Further investigation of the effects of 5-hydroxytryptamine, 8-OH-DPAT and DOI to mediate contraction and relaxation responses in the intestine and emesis in *Suncus murinus*

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ABSTRACT

5-HT receptors are implicated in many gastrointestinal disorders. However, the precise role of 5-HT in mediating GI responses in *Suncus murnius* is still unclear. Therefore in this study, the effects of 5-HT and its agonists were investigated in *Suncus*. The involvement of 5-HT_{2C} receptors in mediating emesis was also investigated. The ability of 5-HT and its agonists/antagonists at 5-HT_{1A} and 5-HT_2 to modify GI motility was investigated *in vitro* and *in vivo*.

WAY100635 (a 5-HT_{1A} antagonist) inhibited the contraction response to 5-HT in the proximal segments without affecting the maximum response; whilst enhancing the contraction to 5-HT (>30.0 nM) in the distal intestine. The selective 5-HT_{2A} and 5-HT_{2B} receptor antagonists MDL-100907 and RS-127445 attenuated 5-HT-induced contractions (<10.0 μM) in the distal segments. RS-127445 also attenuated 5-HT-induced contractions in the central segments. The selective 5-HT_{2C} receptor antagonist SB-242084, attenuated the responses to 5-HT (> 3.0 nM) in the proximal and central but not the distal regions. 8-OH-DPAT-induced relaxation was resistant to the antagonism by 5-$HT_{1A/7}$ antagonists. DOI in the presence of 5-$HT_{1A/2A/2B/2C}$ antagonists induced greater contraction responses (≥1.0 μM) in most tissues, whilst RS-127445, or SB-242084, reduced the responses to DOI (≤ 1.0 μM) in some tissues. SB-242084 also suppressed emesis-induced by motion and intragastric $CuSO_4$.

In conclusion, within different regions of intestine, 5-HT_2 receptors are differently involved in contraction and emetic responses and that 8-OH-DPAT induces relaxation via non-5-$HT_{1A/7}$ receptors. *Suncus* could provide a model to investigate these diverse actions of 5-HT.

Keywords: 5-Hydroxytryptamine; 8-OH-DPAT; DOI; 5-HT_{1A} and 5-HT_2 receptor subtypes; *Suncus murinus* intestine

1. Introduction

The diversity of the actions of serotonin (5-HT) is revealed by its involvement in behavioural, endocrine, temperature, sleep, and gastrointestinal functions (Aghajanian, 1995; Cowen et al., 1991; Dubovsky, 1994; Glennon and Dukat, 1995; Jacob and Fornal, 1995; Keppel and Sambunaris, 1995; Sandler et al., 1991; Shih et al., 1995; Javid and Naylor, 2002, 1999a, 1999b).

Approximately 95% of mammalian 5-HT is produced in the gastrointestinal tract. Within the gut, the net effect of 5-HT in a given smooth muscle preparation will involve a summation of its potential excitatory and inhibitory actions (Fozard, 1985; Mir et al., 1988). Previous studies in the guinea pig and rat have shown the involvement of 5-HT receptors in mediating relaxation (5-HT_4 and 5-HT_7 receptors), contraction (5-HT_2 and 5-HT_3 receptors), and secretion (5-HT_3 and 5-HT_4 receptors) to the application of exogenous serotonin (Hoyer et al., 2002). Whether or not these receptors are responsive to endogenously released 5-HT is uncertain; but it is likely that 5-HT, through 5-HT_{1A}, 5-HT_2, 5-HT_3, 5-HT_4 and 5-HT_7 receptors, plays a key role in gastrointestinal motility patterns (Dhasmana et al., 1993; Buchheit et al., 1985; Chahal, 1983; Fozard, 1985, 1990; Sanger, 1987; Butler et al., 1990; Bradley et al., 1986). Agonists and antagonists for various 5-HT receptors have been used to treat gastrointestinal disorders, including IBD, functional dyspepsia, and chronic constipation. For example, 5-HT_1 receptor agonists and antagonists have been developed for functional dyspepsia and IBS diarrihoea-predominant (IBS-D) and gastroesophageal reflux disease (GERD), and 5-HT_2 receptor antagnonists for IBS-D (Gershon and Tack, 2007; Beattie and Smith, 2008). There is evidence that diarrhoea-predominant IBS is associated with an elevated level of 5-HT, whereas constipation-predominant IBS is associated with reduction in 5-HT in the colon mucosa (Spiller,

2008; Camilleri, 2009). Alterations in gut motility are paramount to this disorder and contribute to prevalence of both diarrhoea and constipation.

There are several studies, both in humans and in experimental models, that report associations of symptoms of IBS and 5-HT levels in mucosal biopsies (Coates et al., 2004; Miwa et al., 2001). Changes in the 5-HT content are also associated with IBD, both in Crohn's disease and Ulcerative colitis (Wheatcroft et al., 2005; Belai et al., 1997). Approximately 50% of patients with IBD in long-term remission have IBS-like symptoms related to changes in 5-HT signaling in the gut (Fernandez-Banares et al., 2007). Moreover, 5-HT content is increased in an experimental model of colitis (Oshima et al., 1999; Linden et al., 2003). Therefore, extensive research has indicated a privitol role for 5-HT and its receptors in many GI pathophysiological conditions with symptoms of a disturbed pattern of motility and emesis (Spiller, 2008; Camilleri et al., 2009; Gershon, 1991, Saha, 2014; Noddin et al., 2005; Crowell, 2004).

Linking emesis to disturbed patterns of motility in rodents and guinea pigs is not possible; such species do not possess the emetic reflex (Horn et al., 2013). However, advances in emesis research have revealed the value of the House Musk Shrew, *Suncus murinus* (Ito et al., 1995; Javid and Naylor, 2002). The animal shares several similarities with humans in its morphological, physiological, and genetic features (Hoyle et al., 2003; Ishiguro et al., 1989; Kimura et al., 1996) and is considered as an available model for the study of human physiology and pathology (Takata et al., 1999; Javid and Naylor, 1999a, 1999b, 2001, 2002, 2006, 2013; Cluny et al., 2008), particularly in field of gastroinestinal motility (Sanger et al, 2011).

Understanding the precise role of 5-HT and its related receptors in intestinal pathology would ultimately lead to imporoved therapeutic strategies in a variety of GI disorders. Therefore, the aim of the present study is to achieve further understanding

of the role of serotonin receptors in the intestine of *Suncus murinus* which play important roles in the mediation of disturbed patterns of gastrointestinal motility in many pathophysiological conditions. We have previously reported that 5-HT_2 and 5-HT_3 receptors play an important role in mediating a response to exogenously applied 5-HT and its related agonists in the gastrointestinal segments of *Suncus murinus* (Javid and Naylor, 1999a, 1999b). However, the role of 5-HT_2 receptor subtypes and other 5-HT receptors have not been investigated. Thus the present study was designed to further evaluate the action of 5-HT, 8-OH-DPAT and DOI using selective antagonists for 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_6 and 5-HT_7 receptors in different regions of the *Suncus murinus* intestine. Further *in vivo* experiments were also designed to investigate the role of 5-HT_{2C} receptors in emesis-induced by motion and intragastric $CuSO_4$.

2. Materials and Methods

2.1.Animals and housing conditions

Adult House Musk shrews (*Suncus murinus*) of either sex from the Bradford University strain were used; animals used for the vomiting experiments were not re-used for the *in vitro* experiments. The shrews were individually housed and allowed food (AQUATIC 3, trout pellets) and water *ad libitum*. Animals were also fed with mealworms or cat food three times per week. The floor of the cages were covered with sawdust and cleaned twice a week. The animal room was maintained at humidity between 45 to 55% at 19-21°C. All *in vitro* experimental procedures were in compliance with the UK Animals (Scientific Procedures) Act 1986, and were carried out using age-matched animals adult male (72.1$\pm$1.6g). Emesis testing was conducted

at the University of Pittsburgh. In Pittsburgh, adult male animals (83.1 ± 2.1 g, mean ± SEM) were housed individually in clear plastic cages (28 × 17 × 12 cm; length x width x height), with a filtered air supply, under a 12:12 h light:dark cycle (lights on at 0700 h), in a temperature (~ 23 °C) and humidity (~ 40%) controlled environment. Food consisted of a mixture of 75% Purina Cat Chow Complete Formula and 25% Complete Gro-Fur mink food pellets. Food and drinking water were freely available.

All experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Animals were housed in an animal care facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

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 10-15 cm. 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, Two intact segments (each segment was 1.0 cm in length) were taken from 3 different regions of the intestine: the 'proximal' region, 1.0 cm distal to the pyloric sphincter, the 'central' region of the intestine, approximately

5.0-7.0 cm from the pyloric sphincter, and the 'terminal' section of the intestine, 2.0 cm proximal to the anal region respectively.

The whole intact segments were bathed in 10.0 ml water-jacketed organ baths and placed longitudinally under 0.5 g tension (basal or resting tension, 'g' is the unit of change in tension). The Krebs' solution was maintained at a temperature of 37.0±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.Experimental design: In vitro small intestine

Using a paired experimental design, the effects of 5-HT agonists in the absence (control) or presence of antagonists were investigated. Preliminary experiments revealed that paired tissues, 2 adjacent segments taken from each intestinal region (one control tissue and one test tissue), showed the same response, and, therefore, all three regions of the intestine were selected.

Non-cumulative dose-response curves to 5-HT (1.0 nM-30.0 μM), DOI (3.0 nM-10.0 μM) in the absence (control) or presence of antagonists were constructed. This method includes the single addition of each dose of drugs with a contact time between tissue and each agonist of 1 min duration which was followed by two washings with 1 min between each wash. Each single dose of 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 WAY-100635 (a 5-HT_{1A} receptor antagonist, 1.0 μM) (Fletcher et al., 1996), MDL-100907 (a 5-HT_{2A} receptor antagonist, 1.0 μM) (Ullrich and Rice, 2000), RS-127445 (a 5-HT_{2B} receptor antagonist, 1.0 μM) (Bonhaus et al., 1999), SB-242084 (a 5-HT_{2C} receptor antagonist, 1.0 μM) (Kennett et al., 1997), SB258585 (a 5-HT_6 receptor antagonist, 1.0 μM) [33] or SB269970A (a 5-HT_7 receptor antagonist, 1.0 μM) (Hagan et al., 2000) for 1.0 hour before the application of agonist. The antagonists were constantly present in the organ bath during the construction of the dose-response curves. Also, the effects of 8-OH-DPAT (3.0, 10.0 and 30.0 μM) in the absence and presence of WAY-100635 or SB269970A (a 5-HT_7 receptor antagonist, 1.0 μM) were studied.

A control contraction to KCl (0.12 M) was also established in each tissue at the end of the experiments. 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. None of the 5-HT receptor antagonists showed any effect on the spontaneous activity of the tissues examined. In separate experiments the integrity of the preparation were evaluated for the duration of the experiments. In such experiments KCl induced reproducible contraction responses for the whole duration of the experiments. The effective concentration of the antagonists were chosen according to previous published studies. The number of observations is shown by ‘n’, which represents the number of animals used.

2.4. 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 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, and B represents the concentration of antagonist, and is expressed as $pA_2 \pm$ S.E.M. 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. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtise et al., 2015).

2.5.Experimental design: Emesis testing

All experimental procedures were in compliance with the US Animals (Scientific Procedures) Act 1986, and were carried out using age-matched animals of either sex (female 36.5±1.2 g; adult male 71.1±1.2 g).

Ten experimentally naive male shrews were tested to determine SB-242084 is anti-emetic. Intially, shrews were injected with vehicle (saline with 8% hydroxy-propyl-B-cyclodextrin and 25 mmol/l citric acid (Jones et al., 2002); n=5) or SB-242084 (1 mg/kg/2 ml, ip; n=5) 40 min prior to exposure to provocative motion (10 min, 1 Hz, 4 cm lateral displacement). After the administration of a drug or vehicle, each animal was placed individually in a transparent cage (100 W x 150 L x 150 H mm) and observed for any behavioural change. After a described time, a horizontal

motion stimulus of 1 Hz and a 40 mm lateral displacement of the shaker platform was commenced for 10 min.

Previous experiments showed that these parameters were suitable to induce a reliable and reproducible emetic response ((Javid and Naylor 1999, Chan et al., 2007; Tu et al., 2017). Animals were observed for emesis during motion exposure and for 20 min after exposure. Emesis (with and without vomiting) was recorded to computer by an observer using software (JWatcher). Twelve days after testing with motion animals were divided into two groups (n=5 per group), receiving vehicle or SB-242084 injections 40 min prior to intragastric delivery of $CuSO_4$ (120 mg/kg/5 ml, gavage). To control for potential carryover effects, those animals receiving the vehicle during the motion testing (n=5) were assigned to receive SB-242084 for $CuSO_4$ testing. This was conducted to determine if the observed effects carried over from one experimental condition to the next. Animals were observed for 30 min post-treatment for the occurrence of emesis. In all experiments, the number of emetic episodes (defined as productive vomiting or as dry retching) and the latency of onset to the first emetic episode were recorded. It should be noted that the animals were kept and tested in exactly the same environment to obviate confounding differences of olfactory, visual and other cues. All the experiments were conducted at the same time every day.

2.6. *Drugs*

5-Hydroxytryptamine maleate (Sigma), 8-OH-DPAT (8-hydroxy-2(di-n-dipropyl-2-aminotetralin hydrobromide) (BRI), DOI(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (Sigma), WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]- N- (2-pyridinyl) cyclohexanecarboxamidetrihydrochloride) (Research

Biochemicals Inc), SB269970 (®)-3-(2-(2-(4-Methyl-piperidin-1-yl)ethyl)-pyrolidine-1-sulphonyl)-phenol) and SB258585 (4-Iodo-N-[4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-benzenesulphonamide) (Glaxosmithkline), MDL-100907 (®-(+)-alpha-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl) ethyl]-4-piperidinemethanol) (Vernalis), RS-127445 (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine) (Vernalis), SB-242084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] (Vernalis), hydroxy-propyl-B-cyclodextrin and citric acid (Sigma),$CuSO_4$ (Sigma). All drugs were dissolved in distilled water except for SB-242084, RS-127445 and MDL-100907 which were dissolved in 50% DMSO at 1 mM concentration with distilled water being used for further dilutions. Preliminary experiments established that the vehicles used did not show any effect on the tissues.

3.0. Results

3.1. The ability of 5-HT receptor antagonists to modify 5-HT induced contractions

The non-cumulative addition of 5-HT (10.0 nM-30.0 µM) induced concentration-dependent contraction response curves in all segments examined.

In tissues taken from the proximal region but not the central region of the intestinal tract, pre-treatment with WAY-100635 (1.0 µM) significantly ($P<0.05$) reduced the contractions induced by concentrations of 5-HT lower than 0.1 µM (Figure 1). The concentration response curves to 5-HT were shifted to the right with the maximum responses being comparable to the control values. Estimated pA_2 value for WAY-100635 to antagonise the effects of 5-HT was 6.5 ± 0.1 in the proximal region. In contrast, in the terminal segments the contraction responses to 5-HT at concentrations higher than 30.0 nM were significantly ($P<0.01$) greater than those in control tissues (Fig. 1).

MDL-100907 (1.0 μM) induced a rightward shift of the concentration response curve to 5-HT without affecting the maximum response in segments taken from the terminal but not the proximal and central regions of the intestine (Fig. 2). An estimated pA_2 value for MDL-100907 to antagonise the effects of 5-HT was 7.3±0.4.

RS-127445 (1.0 μM), in tissues taken from the central and terminal regions of the intestine, attenuated the contraction responses to 5-HT (30 nM to 1.0 μM in the central region and 3.0 nM to 0.3 μM in the terminal region) significantly ($P<0.05$, 0.01) without reducing the maximal response (Fig. 3). Estimated pA_2 values for RS-127445 to antagonise the effects of 5-HT were 7.4±0.6 and 7.2±0.4 in tissues taken respectively from the central and terminal regions of the intestine. RS-127445 (1.0 μM), failed to modify the effects of 5-HT in the proximal region.

SB-242084 (1.0 μM), attenuated the contraction responses to 5-HT in the concentration range of 10.0 nM to 100/300 nM significantly ($P<0.05$, 0.01) in tissues taken from the proximal and central (but not consistently in the terminal) regions of the intestine without significantly reducing the maximum response (Fig. 4). Estimated pA_2 values for SB-242084 to antagonise the effects of 5-HT were 7.1±0.6 and 7.8±0.4 in tissues taken from the proximal and central regions of the intestine.
The application of SB258585 (1.0 μM) and SB269970A (1.0 μM) failed to modify 5-HT-induced contractions in any tissue examined (data not shown).

3.2. Effect of 8-OHDPAT in the absence and presence of 5-HT_{1A} and 5-HT_7 receptor antagonists

The non-cumulative addition of 8-OH-DPAT (10.0 nM- 30.0 μM) failed to induce a contraction or relaxation response in any tissues under basal tension.

However, when tissues were pre-contracted with KCl (0.12 M), 8-OH-DPAT at concentration range of 3.0- 30.0 µM induced a relaxation response in all regions of the intestine. The application of WAY-100635 (1.0 µM) or SB269970A (1.0 µM) failed to modify the relaxation response to 8-OH-DPAT in all regions examined (data not shown).

3.3. The ability of the 5-HT receptor antagonists to modify DOI- induced contractions

The application of DOI at concentrations of 10.0 nM to 300.0 nM induced a concentration-dependent contraction. The contraction responses achieved in the terminal regions of the intestine were smaller compared to those achieved in the proximal and central regions (Fig. 5-9). However, at concentrations of 1.0 µM and higher the contraction responses decreased, revealing a bell-shaped concentration response curve in all regions of the intestine (Fig. 5-8).

The application of WAY-100635 (1.0 µM) failed to modify the DOI-induced contraction responses up to 0.3 µM in all tissues examined. However, the higher concentrations of DOI ($\leq$10.0 µM) induced significantly ($P<0.05$) greater contraction responses in tissues taken from the proximal and terminal but not the central region of the intestine. At a concentration of 10.0 µM or higher the responses were comparable to those in control tissues (Fig. 5). In the presence of MDL-100907 (1.0 µM) the contraction responses to DOI at concentrations higher than 1.0 µM were significantly ($P<0.05$) greater as compared to the control values in all tissues examined (Fig. 6). MDL-100907 failed to modify the contractions induced by the lower concentrations of DOI (<1.0 µM).

RS-127445 (1.0 µM) significantly ($P<0.05, 0.01$) reduced the contraction responses to lower concentrations of DOI ($\geq$1.0 nM –1.0 µM) in segments taken from the central and terminal but not from the proximal region of the intestine (Fig. 7), but increased the contractions to higher concentrations of DOI (10.0-30.0 µM) (Fig. 7).

SB242084 (1.0 µM) significantly ($P<0.05, 0.01$) antagonized the contraction responses to lower concentrations of DOI (0.1-0.3 µM) in the central region, and (30.0 nM- 0.1 µM) in the terminal region, but not in the proximal region (Fig. 8). SB242084 enhanced the contractions to the higher concentrations of DOI and is achieved significance ($P<0.05, 0.001$) mainly in the central and terminal regions of the intestine (Fig. 8).

3.4. Effect of SB-242084 on emesis

SB-242084 had no statistically significant effects on the total number of emetic episodes to motion exposure (Cox regression, $P<0.05$; Fig. 9); however, this agent did produce signficantly longer lantency to the first emetic episode (Fig. 9). In contrast to motion testing, SB-242084 pre-treatment resulted in a statistically significant decrease in total number of emetic episodes produced by intragastric $CuSO_4$ (t-test, $P<0.05$; Fig. 10).

4.0 Discussion

The present study investigated the involvement of 5-HT_{1A} , 5-HT_{2A} , 5-HT_{2B} , 5-HT_{2C} receptors in mediating responses to 5-HT, DOI, and 8-OH-DPAT. The non-cumulative addition of 5-HT induced concentration related contraction responses in all segments of the intestine, and relaxation responses were not observed. WAY-

100635 is a potent and selective 5-HT_{1A} receptor antagonist with an IC_{50} of 1.35 nM in the rat hippocampus and over 100-fold selectivity for the 5-HT_{1A} receptors over other receptors (Fletcher et al., 1996). The antagonism afforded by WAY-100635 indicates the involvement of 5-HT_{1A} receptors in the contraction induced by nanomolar concentrations of 5-HT in the proximal region of the intestine. This profile of action was not seen in the central region of the intestine as the responses were comparable to those in control tissues. However, this profile of action was quite different in segments taken from the terminal region of the intestine since WAY-100635 facilitated a greater contraction response to 5-HT. The present results suggest both a regional difference within the intestine of *Suncus murinus* in terms of a 5-HT_{1A} receptor involvement in mediating the responses to 5-HT and in the nature of the response- contraction versus relaxation.

Classical subtypes of 5-HT receptor that have been identified in the gut include 5-HT_{1A} receptors which are present in enteric nerves and ganglia (Kirchgessner et al., 1993). 5-HT_{1A} receptors function to presynaptically inhibit the release of acetylcholine at nicotinic synapses and the secretion of tachykinins (Broad et al., 1993), and postsynaptically to hyperpolarize enteric neurones (Galligan and North, 1991; Galligan et al., 1988; Pan and Galligan, 1994). If 5-HT_{1A} receptors are involved in mediating relaxation or inhibitory responses in the intestine of *Suncus murinus,* it is a complex mechanism because the application of WAY-100635 revealed a greater contraction response to 5-HT in the terminal region of the intestine. Whether this effect involves a different action on post- or presynaptic receptors in the different regions of the intestine remains to be established.

In the present study, and in line with previous experiments, the non-cumulative addition of DOI revealed a bell-shaped response; a contraction developing at low

concentrations (first phase) (10.0 nM - 0.1 µM), followed by a decreasing contraction (second phase) (1.0 – 10.0 µM). It has been shown that DOI has agonistic activity at 5-HT_2 receptors 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). DOI also has low affinity (6,938 nM) for the 5-HT_{1A} receptors (Zifa and Fillion, 1992).

The ability of WAY 100635 to induce a greater contraction response to DOI in the declining contraction phase, in the proximal and terminal segments but not in the central region of the intestine, suggests the involvement of 5-HT_{1A} receptors in mediating the second phase of DOI. 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). However, our further experiments using atropine revealed no involvement of the cholinergic component in mediating a response to DOI (unpublished data).

In previous experiments it has been shown that 5-HT_2 receptors play an important role in mediating a contraction response to 5-HT and DOI (Javid and Naylor, 1999b). In the present study, attempts were made to investigate further the 5-HT_2 receptor subtypes using more selective and specific antagonists. MDL 100907 has nanomolar affinity (Ki= 0.85 nM, as an average value) for 5-HT_{2A} receptors in various assays and shows >100 fold separation from all other receptors measured (Kehne et al., 1996). In the present investigation, the ability of MDL 100907 to induce a greater contraction response to micromolar concentrations but not lower concentrations of DOI suggests the involvement of 5-HT_{2A} receptors in the declining contraction response in all intestinal regions. Therefore, this suggests that the contraction

responses achieved at lower concentrations of DOI could be either 5-HT_{2B} or 5-HT_{2C} receptor mediated.

To test this hypothesis further, experiments using selective 5-HT_{2B} or 5-HT_{2C} receptor antagonists were conducted. Pre-treatment with the 5-HT_{2B} and 5-HT_{2C} receptor antagonists, RS-127445 (Bonhaus et al., 1999) and SB242084 (Kennett et al., 1997) respectively antagonized the contractions induced by DOI at concentrations lower than 1.0 μM, in segments taken from the central and terminal regions of intestine, whilst inducing a greater contraction response to higher concentrations of DOI in all regions of the intestine. RS-127445 has been shown to have nanomolar affinity for the 5-HT_{2B} receptors (pKi= 9.5 $\pm$ 0.1) and 1000-fold selectivity for this receptor as compared to other receptors and ion channel binding sites (Bonhaus et al., 1999). On the other hand, SB242084 has high affinity (pKi= 9.0) for the cloned human 5-HT_{2C} receptor and 100 to 158-fold selectivity over the closely related cloned human 5-HT_{2B} and 5-HT_{2A} receptors, respectively. It also has over 100-fold selectivity over a range of other 5-HT, dopamine, and adrenergic receptors (Kennett et al., 1997). There is some discrepancy between the antagonist apparent pA_2 values in this study and their corresponding binding affinity values in other studies (Kehne et al., 1996; Bonhaus et al., 1999; Kennett, 1997). However, this could be due to species differences and also the fact that brain tissues or cell lines were used in other studies to estimate the affinity of antagonists.

Several studies have shown that 5-HT_{2A} and 5-HT_{2C} receptor antagonism may lead to opposite functional effects (Fletcher et al., 2002; Eberle-Wang et al., 1996; Meltzer et al, 1989a, 1989b; Reavill et al., 1999). For example, cocaine-induced locomotor activity was attenuated by the antagonism of 5-HT_{2A} receptors, whilst potentiated by a 5-HT_{2C} receptor antagonist (Fletcher et al., 2002). In other

experiments, orofacial dyskinesias, a severe side effect associated with the prolonged use of anti-psychotic drugs, which is also present in a number of hyperkinetic disorders of the basal ganglia in humans, has been attenuated by 5-HT_{2C} receptor antagonism in rats (Eberle-Wang et al., 1996). More recent studies by Creed-Carson et al (2011), suggested that the antagonism of 5-HT_{2C} receptors could be targeted for the development of new anti-psychotic drugs. In line with the above studies, the present studies indicated a differential effects to the application of DOI when the central and terminal regions of the intestine were antagonized by the 5-HT_{2A} receptor antagonist as compared to the antagonism afforded by the 5-HT_{2C} receptor antagonist in the same regions of the intestine. Additionally, the 5-HT_{2B} receptor antagonist induced the same profile of action on the responses to DOI as the 5-HT_{2C} receptor antagonist. Furthermore, an opposite profile of action was observed when the contractile responses to 5-HT in the central region of the intestine were antagonized by the 5-HT_{2A} receptor antagonist as compared with the antagonism by 5-HT_{2B} or 5-HT_{2C} receptor antagonists. These observations provide evidence for the *Suncus murinus* intestine as a useful model for characterizing the action of novel therapeutics, including anti-psychotics.

As an initial test of novel therapeutics, we investigated the action of the selective 5-HT_{2C} receptor antagonist SB-242084 on emesis. SB-242084 had inhibitory effects on emesis provoked by motion exposure and intragastric $CuSO_4$. We used a broad set of stimuli to activate three peripheral emetic inputs, including the action of provocative motion on the vestibular system and intragastric $CuSO_4$ on vagal and area postrema pathways (Horn et al., 2014). Our experiments suggest a broad therapeutic action of 5-HT_{2C} receptors on emesis. Further experiments, including SB-242084, and

other 5-HT agents, should be conducted to establish the site of functional effects on emesis and other therapeutic modalities.

In summary, the present data strongly suggests different effects for 5-HT_{2B} and 5-HT_{2C} receptor activation in *Suncus murinus* intestine: a contraction response to lower concentrations of DOI and probably an inhibitory response to higher concentrations of DOI. The results provide evidence for a potential role for 5-HT_{2C} receptors in the intestine to moderate contraction responses. The present study also indicates regional differences for the involvement of 5-HT receptors in inducing such effects. Activation of 5-HT_{1A} receptors may attenuate or potentiate the responses to 5-HT depending on the region of the intestine studied. Indeed the 5-HT_{1A} and 5-HT_2 receptors are involved in mediating diarrhoea in IBD patients (Chen et al., 2001; Kadowaki et al., 1996; Margo et al., 2002). Thus *Suncus murinus* intestine would provide a good model to substantiate the role of 5-HT receptor subtypes in designing novel therapeutics.

5.0 Acknowledgement

We would like to thank Vernalis for generous donation of 5-HT receptor antagonists.

6.0 Conflict of interests

The authors declare no conflict of interests

7.0 References

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Fig. 1. The contractile response to 5-HT (10 nM- 30 uM) in the absence and presence of WAY-100635 (1uM) in segments taken from the proximal (1-3 cm distal to the pyloric sphincter) , central (6-7 cm distal to the pyloric sphincter), and terminal (1-3 cm proximal to the anal region) regions of the *Suncus murinus* intestine. Each point represents the mean± S.E.M.; n=6. * $P<0.05$, ** $P<0.01$ compared to the 5-HT control values.

Fig. 2. The contraction response to 5-HT (1 nM-30 uM) in the absence and presence of 5-HT_{2A} receptor antagonist, MDL 100907 (1uM), in the segments taken from the proximal (1-3 cm distal to the pyloric sphincter) , central (6-7 cm distal to the pyloric sphincter), and terminal (1-3 cm proximal to the anal region) region of the Suncus murinus intestine. Each point represents the mean$\pm$ S.E.M.; n=4. * $P<0.05$ compared to the control values.

Fig. 3. The contraction response to 5-HT (1 nM-30 uM) in the absence and presence of 5-HT_{2B} receptor antagonist, RS-127445 (1uM), in the segments taken from the proximal (1-3 cm distal to the pyloric sphincter) , central (6-7 cm distal to the pyloric sphincter), and terminal (1-3 cm proximal to the anal region) region of the Suncus murinus intestine. Each point represents the mean$\pm$ S.E.M.; n=4. * $P<0.05$ and ** $P<0.01$ compared to the control values.

Fig. 4. The contraction response to 5-HT (1 nM-30 uM) in the absence and presence of 5-HT_{2C} receptor antagonist, SB242084 (1uM), in the segments taken from the proximal (1-3 cm distal to the pyloric sphincter) , central (6-7 cm distal to the pyloric sphincter), and terminal (1-3 cm proximal to the anal region) region of the Suncus murinus intestine. Each point represents the mean$\pm$ S.E.M.; n=4. * $P<0.05$ and ** $P<0.01$ compared to the control values.

Fig. 5. Representative data showing the contractile response to DOI (10 nM- 30 uM) in the absence and presence of WAY-100635 (1uM) in the segments taken from proximal and terminal region of the *Suncus murinus* intestine. Each point represents the mean± S.E.M.; n=6. * $P<0.05$ compared to the DOI control values.

Fig. 6. The contraction response to DOI (3 nM-30 uM) in the absence and presence of 5-HT_{2B} receptor antagonist, MDL 100907 (1uM), in the segments taken from the proximal (1-3 cm distal to the pyloric sphincter) , central (6-7 cm distal to the pyloric sphincter), and terminal (1-3 cm proximal to the anal region) region of the S*uncus murinus* intestine. Each point represents the mean $\pm$ S.E.M.; n=5. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ compared to the control values.

Fig. 7. The contraction response to DOI (3 nM-30 uM) in the absence and presence of 5-HT_{2B} receptor antagonist, RS-127445 (1uM), in the segments taken from the proximal (1-4 cm distal to the pyloric sphincter) , central (6-7 cm distal to the pyloric sphincter), and terminal (1-3 cm proximal to the anal region) region of the Suncus murinus intestine. Each point represents the mean$\pm$ S.E.M.; n=5. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ compared to the control values.

Fig. 8. The contraction response to DOI (3 nM-30 uM) in the absence and presence of 5-HT_{2C} receptor antagonist, SB242084 (1uM), in the segments taken from the proximal (1-3 cm distal to the pyloric sphincter) , central (6-7 cm distal to the pyloric sphincter), and terminal (1-3 cm proximal to the anal region) region of the *Suncus murinus* intestine. Each point represents the mean$\pm$ S.E.M; n=5. * $P<0.05$ and ** $P<0.01$ compared to the control values.

Fig. 9. Effect of pre-treatment with vehicle or SB242084 (1 mg/kg) on motion-induced emesis (10 min, 1 Hz). Top for panel show the total number of emetic episodes, duration of emesis (from the 1st to the last episode), episodes with vomiting, and episodes without vomiting. Data represent the mean $\pm$ S.E.M.; n=5. Bottom plot shows the cummulative latency to the first emetic episode. * $P<0.05$, Cox regression.

Fig. 10. Effect of pre-treatment with vehicle or SB242084 (1 mg/kg) on intragastric $CuSO_4$-induced emesis (120 mg/kg, gavage). Top for panel show the total number of emetic episodes, duration of emesis (from the 1st to the last episode), episodes with vomiting, and episodes without vomiting. Data represent the mean $\pm$ S.E.M.; n=5. * $P<0.05$, t-test. Bottom plot shows the cummulative latency to the first emetic episode.