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Characterization of the 5-hydroxytryptamine receptors mediating contraction in the intestine of *Suncus murinus*

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1 The effects of 5-HT and 5-HT agonists to induce contraction and the 5-HT receptors mediating these effects were investigated in the proximal, central and terminal intestinal segments of *Suncus murinus*.

2 The contraction curves to 5-HT (3 nM – 30 μM) were shifted to the right by methysergide (1 μM) and ritanserin (0.1 μM), without affecting the maximum response.

3 In the central and terminal segments (but not the proximal segments) ondansetron (1 μM) and atropine (1 μM) significantly attenuated the contractions to higher concentrations of 5-HT. The selective 5-HT_4 receptor antagonist SB204070 (1 nM), failed to modify 5-HT induced contractions in any segment examined.

4 5-carboxamidotryptamine, α-methyl-5-HT and 5-methoxytryptamine (0.003 – 3.0 μM) induced contractions but unlike 5-HT, higher concentrations of these three agents failed to increase the response or were associated with a decrease in response. 2-methyl-5-HT (0.03 – 1.0 μM) was ten times less potent than 5-HT to induce contraction but achieved the same maximum response.

5 The contractions induced by the lower concentrations of 2-methyl-5-HT (0.03 – 1.0 μM) in all segments were markedly reduced or abolished by methysergide (1.0 μM); the response to the higher concentrations of 2-methyl-5-HT (3 – 30.0 μM) were markedly reduced by atropine (1.0 μM) and ondansetron (1.0 μM).

6 In all segments examined, tetrodotoxin (1 μM) significantly reduced the 5-HT-induced contraction.

7 It is concluded that the 5-HT-induced contraction was mediated *via* 5-HT_2 (ritanserin sensitive) receptors in all regions of the intestine, with 5-HT_3 (ondansetron sensitive) receptors mediating an additional major component in the central and terminal regions.

Keywords: 5-Hydroxytryptamine; contraction response; 5-HT agonists; 5-HT_2 and 5-HT_3 receptors; *Suncus murinus* intestine

Abbreviations: 5-CT, 5-carboxamidotryptamine; 5-HT, 5-hydroxytryptamine; α-methyl-5-HT, α-methyl-5-hydroxytryptamine; 5-MeOT, 5-methoxytryptamine; TTX, tetrodotoxin

Introduction

Large amounts of 5-HT are present in the gastro-intestinal tract of many species such as the guinea-pig, mouse, rat and dog (Thompson, 1971; Gabella, 1971; Juorio & Gabella, 1974; Robinson & Gershon, 1971; Gershon *et al*., 1965; Thompson, 1966; Feldberg & Toh, 1953; Dalgliesh *et al*., 1953), most of which is present in the enterochromaffin cells of the mucosa (Erspamer, 1954) with smaller amounts being found in the myenteric plexus (Branchek & Gershon, 1987).

5-HT can induce muscle contraction or relaxation responses and enhance secretion in the intestinal tract through a multiplicity of 5-HT receptor subtypes (Buchheit *et al*., 1985b; Chahl, 1983; Fozard, 1985; 1990; Sanger, 1987; Butler *et al*., 1990; Bradley *et al*., 1986; Costall & Naylor, 1990; Dhasmana *et al*., 1993). For example, 5-HT is known to mediate contraction, relaxation or secretory responses in the guinea-pig ileum, rat oesophagus and intestine and human jejunal mucosa *via* 5-HT_2, 5-HT_3, 5-HT_4 and 5-ht_7 receptors (Craig & Clarke, 1990; Baxter *et al*., 1991; Reeves *et al*., 1991; Kellum *et al*., 1994; Beubler & Horina, 1990; Carter *et al*., 1995). Stimulation of 5-HT_3 receptors located on the vagus afferent nerve terminals of the ferret and dog is also considered to trigger the emetic reflex, evoking a reverse peristalsis in response to challenge with chemotherapeutic agents and radiation treatment (Costall *et al*., 1986; Miner & Sanger, 1986; Haga *et al*., 1993; Kamato *et al*., 1991; King & Sanger, 1989; Leo *et al*., 1992). In such studies the cat, dog, pigeon, ferret and primate have been the species most frequently used to establish the site(s) and mechanisms of action of emetogenic and antiemetic drugs (Milano *et al*., 1991; Lucot, 1989; Smith *et al*., 1986; Navarra *et al*., 1992; Sancilio *et al*., 1990; Costall *et al*., 1986; Wilpizeski *et al*., 1987), which inevitably effect gastro-intestinal function.

Recent experiments have also described the value of *Suncus murinus* as a species useful for the study of the actions of many types of emetogenic challenge and including those procedures or drug treatments which effect the 5-HT systems (Ueno *et al*., 1987; Ito, 1995; Okada, 1994). The role of the 5-HT system in moderating gastro-intestinal function in *Suncus murinus* has received little investigation (Muraki *et al*., 1988). The aim of this study was to investigate the potential of 5-HT to induce changes in muscle tension in *Suncus murinus* intestine and to characterize the receptors that mediate the response.

Methods

Animals and housing conditions

The experiments were carried out using adult Japanese House Musk shrew, *Suncus murinus* (Bradford University strain)

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(38–88 g) of either sex. Animals were housed in groups of not more than six in each cage and were allowed food (AQUATIC 3, trout pellets) and water '*ad libitum*'. Animals were also fed with cat food three times per week. The cages were floored with sawdust and cleaned twice a week; water and food were checked daily. The animal room was maintained at a humidity between 45–50% at 24°C and illuminated between 21.00 h to 07.00 h on a reversed light–dark cycle.

Preparation of isolated tissues

Animals were killed by cervical dislocation following a blow to the head. The whole intestine was removed and immediately placed in freshly prepared Krebs' solution (composition mM: NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 10) and gassed with 95% O_2 and 5% CO_2 at room temperature. The mesentery and fatty tissue were removed and the intestine was emptied of its contents by flushing Krebs' solution gently through the intestine using a narrow tipped pipette. The length of the intestine was approximately 25–35 cm in its relaxed form and 10–15 cm in its contracted form. The intestine of the *Suncus murinus* lacks a caecum and it is difficult to distinguish between a duodenum, jejunum, ileum and colon. In order to create a reproducible dissection of specified segments, the intestine was cut into eight segments defined as S1 to S8 respectively of approximately 1 cm length (in contracted form) taken 1–8 cm distal to the pyloric sphincter (S1 to S7) and also 2 cm proximal to the anal region (S8). Segments S1, 2, 3 and 4 (taken 1, 2–3, 3–4 and 4–5 cm from the pyloric sphincter, respectively) are referred to as 'proximal', segments S5, 6 and 7 (taken 5–6, 6–7 and 7–8 cm from the pyloric sphincter) as 'central' and segment S8 as the 'terminal' region of the intestine. In this study, since the profiles of response to 5-HT in the absence and presence of different antagonists were revealed to be similar for segments taken from the four proximal regions of the intestine, only representative data taken from segment S1 are shown. For similar reasons, representative data taken from segment S6 are shown for the three central regions of the intestine.

Tissues were bathed in 10 ml water-jacketed organ baths and placed under 0.5 g tension. The Krebs' solution was maintained at a temperature of 37±0.5°C and gassed continuously with a mixture of 95% O_2 and 5% CO_2. Each tissue was left to equilibrate in the presence or absence of antagonist for 1 h, and washed every 20 min. The resting tension was re-adjusted to 0.5 g when required throughout the experiment. Responses were recorded using isometric Grass transducers which were connected to an Apple Macintosh Computer Performa 630 using MacLab Software 3.5v.

Application of drugs

In preliminary experiments two non-cumulative concentration-response curves to 5-HT were constructed from the same tissue using a 22 min cycle, a 1 min contact time with a 1 h interval between the construction of the response curves and with washing every 15 min. In such studies the magnitude of response to 5-HT changed in the second curve as compared to

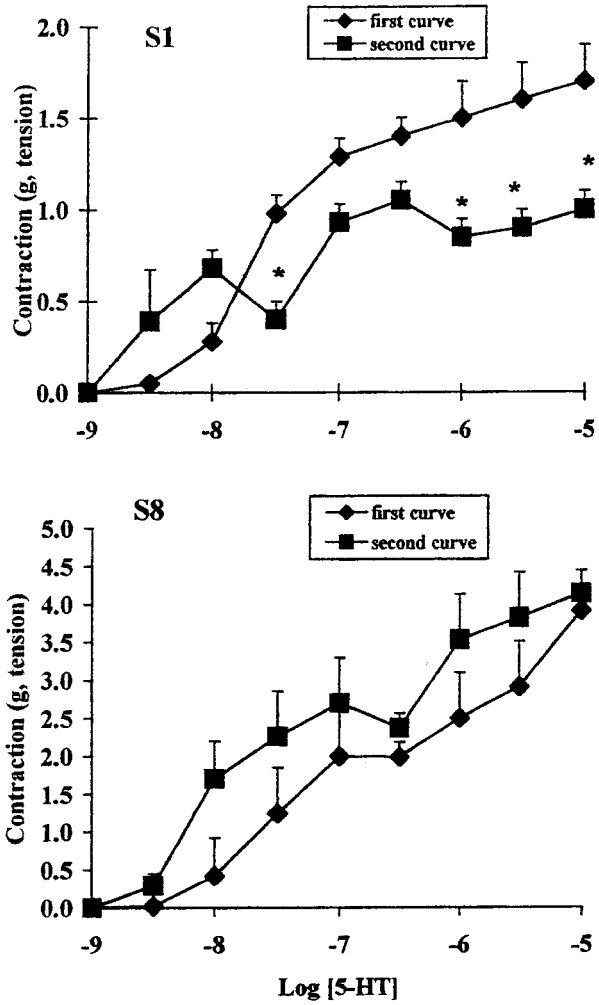

Figure 1 Representative data showing contraction responses to the non-cumulative addition (1 min contact time 22 min cycle) of 5-HT (30 nM–10 μM) in the proximal S1 and terminal S8 segments of *Suncus murinus* intestine. The second curve was constructed in the tissues 60 min after the first. Each point represents the mean± s.e.mean, $n=3$; $^*P<0.05$ taken as significant difference between the two curves.

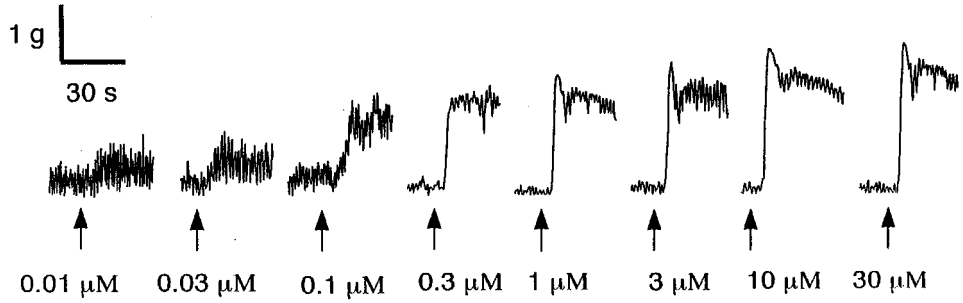

Figure 2 Representative tracing showing the contractile response induced by increasing concentrations of 5-HT (0.3 nM–30 μM) in the isolated proximal segments of *Suncus murinus* intestine.

the first curve. A consistent reduction in the sensitivity of the segments to 5-HT was observed which achieved significance in some tissues and concentrations, with the exception of the most terminal segment (S8). Indeed in segment S8 there was a trend for the contractile response to 5-HT in the second curve to be greater than that in the first curve although it was not statistically significant (Figure 1). These results negated the use of tissues to construct more than a single concentration response curve. Therefore in the subsequent experiments only one concentration-response curve to 5-HT was established in each tissue.

Non-cumulative dose-response curves to 5-HT (1 nM–30 μM) in the absence (control) or presence of antagonists were constructed. The contact time between tissue and agonist was 1 min which was followed by two washings with 1 min between each wash. 5-HT was added at 22 min intervals between doses. Preliminary experiments showed that the interval was sufficient to avoid either tachyphylaxis or enhancement of the response to a subsequent challenge. The addition of agonist did not exceed 0.3% of bath volume. Tissues were left to equilibrate with the antagonists methysergide (1 μM), ritanserin (0.1 μM), ondansetron (1 μM), atropine (1 μM) or SB204070 (1 nM) for 1 h before the application of agonist. The antagonists were constantly present in the organ bath during the construction of the dose-response curves.

A control contraction to KCl (0.12 M) was also established in each tissue. The effect of agonists in the absence or presence of antagonists were also compared as a percentage of the maximum contractions obtained with KCl (0.12 M). Initial analysis using an internal standard (KCl) revealed that expression of the contractions as a percentage of the KCl control made no discernible difference to the results obtained and comparisons made to the absolute values. Hence most data are expressed in absolute values. None of the 5-HT receptor antagonists or atropine showed any effect on the spontaneous activity of the tissues or the contractions induced by KCl in any segment examined.

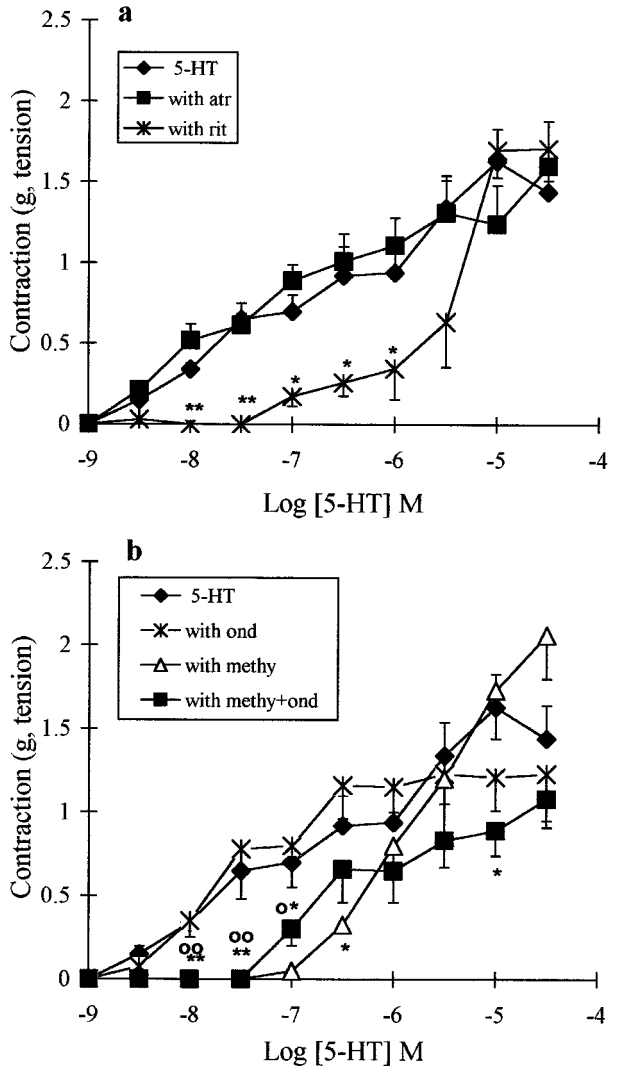

Figure 3 The contractile response to 5-HT (10 nM–30 μM) in the absence and presence of (a) atropine (atr, 1 μM), ritanserin (rit, 0.1 μM), and (b) methysergide (methy, 1 μM), ondansetron (ond, 1 μM) and a combination of methysergide (1 μM) plus ondansetron (1 μM) in proximal segments taken 1 cm distal to the pyloric sphincter of the *Suncus murinus* intestine. Each point represents the mean ± s.e.mean, $n=8$; $^{*}P<0.05$ and $^{**}P<0.01$ compared to the 5-HT control values. $^{\circ}P<0.05$, $^{\circ\circ}P<0.01$ compared to the ondansetron treatment values.

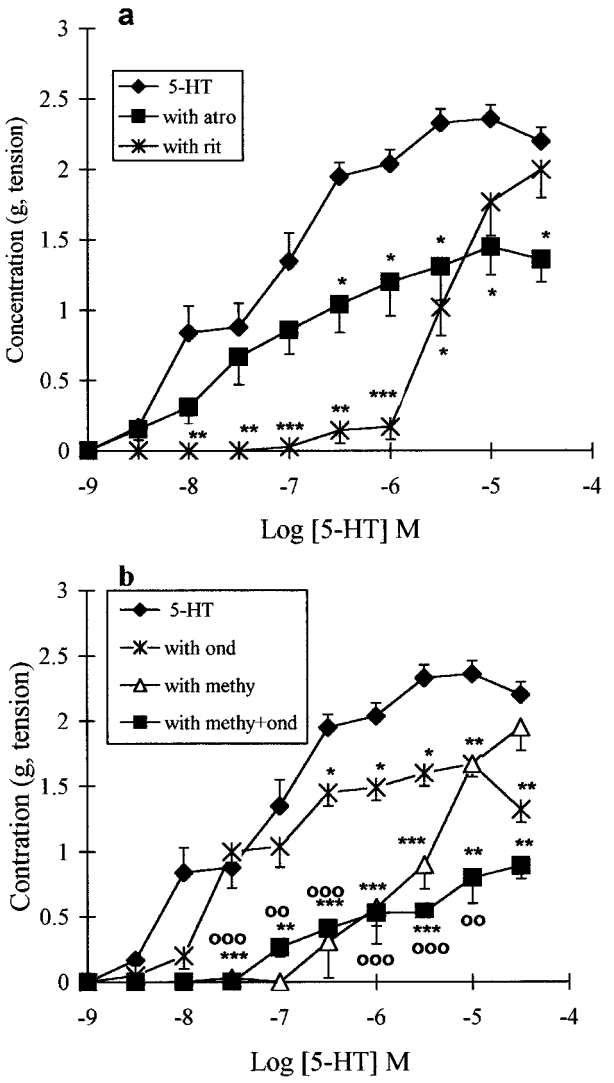

Figure 4 The contractile response to 5-HT (10 nM–30 μM) in the absence and presence of (a) atropine (atro, 1 μM), ritanserin (rit, 0.1 μM), and (b) methysergide (methy, 1 μM), ondansetron (ond, 1 μM) and a combination of methysergide (1 μM) plus ondansetron (1 μM) in the central segments taken 6–7 cm distal to the pyloric sphincter of the *Suncus murinus* intestine. Each point represents the mean ± s.e.mean, $n=8$; $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to the 5-HT control values. $^{\circ\circ}P<0.01$; $^{\circ\circ\circ}P<0.001$ compared to the ondansetron treatment values.

The number of observations is shown by '*n*', which represents the number of animals used.

Experimental design

Four different segments (i.e. S_1 to S_4) of the intestine were taken from any one animal and subjected to one of the following treatments: 5-HT alone and in the presence of ritanserin, methysergide, atropine, ondansetron, a combination of methysergide and ondansetron, SB204070 and a combination of methysergide and SB204070. Also, the response to 2-methyl-5-HT was determined in the absence and presence of methysergide, atropine or ondansetron. The effects of different 5-HT agonists 5-carboxamidotryptamine, 5-methoxytryptamine and α-Methyl-5-HT were also studied. A Latin Square design was used to randomize administrations of the treatments to any one segment.

Analysis of results

Changes in g tension were expressed as either a percentage of the maximal response to KCl (0.12 M) or the mean of the absolute values. The potencies of the 5-HT receptor agonists were expressed as a pEC value and the E_{max} as the percentage response compared to the KCl induced contraction response. The apparent pA_2 values for antagonists were calculated from the Furchgott (1972) equation: $pA_2 = \log (CR-1) - \log [B]$, where CR is the concentration ratio of agonist used in the presence and absence of antagonist (B) and is expressed as $pA_2 \pm$ s.e.mean. The significance of differences between the control and the test responses was determined using analysis of variance (ANOVA) which was followed by Bonfferroni-Dunnett's *t*-test, where *P* values less than 0.05 were taken as significant.

Drugs

5-Hydroxytryptamine maleate (Sigma), 5-carboxamidotryptamine maleate (Research Biochemicals Inc), 5-methoxytryptamine HCl (Sigma), α-methyl-5-hydroxytryptamine (Research Biochemicals Inc), 2-methyl-5-hydroxytryptamine HCl (Tocris), atropine sulphate (Sigma), ondansetron dihydrochloride (Glaxo-Welcome), methysergide maleate (Sandoz), tetrodotoxin (Sigma) and SB204070 hydrochloride (8-amino-7-chloro(N-butyl-4-piperidyl) methylbenzo-1,4-dioxan-5-carboxylate hydrochloride) (Smith Kline Beecham) were dissolved in distilled water; ritanserin (Research Biochemicals Inc) was dissolved in methanol with distilled water being used for further dilutions. Preliminary experiments established that the vehicles used did not show any effect on the tissues.

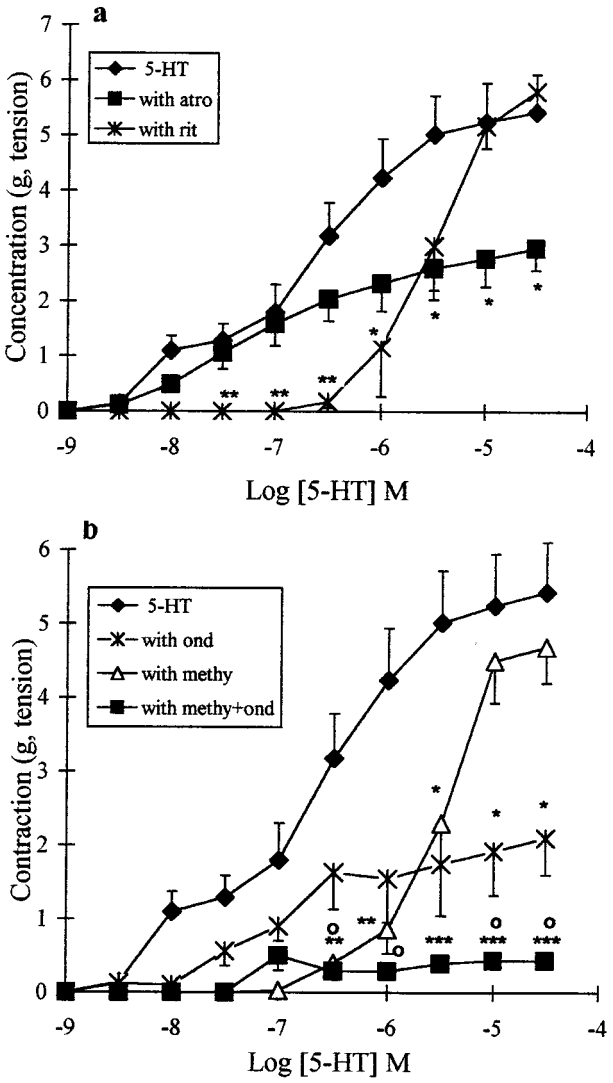

Figure 5 The contractile response to 5-HT (10 nM – 30 μM) in the absence and presence of (a) atropine (atro, 1 μM), ritanserin (rit, 0.1 μM) and (b) methysergide (methy, 1 μM), ondansetron (ond, 1 μM) and a combination of methysergide (1 μM) plus ondansetron (1 μM) in the terminal segments taken 2 cm proximal to the anal region of the *Suncus murinus* intestine. Each point represents the mean ± s.e.mean, $n=8$; $^*P<0.05$, $^{***}P<0.001$ compared to the 5-HT control values. $^\circ P<0.05$ compared to the ondansetron treatment values.

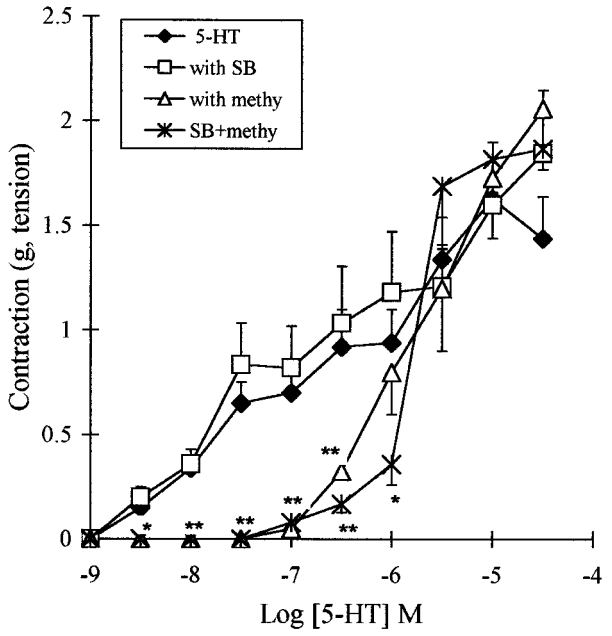

Figure 6 The contractile response to 5-HT (1 nM – 30 μM) in the absence and presence of SB204070 (SB, 1 nM), methysergide (methy, 1 μM) and a combination of methysergide (1 μM) plus SB204070 (1 nM) in the proximal segments taken 1 cm distal to the pyloric sphincter of the *Suncus murinus* intestine. Each point represents the mean ± s.e.mean, $n=8$; $^*P<0.05$ and $^{**}P<0.01$ compared to the 5-HT control values.

Results

General observations

The isolated intestine of *Suncus murinus* showed spontaneous activity under a basal 0.5 g resting tension. The magnitude of the contractions was generally between 0.1–0.8 g and the rate of the spontaneous activity was approximately 25 to 30 cycles per min (Figure 2).

5-HT induced contraction in the intestinal tissues

The non-cumulative addition of 5-HT (10 nM–30 μM) produced concentration-dependent contraction-response curves in all segments. The contractile responses to 5-HT at concentrations below 0.3 μM developed slowly, higher concentrations produced a rapid contraction which was followed by a contraction maintained at a slightly lower level (Figure 2).

The maximum tension changes produced by 5-HT were in the range of 1.5–2.5 g in tissues taken from the proximal and central segments of the intestine; a 5.4±0.7 g maximum tension change was obtained in the terminal segments (Figures 3–5).

In experiments designed to determine whether tachyphylaxis developed over the time period required to construct the non-cumulative concentration-response curve to 5-HT, the repeated application of 5-HT (1 μM) at 22 min intervals produced reproducible responses over a 4 h period. 5-HT did not produce relaxation in any of the tissues examined.

The ability of the 5-HT receptor antagonists and atropine to modify 5-HT induced contractions

In tissues taken from all three regions of the intestinal tract, methysergide (1 μM) and ritanserin (0.1 μM) abolished or significantly ($P<0.05$) reduced the contractions induced by concentrations of 5-HT lower than 1 μM ($F_{(3,28)}$ ratios were in the range of 3.9–16.2 with P values of 0.0331–0.0017 for methysergide; $F_{(2,21)}$ ratios were in the range of 5.7–11.3 with P values of 0.0003–0.0048 for ritanserin). In the presence of methysergide or ritanserin higher concentrations of 5-HT induced contractions where the maximum response was comparable to that obtained in control tissues (Figures 3–5). Estimated pA_2 values for methysergide and ritanserin to antagonise the effects of 5-HT in tissues taken from the three regions of the intestine were in the range of 7.78+0.03–8.3+0.1 and 8.8+0.2–9.3+0.2, respectively.

In tissues taken from the proximal region of the intestine, (i.e. S_1 segments) atropine (1 μM) and ondansetron (1 μM) alone had no consistent actions to modify the 5-HT-induced contractions (Figure 3). However, in tissues taken from the central and terminal regions, both atropine and ondansetron

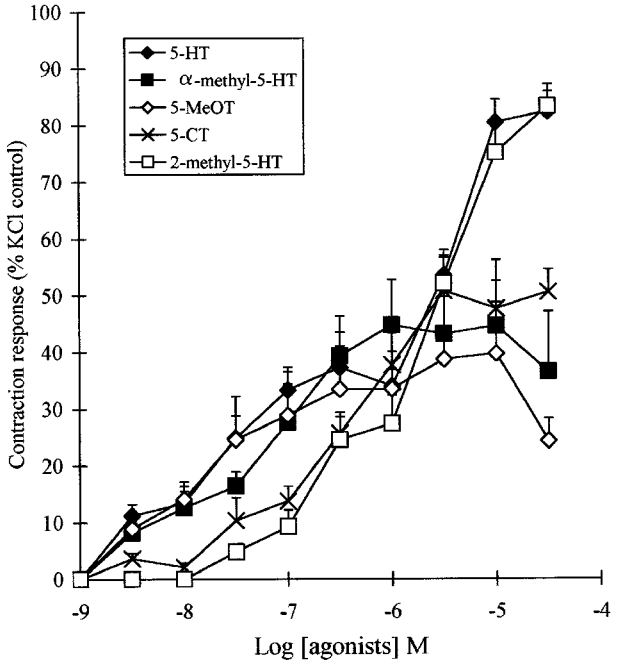

Figure 7 The contractile effect of 5-HT, α-methyl-5-HT, 5-methoxytryptamine (5-MeOT), 2-methyl-5-HT and 5-carboxamidotryptamine (5-CT) (30 nM–30 uM) on central segments taken 6–7 cm distal to the pyloric sphincter from the *Suncus murinus* intestine. Responses are expressed as a percentage of the KCl (0.12 M) induced contraction. Each point represents the mean±s.e.mean, $n=6$.

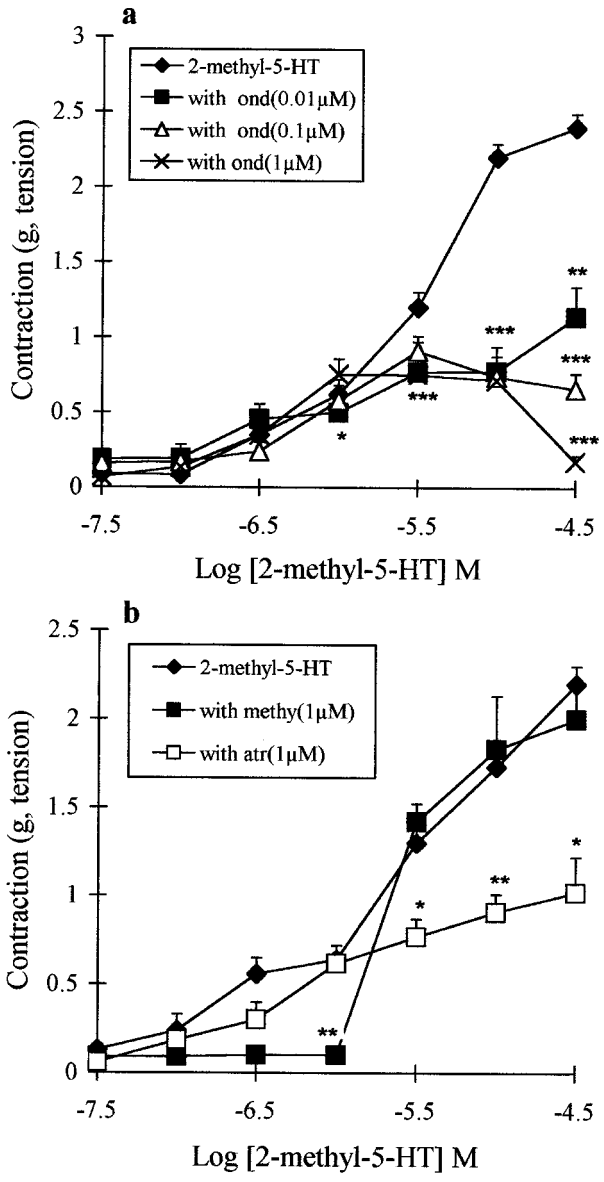

Figure 8 The contractile response to 2-methyl-5-HT (30 nM–30 μM) in the absence and presence of (a) ondansetron (ond, 0.01, 0.1 and 1 μM), and (b) atropine (atr, 1 μM) and methysergide (methy, 1 μM) in the central segments taken 6–7 cm distal to the pyloric sphincter of the *Suncus murinus* intestine; Each point represents the mean± s.e.mean, $n=8$; $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to the 2-methyl-5-HT control values.

caused a significant reduction in the contractions induced by concentrations of 5-HT greater than 1 μM ($F_{(3,32)}$ ratios were in the range of 10.5–23.4 with P values of 0.0001 for ondansetron; $F_{(2,24)}$ ratios were in the range of 4.9–26.1 with P values of 0.0001–0.0166 for atropine) (Figures 4 and 5). This reduction was significantly enhanced by the combined administration of methysergide with ondansetron in the central and terminal regions ($F_{(3,28)}$ ratios were in the range of 10.5–21.2 with P values of 0.0001 for the central segments; $F_{(3,28)}$ ratios were in the range of 12.0–23.4 with P values of 0.0001–0.03 for the terminal segments) (Figures 4 and 5). Furthermore, there was also a trend for the combined methysergide-ondansetron treatment to reduce the contractile response to the higher concentrations of 5-HT in the proximal segment (Figure 3).

SB204070 administered alone failed to modify 5-HT induced contractions in any tissue ($F_{(3,28)}$ ratios were in the range of 1.2–16.7 with P values of 0.10–0.97). When SB204070 was administered with methysergide, the antagonism afforded was indistinguishable from that obtained to methysergide alone. Representative data obtained from the proximal segments are shown in Figure 6.

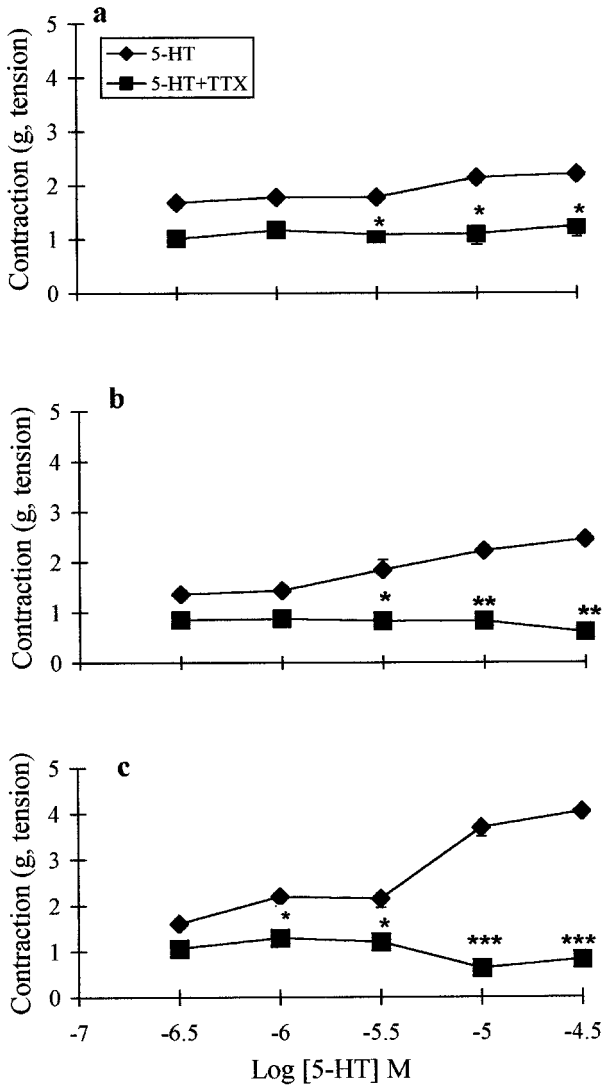

Figure 9 The contractile effect of 5-HT (0.3 μM–30 μM) in the absence and presence of TTX (1 μM) on the (a) proximal segments (1 cm distal to the pyloric sphincter), (b) central segments (6–7 cm distal to the pyloric sphincter) and (c) terminal segments (2 cm proximal to the anal region) of the *Suncus murinus* intestine. Each point represents the mean ± s.e.mean, $n=7$; *$P<0.05$, **$P<0.01$ and ***$P<0.001$ compared to the 5-HT control values.

The effect of 5-HT receptor agonists to modify intestinal muscle tension

The 5-HT agonists 2-methyl-5-HT, 5-carboxamidotryptamine, 5-methoxytryptamine and α-methyl-5-HT (3 nM–30 μM) induced concentration-dependent contractions in all tissues examined. Representative data obtained in the central segments are shown in Figure 7. There was no evidence of a relaxant response with any of the agonists tested. In all the segments examined, 5-HT produced its contractile response with a mean pEC_{50} of 6.2 ± 0.1 and E_{max} of 81.02 ± 2.5% of the KCl-induced contraction. 5-methoxytryptamine (pEC_{50} = 7.3 ± 0.1, E_{max} = 35.6 ± 4.2%) and α-methyl-5-HT (pEC_{50} = 7.4 ± 0.1, E_{max} = 21.2 ± 3.4%) over a range of 3 nM–3.0 μM produced similar contraction responses, although the higher concentrations failed to achieve the maximum response attained by 5-HT. 5-carboxamidotryptamine (pEC_{50} = 6.2 ± 0.1, E_{max} = 51.4 ± 3.6%) and 2-methyl-5-HT (pEC_{50} = 5.8 ± 0.1, E_{max} = 82.5 ± 1.8%) (0.03–1.0 μM) were approximately ten times less potent but whereas 5-carboxamidotryptamine failed to produce the same maximum response as 5-HT, this was achieved using 2-methyl-5-HT in all the segments examined.

The effect of 5-HT receptor antagonists to inhibit the contractions induced by 2-methyl-5-HT

Neither atropine (1 μM) nor ondansetron (0.01, 0.1, and 1 μM) reduced the contractions induced by the 1 μM and lower concentrations of 2-methyl-5-HT. Both atropine and ondansetron caused a non-surmountable reduction in the contractions induced by concentrations of 2-methyl-5-HT higher than 1 μM ($F_{(3,\ 28)}$ ratios were in the range of 2.6–65.6 with P values of 0.0001–0.05 for ondansetron; $F_{(2,\ 21)}$ ratios were in the range of 3.4–18.7 with P values of 0.0016–0.05 for atropine). In contrast, methysergide (1 μM) significantly ($P<0.05$) antagonized the contractions induced by concentrations of 2-methyl-5-HT lower but not higher than 1.0 μM ($F_{(2,\ 21)}$ ratios were in the range of 7.1–18.7 with P values of 0.0001–0.0044 for methysergide). These profiles of action were recorded in proximal, central and terminal segments; representative data from the proximal segments are shown in Figure 8.

The effect of tetrodotoxin (TTX) to modify the contractile response to 5-HT

TTX (1 μM) significantly reduced by approximately 40% the contractions induced by lower concentrations of 5-HT (0.03–3.0 μM) in the proximal, central and terminal regions of the intestine ($F_{(1,\ 12)}$ ratios were in the range of 8.5–300.9 with P values of 0.0001–0.01) (Figure 9). However, the contractions to higher concentrations of 5-HT (10 and 30 μM) were maximally reduced by approximately 45, 80 and 85 in the proximal, central and terminal regions of the intestine respectively.

Discussion

This study has shown that the non-cumulative addition of 5-HT induced concentration related contraction responses in all segments of the *Suncus murinus* intestine examined, relaxation was never observed. A single response curve was constructed in

each tissue since attempts to use the tissues to construct a second curve were compromised by an increase in response to 5-HT. The mechanisms mediating the contraction response to 5-HT were shown to be complex, involving both tetrodotoxin sensitive and insensitive components, and different 5-HT receptors.

The antagonism afforded by methysergide and ritanserin of the contractions induced by concentrations of 5-HT lower than one micromolar in all the segments suggests that the effects of 5-HT are mediated *via* 5-HT_2 receptors (Bradley *et al*., 1986; Zifa & Fillion, 1992). 5-HT_2 receptors have previously been shown to mediate 5-HT induced contractions in the guinea-pig ileum and colon and the rat stomach fundus (Adam-Vizi & Vizi, 1978; Costa & Furness, 1979; Engel *et al*., 1984; Eglen *et al*., 1992). However, the concentrations of 5-HT required to induce contractions in the intestine of *Suncus murimus* were lower than those required to induce 5-HT_2 mediated contractions in the guinea-pig ileum (greater than three micromolar) (Buchheit *et al*., 1985b). Also, they were lower than required in binding and functional assays, where 5-HT has micromolar affinity for the 5-HT_2 antagonist recognition sites (Zifa & Fillion, 1992; Muramatsu *et al*., 1988; Chahl, 1983). Whether or not the 5-HT_2 receptors in *Suncus murinus* intestine are linked to a more effective transduction mechanism is not known, and elucidation of the 5-HT_2 receptor subtype mediating the response will require further experiments using more selective 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor antagonists (Baxter *et al*., 1995).

Ondansetron when administered alone in a concentration known to cause a highly specific and selective blockade of 5-HT_3 receptors (Butler *et al*., 1990) failed to modify the contractions induced by 5-HT in the proximal segments (one to four centimeters distal to the pyloric sphincter) of the *Suncus murinus* intestine. It could therefore be concluded that 5-HT_3 receptors have no involvement in mediating the contractile actions of either high or low concentrations of 5-HT in the proximal segments of the *Suncus murinus* intestine. However, this conclusion may be premature, since there was a trend for a combined treatment with methysergide and ondansetron to cause a greater antagonism of the contractile actions of micromolar concentrations of 5-HT than observed to methysergide alone. In addition, the contractile effect obtained by concentrations of 2-methyl-5-HT higher than one micromolar and shown to stimulate 5-HT_3 receptors (Buchheit *et al*., 1985b; Clarke *et al*., 1989) was antagonized by ondansetron, suggesting the presence of 5-HT_3 receptors in the proximal segments to mediate a contraction response (Ismaiel *et al*., 1990; Wilson *et al*., 1990). Atropine also reduced the contraction induced by concentrations of 2-methyl-5-HT higher than one micromolar, providing further support for the involvement of 5-HT_3 receptors mediating the release of acetylcholine as has been reported from other species (Bianchi *et al*., 1970; Buchheit *et al*., 1985a).

It remains more likely that in the proximal tissues of the *Suncus murinus* intestine the 5-HT_3 receptors play a minor role in mediating the contractile effects of 5-HT. This contrasts with data obtained in the guinea-pig where 5-HT_3 receptors in the intestine mediate a major contraction response that is sensitive to 5-HT_3 receptor blockade (Costa & Furness, 1979; Buchheit *et al*., 1985b). In the guinea-pig ileum, a 5-HT_3 receptor-mediated depolarization of cholinergic neuronal terminals causes a release of acetylcholine to induce a contraction response which is antagonized by atropine (Bianchi *et al*., 1970; Buchheit *et al*., 1985a; Cohen *et al*., 1985; Fozard, 1990; Fox & Morton, 1989; 1990). It is relevant that in the proximal segments of the *Suncus murinus* intestine, atropine failed to antagonize 5-HT induced contractions, providing further support for a minor involvement of 5-HT_3 receptors to mediate a cholinergic response. The presence of a limited neuronal involvement in the proximal segment also came from the modest actions of TTX to attenuate the contraction induced by concentrations of 5-HT higher than one micromolar.

The necessity for the use of a combined 5-HT_2 and 5-HT_3 receptor blockade to indicate a 5-HT_3 receptor involvement in 5-HT induced contraction responses in the proximal segments, contrasts with the data obtained in the central and terminal segments. When using micromolar concentrations of 5-HT appropriate for 5-HT_3 receptor stimulation (Buchheit *et al*., 1985b; Butler *et al*., 1988), both ondansetron and atropine when administered alone antagonized the 5-HT induced contractions in the central and terminal segments of *Suncus murinus* intestine. In both the central and terminal segments examined, the antagonism afforded by ondansetron appeared non-competitive, the maximum response being reduced to approximately 50% of the control values. The apparent non-competitive antagonism afforded by 5-HT_3 receptor antagonists is well established (Woollard *et al*., 1994; Sanger, 1987), although ondansetron normally exerts a competitive antagonism (Butler *et al*., 1988; 1990). The data remains strongly supportive that a significant component of the 5-HT induced contraction in the central and terminal segments of *Suncus murinus* is induced *via* a 5-HT_3 receptor stimulation involving an enhanced cholinergic function (Fox & Morton, 1989). A significant component of the residual contraction reflected activation of the 5-HT_2 receptor, since a combined methysergide plus ondansetron treatment further reduced the contraction as compared to the ondansetron treatment alone. Further evidence for the involvement of 5-HT_3 receptors in mediating a contractile response came from the antagonism by ondansetron and atropine of the responses induced by 2-methyl-5-HT in the central and terminal regions, similar to the antagonism observed in the proximal region of the intestine.

2-methyl-5-HT was used as a ligand with a modest selectivity and affinity for 5-HT_3 receptors and a lower affinity for 5-HT_2, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1E} receptor sites (Ismaiel *et al*., 1990; Wilson *et al*., 1990; Zifa & Fillion, 1992). In the present study, methysergide was able to antagonize the contractions induced by concentrations of 2-methyl-5-HT lower than one micromolar. The results may reflect the known affinity of 2-methyl-5-HT for 5-HT_2 receptors (Zifa & Fillion, 1992). The possibility that in *Suncus murinus* 2-methyl-5-HT has an unusual high affinity for 5-HT receptors other than 5-HT_3 receptors remains to be investigated.

The 5-HT_4 receptor has an established role to mediate contraction responses *via* a cholinergic mechanism in the guinea-pig intestine and colon (Craig & Clarke, 1990; Elswood *et al*., 1991). SB204070 is a highly selective and specific 5-HT_4 receptor antagonist (Wardle *et al*., 1994) which failed when administered alone to attenuate the contractile effects of any concentration of 5-HT in any of the *Suncus murinus* tissues examined. Also, when SB204070 was administered with methysergide, the antagonism afforded was indistinguishable from that afforded by methysergide alone in any segment. Therefore it is unlikely that 5-HT_4 receptors are involved in mediating the contraction response to 5-HT. Further evidence for the unlikely involvement of 5-HT_4 receptors in mediating a contraction response came from the failure of GR125487D, another very potent 5-HT_4 receptor antagonist (Gale *et al*., 1993), to antagonize the response to 5-HT in any of segments taken from *Suncus murinus* intestine (unpublished observations).

The contractile effect of 5-HT was mimicked by a number of indole 5-HT agonists. The agonist potency values in the present study are in general agreement with those recorded by McLean & Coupar (1996) studying a contraction response in the rat jejunum; 5-HT (6.94 ± 0.19), 5-methoxytryptamine (6.95 ± 0.21), α-methyl-5-HT (6.84 ± 0.44) and 5-carboxamidotryptamine (7.56 ± 0.47). 5-carboxamidotryptamine appeared to be less potent in *Suncus murinus* intestine. The potency of 5-methoxytryptamine and 2-methyl-5-HT in the present study is in good agreement with that in guinea-pig ileum (5-methoxytryptamine (7.4, 6.7), 2-methyl-5-HT (5.9) Buchheit *et al*., 1991; 1985b; Fozard, 1990). Further experiments are required to investigate the affinity of these agonists for a particular 5-HT receptor type or subtype in *Suncus murinus* intestine.

The attenuation of the contractile response to 5-HT by TTX suggests the existence of a neuronal mechanism to mediate 5-HT induced contractions. The involvement of a non-neuronal mechanism in response to 5-HT is also apparent since TTX failed to abolish the response completely. The residual response is likely to be a direct effect of 5-HT on the muscle in the isolated segments. Furthermore, the greater attenuation afforded by TTX of the response in the central and terminal segments to 5-HT at concentrations higher than three micromolar may suggest the greater contribution of neuronal over non-neuronal mechanism/s in mediating 5-HT induced contractions than in the proximal region of the intestine. However, the profile of attenuation of response afforded by TTX to 5-HT at concentrations lower than three micromolar appeared to be similar in all the regions of the intestine.

In the present study the contractions induced by 5-HT appeared to be sensitive to the antagonism afforded by methysergide and ritanserin suggesting a dominant role for 5-HT_2 in mediating 5-HT induced contraction in all sections of the intestine of *Suncus murinus*. However, both methysergide and ritanserin have recently been shown to have moderate affinity (7.1 – 7.9, 7.1 – 7.7, respectively; Eglen *et al*., 1997) for 5-HT_7 receptors which relates closely to the apparent affinity values of methysergide (7.78 ± 0.03 to 8.3 ± 0.1) and ritanserin (8.8 ± 0.2 to 9.3 ± 0.1) shown in the present study. The development of more selective 5-HT_7 receptor antagonists will be required to investigate the role of 5-HT_7 receptors in mediating contraction responses in the *Suncus murinus* intestine.

It is concluded that the contractile response to 5-HT involves 5-HT_2 receptors which play a dominant non-neuronal role in mediating the contractile actions in the proximal, central and terminal regions of the intestine of *Suncus murinus*. 5-HT_3 receptors play a relatively minor role in the proximal region and, with 5-HT_2 receptors, an important neuronal role in the central and distal regions.

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