204070, ondansetron and granisetron. 5-HT (10 μM) was added first to the mucosal side which was followed 10 min after, and in continuous presence of 10 μM 5-HT on the mucosal side, by a further addition of 10 μM 5-HT to the serosal side on four tissues; without any antagonist (Control), one with 0.1 μM SB 204070 (SB), one with 0.1 μM SB 204070 + 5 μM ondansetron (SB + Ond) and the final tissue with 0.1 μM SB 204070 + 1 μM granisetron (SB + Ond). SB 204070, ondansetron and granisetron were added only to the serosal side of the ileum. (a) Reduction (□) in the threshold for peristalsis due to the mucosal addition of 5-HT, and (b) reduction (■) in the threshold on serosal addition of 10 μM 5-HT on the same tissues. The reduction in the threshold is expressed as the reduction from the minimum threshold in the 2 min period before the addition of 5-HT in each case. Values are means from five animals with s.e.mean shown by vertical bars. *Significant difference from the control, $P<0.01$; ANOVA followed by Bonferroni/Dunnett's t test.

When 10 μM 5-HT was first added to the serosal side, an initial reduction in peristaltic threshold was soon followed by an increase in the threshold leading to a loss of peristalsis in some tissues and peristalsis with a higher threshold in others. In such tissues a smaller concentration of 3 μM 5-HT applied mucosally either restored peristalsis in tissues in which peristalsis was completely inhibited or reduced the threshold for peristalsis in the tissues in which threshold was increased but peristalsis was still present ($n=4$) (Figure 6). The effect of mucosally applied 5-HT in these experiments also lasted only for 2–4 min after which the ileum either lost peristalsis or the threshold returned to the one before the addition of 5-HT to the mucosal side.

Discussion

The potency of the mucosally applied 5-HT (pD_2 5.61) found in this study to facilitate peristalsis is approximately 50 times lower than the potency, we have reported following serosally-applied 5-HT (pD_2 7.36) (Costall *et al*., 1993). The lower potency of mucosally applied 5-HT compared to the serosal application in the guinea pig ileum is in general agreement with the previous findings of Bülbring & Crema (1958). They reported that approximate equiactive concentrations of 5-HT to facilitate peristalsis from serosal and mucosal sides were 80 nM and 4 μM, respectively. Such a difference in potency of 5-HT could, among other reasons, be due to differences in the receptors mediating the effects.

The failure of mucosally applied methiothepine, ritanserin and SB 204070 to antagonize the effects of mucosally applied 5-HT indicates the lack of involvement of 5-HT$_1$, 5-HT$_2$ and 5-HT$_4$ receptors in the facilitatory effects of mucosally applied 5-HT. The inability of the 5-HT$_4$ receptor selective antagonist SB 204070 (Wardle *et al*., 1994) to affect the concentration–response curve to mucosally applied 5-HT is particularly interesting because the 5-HT$_4$ receptor has been shown to be involved in the effect of serosally applied 5-HT in both the guinea pig and marmoset ileum to reduce the peristaltic threshold (Craig & Clarke, 1991; Costall *et al*., 1993; Tuladhar *et al*., 1996).

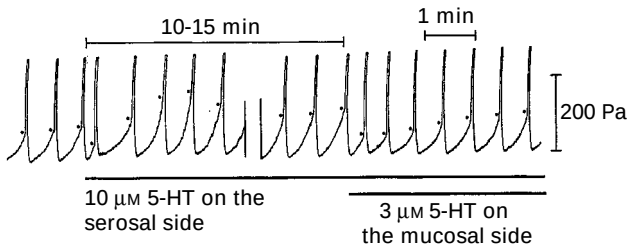

Figure 6 Example of intraluminal pressure recording showing a reduction followed by an increase in the threshold for peristalsis with a serosal application of 10 μM 5-HT and the further reduction in the threshold on the mucosal addition of 3 μM 5-HT with the continuous presence of 5-HT on the serosal side. Dots indicate the threshold for peristalsis.

The antagonism of the facilitatory response to mucosally applied 5-HT by the mucosally applied selective 5-HT$_3$ receptor antagonists ondansetron and granisetron indicates an involvement of the 5-HT$_3$ receptor. It is unlikely that ondansetron or granisetron is having an effect at any other 5-HT or other receptor types at the concentrations used in the present study (Butler *et al*., 1988; Sanger & Nelson, 1989; Hoyer *et al*., 1994). The lower potency of 5-HT to facilitate peristalsis on mucosal application is also in agreement with a 5-HT$_3$ receptor involvement. Thus, 5-HT$_3$ receptors have a much lower affinity for 5-HT than 5-HT$_4$ receptors (Craig *et al*., 1990) and 5-HT$_4$ receptors mediate the facilitatory effect of lower concentrations of 5-HT on a serosal application (Craig & Clarke, 1991; Costall *et al*., 1993).

The apparent pK_B values obtained in the present study for both ondansetron and granisetron are less than the reported values of 7.3 and 8.1 for ondansetron (Butler *et al*., 1988) and granisetron (Sanger & Nelson, 1989) respectively, in the guinea pig ileum. This is likely to have resulted from a lack of an equilibrium condition which is assumed in such calculations. Many factors might contribute to the non-equilibrium condition resulting in lower pK_B values than expected. For example, there is a possibility that 5-HT released from enterochromaffin cells might complicate the response to mucosally applied exogenous 5-HT; a 5-HT$_4$ receptor-mediated decrease and 5-HT$_3$ receptor-mediated increase in 5-HT release from the enterochromaffin cells have been reported in the guinea pig ileum (Gebauer *et al*., 1993). The presence of a saturable 5-HT uptake mechanism could be another factor. Similarly, it is likely that 5-HT might have non-selective effects at the high concentrations required to stimulate peristalsis in the presence of the 5-HT$_3$ receptor antagonists. Further investigations are required to understand the reason for the lower pK_B values observed in the present study.

The lack of any effect on spontaneous peristalsis of ondansetron or granisetron, either applied mucosally or serosally, indicates that there is no endogenous 5-HT$_3$ receptor-mediated tone in this isolated preparation. The findings also indicate that the 5-HT$_3$ receptor plays a modulatory role rather than a direct involvement in the circuitry of peristalsis itself. Similarly, a modulatory role has been proposed for the 5-HT$_4$ receptor, a stimulation of the receptor following the serosal addition of 5-HT facilitating peristalsis whilst the administration of 5-HT$_4$ receptor antagonists alone fails to modify peristalsis (Craig & Clarke, 1991; Costall *et al*., 1993). The receptor mediating the inhibitory effect of higher concentrations of serosally applied 5-HT is not known, but again, 5-HT probably plays only a modulatory role as the loss of peristalsis due to high concentrations of 5-HT is known to recover in 20–30 min with 5-HT remaining in contact with the tissue (Bülbring & Lin, 1958). It is thus likely that 5-HT has a general modulatory role in peristalsis in the guinea pig ileum.

The ability of a lower concentration of 5-HT applied mucosally to reduce the threshold for peristalsis in the presence of a higher and desensitizing concentration of 5-HT on the serosal side of the ileum confirms the previous findings of Bülbring & Crema (1958). It supports the hypothesis that the mucosal and serosal application of 5-HT stimulates different sites in the ileum (Bülbring & Crema, 1958; Gershon & Tamir, 1981). This is important as the stimulation of 5-HT receptors on a serosal application also results in a facilitation of peristalsis. The ability of serosally applied ondansetron and granisetron to block the serosally applied 5-HT mediated facilitation (in the presence of SB 204070), but their inability to affect the concentration–response curve to mucosally-applied 5-HT, shows that 5-HT$_3$ receptors must exist at at least two sites accessible to mucosal and serosal 5-HT, respectively. The inability of combination of SB 204070 and ondansetron or granisetron applied to the serosal side to alter the facilitatory effect of mucosally applied 5-HT also shows that the facilitatory effect of mucosally applied 5-HT does not involve the stimulation of the 5-HT$_4$ or 5-HT$_3$ receptors on the serosal side, a stimulation of either which can also facilitate peristalsis (Craig & Clarke, 1991; Costall *et al*., 1993; Tuladhar *et al*., 1995).

In summary, the present study indicates that the long reported facilitatory effect of mucosally applied 5-HT on the peristaltic threshold (Bülbring & Crema, 1958) is mediated by a 5-HT$_3$ receptor which is inaccessible to 5-HT added to the serosal side.

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