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Characterisation of 5-HT_2 receptor subtypes in the *Suncus murinus* intestine

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Abstract

The involvement of 5-HT_2 receptor subtypes in mediating a contraction response in the isolated intestine of *Suncus murinus* was investigated using DOI ((±)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane, a 5-HT_2 receptor agonist) which produced a bell-shaped concentration response curve that was significantly ($p < 0.05$) reduced by methysergide (a 5-$HT_{1/2}$ receptor antagonist, 1 μM) but not ketanserin (a 5-HT_{2A} receptor antagonist, 1 μM), yohimbine (a 5-HT_{2B} receptor antagonist, 1 μM) or a combination of ondansetron (a 5-HT_3 receptor antagonist, 1 μM) plus SB204070 (8-amino-7-chloro(*N*-butyl-4-piperidyl) methylbenzo-1,4-dioxan-5-carboxylate hydrochloride, a 5-HT_4 receptor antagonist, 1 nM). The contraction response to the lower concentrations of DOI (10 nM–0.3 μM) was reduced in the presence of SB206553 (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole, a 5-$HT_{2B/2C}$ receptor antagonist, 1 μM), whilst conversely, the reducing response to the higher concentrations of DOI (1–30 μM) was prevented. A repeated challenge with 3 μM DOI produced a smaller response (desensitisation) and also reduced the response to 5-HT (5-hydroxytryptamine, 0.3 μM) that was inhibited by SB206553 (1 μM). Data indicate that 5-HT_{2C} receptors are likely candidates to mediate the contractile response to DOI and demonstrate desensitisation to repeated challenges.

Keywords: 5-HT_2 receptor; Desensitisation; Intestine; *Suncus murinus*

1. Introduction

Current knowledge, based on functional, structural and transductional data, provides evidence for the existence of three receptor subtypes for the 5-HT_2 receptor family; 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors (Martin, 1998). The latter receptor was previously termed the 5-HT_{1C} receptor before its structural similarity to the 5-HT_2 family members was recognised (Hoyer et al., 1994).

All three subtypes are thought to be linked to the phosphoinositol hydrolysis signal transduction system via the α subunit of the Gq GTP binding protein (Hoyer et al., 1994). However, there is evidence in the human pulmonary artery endothelial cells and rat stomach fundus, that 5-HT_{2B} receptor stimulation causes intracellular calcium release via a mechanism independent of phosphatidylinositol hydrolysis (Cox and Cohen, 1996; Ullmer et al., 1996). 5-HT_{2A} receptors are widely distributed in the peripheral tissues where it mediates a contractile response in many vascular, urinary, gastrointestinal and uterine smooth muscle preparations, platelet aggregation and increased capillary permeability in both rodent and human tissues (Hoyer et al., 1994). The 5-HT_{2B} mRNA transcript has been identified in the rat and guinea-pig colon and small intestine and also in the rat stomach fundus, where it may mediate a contraction response (Forguet et al., 1992; Wainscott et al., 1993; Choi and Maroteaux, 1996). Furthermore, the endothelium dependent relaxation of the rat and cat jugular veins and possibly of the pig pulmonary artery via nitric oxide release is attributed to the activation of the 5-HT_{2B} receptor (Baxter et al., 1995).

The presence of 5-HT_{2C} receptors has been shown to have the highest density in the choroid plexus and at a lower level in the cerebral cortex, hippocampus, striatum and substansia nigra of rat with a similar distribution in man (Hoyer et al., 1994). There is, at present, no evidence of the existence of this receptor or its mRNA in peripheral tissues (Hoyer et al., 1994). Indeed, the pharmacological congruity of both 5-HT_{2B} and 5-HT_{2C} receptor subtypes is so strong that it is conceivable that disorders (e.g., migraine) and functions such as vasodilatation in the periph-

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eral blood vessels previously thought to involve 5-HT_{2C} receptors may actually involve the 5-HT_{2B} receptor subtype (Bodelsson et al., 1992; Kalkman, 1994; Ellis et al., 1995).

Recently, the availability of more selective antagonists has provided a better tool to discriminate between the 5-HT_2 receptor subtypes (Baxter et al., 1995). In previous studies we have shown that in the *Suncus murinus* intestine, 5-HT_2 receptors play a major role in mediating a contractile response to 5-HT. In the present study using DOI ((±)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane) as a 5-HT_2 receptor agonist and different 5-HT_2 receptor antagonists, attempts were made to characterise further the 5-HT_2 receptor subtypes in the *S. murinus* intestine mediating a contraction response.

2. Methods

2.1. Animals and housing conditions

The experiments were carried out using adult female (38–46 g) and male (62–88 g) Japanese House Musk shrew, *S. murinus* (Bradford University strain). 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 floor of the cages were covered with sawdust and cleaned twice a week; water and food were checked daily. The animal room was maintained at a humidity between 45 to 50% at 24°C and illuminated between 2100 and 0700 on a reversed light–dark cycle.

2.2. 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 *S. 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 to 8 cm distal to the pyloric sphincter (S1 to S7) and also 2 cm proximal to the anal region (S8). Segments S1, S2, S3, S4 (taken 1, 2–3, 3–4 and 4–5 cm from the pyloric sphincter, respectively) are referred to as 'proximal', segments S5, S6 and S7 (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 DOI and 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. Also, representative data are shown when similar profiles of response were obtained in all 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.

2.3. Application of drugs

In our previous study, preliminary experiments had revealed a change in the sensitivity of the segments challenged with 5-HT when attempts were made to establish two non-cumulative concentration–response curves to 5-HT from the same tissue (Javid and Naylor, 1999). The results negated the use of tissues to construct more than a single concentration response curve. Therefore, in this study only one concentration–response curve to DOI was established in each tissue.

Non-cumulative dose–response curves to DOI (10 nM–30 μM), 5-HT (10 nM–30 μM) and α-methyl-5-HT (10 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. DOI was added at 22 min intervals between doses. The addition of agonist did not exceed 0.3% of bath volume. Tissues were left to equilibrate with the antagonists methysergide (1 μM), ketanserin (1 μM), yohimbine (1 μM), SB206553 (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole) (0.01, 0.1 and 1 μM), a combination of ondansetron (1 μM) plus SB204070 (8-amino-7-chloro(*N*-butyl-4-piperidyl) methylbenzo-1,4-dioxan-5-carboxylate hydrochloride) (1 nM) or a combination of methysergide (1 μM) plus SB206553 (1 μM), 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.

The effect of SB206553 (1 μM) on the contractile response to 5-HT and α-methyl-5-HT (1 nM–30 μM) was also investigated.

Also, a series of experiments was carried out to investigate whether tachyphylaxis occurs in segments challenged with a repeated administration of DOI. Segments were set up and challenged with three successive additions of 3 μM DOI; each concentration was in contact with the tissues for 1 min and this was followed by two washings. A 20 min drug cycle was applied. Similar protocols were used in which segments were challenged with three additions of 3 μM DOI in the presence of SB206553 (1 μM).

A further series of experiments was carried out to investigate whether tachyphylaxis which was shown to occur in segments challenged with a repeated addition of DOI would also affect the contractile response to 5-HT. Segments were set up and two concentrations of 5-HT (0.3 and 10 μM) were added and taken as the control responses. Each concentration was in contact with the tissues for 1 min which was followed by 2 washings and a 20 min drug cycle. Tissues were then challenged with three additions of 3 μM DOI with the same drug cycle and 2 washings which was followed after 1 min by the addition of DOI. After establishing tachyphylaxis to DOI, attempts were made to re-establish the contractile response to 5-HT (0.3 and 10 μM). In another set of experiments, the same procedure was repeated in the presence of SB206553 (1 μM).

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 was also compared as a percentage of the maximum contractions obtained with KCl (0.12 M). None of the DOI receptor antagonists showed any effect on the spontaneous activity of the tissues or the contractions induced by KCl in any segment examined.

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

2.4. Experimental design

Segments (i.e., S_1 to S_8) of the intestine were taken from any one animal and subjected to one of the following treatments: DOI alone and in the presence of methysergide, a combination of ondansetron and SB204070, ketanserin, yohimbine and SB206553. Also the responses to 5-HT and α-Methyl-5-HT were determined in the absence and presence of SB206553. The effects of pre-treatment with DOI on the responses to 5-HT in the absence and presence of SB206553 were also studied. A Latin Square design was used to randomise administrations of the treatments to any one segment.

2.5. 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 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.

2.6. Drugs

5-Hydroxytryptamine maleate (Sigma), DOI(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (Sigma), α-methyl-5-hydroxytryptamine (Research Biochemicals), ondansetron dihydrochloride (Glaxo-Welcome), methysergide maleate (Sandoz), tetrodotoxin (Sigma), SB204070 hydrochloride (8-amino-7-chloro(*N*-butyl-4-piperidyl) methylbenzo-1,4-dioxan-5-carboxylate hydrochloride) (Smith Kline Beecham), yohimbine hydrochloride (Sigma), were dissolved in distilled water; SB206553 hydrochloride (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole) (Smith Kline Beecham) was dissolved in 50% dimethyl sulfoxide plus 50% distilled and water being used for further dilutions. Preliminary experiments established that the vehicles used did not show any effect on the tissues.

3. Results

3.1. The contractile effects of DOI and the modification of response by 5-HT receptor antagonists

In all segments, DOI at a concentration range of 10 nM to 1 μM produced a concentration-dependent contraction. However, at higher concentrations of DOI (> 1 μM) the contraction clearly decreased and at concentrations of 10 and 30 μM DOI failed to produce a contraction response. Indeed in some segments a small relaxation or reduction of spontaneous activity was obtained. A representative tracing taken from a proximal segment is shown in Fig. 1 and quantified in Fig. 2.

The maximum tension changes produced by DOI at concentrations of 0.3–1 μM were similar at 0.81 ± 0.1, 0.93 ± 0.1 and 0.63 ± 0.07 g in the proximal, central and terminal regions of the intestine, respectively.

In the presence of methysergide (1 μM), the response to DOI (30 nM–3 μM) was abolished or significantly reduced ($F(2,24)$ ratios were in the range of 70.3–9.6 with *P* values of 0.001–0.0009) (Fig. 2). A combination of ondansetron (1 μM) plus SB204070 (1 nM) failed to modify the responses to DOI. Ketanserin (1 μM) failed to antagonise the contraction response induced by the lower concentrations of DOI (30 nM–3 μM) with a trend to attenuate the decline in contraction induced by the higher concentrations of DOI; at some concentrations this achieved

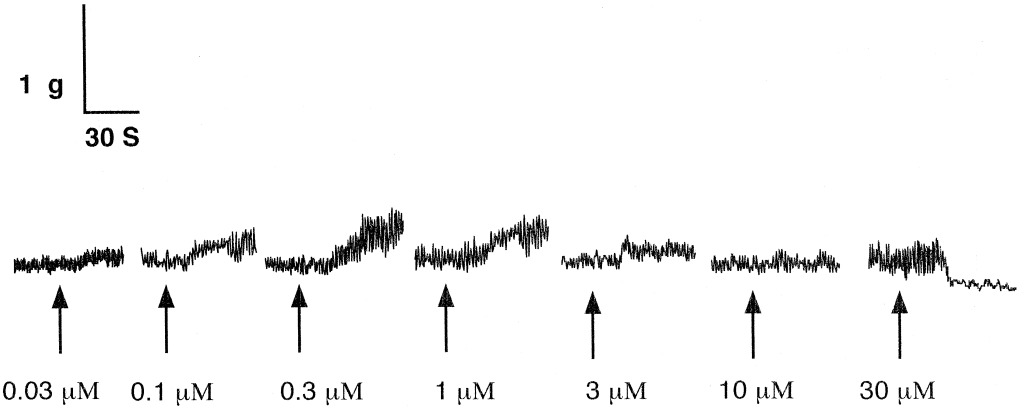

Fig. 1. Representative tracing showing the responses induced by increasing concentrations of DOI in an isolated proximal segment of *S. murinus* intestine; an increasing contraction, decreasing effect and finally relaxation.

significance ($F(2,24)$ ratios were in the range of 6–4 with P values of 0.01–0.03) (Fig. 2). This profile of response was also recorded in the presence of yohimbine ($F(2,24)$ ratios were in the range of 4.6–6 with P values of 0.02–0.0036) (Fig. 2).

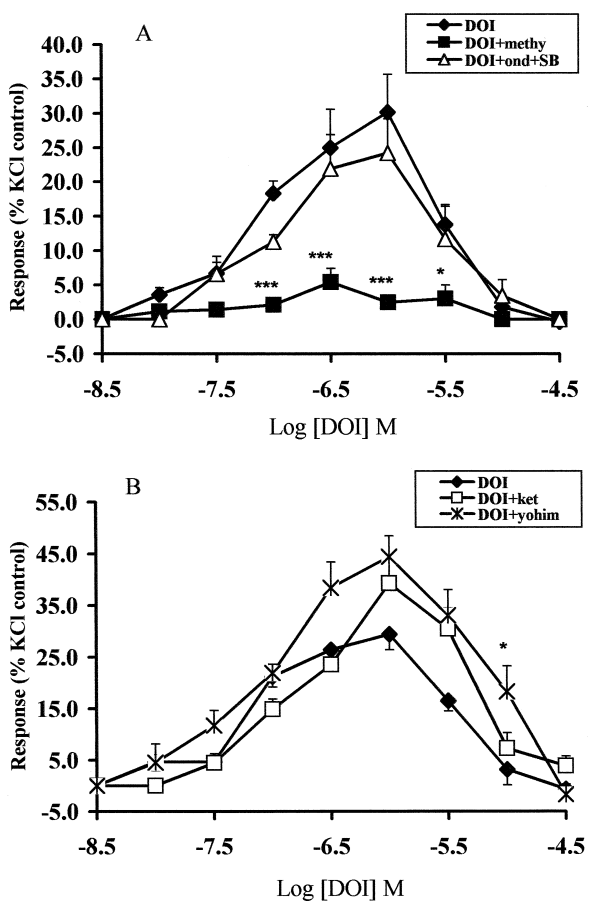

Fig. 2. The contractile effect of DOI (10 nM–30 μM) in the absence and presence of (A) methysergide (methy, 1 μM) and a combination of ondansetron (ond, 1 μM) plus SB204070 (SB, 1 nM) and (B) ketanserin (ket, 1 μM) or yohimbine (yohim, 1 μM) on segments taken 1 cm (S1) distal to the pyloric sphincter of the *S. murinus* intestine. Each point represents the mean ± s.e.m.; $n = 9$. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the DOI control values.

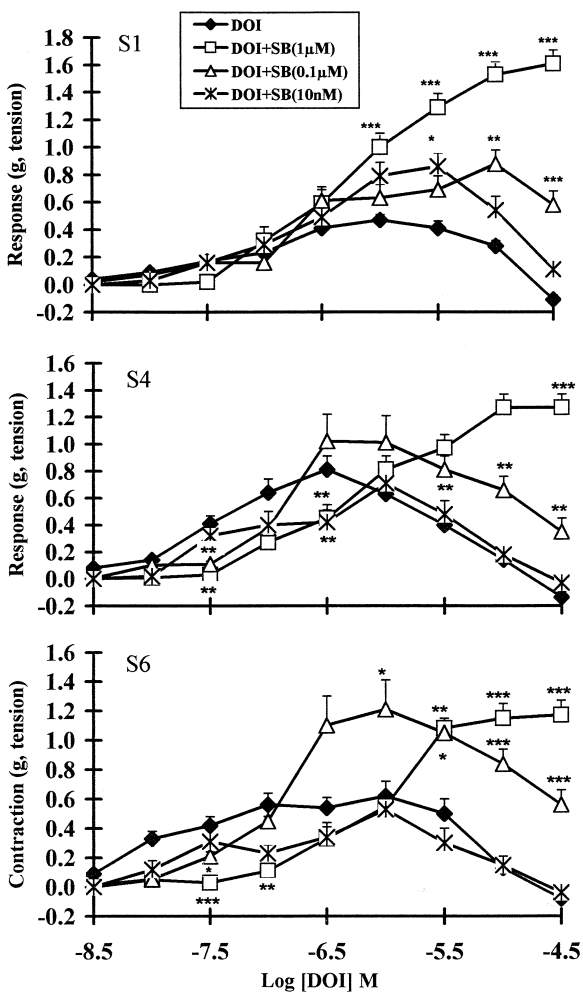

Fig. 3. The contractile effect of DOI (30 nM–30 μM) in the absence and presence of SB206553 (SB, 0.01, 0.1 and 1 μM) in segments taken 1 cm (S1), 4–5 cm (S4) and 6–7 cm (S6) distal to the pyloric sphincter of the *S. murinus* intestine. Each point represents the mean ± s.e.m.; $n = 8$. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the DOI control values.

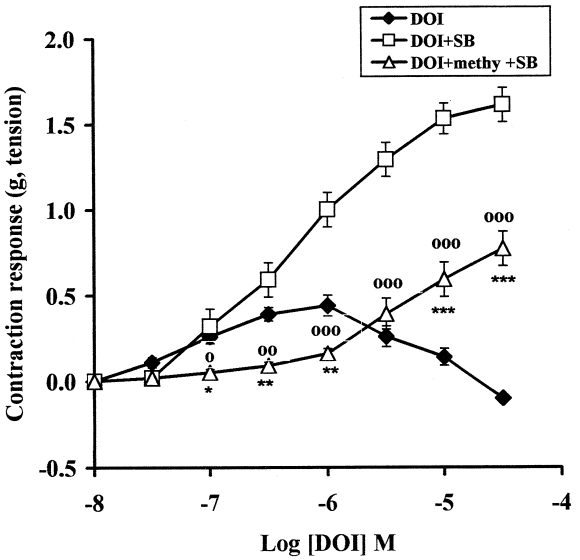

Fig. 4. The contractile response to DOI (30 nM–30 μM) in the absence and presence of SB206553 (SB, 1 μM) and a combination of methysergide (methy, 1 μM) plus SB206553 (SB, 1 μM) in the segments taken 1 cm (S1) distal to the pyloric sphincter of the *S. murinus* intestine. Each point represents the mean ± s.e.m.; $n = 8$. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the DOI control values; $^{\circ}P < 0.05$, $^{\circ\circ}P < 0.01$ and $^{\circ\circ\circ}P < 0.001$ compared to the DOI + SB206553 values.

SB206553 modified the contraction response to DOI in S4 to S8 segments (but not S1) in a similar way. There were two effects, the first being for SB206553 (1 μM) to significantly antagonise the contraction response induced by the lower concentrations of DOI (10 nM–0.3 μM) ($F(3,28)$ values were in the range of 7.8–16.7 with P values of 0.0001–0.01 as compared to the control curve); representative data obtained in S1, S4 and S6 segments is presented in Fig. 3. The second effect was to attenuate the decline in contraction observed to the higher concentrations of DOI. This occurred in all eight segments (S1 to S8) and was highly significant ($F(3,28)$ ratios were in the range of 9.6–42.1 with P values of 0.0001–0.04), the normal 'bell-shaped' concentration response curve to DOI being converted to a sigmoid curve (Fig. 3). The effect of SB206553 was concentration related, with the concentrations of 0.1 and 0.01 μM SB206553 being less effective ($F(3,28)$ ratios were in the range of 7.8–42.1 with P values of 0.0001–0.7) (Fig. 3). The first and second effects were also interrelated. For example, for data presented for S6, a concentration of SB206553 0.1 μM was capable of some antagonism of the initial contraction and partly able to antagonise the declining effect of the higher concentrations of DOI, effectively displacing the 'bell-shaped' concentration response curve to the right (Fig. 3).

The ability of SB206553 (1 μM) to enhance the contraction response to higher concentrations of DOI was sensitive to antagonism by methysergide (1 μM). For example, the maximum contraction responses induced by DOI (10 and 30 μM) in the presence of SB206553 (1 μM) were 1.6 ± 0.1 (S1), 2.0 ± 0.2 (S6) and 1.7 ± 0.2 (S8) g in the proximal, central and terminal regions of the intestine were reduced by a combination of SB206553 plus methysergide to 0.7 ± 0.2 (S1), 0.5 ± 0.1 (S6) and 0.4 ± 0.1 (S8) g respectively ($F(1,14)$ ratios were in the range of 10.5–42.5 with P values of 0.0001–0.04). Representative data from the S1 segment showing a rightward shift in the concentration curve is shown in Fig. 4.

3.2. The ability of SB206553 to modify the contractile responses to 5-HT and α-methyl-5-HT

In all segments of the intestine, 5-HT induced a sigmoid concentration response curve. SB206553 (1 μM) significantly ($F(1,10)$ ratios were in the range of 7.4–26.3 with P values of 0.004–0.021) antagonised the contractions induced by only the lower concentrations of 5-HT (10

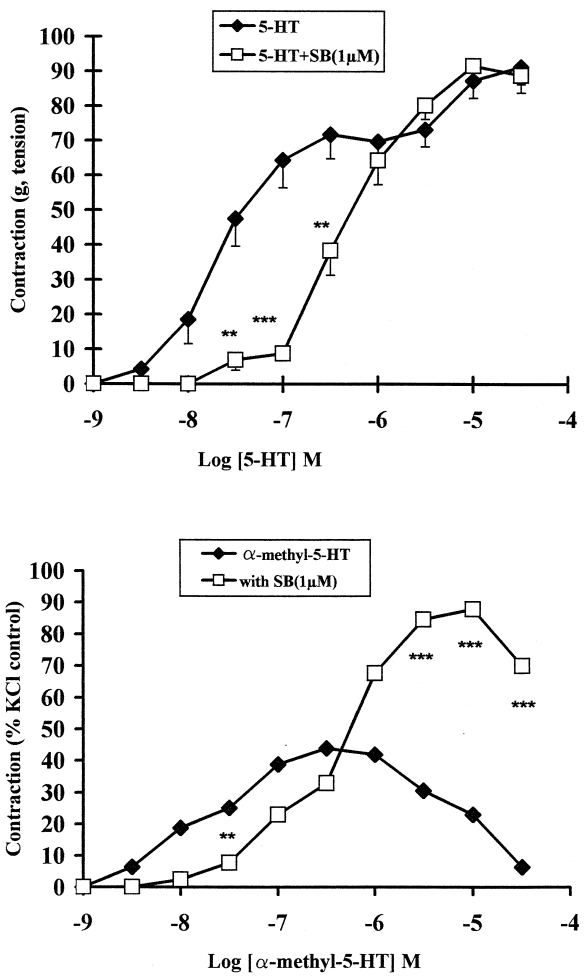

Fig. 5. The contractile response to 5-HT (10 nM–30 μM) and α-methyl-5-HT (10 nM–30 μM) in the absence and presence of SB206553 (SB, 1 μM) in the segments taken 6–7 cm (S6) distal to the pyloric sphincter of the *S. murinus* intestine. Each point represents the mean ± s.e.m.; $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the 5-HT and α-methyl-5-HT control values.

nM–3 μM) in all eight segments examined; representative data taken from S1 is shown in Fig. 5. In the presence of SB206553 the contractile responses to 5-HT at concentrations greater than 3 μM were comparable to that obtained in the control tissues.

α-methyl-5-HT (10 nM–30 μM) produced a bell-shaped contraction curve. But unlike DOI, no relaxation response was observed when applied at the highest concentration of 30 μM in any of the tissues examined. The profile of action of SB206553 (1 μM) on the contractile response to α-methyl-5-HT was similar to that recorded for DOI (Fig. 5). SB206553 caused a significant ($F(1,14)$ ratios were in the range of 7.8–22.8 with P values of 0.0003–0.014) reduction in the contractions induced by α-methyl-5-HT at concentrations lower than 0.3 μM, and in the presence of SB206553, the higher concentrations of α-methyl-5-HT induced a significantly greater contraction ($F(1,14)$ ratios were in the range of 42.2–54.9 with $P < 0.0001$) (Fig. 5).

3.3. Tachyphylaxis of response to repeated challenge with DOI

The first challenge to 3 μM DOI produced a robust contraction response of 55 ± 11% (S1), 55 ± 7% (S4), 47 ± 7% (S6) and 27 ± 9% (S8) of the KCl control values in the proximal, central and terminal region of the intestine (Fig. 6). However, subsequent challenges with 3 μM DOI after 20 and 40 min produced a significantly ($F(2,18) =$ 7.3–24.2 with $P = 0.0001$–0.04) smaller contraction response of 17.9 ± 7% and 10.5 ± 2% (S1), 15.5 ± 4% and 9.6 ± 5% (S4), 14.2 ± 4% and 5.8 ± 3% (S6) and 11.2 ± 1% and 4.9 ± 1.4% of the KCl control values in all the segments examined respectively. Representative data for S1 segments is shown in Fig. 6. SB206553 (1 μM) did not significantly influence the contractile response to the first challenge to DOI, but prevented the attenuation of the contraction to the second and third challenge to DOI (Fig. 6).

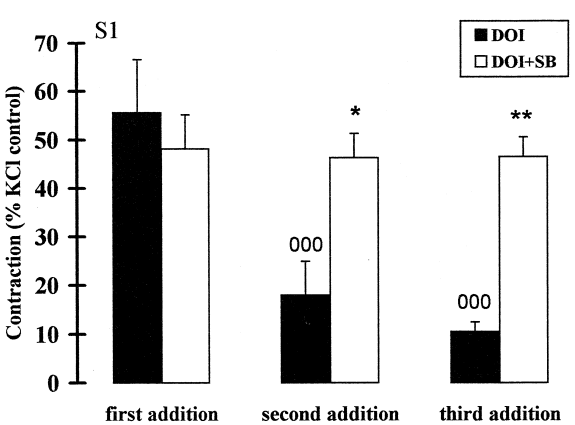

Fig. 6. The contractile response induced by three successive additions of DOI (3 μM), with an interval of 22 min between each addition, in the absence (control) and presence (test) of SB206553 (SB, 1 μM) in segments taken 1 cm (S1) distal to the pyloric sphincter of the *S. murinus* intestine. Each histogram represents the mean ± s.e.m.; $n = 7$. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to the DOI control values; $^{\circ\circ\circ}P < 0.001$ compared to the first addition of DOI.

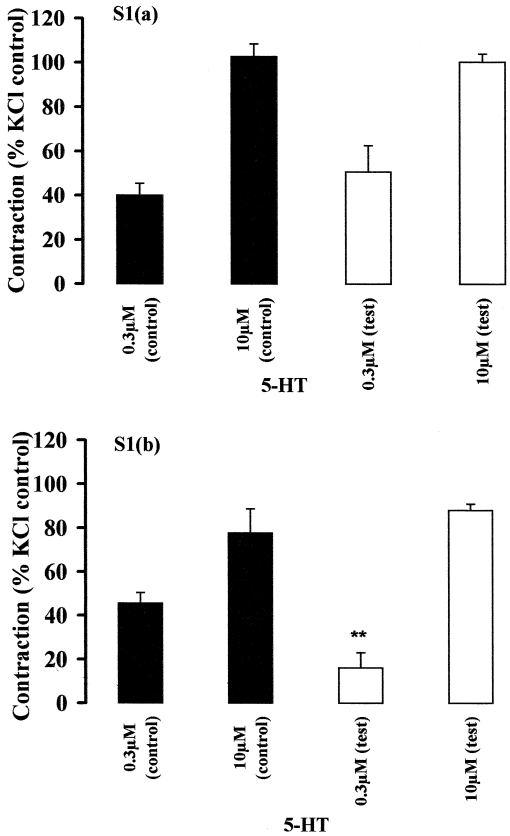

Fig. 7. The contractile response to 5-HT (0.3 and 10 μM) before (control) and after (test) desensitisation to DOI (3 μM) in the (a) presence and (b) absence SB206553 (1 μM) in segments taken 1 cm (S1) distal to the pyloric sphincter of the *S. murinus* intestine. Each point represents the mean ± s.e.m.; $n = 6$. $^{**}P < 0.01$ compared to the 5-HT control values.

3.4. Effect of tachyphylaxis of the DOI response on the contractions induced by 5-HT

The administration of 5-HT 0.3 μM and 10 μM caused a contraction response of 40–45% and 75–100% respectively of the KCl control values. In tissues that had been subjected to repeated challenges to DOI the contractile response induced by 0.3 μM 5-HT was reduced ($F(1,10)$ $= 8.9$–31.9 with $P = 0.003$–0.01), but not to 10 μM 5-HT. The contractile response to 0.3 μM 5-HT after a repeated challenge to DOI was 16.1 ± 7.0% (S1), 19.2 ± 2.6% (S4), 9.0 ± 2.4% (S6) and 5.5 ± 3.4% (S8) as compared to the control response to 5-HT (before treatment with DOI) of 45.5 ± 4.9% (S1), 52.1 ± 4.6% (S4), 51.4 ± 6.4% (S6) and 42.5 ± 6.3% (S8). Representative data is shown in Fig. 7. SB206553 prevented the attenuation of

response to 0.3 μM 5-HT in all tissues. Representative data for S1 segment is shown in Fig. 7.

4. Discussion

In the present study, DOI was chosen to further investigate and characterise the 5-HT_2 receptor subtypes in the *S. murinus* intestine mediating a contraction response (Javid and Naylor, 1999). DOI has agonistic activity at 5-HT_2 receptor with pEC_{50} values of 7.3, 7.4 and 7.8 for the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors respectively (Baxter et al., 1995). It is also specific for the 5-HT_2 receptor with a low affinity (6938 nM) for the 5-HT_{1A} receptors (Zifa and Fillion, 1992). The results obtained using a non-cumulative addition of DOI revealed a complex action; a contraction developing at low concentrations (10 nM–0.1 μM), maximising at 0.3 μM, followed by a decreasing contraction (1–10 μM) with a relaxation response being recorded at the highest concentration (30 μM) used. Therefore, DOI was revealed as an interesting pharmacological probe whose use raised perhaps as many questions as answers in an analysis of the 5-HT receptors mediating contraction or relaxation processes in the intestine.

The bell-shaped concentration response curve observed to DOI in all segments of *S. murinus* intestine was not modified by ketanserin, ondansetron or SB204070. These negative results could be cautiously interpreted to reveal an absence of effect of DOI on 5-HT_{2A}, 5-HT_3 and 5-HT_4 receptors to mediate the contraction phase, the declining contraction or relaxation response. However, methysergide, a 5-$HT_{1/2}$ receptor antagonist, was shown to abolish the bell-shaped concentration response curve, indicating the involvement of 5-$HT_{1/2}$ receptors in the contraction/declining contraction profile. The possibility that the inhibitory action of methysergide was due to a 5-HT_2 receptor blockade at the 5-$HT_{2B/2C}$ receptor was first investigated using yohimbine which has affinity for the 5-HT_{2B} receptor in the rat stomach fundus (pA_2 7.9, Baxter et al., 1994). However, yohimbine failed to antagonise the contraction/declining contraction response and indeed, there was a consistent trend for yohimbine to increase the contraction response to both the lower and higher concentrations of DOI, this failed to achieve significance. It was cautiously concluded that the 5-HT_{2B} receptor does not play an important role in the contractile profile of action of DOI in *S. murinus* intestine.

If the 5-HT_{2A} (ketanserin sensitive) and 5-HT_{2B} (yohimbine sensitive) receptors are not involved in the contractile actions of DOI, it could be hypothesised that the contraction is mediated via 5-HT_{2C} receptors. However, no selective 5-HT_{2C} receptor antagonists are available. But SB206553 has been shown to have high affinity for the 5-HT_{2C} (pK_i 8.0) and 5-HT_{2B} (pK_i 8.5) receptors with more than a 1000 fold selectivity over the 5-HT_{2A} receptors ($pK_i < 6.0$) (Forbes et al., 1995). SB206553 was found to influence the bell-shaped contraction response curve to DOI in a distinctive and reproducible manner. Firstly, in all tissues examined from *S. murinus* intestine, SB206553 antagonised the contraction response induced by lower concentrations of DOI in a competitive manner. SB206553 also caused rightward shifts in the concentration–response curves to α-methyl-5-HT and to 5-HT itself. A second and unexpected phase was to attenuate the declining response but also to clearly enhance the contraction response to the higher concentrations of DOI (and α-methyl-5-HT). The enhancement was shown to be concentration-related, a sigmoid contraction response curve being compared to a declining response in control DOI treated tissues.

The antagonism of the first contractile phase of DOI by methysergide (5-HT_1 and 5-HT_2 receptor antagonist) and SB206553 (5-$HT_{2B/2C}$ receptor antagonist) but not yohimbine (5-HT_{2B} receptor antagonist) indicates a 5-HT_{2C} receptor involvement. If this hypothesis is correct, the results provide the first evidence for a 5-HT_{2C} receptor to mediate a contraction response in the gut. The actions of SB206553 to *reverse* the second phase of a declining response to an *enhanced* contraction are more difficult to interpret.

The initial challenge is to attempt an understanding of the bell-shaped response curve, or why does the DOI induced contraction decline at higher concentrations? Three possibilities appear important. An action of higher concentrations of DOI may begin to mediate, (a) an inhibitory action to antagonise the contractile mechanism, (b) a relaxation response which may oppose the contraction, and (c) a desensitisation of the 5-HT receptor system mediating contraction.

An inhibitory action of DOI could relate to an action mediated via 5-HT receptors or through unspecified mechanisms. Whilst the latter remains speculative, there is evidence that activation of 5-HT_2 receptors (the receptor subtype has not been identified) located on GABA neurones in the rat thalamic reticular nucleus have excitatory actions to enhance GABA release. This post-synaptic response appeared to result from suppression of a potassium current (McCormick and Wang, 1991). Whether DOI may activate a similar inhibitory mechanism in the gut remains to be established. There may exist a further mechanism involving a DOI induced activation of the 5-HT_{1A} receptor. Although DOI has a relatively low affinity for the 5-HT_{1A} as compared to the 5-HT_2 receptors (see above), with increasing concentration this may become significant. Stimulation of the 5-HT_{1A} receptors located on cholinergic nerves in the gut may reduce acetylcholine release (Kirchgessner et al., 1993). The role of the cholinergic system to mediate the effects of DOI could be investigated in further experiments, using selective 5-HT_{1A} and muscarinic receptor antagonists.

There is limited evidence for a relaxation effect of DOI. In the present studies a small relaxation response was recorded in *S. murinus* intestinal tissues. But this occurred

only at the very highest concentration of DOI tested and its relevance to the use of lower concentrations of DOI is unknown.

Possibly the most convincing evidence to explain the DOI induced bell-shaped concentration response curve relates to changes in 5-HT_2 receptor function on persistent challenge. 5-HT receptor desensitisation has been observed in many studies. Thus, chronic treatment with 5-$HT_{2A/2C}$ receptor agonists such as DOI reduces 5-$HT_{2A/2C}$ receptor number as measured by receptor binding (Buckholtz et al., 1988; Chaouloff et al., 1995), reduces 5-$HT_{2A/2C}$ receptor agonist-mediated phosphoinositol hydrolysis (Dillon-Carter and Chuang, 1989; Apud et al., 1992) and produces behavioural sub-sensitivity (Darmani and Gerdes, 1995; Darmani et al., 1992). Following agonist withdrawal, supersensitivity to a 5-$HT_{2A/2C}$ receptor agonist can develop (Darmani and Gerdes, 1995). Similar findings are observed following treatment with ketanserin (Darmani et al., 1992).

Behavioural and binding changes have been shown to develop over hours to days following treatment with 5-$HT_{2A/2C}$ receptor agonists and may last several days (Blackshear et al., 1986; Darmani et al., 1992). 5-HT_{2C} receptors are known to couple to phospholipase C which induces increased phosphoinositide hydrolysis leading to the elevation of intracellular calcium (Boddeke et al., 1993). In general, changes in 5-$HT_{2A/2C}$ receptor-elicited phosphoinositol hydrolysis usually occur more rapidly (Apud et al., 1992) than behavioural sub-sensitivity; recovery also is faster (e.g., within hours) than the change in the receptor number and/or behavioural response (Leysen et al., 1989).

In vitro studies have also demonstrated that the acute desensitisation of 5-HT receptor subtypes is mediated via protein kinase activation (Boddeke et al., 1993; Rahman and Neuman, 1993). If the desensitisation mechanism involves a receptor mediated stimulation of adenylyl cyclase and subsequent activation of c-AMP-dependent protein kinase C, then an increase in the level of this enzyme should facilitate or mimic the desensitisation process; in contrast, inhibition of the activity of this enzyme should attenuate the tachyphylaxis. The involvement of protein kinase C in the desensitisation of 5-HT_2 receptors has been demonstrated using staurosporine, an inhibitor of protein kinase C (Tamaoki et al., 1986; Davis et al., 1992). Staurosporine reduced the DOI-induced desensitisation in the rat brain (Rahman and Neuman, 1993). On the other hand, protein kinase C activation resulted in a negative feedback which consequently reduced 5-HT_2 receptor-mediated responses.

The totality of this evidence is to clearly indicate that in many tissues the persistent stimulation of 5-HT_2 receptors will result in a desensitisation response. This appears in general, to reduce agonist effectiveness from a reduction in the availability of receptors (i.e., down-regulation) and in other cases from agonist-induced modification of receptor function (i.e. desensitisation). Attempts were made to further investigate whether the decreasing contraction to increasing concentrations of DOI was due to a desensitisation process.

It was observed in *S. murinus* intestine that whereas a single addition of a 3 micromolar concentration of DOI produced a robust contraction response, the repeated challenges with two further concentrations of these micromolar DOI produced a smaller response. SB206553 did not influence the contractile response to the first challenge to DOI, but prevented the attenuation of the contractions to the subsequent challenges to DOI. Furthermore, the possibility that pre-exposure to DOI affects the 5-HT receptor system was tested with a subsequent challenge to either 0.3 or 10 micromolar 5-HT. The desensitisation to DOI did not affect the response to the higher concentration of 5-HT (10 micromolar) but reduced the response to the low concentration of 5-HT. Pre-treatment with SB206553 also prevented the attenuation of response to the low concentration of 5-HT.

In conclusion, the results recorded in the present studies strongly indicate that the response to DOI is decreased on a repeated challenge and may contribute significantly to the bell-shaped responses curve. Since this effect was prevented by SB206553 but not by yohimbine, 5-HT_{2C} rather than 5-HT_{2B} receptors are implicated in the desensitisation response. This study is the first report to show that of the 5-HT_2 receptor subtypes, 5-HT_{2C} receptors are likely candidates to play a dominant role in the contraction response to DOI and demonstrate desensitisation to repeated challenge.

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