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Pharmacological Research 53 (2006) 353–358

Pharmacological research

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The 5-HT_{2B} antagonist and 5-HT_4 agonist activities of tegaserod in the anaesthetized rat

J.L. McCullough, S.R. Armstrong, S.S. Hegde, D.T. Beattie *

Department of Pharmacology, Theravance Inc., 901 Gateway Boulevard, South San Francisco, CA 94080, USA

Accepted 12 January 2006

Abstract

The 5-HT_4 receptor agonist and gastroprokinetic, tegaserod, possesses 5-HT_{2B} receptor antagonist activity. However, the relevance of such activity is unclear. In this study, the 5-HT_{2B} receptor antagonist and 5-HT_4 agonist activities of tegaserod were investigated.

Two piezoelectric crystals were implanted on the stomach fundus or oesophagus of anaesthetized Sprague–Dawley rats. Measurement of the transmission time of ultrasonic pulses between the implanted crystals provided a continuous record of inter-crystal distance, and thus of muscle length.

In the stomach fundus, tegaserod (1 and 3 mg kg^{-1}), administered subcutaneously (s.c.), inhibited the contractile response evoked by the 5-HT_{2B} receptor agonist, BW 723C86 (0.01–1 mg kg^{-1} intravenously (i.v.)). SB 206553 (1 mg kg^{-1} s.c.), a selective 5-$HT_{2B/2C}$ receptor antagonist, also inhibited the BW 723C86-mediated responses. In the rat oesophagus, tegaserod (0.001–0.3 mg kg^{-1} i.v. or 0.003–3 mg kg^{-1} s.c.) increased inter-crystal distance, consistent with smooth muscle relaxation; the responses were inhibited by the 5-HT_4 antagonist, piboserod (0.1 mg kg^{-1} s.c.).

Data from this in vivo rat study are consistent with tegaserod-induced 5-HT_4 receptor-mediated oesophageal relaxation, and antagonism of 5-HT_{2B} receptor-mediated stomach fundus contraction. The clinical relevance of the 5-HT_{2B} receptor antagonism of tegaserod remains to be determined.

Keywords: Tegaserod; 5-HT_{2B}; 5-HT_4; Stomach fundus; Oesophagus

1. Introduction

5-Hydroxytryptamine (5-HT), which originates in enterochromaffin cells and intrinsic neurons of the alimentary tract, plays a pivotal physiological role in regulating gastrointestinal motility via an interaction with 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors [1].

Activation of 5-HT_4 receptors, located on motor neurons and interneurons in the alimentary tract results in facilitation of gastrointestinal motility by augmenting cholinergic and non-adrenergic, non-cholinergic neurotransmission [2]. Additionally, stimulation of 5-HT_4 receptors on smooth muscle inhibits colonic circular muscle contractile activity in humans [3]. With respect to the role of the 5-HT_{2B} receptor in gastrointestinal physiology, preclinical data indicate an involvement in the development and function of the enteric nervous system and alimentary tract [4–6]. 5-HT_{2B} receptor mRNA and protein are expressed in the human and rodent gastrointestinal tract [5,6], and their activation enhances neurogenically mediated contraction of colonic longitudinal smooth muscle [5].

Drug discovery efforts have identified several 5-HT ligands with clinical efficacy in gastrointestinal disorders. The 5-HT_4 receptor agonists, cisapride (Propulsid®), prucalopride, renzapride, mosapride and tegaserod (Zelnorm®) have demonstrated efficacy in patients suffering disorders characterized by reduced gastrointestinal motility [7–9]. Notable amongst these agents is tegaserod [10–12], which is approved in several countries for the treatment of constipation-predominant irritable bowel syndrome in females and chronic constipation in males and females. Clinical studies have demonstrated that tegaserod increases gastric emptying and accelerates small intestinal and colonic transit in humans [13,14]. Although the gastrointestinal actions of tegaserod are attributed to 5-HT_4 receptor activation, the compound binds to 5-HT_1 and 5-HT_2 receptors, and is a potent

* Corresponding author. Tel.: +1 650 808 6105; fax: +1 650 808 6441.
E-mail address: dbeattie@theravance.com (D.T. Beattie).

1043-6618/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phrs.2006.01.003

5-HT_{2B} receptor antagonist [15]. The 5-HT_{2B} receptor antagonist activity of tegaserod could have clinical significance.

The objective of this study was to investigate the 5-HT_{2B} receptor antagonist activity of tegaserod in vivo, and to compare its potency in this respect to its 5-HT_4 agonist activity. While the rat stomach fundus [16] and oesophagus [17] exist for assaying, respectively, 5-HT_{2B} and 5-HT_4 receptor agonist potency and efficacy in vitro, investigation of such activity in vivo, in rodents, is generally limited to intestinal transit or gastric emptying of test meals containing a marker [18,19]. Implanted strain gauge force transducers have been used successfully in guinea pigs [20] to measure "real-time" gastrointestinal contractility, although due to the technical challenges, are more commonly used in larger species. In this study, digital sonomicrometry was used in anticipation that it would provide a sensitive means of recording real time changes in oesophageal and stomach fundus responsiveness in a less invasive manner than utilization of strain gauges or other forms of tissue tension measurement. Traditionally used in cardiovascular physiology and pharmacology studies, digital sonomicrometry utilizes miniature piezoelectric crystals to transmit and receive ultrasonic pulses, and the "time of flight" of the sound wave is measured with high sensitivity. As the speed of sound in biological tissue is fixed, a continuous record of inter-crystal distance is provided, and changes in muscle length are recorded. The in vivo sonomicrometry output (i.e. inter-crystal distance) is considered equivalent to that generated by traditional tissue bath methodology in which changes in muscle length are recorded isotonically. The application of this technique to the gastrointestinal field has been reported recently for measuring stomach motility in response to intravenous cholecystokinin [21]. Piboserod (SB-207266) and SB 206553, selective 5-HT_4 and 5-$HT_{2B/2C}$ receptor antagonists, respectively [22,23] and BW 723C86 and α-methyl 5-HT, selective 5-HT_{2B} receptor agonists, were used to characterize definitively the pharmacological responses evoked by tegaserod. The oesophageal activity of tegaserod was compared to that produced by cisapride and mosapride, both of which are 5-HT_4 receptor agonists [19,24].

2. Materials and methods

All experiments were conducted in accordance with the principles of good laboratory animal care provided by the Animal Care and Usage Committee of Theravance Inc. Adult, male Sprague–Dawley rats (250–350 g, Harlan) were acclimated to the colony room (temperature controlled at $21 \pm 1\,^{\circ}C$ and 12:12 h light-dark cycle commencing at 7:00 am) for at least 5 days following arrival. During this period, standard rat diet (Harlan Teklad) and drinking water were available ad libitum.

Rats were anaesthetized with isoflurane (2–3%) via a nose cone for the duration of each experiment. Animals were placed on a heated pad to maintain body temperature at 37–38 °C (monitored rectally with a sensor (Physitemp BAT-12)). The right femoral vein was exposed, and a micro-renathane cannula (MRE-033) was inserted to permit i.v. administration of test agents. A midline incision was made in the skin and muscle layers of the abdomen to expose the stomach and oesophagus. Two piezoelectric crystals (1 mm diameter; Sonometrics Corp.) were gently glued, approximately 2 mm apart, in a longitudinal orientation, to the stomach fundus or distal oesophagus using Vetbond tissue adhesive. The wires connecting the crystals to the measurement device (Sonometrics Corp. TRX series 8) were exteriorized through the abdominal incision site.

The settings for the Sonometrics system were fixed within the Sonoview software (Sonometrics Corp. version 3.2.1) as follows: sampling rate = 99.4 Hz, transmit pulse = 375 ns, inhibit delay = 1.2–1.5 mm, velocity of sound through biological tissue = 1.59 mm μs^{-1}. Drug vehicle, followed by increasing doses of tegaserod, BW 723C86 or α-methyl 5-HT (dosing volumes: 0.3–1 ml kg^{-1} over 3 s, i.v. or s.c.), were administered cumulatively (i.v.) or non-cumulatively (s.c., in the right flank). Piboserod and SB 206553 were administered s.c., 15 min prior to i.v. administration of tegaserod or BW 723C86.

Changes in inter-crystal distance (in mm) were averaged for each treatment group. From the dose–response relationship for each agonist, an ED_{50} value (i.e. the dose resulting in 50% of the maximum response) was calculated using Prism graphics software 3.0 (Graphpad Inc.). The data were fitted to a sigmoidal dose–response relationship with variable slope according to the following equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom})/(1 + 10\text{^}((\log \text{ED50} - X) \times \text{HillSlope})),$$

where X is the logarithm of the agonist dose, Y is the response, and the bottom and top are the curve asymptotes.

Tegaserod and cisapride were purchased (Apin Chemicals and Sequoia Research Products, respectively). BW 723C86, α-methyl 5-HT and SB 206553 were purchased from Tocris Cookson. Piboserod and mosapride were prepared using published synthetic procedures [22,23]. Piboserod, α-methyl 5-HT and SB 206553 were dissolved in 0.9% saline or 5% dextrose in water (D5W), while tegaserod and mosapride were prepared in 10 % sulfobutyl ether-beta cyclodextrin (10% SBE/CD). BW 723C86 was formulated in DMSO (1% by volume) in D5W. Cisapride was formulated in 10% SBE-CD and citric acid (20 mM). Doses were expressed in terms of the free base weights of each compound.

3. Results

3.1. Stomach fundus

Following crystal placement, 30 min proved sufficient to establish a stable sonomicrometry recording of inter-crystal distance. No spontaneous changes in stomach fundus muscle length were observed after this stabilization period, and preparations remained viable for up to 2 h thereafter. Following i.v. administration, the selective 5-HT_{2B} receptor agonist, BW 723C86 (0.01–1 mg kg^{-1}), but not its vehicle, produced a dose-dependent reduction in fundus inter-crystal distance, consistent with longitudinal smooth muscle contraction (Fig. 1a and b). The mean ED_{50} value (with 95% confidence intervals) for BW 723C86 ($n = 7$) was 65 (23–190) μg kg^{-1} and the mean maximum response was 0.24 (0.15–0.32) mm (equivalent to a

maximum change from resting levels of 8.2 (4.3–12.1)%). The 5-HT_{2B} receptor agonist, α-methyl 5-HT (0.01–0.1 mg kg^{-1} i.v.), also produced a dose-dependent reduction in fundus inter-crystal distance (data not shown). The responses evoked by BW 723C86 and α-methyl 5-HT had a rapid onset (of typically <1 min after dosing) and reached a maintained maximum response typically within 2 min. Responses evoked by α-methyl 5-HT were more transient than those induced by BW 723C86, and more prone to desensitization. In subsequent studies, BW 723C86 was used routinely, rather than α-methyl 5-HT, as the former was considered likely to provide a more accurate representation of 5-HT_{2B} receptor agonist activity. Pretreatment of rats with the selective 5-$HT_{2B/2C}$ receptor antagonist, SB 206553 (1 mg kg^{-1} s.c., $n = 10$) attenuated the BW 723C86-mediated responses. SB 206553 produced a rightward shift in the dose–response curve to BW 723C86, with a reduction in the maximum agonist response (Fig. 1b). Pretreatment with piboserod (0.1 mg kg^{-1} s.c., $n = 8$), a selective 5-HT_4 receptor antagonist, was associated with only a small (i.e. approximately twofold) rightward shift in the BW 723C86 dose response curve (Fig. 1b). Neither piboserod (0.1 mg kg^{-1} s.c.) nor SB 206553 (1 mg kg^{-1} s.c.) had any effect on the resting inter-crystal distance per se.

Intravenous administration of tegaserod (0.001–1 mg kg^{-1} i.v.) had no effect on the resting activity of the stomach fundus, with or without prior treatment with SB 206553 (1 mg kg^{-1} s.c.). Similarly tegaserod (1 and 3 mg kg^{-1} s.c.) had no effect on the resting activity of the fundus. However, tegaserod (1 and 3 mg kg^{-1} s.c., $n = 10$ for each), 5 min after piboserod treatment (0.1 mg kg^{-1} s.c., to avoid any potential influence of 5-HT_4 receptor interaction), inhibited the contractile response evoked by BW 723C86 (0.01–1 mg kg^{-1} i.v.) in a dose-dependent manner. Tegaserod produced a rightward shift in the BW 723C86 dose–response curve, with a reduction in the maximum agonist response (Fig. 2).

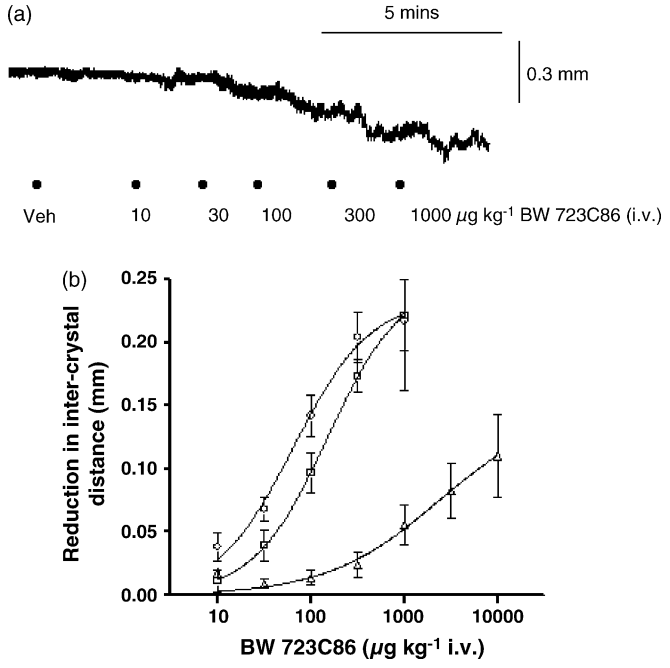

Fig. 1. (a) Representative digital sonomicrometry recording of stomach fundus mechanical activity in the anaesthetized rat. BW 723C86 (0.01–1 mg kg^{-1} i.v.), but not its vehicle (Veh), produced a dose-dependent reduction in inter-crystal distance. (b) BW 723C86-mediated reduction in inter-crystal distance (mean ± S.E.M., mm) following pretreatment with vehicle (open circles), piboserod (0.1 mg kg^{-1} s.c.; open squares) or SB 206553 (1 mg kg^{-1} s.c.; open triangles).

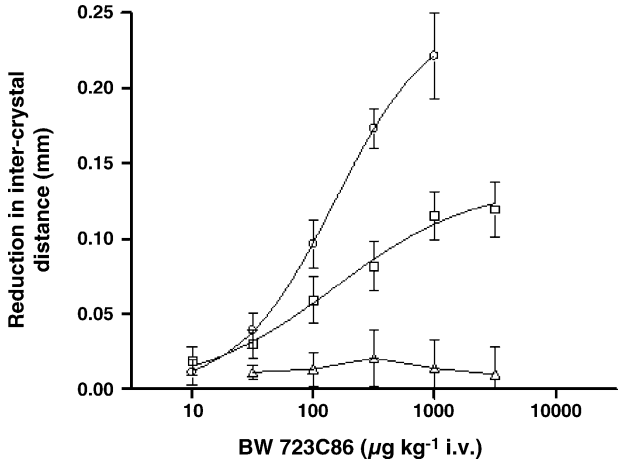

Fig. 2. BW 723C86-mediated reduction in stomach fundus inter-crystal distance (mean ± S.E.M., mm) in rats pretreated with vehicle (open circles) or tegaserod (1 and 3 mg kg^{-1} s.c.; open squares and triangles, respectively). Rats were dosed with piboserod (0.1 mg kg^{-1} s.c.) 5 min prior to administration of tegaserod or its vehicle (1 ml kg^{-1}).

3.2. Rat oesophagus

Following crystal placement on the rat oesophagus, 30 min proved sufficient to establish a stable sonomicrometry recording. No spontaneous changes in oesophageal muscle length were observed after this stabilization period, and preparations remained viable for up to 2 h. Following i.v. administration, tegaserod (0.001–0.3 mg kg^{-1}, $n = 4$), cisapride (0.03–3 mg kg^{-1}, $n = 3$) and mosapride (0.1–10 mg kg^{-1}, $n = 5$) but not their vehicles (0.5 ml kg^{-1}), evoked dose-dependent increases in inter-crystal distance, consistent with relaxation of the oesophagus (Fig. 3a and b). The responses had a rapid onset (typically 0.5–1 min after dosing) and reached a maintained maximum response within 2–3 min. The calculated mean ED_{50} values (with 95% confidence limits) for tegaserod, cisapride and mosapride were 11 (7–15) μg kg^{-1}, 142 (46–434) and 1824 (240–14,048) μg kg^{-1}, respectively. As a result of the low potency of mosapride and solubility limitations, it was unclear whether a maximum response to the compound was achieved. It is considered that a combination of these issues, together perhaps, with an inherently high degree of inter-tissue variability, contributed to the large confidence interval in the mosapride potency determination. The mean maximum responses evoked by tegaserod and cisapride were similar (0.19 (0.17–0.20) mm and 0.18 (0.12–0.25) mm, respectively; equivalent to a maximum change from resting levels of 8.7 (8.0–9.4) and 8.3 (5.4–11.2)%, respectively). Following s.c. dosing, tegaserod (0.003–3 mg kg^{-1}) increased the inter-crystal distance in a dose-dependent manner (ED_{50} = 17.2 (9.7–30.4) μg kg^{-1}, maximum

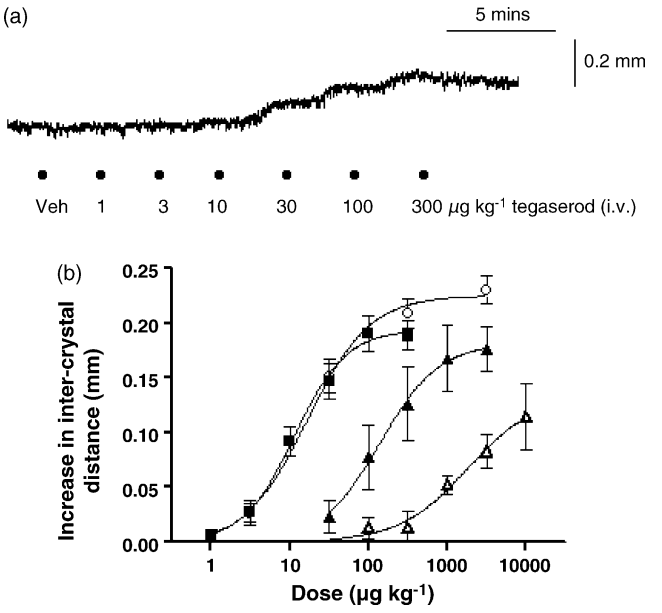

Fig. 3. (a) Representative digital sonomicrometry recording of oesophageal mechanical activity in the anaesthetized rat. Tegaserod (0.001–0.3 mg kg^{-1} i.v.), but not its vehicle (Veh), produced a dose-dependent increase in inter-crystal distance. (b) Tegaserod (0.001–0.3 mg kg^{-1} i.v. and 0.003–3 mg kg^{-1} s.c.; filled squares and open circles, respectively), cisapride (0.03–3 mg kg^{-1} i.v.; filled triangles) and mosapride (0.1–10 mg kg^{-1} i.v.; open triangles)-mediated increases in oesophageal inter-crystal distance (mean ± S.E.M., mm) in anaesthetized rats.

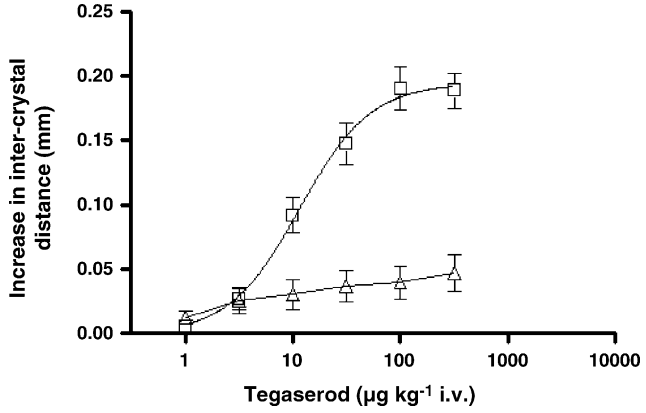

Fig. 4. Tegaserod-mediated increases in oesophageal inter-crystal distance (mean ± S.E.M., mm) following pretreatment with vehicle (1 ml kg^{-1} s.c.; open squares) or piboserod (0.1 mg kg^{-1} s.c.; open triangles).

response of 0.23 (0.20–0.25) mm; Fig. 3). Tegaserod (s.c.)-mediated responses were slower in onset (4–5 min after dosing, reaching a plateau generally by 10–15 min) than those following i.v. dosing. Pretreatment of rats with piboserod (0.1 mg kg^{-1} s.c.) abolished the tegaserod (0.001–0.3 mg kg^{-1} i.v.)-mediated oesophageal responses (Fig. 4). Piboserod (0.1 mg kg^{-1} s.c.) had no effect on resting inter-crystal distance per se.

4. Discussion

Tegaserod (Zelnorm®) was introduced recently for the treatment of constipation-predominant irritable bowel syndrome and chronic constipation, and is currently under clinical evaluation in diabetic gastroparesis, gastro-oesophageal reflux disease and functional dyspepsia [7]. The clinical efficacy of tegaserod is attributed to its 5-HT$_4$ receptor agonist activity, although it has affinity for other, non-5-HT$_4$ receptors relevant to gastrointestinal function, such as the 5-HT$_2$ receptor family [11]. Recently, tegaserod was shown to possess high 5-HT$_{2B}$ receptor binding affinity and potent 5-HT$_{2B}$ antagonist activity. Localization of 5-HT$_{2B}$ receptors in the smooth muscle and myenteric plexus of the gastrointestinal tract, and their role in augmenting neuronally-mediated contraction of the human colon has been demonstrated [4].

This study examined the 5-HT$_{2B}$ receptor antagonist and 5-HT$_4$ agonist activities of tegaserod. As the rat isolated stomach fundus [16] and oesophagus [17] have been used widely for assaying, respectively, 5-HT$_{2B}$ and 5-HT$_4$ receptor agonist-mediated changes in isometric tension, these tissues were selected for this in vivo digital sonomicrometry investigation. In addition to tegaserod, the selective 5-HT$_{2B}$ receptor agonists, α-methyl 5-HT and BW 723C86, and the selective 5-HT$_4$ or 5-HT$_{2B/2C}$ receptor antagonists, piboserod [25] or SB 206553, respectively, were used as pharmacological tools to validate the study findings. BW 723C86 and α-methyl 5-HT possess at least 80 or 10-fold higher affinity, respectively, for human and rat 5-HT$_{2B}$ receptors over the 5-HT$_{2A}$ and 5-HT$_{2C}$ subtypes and considerably greater selectivity over other non-5-HT$_2$ receptors [26,27]. SB 206553 is a potent, mixed 5-HT$_{2B/2C}$ receptor antagonist with at least 100-fold selectivity over 5-HT$_{2A}$ and non-5-HT$_2$ receptors [22].

The data from this study are consistent with the documented role for 5-HT$_{2B}$, but not 5-HT$_4$ receptor agonist activity, in contraction of the rat stomach fundus. Thus, BW 723C86 and α-methyl 5-HT, but not tegaserod, produced dose-dependent reductions in inter-crystal distance indicative of smooth muscle contraction. With the oesophagus, following i.v. administration of tegaserod, a dose-dependent increase in inter-crystal distance was observed, consistent with 5-HT$_4$ receptor agonist-mediated relaxation of this preparation in vitro [28,29]. In support of this conclusion, the 5-HT$_4$ receptor agonists, cisapride and mosapride, also increased inter-crystal distance and pretreatment with the selective 5-HT$_4$ receptor antagonist, piboserod, inhibited the tegaserod-induced responses. The rank order of agonist potency following i.v. administration was tegaserod > cisapride > mosapride, consistent with their relative potencies at the 5-HT$_4$ receptor [29,30].

This study provided further evidence that tegaserod is not a selective 5-HT$_4$ receptor agonist, but rather is a mixed 5-HT$_4$ agonist/5-HT$_{2B}$ antagonist. Thus, tegaserod (1 and 3 mg kg^{-1} s.c.), in animals pretreated with piboserod, at a dose (0.1 mg kg^{-1} s.c.) which abolished 5-HT$_4$ receptor agonist-mediated oesophageal responses, inhibited the contractile response evoked by BW 723C86 (0.01–1 mg kg^{-1} i.v.) in the stomach fundus. SB 206553, a selective 5-HT$_{2B/2C}$ receptor antagonist, also inhibited the BW 723C86-mediated responses.

Rodent studies implicate activation of 5-HT$_{2B}$ receptors in the development of the enteric nervous system [6]. It is evident that mRNA for the 5-HT$_{2B}$ receptor is expressed throughout the

human and rodent gastrointestinal tract [4,6]. In humans, 5-HT_{2B} receptor protein is localized in circular and longitudinal muscle layers, and in myenteric neurons of the colon [4]. Furthermore, 5-HT_{2B} receptor activation is associated with augmentation of neurogenic contractions of human isolated colonic longitudinal smooth muscle [4], consistent with intestinal prokinetic activity in humans.

The observations that tegaserod has identical binding affinity (K_i = 4 nM) for the human 5-HT_{2B} and 5-HT_4 receptor subtypes [15], and that the maximum steady-state plasma concentration is approximately 9 nM after oral administration of the approved clinical dose in humans [31], suggest that 5-HT_{2B} receptors are occupied by tegaserod therapeutically. In this rodent study, the 5-HT_4 receptor agonist potency of tegaserod exceeded its 5-HT_{2B} antagonist potency when administered via the same, s.c., route, despite the identical affinity of tegaserod at these subtypes [15]. This discrepancy may be a consequence of high 5-HT_4 receptor efficacy of tegaserod, a large 5-HT_4 receptor reserve in oesophageal tissue and/or differences in penetration of tegaserod to the 5-HT_{2B} and 5-HT_4 receptors in the stomach and oesophagus, respectively. A disparity in tissue penetration is unlikely; in isolated rat oesophagus and stomach fundus, there was good agreement between the corresponding pEC_{50} and pK_b values [15]. As the 5-HT_{2B} and 5-HT_4 receptor functional potencies will be dependent clinically on the nature and degree of a pathophysiological role for 5-HT at each receptor subtype, the significance of the potency differential in the rat is unclear. The relevance of the 5-HT_{2B} receptor antagonist activity of tegaserod in the human gastrointestinal tract should become clear when highly selective 5-HT_{2B} receptor antagonists or 5-HT_4 agonists have been evaluated therapeutically. Such compounds are now in clinical development and data are awaited with interest.

Acknowledgements

We thank Drs Patrick Humphrey, Roger Thomas and Jan Smith for their comments on the manuscript during its preparation.

References

[1] Gershon MD. Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel. Aliment Pharmacol Ther 1999;13:15–30.
[2] Jin JG, Foxx-Orenstein AE, Grider JR. Propulsion in guinea pig colon induced by 5-hydroxytryptamine (5-HT) via 5-HT_4 and 5-HT_3 receptors. J Pharmacol Exp Ther 1999;288:93–7.
[3] Hillier K, Tam FSF, Bunce KT, Grossman C. Inhibition of motility induced by the activation of 5-HT_1 and 5-HT_4 receptor types in isolated human colon smooth muscle. Br J Pharmacol 1994;112:102.
[4] Borman RA, Tilford NS, Harmer DW, et al. 5-HT(2B) receptors play a key role in mediating the excitatory effects of 5-HT in human colon in vitro. Br J Pharmacol 2002;135:1141–51.
[5] Borman RA, Burleigh DE. Functional evidence for a 5-HT_{2B} receptor mediating contraction of longitudinal muscle in human small intestine. Br J Pharmacol 1995;114:1525–7.
[6] Fiorica-Howells E, Maroteaux L, Gershon MD. Serotonin and the 5-HT_{2B} receptor in the development of enteric neurones. J Neurosci 2000;20:294–305.
[7] Camilleri M. Review article: tegaserod. Aliment Pharmacol Ther 2001;15:277–89.
[8] Deruyttere M, Lepoutre L, Heylen H, Samain H, Pennoit H. Cisapride in the management of chronic functional dyspepsia: a multicenter, double-blind, placebo-controlled study. Clin Ther 1987;10:44–51.
[9] Muller-Lissner SA. Treatment of chronic constipation with cisapride and placebo. Gut 1987;28:1033–8.
[10] Hoyer D, Fehlmann D, Langenegger D, et al. High affinity of SDZ HTF-919 related molecules for calf and human caudate 5-HT_4 receptors. Annals New York Acad Sci 1998;861:267–8.
[11] Buchheit KH, Gamse R, Giger R, et al. The serotonin 5-HT_4 receptor. 2. Structure-activity studies of the indole carbazimidamide class of agonists. J Med Chem 1995;38:2331–8.
[12] Grider JR, Foxx-Orenstein AE, Jin JG. 5-Hydroxytryptamine$_4$ receptor agonists initiate the peristaltic reflex in human, rat, and guinea pig intestine. Gastroenterol 1998;115:370–80.
[13] Degen L, Matzinger D, Merz M, et al. Tegaserod, a 5-HT_4 receptor partial agonist, accelerates gastric emptying and gastrointestinal transit in healthy male subjects. Aliment Pharmacol Ther 1745-51;15.
[14] Prather CM, Camilleri M, Zinsmeister AR, McKinzie S, Thomforde G. Tegaserod accelerates orocecal transit in patients with constipation-predominant irritable bowel syndrome. Gastroenterology 2000;118:463–8.
[15] Beattie DT, Smith JA, Marquess D, et al. The 5-HT_4 receptor agonist, tegaserod, is a potent 5-HT_{2B} receptor antagonist in vitro and in vivo. Br J Pharmacol 2004;143:549–60.
[16] Baxter GS, Murphy OE, Blackburn TP. Further characterization of 5-hydroxytryptamine receptors (putative 5-HT_{2B}) in rat stomach fundus longitudinal muscle. Br J Pharmacol 1994;112:323–31.
[17] Bieger D, Triggle C. Pharmacological properties of mechanical responses of the rat oesophageal muscularis mucosae to vagal and field stimulation. Br J Pharmacol 1985;84:93–106.
[18] Nagakura Y, Ito H, Kiso T, Naitoh Y, Miyata K. The selective 5-hydroxytryptamine (5-HT)4-receptor agonist RS67506 enhances lower intestinal propulsion in mice. Jpn J Pharmacol 1997;74:209–12.
[19] Yoshida N, Omoya H, Kato S, Ito T. Pharmacological effects of the new gastroprokinetic agent mosapride citrate and its metabolites in experimental animals. Arzneimittelforschung 1993;43:1078-L1083.
[20] Inui A, Yoshikawa T, Nagai R, Yoshida N, Ito T. Effects of mosapride citrate, a 5-HT_4 receptor agonist, on colonic motility in conscious guinea pigs. Jpn J Pharmacol 2002;90:313–20.
[21] Adelson DW, Million M. Tracking the moveable feast: sonomicrometry and gastrointestinal motility. News Physiol Sci 2004;19:27–32.
[22] Kennett GA, Wood MD, Bright F, et al. In vitro and in vivo profile of SB 206553, a potent 5-HT_{2C}/5-HT_{2B} receptor antagonist with anxiolytic-like properties. Br J Pharmacol 1996;117:427–34.
[23] Sanger GJ, Banner SE, Smith MI, Wardle KA. SB-207266: 5-HT_4 receptor antagonism in human isolated gut and prevention of 5-HT-evoked sensitization of peristalsis and increased defaecation in animal models. Neurogastroenterol Motil 1998;10:271–9.
[24] Kato S, Morie T, Harada H, Yoshida N, Karasawa T, Matsumato JI. Novel benzamides as selective and potent gastrokinetic agents. III. Synthesis and structure activity relationships of 4-amino-5-chloro-2-methoxy- and 2-ethoxy-*N*-[(4-substituted 2-morpholinyl0methyl]benzamides. Chem Pharm Bull 1992;40:652–60.
[25] Gaster LM, Joiner GF, King FD, et al. *N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carboxamide hydrochloride: the first antagonist amide with oral activity. J Med Chem 1995;38:4760–3.
[26] Baxter GS. Novel discriminatory ligands for 5-HT_{2B} receptors. Behav Brain Res 1996;73:149–52.
[27] Porter RH, Benwell KR, Lamb H, et al. Functional characterization of agonists at recombinant human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors in CHO-K1 cells. Br J Pharmacol 1999;128:13–20.
[28] Pindon A, Van Hecke G, Van Gompel P, Lesage AS, Leysen JE, Jurzak M. Differences in signal transduction of two 5-HT_4 receptor splice variants: compound specificity and dual coupling with $G_{\alpha s}$- and $G_{\alpha i/o}$-proteins. Mol Pharm 2001;61:85–96.

[29] Baxter GS, Craig DA, Clarke DE. 5-Hydroxytryptamine4 receptors mediate relaxation of the rat oesophageal tunica muscularis mucosae. Naunyn-Schmiedebergs Arch Pharmacol 1991;343:439–46.

[30] Mine Y, Yoshikawa T, Oku S, Nagai R, Yoshida N, Hosoki K. Comparison of effect of mosapride citrate and existing 5-HT_4 receptor agonists on gastrointestinal motility in vivo and in vitro. J Pharm Exp Ther 1997;283:1000–8.

[31] Appel-Dingemanse S, Hirschberg Y, Osborne S, Pommier F, McLeod J. Multiple-dose pharmacokinetics confirm no accumulation and dose proportionality of the novel promotile drug tegaserod (HTF 919). Eur J Clin Pharmacol 2001;56:889–91.