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ARTICLE *in* BRITISH JOURNAL OF PHARMACOLOGY · DECEMBER 2004

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British Journal of Pharmacology (2004) 143, 549–560
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The 5-HT_4 receptor agonist, tegaserod, is a potent 5-HT_{2B} receptor antagonist *in vitro* and *in vivo*

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1 Tegaserod (Zelnorm®) is a potent 5-hydroxytryptamine$_4$ (5-HT_4) receptor agonist with clinical efficacy in disorders associated with reduced gastrointestinal motility and transit. The present study investigated the interaction of tegaserod with 5-HT_2 receptors, and compared its potency in this respect to its 5-HT_4 receptor agonist activity.

2 Tegaserod had significant binding affinity for human recombinant 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors (pK_i = 7.5, 8.4 and 7.0, respectively). The 5-HT_{2B} receptor-binding affinity of tegaserod was identical to that at human recombinant 5-$HT_{4(c)}$ receptors (mean pK_i = 8.4) in human embryonic kidney-293 (HEK-293) cells stably transfected with the human 5-$HT_{4(c)}$ receptor.

3 Tegaserod (0.1–3 μM) inhibited 5-HT-mediated contraction of the rat isolated stomach fundus potently (pA_2 = 8.3), consistent with 5-HT_{2B} receptor antagonist activity. Tegaserod produced, with similar potency, an elevation of adenosine 3′,5′ cyclic monophosphate in HEK-293 cells stably transfected with the human 5-$HT_{4(c)}$ receptor (mean pEC_{50} = 8.6), as well as 5-HT_4 receptor-mediated relaxation of the rat isolated oesophagus (mean pEC_{50} = 8.2) and contraction of the guinea-pig isolated colon (mean pEC_{50} = 8.3).

4 Following subcutaneous administration, tegaserod (0.3 or 1 mg kg^{-1}) inhibited contractions of the stomach fundus in anaesthetized rats in response to intravenous dosing of α-methyl 5-HT (0.03 mg kg^{-1}) and BW 723C86 (0.3 mg kg^{-1}), selective 5-HT_{2B} receptor agonists. At similar doses, tegaserod (1 and 3 mg kg^{-1} subcutaneously) evoked a 5-HT_4 receptor-mediated increase in colonic transit in conscious guinea-pigs.

5 The data from this study indicate that tegaserod antagonizes 5-HT_{2B} receptors at concentrations similar to those that activate 5-HT_4 receptors. It remains to be determined whether this 5-HT_{2B} receptor antagonist activity of tegaserod contributes to its clinical profile.
British Journal of Pharmacology (2004) **143,** 549–560. doi:10.1038/sj.bjp.0705929

Keywords: Tegaserod; 5-HT_4; 5-HT_{2B}; gastrointestinal motility; stomach fundus

Abbreviations: cAMP, adenosine 3′,5′ cyclic monophosphate; CHO-K1, Chinese hamster ovary-K1; cIBS, constipation-predominant irritable bowel syndrome; HEK-293, human embryonic kidney-293; 5-HT, 5-hydroxytryptamine; IA, intrinsic activity; LSD, lysergic acid diethylamide; 10% SBE/CD, sulphobutyl ether-beta cyclodextrin

Introduction

5-Hydroxytryptamine (5-HT) plays a pivotal physiological role in the regulation of gastrointestinal motility (Read & Gwee, 1994). Of the recognized 5-HT receptor subtypes (Hoyer & Martin, 1997), 5-HT_1, 5-HT_{2A}, 5-HT_{2B}, 5-HT_3 and 5-HT_4 receptors mediate many of the gastrointestinal responses to 5-HT. Activation of 5-HT_4 receptors, for example, on motor neurones and interneurones within the gut wall, is associated with facilitation of cholinergic and nonadrenergic, noncholinergic neurotransmission. Release of acetylcholine, substance P and calcitonin gene-related peptide results in propulsion of contents along the gastrointestinal tract (Jin *et al*., 1999). Additionally, 5-HT_4 receptors are expressed on smooth muscle cells, activation of which results in, for example, oesophageal relaxation in rats (Briejer *et al*., 2001) and inhibition of human colonic circular muscle contractile activity (Hillier *et al*., 1994). Furthermore, activation of 5-HT_4 receptors on enteric neurones or enterocytes promotes fluid secretion in the gastrointestinal tract in a variety of species, including man (Hansen & Skadhauge, 1997).

In support of the postulated role of 5-HT_4 receptor activation in the regulation of gastrointestinal motility, 5-HT_4 receptor agonists such as cisapride (Propulsid®), prucalopride, renzapride and tegaserod have demonstrated efficacy in patients with constipation-predominant irritable bowel syndrome (cIBS), chronic constipation, functional dyspepsia or gastroparesis (Deruyttere *et al*., 1987; Muller-Lissner, 1987; Abell *et al*., 1991; Camilleri, 2001). Tegaserod was recently approved for the short-term treatment of cIBS in females and chronic constipation in males and females, in the United States and some other countries. Preclinical investigation has demonstrated that tegaserod has high binding affinity at human 5-HT_4 receptors (Hoyer *et al*., 1998). Tegaserod produces relaxation of canine rectal circular smooth muscle (Prins *et al*., 1999), neuronally mediated contraction of the guinea-pig ileum (Buchheit *et al*., 1995) and initiation or

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Advance online publication: 4 October 2004

facilitation of the peristaltic reflex in guinea-pig, rat and human intestine (Grider *et al*., 1998; Ji *et al*., 2004). All of these effects are attributed to 5-HT$_4$ receptor agonist activity. Tegaserod also attenuates sensory neurotransmission in the human rectum (Coffin *et al*., 2003), consistent with its ability to relieve the sensory symptoms of cIBS.

It is evident from limited published data that tegaserod and structurally related analogues are not selective for the 5-HT$_4$ receptor, having some affinity for 5-HT$_1$ and 5-HT$_2$ receptors (Buchheit *et al*., 1995; Briejer *et al*., 2001). An interaction of tegaserod with the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor subtypes would be expected to influence gastrointestinal activity. 5-HT$_{2A}$ receptors are located on enteric neurones and smooth muscle cells of the gastrointestinal tract, and their activation is associated with, for example, secretion from isolated human colonic mucosa, and contraction of canine stomach or canine and guinea-pig colonic longitudinal smooth muscle (Borman & Burleigh, 1996; Prins *et al*., 1997; Fiorica-Howells *et al*., 2002). 5-HT$_{2B}$ receptors are located in the longitudinal and circular smooth muscle layers, and in the myenteric nerve plexus of the gastrointestinal tract in a variety of species, including man (Engel *et al*., 1984; Borman *et al*., 2002). Furthermore, 5-HT$_{2B}$ receptor activation is associated with a neuronally mediated enhancement of electrically evoked contractile activity of human isolated colonic longitudinal smooth muscle (Borman *et al*., 2002). It is postulated that 5-HT$_{2B}$ receptor antagonism may result in inhibition of both 5-HT-mediated gastrointestinal motility and visceral hypersensitivity.

The primary objective of the present investigation was to study the interaction of tegaserod with 5-HT$_2$ receptors. Following demonstration of high binding affinity for tegaserod at 5-HT$_{2B}$ receptors (*vide infra*), its functional activity at this subtype was characterized *in vitro* and *in vivo*. In addition to determining the extent of tegaserod binding at human recombinant 5-HT$_{2B}$ receptors, its 5-HT$_{2B}$ functional activity was evaluated in the rat isolated stomach fundus, a tissue that contracts in response to 5-HT$_{2B}$ receptor activation (Bonhaus *et al*., 1999). Contractile activity of the stomach fundus in anaesthetized rats was also recorded to investigate the influence of tegaserod on 5-HT$_{2B}$ receptor-mediated responses *in vivo*. To assess the significance of any 5-HT$_{2B}$ receptor interaction, the agonist activity of tegaserod at human, rat and guinea-pig 5-HT$_4$ receptors was determined. Thus, radioligand binding and cAMP accumulation studies were carried out using human recombinant 5-HT$_{4(c)}$ receptors. The 5-HT$_4$ receptor activity of tegaserod was also investigated *in vitro*, in the rat oesophageal tunica muscularis mucosa and guinea-pig colonic longitudinal muscle/myenteric plexus preparations, and, *in vivo*, in a guinea-pig colonic transit model. In the majority of the assays, piboserod and SB 206553, selective 5-HT$_4$ and 5-HT$_{2B/2C}$ receptor antagonists, respectively (Kennett *et al*., 1996; Sanger *et al*., 1998), were used to establish the nature of the pharmacological responses measured. Preliminary data were presented at the Digestive Disease Week meeting, held in New Orleans, May 15–20, 2004.

Methods

All animal experiments were conducted in accordance with the principles of laboratory animal care provided by the Institutional Animal Care and Usage Committee of Theravance, Inc.

Receptor-binding studies

Cell membrane preparation Radioligand-binding studies were conducted using membranes prepared from cell lines transfected with the respective human recombinant receptor. Membranes prepared from Chinese hamster ovary-K1 (CHO-K1) cells stably transfected with 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors were prepared as described previously (Stam *et al*., 1994; Bonhaus *et al*., 1995). Membranes prepared from CHO-K1 cells stably transfected with 5-HT$_{2B}$ receptors were purchased from Euroscreen (Brussels, Belgium). HEK-293 cells stably transfected with the human recombinant 5-HT$_{4(c)}$ receptor splice variant were cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with D-glucose (4500 mg l^{-1}), 10% foetal bovine serum and 100 U of penicillin (100 μg)–streptomycin ml^{-1} and geneticin (800 μg ml^{-1}) in a 5% CO_2, humidified incubator (37°C). At 20–22 h prior to harvest, cells were washed twice and then cultured in serum-free DMEM. Cells were harvested by gentle mechanical agitation and then centrifugation (1200 × *g* for 5 min). The pellet was resuspended in 50 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulphonic acid *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulphonic acid) (HEPES), pH 7.4, and homogenized with a polytron disrupter (setting 19, 2 × 10 s) on ice. The resultant homogenate was centrifuged (1200 × *g* for 5 min), the pellet discarded and the supernatant centrifuged (40,000 × *g* for 20 min). The pellet was washed once by resuspension in 50 mM HEPES (pH 7.4), and centrifugation (40,000 × *g* for 20 min). The final pellet was resuspended in 50 mM HEPES (pH 7.4) and aliquots stored at −80°C, until required.

Binding assay conditions Human recombinant 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$ and 5-HT$_{4(c)}$ receptor membrane radioligand-binding assays were conducted as described previously (Grossman *et al*., 1993; Stam *et al*., 1994; Bonhaus *et al*., 1995; Pindon *et al*., 2002). Briefly, membranes prepared from cells stably transfected with human recombinant 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$ and 5-HT$_{4(c)}$ receptors were incubated with radiolabelled ligands with a high affinity for the given receptor, that is, [^{3}H]ketanserin, [*N*-methyl-^{3}H]lysergic acid diethylamide (LSD), [^{3}H]mesulergine and [^{3}H]GR113808, respectively. Nonspecific radioligand binding was defined by ketanserin (1 μM), 5-HT (10 μM), SB 242084 (10 μM) and GR113808 (1 μM), respectively.

Competition-binding studies were conducted with increasing concentrations of unlabelled ligand (10 pM–30 μM) and a fixed concentration of radioligand (in nM): [^{3}H]ketanserin (0.5), [*N*-methyl-^{3}H]LSD (1.2), [^{3}H]mesulergine (1) and [^{3}H]GR113808 (0.15). For [^{3}H]GR113808, the radioligand concentration was ~6-fold the K_D value, but for all others the concentration was at, or close to, the K_D. Following an incubation period sufficient to reach equilibrium: 15 min at 37°C, 30 min at 37°C, 30 min at 37°C and 60 min at 22°C, respectively, the membranes were harvested by rapid filtration and bound radioactivity quantitated by liquid scintillation spectroscopy.

Binding data were analysed by nonlinear regression analysis using GraphPad Prism™ software (GraphPad Software, Inc., San Diego, CA, U.S.A.) and a three-parameter model for one-site competition. pK_i (negative decadic logarithm of K_i) values for test compounds were calculated from the best-fit IC_{50} values, and the K_d value of the radioligand, using the

Cheng–Prusoff equation (Cheng & Prusoff, 1973): $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the concentration of the radioligand.

Functional 5-HT$_{2B}$ receptor activity

Rat isolated stomach fundus The rat stomach fundus responds to 5-HT with a 5-HT$_{2B}$ receptor-mediated contraction (Bonhaus *et al*., 1999). Adult, male Sprague–Dawley rats (200–350 g, Harlan, IN, U.S.A.) were euthanized by CO_2 asphyxiation and thoracotamy. The stomach was cut transversely to isolate the fundus, and the contents were removed by cutting along the smaller curvature of the fundus. The tissue was then transferred to a petri dish containing Tyrode's buffer containing (in mM): KCl (2.4), NaH_2PO_4 (0.4), $MgCl_2$ (1.1), NaCl (136.9), D-glucose (5.6), $NaHCO_3$ (11.9), $CaCl_2$ (1.8), corticosterone (0.03, to prevent 5-HT uptake) and pargyline (0.1, to inhibit monoamine oxidase). The tissue was cut into strips of approximately 20 mm in length and 2 mm in width, and the longitudinal muscle in each strip was then gently removed from the circular muscle and mucosa by lifting it away with fine forceps. Each strip was mounted, under a tension of 1 g, in a tissue bath filled with Tyrode's solution. The solution was aerated with 95% O_2/5% CO_2 and maintained at 37°C.

Tissues were allowed to equilibrate for approximately 1 h with washing at 0, 30 and 45 min. A cumulative concentration–effect curve to 5-HT (0.0001–1 μM, in half log increments) was then constructed. Upon completion of the 5-HT concentration–effect curve, the tissues were washed every 5 min for the next 30 min and every 10 min for a further 30 min. Tegaserod was then added and allowed to equilibrate with the tissue for 1 h, and a second concentration–effect curve to 5-HT was then constructed.

Changes in tension of the fundus (and the other *in vitro* tissue preparations used in this study; *vide infra*) were recorded by means of a force transducer (World Precision Instruments, model Fort 100), amplifier (Astro Med., model S48) and a data acquisition system (Biopac MP100, Acknowledge™ Waveform Acquisition and Analysis software). Agonist responses (% maximum contraction) were normalized to the maximum response of the first 5-HT concentration–effect curve. Data were analysed through iterative curve fitting to a logistic equation using Prism™ (GraphPad, Inc., San Diego, CA, U.S.A.). The equation used was as follows:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom})/(1 + 10((\text{LogEC}_{50} - X) * \text{HillSlope}))$$

where X is the logarithm of the drug concentration, and Y is the response (starting from the bottom of the curve and going to the top with a sigmoid shape). For antagonist studies, the concentration ratios (with respect to the 5-HT concentration–effect curves in the absence and presence of antagonist) were calculated, and a pK_b (calculated at a single antagonist concentration) or pA_2 value determined by Schild regression analysis (Arunlakshana & Schild, 1959).

Rat stomach pressure Adult male Sprague–Dawley rats (280–390 g, Harlan, IN, U.S.A.), fasted overnight prior to the study, were anaesthetized with isoflurane (3%) in oxygen. The abdomen was shaved and a midline incision made to expose the duodenum. A small incision was made in the duodenum, approximately 2 cm from the pyloric sphincter, and a water-filled balloon (size no. 6, Harvard), connected to a polyethylene catheter (PE50 tubing), was inserted into the stomach fundus *via* the duodenal incision. The catheter was connected to a pressure transducer and data acquisition system (Biopac MP100, Acknowledge™ Waveform Acquisition and Analysis software) to allow continuous recording of fundus pressure to be made. The incisions in the duodenum and abdomen were closed (4–0 silk suture; Ethicon, Inc., Somerville, NJ, U.S.A.) and a stitch in the skin was used to secure the balloon catheter. The balloon was then filled with 3.0 ml of water from a 10 ml syringe using an infusion pump (0.5 ml min^{-1}, World Precision Instruments, Sarasota, FL, U.S.A. SP230iw). An incision was made in the neck, and the left jugular vein and carotid artery were exposed and catheterized (PE50 tubing). The carotid arterial cannula (prefilled with heparin (50 U ml^{-1}) in 0.9% saline) was connected *via* a pressure transducer (Biopac) to the Biopac data acquisition system to enable the measurement of blood pressure.

Rats were allowed at least 30 min to stabilize following surgery. Typically, spontaneous rhythmical changes in balloon pressure commenced during this period, representing contractility of the stomach fundus. The selective 5-HT$_{2B}$ receptor agonists, α-methyl 5-HT (0.03 mg kg^{-1}) and BW 723C86 (0.3 mg kg^{-1}), or their vehicles, were administered *via* the jugular venous catheter (1 ml kg^{-1}). These doses of α-methyl 5-HT and BW 723C86 were selected, as in initial experiments they were associated with increases in stomach pressure without marked changes in blood pressure. At 15 min after dosing with α-methyl 5-HT (0.03 mg kg^{-1}), tegaserod or its vehicle was administered subcutaneously (1 ml kg^{-1}). The selective 5-HT$_4$ receptor antagonist, piboserod (1 mg kg^{-1}; Sanger *et al*., 1998), was co-administered with tegaserod to exclude any influence of 5-HT$_4$ receptor activation on stomach pressure. After a further 15 min, rats were dosed with α-methyl 5-HT (0.03 mg kg^{-1}). Responses to α-methyl 5-HT were compared, by measuring the amplitude and area of stomach contractions, and data were expressed for the second α-methyl 5-HT challenge as a percentage of the first (statistical significance at $P<0.05$ by ANOVA and Dunnett's *post hoc* test, comparing the tegaserod and vehicle-induced responses). To avoid tachyphylaxis, each rat was challenged only once with BW 723C86, 15 min after subcutaneous co-administration of piboserod (1 mg kg^{-1}) with either tegaserod (1 mg kg^{-1}) or its vehicle, and data were compared by unpaired Student's *t*-test, with statistical significance set at $P<0.05$. The effect of the selective 5-HT$_{2B/2C}$ receptor antagonist, SB 206553 (1 mg kg^{-1}; Kennett *et al*., 1996), on the BW 723C86 responses was also investigated.

Functional 5-HT$_4$ receptor activity

Whole-cell cAMP accumulation studies Whole-cell cAMP accumulation studies were conducted using HEK-293 cells stably transfected with the human recombinant 5-HT$_{4(c)}$ receptor splice variant (B_{max} = 500–800 fmol mg^{-1} protein) and the Flashplate Adenylyl Cyclase Activation Assay System (NEN, SMP004B), as described previously (Pindon *et al*., 2002).

Cell culture Cells were cultured as described above (see Section cell membrane preparation). Cells were grown to

60–80% confluency and, at 20–22 h prior to harvest, washed twice and then cultured in serum-free DMEM. To harvest the cells, the media was aspirated and the cells were incubated for 5 min at room temperature with Versene (Life Technologies, Inc., Grand Island, NY, U.S.A.). The cells were lifted from the flask by gentle mechanical agitation, suspended in pre-warmed (37°C) Dulbecco's phosphate-buffered saline, and then harvested by centrifugation at $1200 \times g$ for 5 min. The supernatant was discarded and the pellet was resuspended in pre-warmed (37°C) 'stimulation buffer' provided with the Flashplate kit.

Measurement of cAMP formation Briefly, cells were diluted to a concentration of 5×10^5 cells ml^{-1} in pre-warmed (37°C) 'stimulation buffer', and preincubated at 37°C for 10 min. Cyclic AMP accumulation assays were performed with increasing concentrations of tegaserod and 5-HT (10 pM–100 μM). Cell suspension (50 μl) was added to each well of the Flashplate for a final assay volume of 100 μl. The cells were incubated, with shaking, at 37°C for 15 min. After the incubation period, a direct radioimmunoassay, using ^{125}I-cAMP, was performed by the addition of 100 μl of ice-cold 'detection buffer' to each well, according to the manufacturer's instructions. The plates were sealed and incubated at 4°C, overnight. Bound radioactivity was quantified by scintillation proximity spectroscopy using the Topcount (Packard BioScience Co., Meriden, CT, U.S.A.).

The amount of cAMP produced was extrapolated from a cAMP standard curve. Data were analysed by nonlinear regression analysis with the GraphPad Prism Software package (GraphPad Software, Inc., San Diego, CA, U.S.A.) using the three-parameter sigmoidal dose model (slope constrained to unity). Potency data were reported as pEC$_{50}$ values (negative decadic logarithm of the effective concentration producing 50% of the maximum response; mean $\pm$ s.d., $n > 3$). To determine the intrinsic activity (IA), relative to 5-HT, the maximum compound-evoked response (minus basal) was expressed as a percentage of the maximum response evoked by 5-HT (minus basal), assayed in parallel on the same plate.

Rat isolated oesophagus The rat precontracted oesophageal tunica muscularis mucosa responds to 5-HT$_4$ receptor activation with relaxation (Briejer *et al*., 2001). Adult, male Sprague–Dawley rats (250–350 g, Harlan, IN, U.S.A.) were euthanized by CO_2 asphyxiation and thoracotomy. The thoracic oesophagus was removed and placed in Tyrode's buffer containing (in μM) indomethacin (3.0; to inhibit prostaglandin synthesis), corticosterone (30.0; to prevent 5-HT uptake) and ketanserin (1.0; to block 5-HT$_2$ receptors). A pair of scissors was inserted between the submucosal layers and the outer striated muscle coat was cut longitudinally, and then gently peeled away leaving the inner tunica muscularis mucosa. Preparations of the tunica muscularis mucosa, each approximately 20 mm in length and 2 mm in width, were mounted longitudinally in 10 ml tissue baths filled with Tyrode's solution, which were aerated continuously with 95% O_2/5% CO_2, and maintained at 37°C.

A tension of 0.5 g was applied to each tissue. During the next 60 min, tissues were washed at 15 min intervals and tension re-applied to 0.5 g as necessary. Carbachol (3 μM; determined to be a submaximal concentration in initial experiments) was then added to the tissue baths to contract the oesophagus. When the contraction had reached a maximum and the tone had stabilized, a cumulative concentration–effect curve to 5-HT (0.001–10 μM in half-log increments) was constructed. Upon completion of the concentration–effect curve, the tissues were washed four times at 5 min intervals and then three more times every 15 min. Carbachol (3 μM) was then added to the tissue baths, and, when the contraction had stabilized, concentration–effect curves to tegaserod or 5-HT (each at 0.001–10 μM), were constructed. Responses (i.e. relaxations of carbachol-induced tone) were allowed to reach a maximum before addition of the next, ascending concentration. The mean potency (pEC$_{50}$) and IA (as a percentage of the 5-HT maximum response observed in the first 5-HT concentration–effect curve) were derived through iterative curve fitting (Prism™; GraphPad, Inc.).

Guinea-pig isolated colon Adult, male Dunkin Hartley guinea-pigs (200–300 g, Harlan, IN, U.S.A.) were euthanized by CO_2 asphyxiation and thoracotomy. The colon was removed, and placed in Krebs-Henseleit physiological buffer, containing (in mM): KCl (4.7), KH_2PO_4 (1.2), $MgSO_4$ anhydrous (1.2), NaCl (118.1), D-glucose (11.1), $NaHCO_3$ (25.0), $CaCl_2$ (2.6), ondansetron (0.003; to block 5-HT$_3$ receptors), methysergide (0.001; to block 5-HT$_1$ and 5-HT$_2$ receptors) and indomethacin (0.001; to inhibit prostaglandin synthesis). The colon was cut into 5 cm lengths, the contents gently removed and each segment then placed on a 10 ml pipette. An incision was made along the length of the colon with a scalpel blade, and the longitudinal muscle was then peeled off carefully using the tip of a thumb. Each longitudinal muscle strip was then mounted, under a tension of 1 g, in a 10 ml tissue bath filled with Krebs-Henseleit buffer. The bathing solution was aerated continuously with 95% O_2/5% CO_2, and maintained at 37°C.

During the next 45 min, tissues were washed three times (at 0, 15 and 30 min after mounting) and tension re-applied to 1 g as necessary. The tissues were then challenged with 5-HT at a concentration (0.3 μM) previously established to evoke a maximal contractile response. Once the contraction had reached its maximum, tissues were washed four times every 2 min and once more 10 min later. An additional priming challenge of 5-HT (0.3 μM) was made 15 min later. Following further washing prior to, and after a third 5-HT (0.3 μM) challenge, a cumulative concentration–effect curve to 5-HT (0.0001–3 μM) or tegaserod (0.0001–10 μM) was constructed. Responses were allowed to reach a maximum before addition of the next, ascending concentration. Contractile responses were normalized to the primed 5-HT (0.3 μM) response in each tissue. Data were analysed through iterative curve fitting to a logistic equation using Prism™ (GraphPad, Inc.). The mean potency (pEC$_{50}$) and IA (as a percentage of the 5-HT maximum response) were derived.

Colonic transit in conscious guinea-pigs Adult, male Duncan Hartley guinea-pigs (220–580 g, Harlan, IN, U.S.A.) were acclimated to the holding room (temperature controlled at 21 $\pm$ 1°C and 12 : 12 h light–dark cycle commencing at 07:00 h) for at least 1 week following arrival.

Guinea-pigs were anaesthetized with isoflurane (2–3%) in an induction chamber. Maintenance of anaesthesia was achieved with isoflurane (2–3%) administered *via* a nose cone.

The mid-scapular area and abdomen were shaved and cleansed with betadine and 70% isopropanol. The proximal colon was exposed, and a small incision made (approximately 2 cm from the cecum). A cannula consisting of micro-renathane (MRE-040) tubing with a 2 cm silicone rubber tip (0.047″ OD × 0.025″ ID) was introduced and advanced approximately 2 cm towards the aboral end. A purse-string suture (6-0 silk) was used to fix the cannula in the colon and an antibiotic (Baytril®; 2.27%) was then applied to the incision. The muscle layer was closed with a 4-0 Vicryl® suture (Ethicon, Inc.). The cannula was then secured to the nearby musculature with a 6-0 silk suture (Ethicon, Inc.) and tunnelled subcutaneously under the skin and exteriorized at the mid-scapular region. The cannula was sealed with a stainless steel pin and secured to the back of the neck with wound clips. The incisions in the peritoneum and abdomen were cleansed of blood and closed with a 3-0 Ethilon® suture (Ethicon, Inc.). Buprenex® (0.03 mg kg^{-1}) was administered subcutaneously immediately after surgery.

At least 5 days post-surgery, guinea-pigs were assigned randomly to a study group. At 5 min after subcutaneous administration of tegaserod (0.03–3 mg kg^{-1}) or vehicle, guinea-pigs were gently restrained and a nonabsorbable marker (0.2 ml) was infused into the proximal colon *via* the implanted cannula. The marker consisted of 6 g of carmine red dye per 15 ml of carboxymethyl cellulose (0.5%). The study personnel were blinded to the treatment that each animal received.

Commencing at 60 min after the infusion of the dye, animal cages were inspected visually for the presence of excreted red faecal pellets. This was repeated at 30 min intervals until each guinea-pig had excreted pellets containing the red marker, or until 10 h had lapsed from the time of the marker injection. In the case that an animal failed to produce red faecal pellets within 10 h, the animal was left overnight and inspected the following morning. If excretion of dye occurred overnight, a value of 10 h was assigned. Any guinea-pig that failed to produce red faecal pellets was removed from the study, and a post-mortem exploration of the abdominal cavity was performed. In these rare instances (<1% of animals), it was generally noted that the colonic catheter had been dislodged from the colon.

The whole colonic transit was defined as the time that lapsed between marker injection and the appearance of dye in the faeces. Data for each treatment group were averaged and expressed as the mean (±s.d.) transit time. Differences between treatment groups were then determined using one-way analysis of variance (ANOVA) with a Dunnett's *post hoc* test ($P<0.05$ considered to be statistically significant).

Materials

5-HT, indomethacin, ketanserin, pargyline, methysergide, SB 242084, carmine red dye and corticosterone were purchased from Sigma-Aldrich, St Louis, MO, U.S.A. Tegaserod and ondansetron were purchased from Apin Chemicals, Abingdon, Oxfordshire, U.K. and Sequoia Research Products, Oxford, Oxfordshire, U.K. respectively, while α-methyl 5-HT, SB 206553, BW 723C86 and GR113808 were purchased from Tocris Cookson, Ellisville, MO, U.S.A. Buprenex and Baytril were purchased from Reckitt & Colman Products, Richmond, VA, U.S.A. and Bayer, Shawnee Mission, KS, U.S.A., respectively. [^{3}H]ketanserin and [*N*-methyl-^{3}H]LSD were purchased from Perkin-Elmer, Boston, MA, U.S.A., while [^{3}H]mesulergine and [^{3}H]GR113808 were purchased from Amersham Biosciences, Newark, NJ, U.S.A. Piboserod was synthesized, as described by Gaster *et al*. (1995).

For radioligand-binding and cAMP accumulation studies, tegaserod and 5-HT were prepared at 10 mM in dimethyl sulphoxide (DMSO), diluted to 400 μM with 50 mM HEPES (pH 7.4) at 25°C, containing 0.1% bovine serum albumin (BSA), and then serially diluted (1 : 5) in the same buffer. For rat oesophagus and stomach, and guinea-pig colon, *in vitro* experiments, tegaserod was prepared at 10 mM in DMSO and then serially diluted in water, while 5-HT was dissolved in water. For *in vivo* experiments, α-methyl 5-HT, piboserod and SB 206553 were dissolved in 0.9% saline or 5% dextrose in water (D5W), while tegaserod was prepared in 10% sulphobutyl ether-beta cyclodextrin (10% SBE/CD). BW 723C86 was formulated in DMSO (1% by volume) in D5W. *In vivo* doses were expressed with respect to the free base weights of each compound.

Results

5-HT$_4$ and 5-HT$_2$ radioligand binding

Tegaserod had high binding affinity for the human 5-HT$_{4(c)}$ receptor (mean pK_i value (±s.d.) of 8.4±0.1; $n>20$) in membranes prepared from HEK-293 cells stably transfected with the human 5-HT$_{4(c)}$ receptor splice variant (Table 1 and Figure 1a). Tegaserod also had significant binding affinity

Table 1 *In vitro* 5-HT$_{2B}$ and 5-HT$_4$ receptor-binding affinity (mean pK_i±s.d.), and functional activity (mean pEC$_{50}$±s.d., IA (% of 5-HT maximum, with 95% confidence limits), pA_2 or pK_b values (±s.d.)) of test compounds

Compound	*Human 5-HT$_{4(c)}$*			*Guinea-pig colon*		*Rat oesophagus*		*Human 5-HT$_{2B}$*	*Rat stomach fundus*
	pK_i	*pEC$_{50}$*	*IA (%)*	*pEC$_{50}$*	*IA (%)*	*pEC$_{50}$*	*IA (%)*	*pK_i*	*pK_b or pA_2*
5-HT	6.9±0.1	8.1±0.2	100	8.1±0.1	98 (94–102)	7.9±0.2	98 (93–103)	8.1±0.3	n/a
Tegaserod	8.4±0.1	8.6±0.2	99 (97–101)	8.3±0.2	54 (49–58)	8.2±0.2	73 (69–80)	8.4±0.1	8.3±0.2
α-Methyl 5-HT	5.8±0.1	6.4±0.1	108 (103–113)	5.7±0.2	80 (68–92)	5.6±0.3	71 (51–91)	8.3±0.2	n/a
BW 723C86	5.5±0.1	<4	0	<4.5	0	<4.5	>25	7.6±0.2	n/a
SB 206553	4.9±0.1	<4	0	<4.5	0	<4.5	0	8.5±0.1	NT
Piboserod	10.4±0.1	NT		<4.5	0	<4.5	0	6.6±0.1	NT

Data represent $n\geqslant 3$ unless indicated otherwise.
n/a–not applicable, NT–not tested.

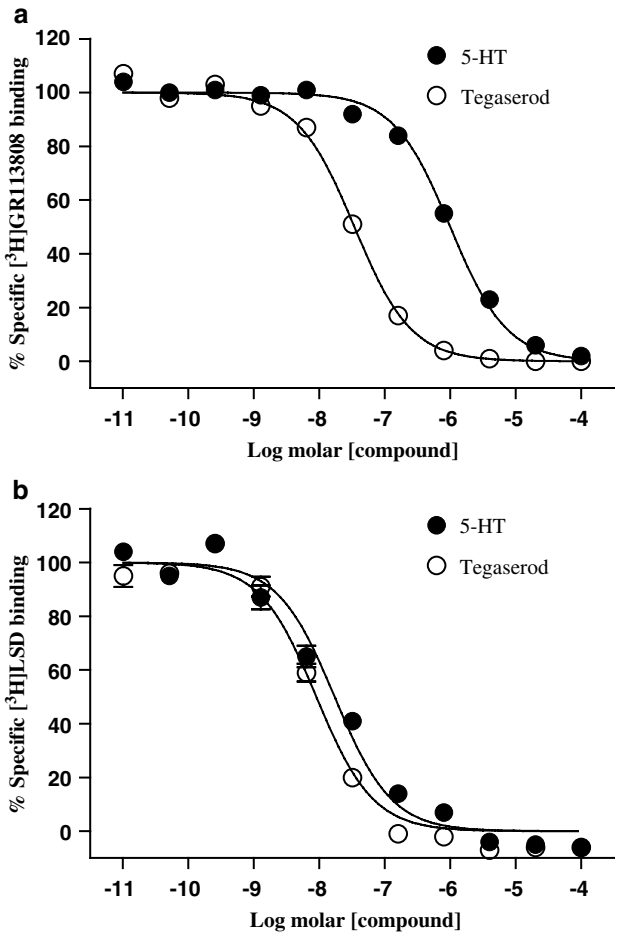

Figure 1 Effect of tegaserod and 5-HT on (a) [^{3}H]GR113808-specific binding to membranes prepared from HEK-293 cells stably transfected with human recombinant 5-HT$_{4(c)}$ receptors, and (b) [^{3}H]LSD-specific binding to membranes prepared from CHO-K1 cells stably transfected with human recombinant 5-HT$_{2B}$ receptors. Results are expressed as fitted curves to the mean (±s.d.) of 3–6 independent experiments.

for human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors (mean (±s.d.) pK_i values = 7.5, 8.4±0.1 and 7.0, respectively) expressed in membranes prepared from CHO-K1 cells stably transfected with the respective receptor (Figure 1b). Tegaserod had selectivities of eight- and 25-fold for the 5-HT$_{4(c)}$ receptor, compared to the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, respectively. Tegaserod had identical affinity for the 5-HT$_{4(c)}$ and the 5-HT$_{2B}$ receptor subtypes. The mean (±s.d.) pK_i values for 5-HT at the human 5-HT$_{4(c)}$ and 5-HT$_{2B}$ receptors were 6.9±0.1 and 8.1±0.3, respectively ($n \geqslant 4$). The 5-HT$_4$ receptor antagonist, piboserod, had higher affinity for the human 5-HT$_{4(c)}$ receptor, compared to the human 5-HT$_{2B}$ subtype (mean (±s.d.) pK_i values = 10.4±0.1 and 6.6±0.1, respectively; $n = 3$). The 5-HT$_2$ receptor agonists, α-methyl 5-HT and BW 723C86, and antagonist, SB 206553, had higher affinity for human 5-HT$_{2B}$ receptors compared to the 5-HT$_4$ subtype (mean pK_i values (±s.d.) = 8.3±0.2, 7.6±0.2 and 8.5±0.1 (5-HT$_{2B}$), and 5.8±0.1, 5.5±0.1 and 4.9±0.1 (5-HT$_4$), respectively ($n = 3$); Table 1).

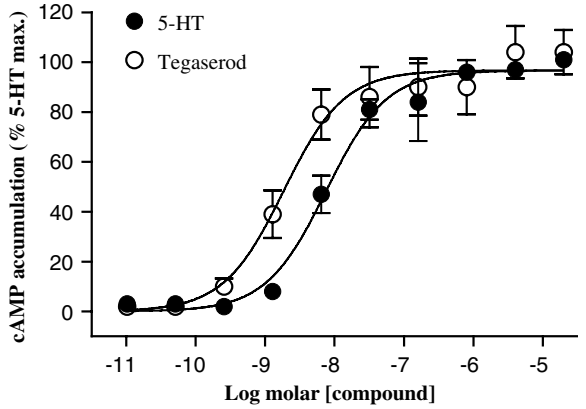

Figure 2 Concentration–effect curves to tegaserod and 5-HT in a whole-cell cAMP accumulation assay using HEK-293 cells stably transfected with human recombinant 5-HT$_{4(c)}$ receptors. Data are expressed as fitted curves to the mean (±s.d.) of three independent experiments, and as a percentage of the maximum cAMP accumulation response to 5-HT.

Functional 5-HT$_4$ receptor activity

Human recombinant 5-HT$_4$ receptors In cAMP accumulation assays, using HEK-293 cells stably transfected with the human 5-HT$_{4(c)}$ receptor, tegaserod had high potency (mean pEC$_{50}$ (±s.d.) = 8.6±0.2; $n > 20$) and high intrinsic agonist activity (mean maximum response of 99% (95% confidence intervals: 97–101%), relative to 5-HT; Table 1 and Figure 2). α-Methyl 5-HT had a mean pEC$_{50}$ value (±s.d.) of 6.4±0.1 and an IA of 108% (95% confidence intervals: 103–113%), relative to 5-HT ($n = 3$), while BW 723C86 and SB 206553 had no agonist activity at concentrations up to 100 μM ($n = 3$).

Rat isolated oesophagus Tegaserod (0.001–10 μM) produced a concentration-dependent relaxation of the carbachol-precontracted rat oesophageal tunica muscularis mucosa (Figure 3), as did 5-HT (0.001–10 μM). Tegaserod had a mean (±s.d.) pEC$_{50}$ value of 8.2±0.2 ($n = 6$) compared to 7.9±0.2 for 5-HT ($n = 7$), and an IA of 73% (95% confidence limits: 69–80%) compared to 98% (93–103%) for 5-HT, with respect to the maximum 5-HT-mediated relaxation of pre-contracted tone (Table 1). Incubation of tissues with the selective 5-HT$_4$ receptor antagonist, piboserod (30 nM), produced a rightward shift in the tegaserod concentration–effect curve (94-fold shift, pK_B = 9.5; data not shown). Piboserod had no agonist activity at 30 μM, while the 5-HT$_{2B}$ receptor agonists, α-methyl 5-HT and BW 723C86, were only weakly active (mean pEC$_{50}$ values = 5.6 and <4.5, with mean IA values of 71 and >25% of the 5-HT maximum, respectively ($n = 3$ or 4; Table 1)). The 5-HT$_{2B/2C}$ receptor antagonist, SB 206553, was inactive at concentrations up to 30 μM.

Guinea-pig isolated colon Tegaserod (0.0001–10 μM) and 5-HT (0.0001–3 μM) produced a concentration-dependent contraction of the guinea-pig isolated colon longitudinal muscle/myenteric plexus preparation (Figure 3b). The pEC$_{50}$ values (±s.d.) for tegaserod and 5-HT were 8.3±0.2 ($n = 6$) and 8.1±0.1 ($n = 12$), respectively (Table 1). Tegaserod had an IA of 54% (95% confidence limits: 49–58%) compared to 98% (94–102%) for 5-HT. α-Methyl 5-HT was only weakly active

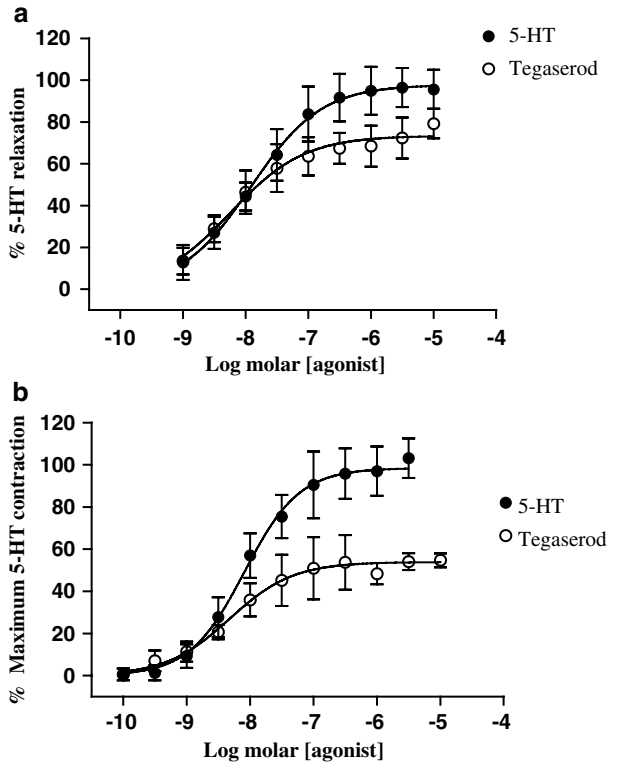

Figure 3 (a) Concentration–effect curves to tegaserod and 5-HT (each 0.001–10 μM; $n=6$ or 7, respectively) in the carbachol (3 μM)-precontracted rat isolated oesophageal tunica muscularis mucosa preparation. Values are expressed as the mean (±s.d.) percentage relaxation of that achieved by 5-HT. (b) Concentration–effect curves to tegaserod (0.0001–10 μM; $n=6$) and 5-HT (0.0001–3 μM; $n=12$) in the guinea-pig isolated colonic longitudinal muscle/myenteric plexus preparation. Values are expressed as the mean (±s.d.) percentage of the response to 5-HT.

(mean pEC_{50} value = 5.7, IA = 80% of the 5-HT maximum; $n=4$), while BW 723C86 had no agonist activity at concentrations up to 30 μM ($n=4$; Table 1). SB 206553 was inactive at concentrations up to 30 μM.

Colonic transit in conscious guinea-pigs In vehicle-treated guinea-pigs, the mean time taken for excretion of the first faecal pellet containing red dye was typically between 220 and 300 min (mean = 257 min; 95% confidence limits: 233–283 min, $n=84$). Subcutaneous administration of tegaserod (0.03, 0.3 and 3 mg kg^{-1}), but not its vehicle (10% SBE/CD, 2 ml kg^{-1}), produced a dose-dependent, prokinetic effect (Figure 4). Tegaserod produced an increase in transit of approximately 40% compared to its vehicle. Co-administration with piboserod (1 mg kg^{-1} subcutaneously), but not its vehicle (D5W, 2 ml kg^{-1}), attenuated the colonic prokinetic activity of tegaserod (1 mg kg^{-1}; Figure 5). In the same study, piboserod itself had no statistically significant effect on colonic transit.

Functional 5-HT$_{2B}$ receptor activity

Rat isolated stomach fundus Tegaserod (0.1–3 μM) had no agonist activity in the rat isolated stomach fundus. There was no change in resting tension of preparations in response to

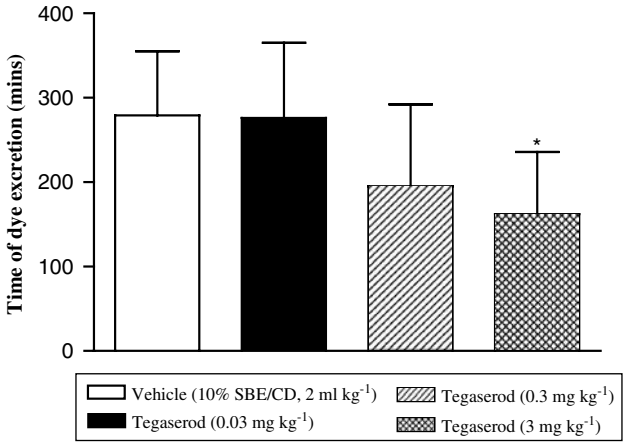

Figure 4 Effects of tegaserod (0.03, 0.3 and 3 mg kg^{-1}) and its vehicle (10% SBE/CD, 2 ml kg^{-1}), each administered subcutaneously, on the colonic transit of dye in conscious guinea-pigs ($n=10$ for each group). The data (mean±s.d.) are expressed in terms of the time at which the first faecal pellet containing dye was excreted (*$P<0.05$, ANOVA and Dunnett's *post hoc* test, compared to vehicle).

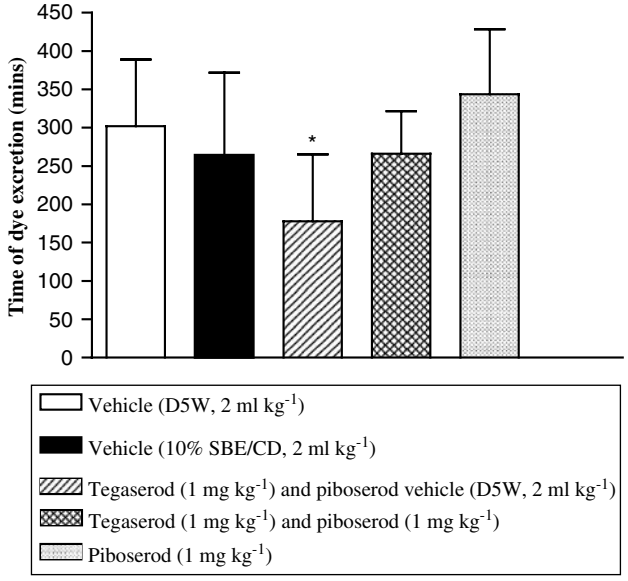

Figure 5 Influence of co-administration of subcutaneous piboserod (1 mg kg^{-1}) on the prokinetic activity of tegaserod (1 mg kg^{-1} subcutaneously) in conscious guinea pigs ($n=12$–15 for each group). The effects of piboserod alone, its vehicle (D5W, 2 ml kg^{-1}) and tegaserod vehicle (10% SBE/CD, 2 ml kg^{-1}) are also shown. The data (mean±s.d.) are expressed with respect to the time at which the first faecal pellet containing dye was excreted (*$P<0.05$, ANOVA and Dunnett's *post hoc* test), for comparison to vehicle.

tegaserod addition to the tissue baths. In the absence of tegaserod, two concentration–effect curves to 5-HT (0.0001–1 μM) were reproducible (data not shown). In the presence of increasing concentrations of tegaserod (0.1, 1 and 3 μM), the 5-HT concentration–effect curve shifted progressively to the right (Figure 6a). Schild regression analysis of these data determined that tegaserod had a pA_2 value of 8.3

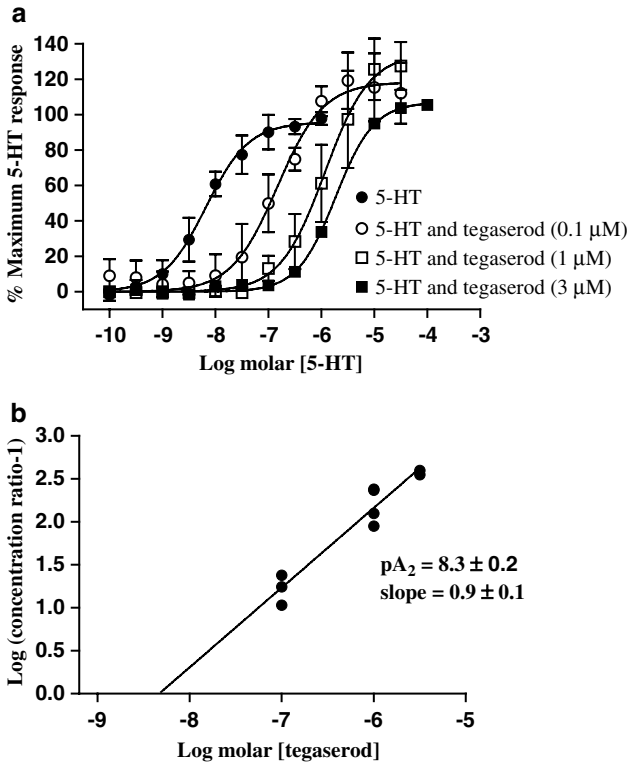

Figure 6 (a) Concentration–effect curves to 5-HT (0.0001–1 μM; $n=9$), and 5-HT (0.0001–100 μM) in the presence of tegaserod (0.1, 1 and 3 μM; $n=3$, 4 and 2, respectively), in the rat isolated stomach fundus preparation. Values are expressed as a mean ($\pm$s.d.) percentage of the 5-HT maximum contraction in each tissue. (b) Schild regression analysis of the tegaserod-mediated antagonism of 5-HT-induced contraction in the rat isolated stomach fundus preparation.

(slope $=0.9\pm0.1$; Table 1 and Figure 6b). In the presence of tegaserod (0.1 and 1 μM), the maximum contractile response to 5-HT was significantly higher than that in its absence, although this potentiation was less evident at the highest concentration of tegaserod (3 μM).

Rat stomach pressure Spontaneous rhythmical gastric activity commenced within 15–30 min of inflation of the balloon placed in the stomach fundus of isoflurane-anaesthetized rats. The selective 5-HT$_{2B}$ receptor agonists, α-methyl 5-HT (0.03 mg kg^{-1}) and BW 723C86 (0.3 mg kg^{-1}), but not their vehicles (0.9% saline and 1% DMSO in D5W, respectively, each 1 ml kg^{-1}) produced an increase in balloon pressure following intravenous administration, consistent with contraction of the stomach fundus. Initial experiments indicated that α-methyl 5-HT was more potent than BW 723C86, an observation that is in agreement with their relative 5-HT$_{2B}$ receptor-binding affinities (Table 1). The doses of α-methyl 5-HT and BW 723C86 selected for further studies (i.e. 0.03 and 0.3 mg kg^{-1}, respectively) were associated with reliable increases in stomach pressure, without marked changes in blood pressure (evident at higher doses of α-methyl 5-HT). Subcutaneous co-administration of piboserod (1 mg kg^{-1}) and the tegaserod vehicle (10% SBE/CD, 1 ml kg^{-1}) had no effect on the spontaneous activity of the stomach (data not shown). The contractile responses to α-methyl 5-HT prior to, and following, piboserod and vehicle co-administration, were similar in magnitude (Figure 7a). While subcutaneous co-administration of tegaserod (0.3 and 1 mg kg^{-1}) and piboserod (1 mg kg^{-1}) had no effect on spontaneous activity, an inhibition of the contractile activity induced by intravenous α-methyl 5-HT (0.03 mg kg^{-1}) was evident (Figure 7a). As responses to BW 723C86 were associated with tachyphylaxis, animals were challenged only once. At 15 min following subcutaneous administration with tegaserod and piboserod (both 1 mg kg^{-1}), the magnitude of the responses to intravenous BW 723C86 was significantly less than that in the absence of tegaserod (Figure 7b). The amplitude of BW 723C86-mediated contractions was significantly smaller following subcutaneous pretreatment of rats with the selective 5-HT$_{2B/2C}$ receptor antagonist, SB 206553 (1 mg kg^{-1}), compared to pretreatment with its vehicle (0.9% saline, 1 ml kg^{-1}; Figure 7b).

Conclusions

Disorders of reduced gastrointestinal motility and transit, such as cIBS, functional dyspepsia and diabetic gastroparesis, are commonly associated with discomfort, nausea and pain, and, as a result, have a debilitating impact on the quality of life of affected individuals. Tegaserod, a potent 5-HT$_4$ receptor agonist, was introduced recently for the treatment of cIBS and chronic constipation, and is currently under clinical evaluation in diabetic gastroparesis, gastro-oesophageal reflux disease and functional dyspepsia. Although the clinical efficacy of tegaserod has been attributed to 5-HT$_4$ receptor agonist activity, it is evident that tegaserod and structurally related analogues have affinity for other, non-5-HT$_4$ receptors relevant to gastrointestinal function (e.g. the 5-HT$_2$ receptor family; Buchheit *et al*., 1995). The present study characterized the *in vitro* and *in vivo* activity of tegaserod at 5-HT$_4$ and 5-HT$_2$ receptors. An interaction of tegaserod with 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors could have clinical significance as these 5-HT receptor subtypes are located on gastrointestinal smooth muscle cells and enteric neurones, where their activation is associated with enhancement of gastrointestinal motility or secretion (Engel *et al*., 1984; Borman & Burleigh, 1996; Prins *et al*., 1997; Borman *et al*., 2002). In addition to tegaserod, the selective 5-HT$_2$ receptor agonists, α-methyl 5-HT and BW 723C86, and the selective 5-HT$_4$ or 5-HT$_{2B/2C}$ receptor antagonists (i.e. piboserod and SB 206553, respectively), were used as pharmacological tools to validate the study findings. The *in vitro* 5-HT$_{2B}$ and 5-HT$_4$ receptor-binding affinities and functional potencies of these compounds, and their observed receptor subtype selectivities, were consistent with published literature (Baxter, 1996; Kennett *et al*., 1996; Sanger *et al*., 1998; Porter *et al*., 1999). Of the 5-HT$_2$ receptor subtypes, α-methyl 5-HT and BW 723C86 possess at least 10- or 80-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 (Baxter, 1996; Porter *et al*., 1999). 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 (Kennett *et al*., 1996).

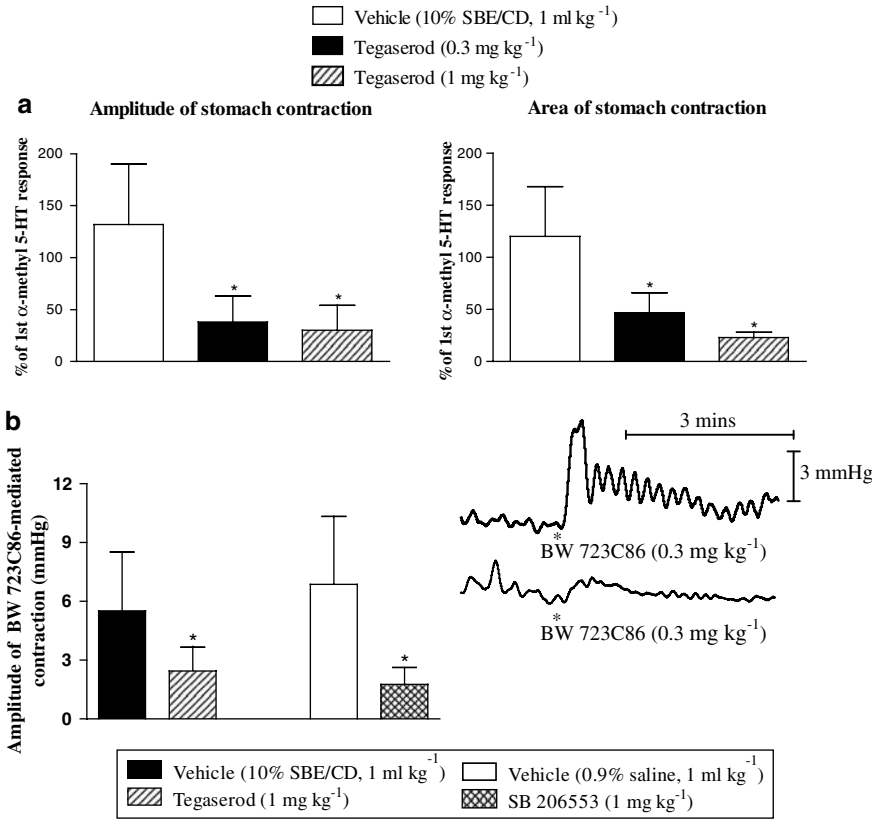

Figure 7 (a) Influence of tegaserod (0.3 or 1 mg kg^{-1}; $n=4$–6) or its vehicle (10% SBE/CD, 1 ml kg^{-1}; $n=5$ or 6), each administered subcutaneously, on intravenous (a) α-methyl 5-HT (0.03 mg kg^{-1})- or (b) BW 723C86 (0.3 mg kg^{-1})-mediated contraction of the stomach fundus in isoflurane-anaesthetized rats. Also shown in (b) is the effect of subcutaneous pretreatment with SB 206553 (1 mg kg^{-1}; $n=3$) or its vehicle (0.9% saline, 1 ml kg^{-1}; $n=3$) on the BW 723C86-mediated responses. Data (mean ± s.d.) are expressed in (a) with respect to the response (area and amplitude of the contraction) to the second α-methyl 5-HT challenge as a percentage of the first, and, in (b), as the amplitude (in mmHg) of the BW 723C86-evoked increase in stomach pressure (*$P<0.05$ by (a) ANOVA and Dunnett's *post hoc* test, or (b) unpaired Student's *t*-test). Representative traces of responses to BW 723C86 (0.3 mg kg^{-1} iv) are shown (inset in (b)) after pretreatment of rats with vehicle (10% SBE/CD; upper trace) or tegaserod (1 mg kg^{-1}; lower trace). The asterisk indicates the time of the BW 723C86 challenge.

In vitro studies, using a cell line stably expressing human recombinant 5-HT$_{4(c)}$ receptors, confirmed that tegaserod is a high-affinity, potent 5-HT$_4$ receptor agonist, consistent with published data (Buchheit *et al*., 1995; Hoyer *et al*., 1998). In the present study, tegaserod was a full agonist (relative to 5-HT) at human 5-HT$_{4(c)}$ receptors, with respect to stimulation of cAMP accumulation. Consistent with this observation, Pindon *et al*. (2002) have reported that tegaserod is a potent, full agonist at both 5-HT$_{4(a)}$ and 5-HT$_{4(b)}$ receptor splice variants. It should be noted that tegaserod is also a full agonist at guinea-pig recombinant 5-HT$_4$ receptors (Vickery *et al*., 2004). The rat tunica muscularis mucosa and guinea-pig colonic longitudinal muscle/myenteric plexus preparations were used to confirm the 5-HT$_4$ receptor agonist potency of tegaserod. A considerable amount of data supports the view that 5-HT$_4$ receptor activation is responsible for the smooth muscle relaxant or neurogenic contractile responses in the rat oesophagus or guinea-pig colon, respectively (Wardle & Sanger, 1993; Briejer *et al*., 2001). In the rat isolated oesophagus, tegaserod relaxed carbachol-precontracted tone with a potency similar to that observed in the human recombinant 5-HT$_{4(c)}$ receptor cAMP accumulation experiments. Similarly, tegaserod behaved as a potent 5-HT$_4$ receptor agonist in the guinea-pig colonic longitudinal muscle/myenteric plexus preparation. In the rat oesophagus and guinea-pig colon preparations, tegaserod had a lower IA than 5-HT (73 and 54% relative to 5-HT, respectively), consistent with its partial agonist profile in guinea-pig ileum (Buchheit *et al*., 1995). Clarification of why tegaserod behaves as a partial agonist in these animal tissues and a full agonist in recombinant cell lines merits investigation. Theoretically, the discrepancy could be a consequence of differences in receptor reserve, splice variant identity, the utilization of alternate signalling pathways and/or differential degrees of receptor desensitization between the two systems. The possibility that tegaserod interacts with non-5-HT$_4$ receptors expressed in animal tissue, thereby influencing the magnitude of the 5-HT$_4$ receptor-mediated response, cannot be excluded.

Tegaserod had high affinity (p$K_i=8.4$) for human 5-HT$_{2B}$ receptors in membranes prepared from CHO-K1 cells stably transfected with 5-HT$_{2B}$ receptors, and significant, although at least 10-fold lower, affinity for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (p$K_i=7.5$ and 7.0, respectively). It is concluded, therefore, that tegaserod is not a selective 5-HT$_4$ ligand, but is rather a mixed 5-HT$_{2B}$/5-HT$_4$ receptor ligand. With respect to its 5-HT$_{2B}$

functional activity, tegaserod (0.1–3 μM) produced a concentration-dependent inhibition of the 5-HT receptor-mediated contraction of the rat isolated stomach fundus. A wealth of data supports the contention that 5-HT-induced contraction of the rat stomach fundus is mediated *via* 5-HT$_{2B}$ receptor activation (Bonhaus *et al*., 1999). The pA_2 value of 8.3 for tegaserod with respect to the antagonism of 5-HT$_{2B}$ receptor-mediated contraction of the stomach fundus was consistent with its high binding affinity at the human 5-HT$_{2B}$ receptor, and similar to its potency for 5-HT$_{4(c)}$ receptor-mediated cAMP accumulation (pEC$_{50}$ = 8.6) in recombinant HEK-293 cells, and 5-HT$_4$-induced relaxation of the rat isolated oesophagus (pEC$_{50}$ = 8.2), or contraction of the guinea-pig colon (pEC$_{50}$ = 8.3). In the rat isolated stomach fundus, tegaserod was associated with an increase in the 5-HT maximum contraction. It is possible that tegaserod attenuates the low-affinity, inhibitory response to 5-HT that exists in the rat stomach fundus (Béjar & Malone, 1995), although this proposal requires further investigation.

Following demonstration that tegaserod possessed significant 5-HT$_{2B}$ receptor antagonist activity *in vitro*, the interaction of tegaserod with 5-HT$_{2B}$ and 5-HT$_4$ receptors was investigated *in vivo*. Tegaserod (1 and 3 mg kg^{-1}) produced a statistically significant, 5-HT$_4$ receptor-mediated, increase in colonic transit, relative to its vehicle, in conscious guinea-pigs, consistent with published data from similar models, with other 5-HT$_4$ receptor agonists (Inui *et al*., 2002). The prokinetic activity of tegaserod was abolished by co-administration of piboserod (1 mg kg^{-1}). This prokinetic activity of tegaserod is consistent with the proposed role of 5-HT and 5-HT$_4$ receptor activation in promoting gastrointestinal motility in animals and man (Muller-Lissner, 1987; Nguyen *et al*., 1997; Jin *et al*., 1999). In order to establish whether tegaserod possessed 5-HT$_{2B}$ receptor antagonist activity *in vivo*, its effect on 5-HT$_{2B}$ receptor agonist-evoked contraction of the stomach fundus was evaluated in anaesthetized rats. Subcutaneous administration of tegaserod (0.3 and 1 mg kg^{-1}) was associated with an inhibition of the α-methyl 5-HT or BW 723C86-mediated contractions. As noted in the *in vitro* assays, the inhibitory doses of tegaserod in this *in vivo* 5-HT$_{2B}$ model were similar to those required for 5-HT$_4$ receptor agonist activity of subcutaneously administered tegaserod in terms of colonic prokinetic activity in guinea-pigs (i.e. a statistically significant increase in transit at 1 and 3 mg kg^{-1}). The sensitivity of the BW 723C86-mediated stomach contraction to inhibition by SB 206553 was consistent with the proposal that 5-HT$_{2B}$ receptor activation is responsible for the contractile response in this *in vivo* system. Furthermore, unpublished findings from our group, using the technique of digital sonomicrometry, have confirmed that tegaserod (in the presence of piboserod) and SB 206553 inhibit BW 723C86-induced contractions of the rat stomach fundus *in vivo*. It is concluded therefore that tegaserod is a potent 5-HT$_{2B}$ receptor antagonist *in vitro* and *in vivo*, and its potency in this respect is similar to its 5-HT$_4$ agonist potency.

The clinical significance of the interaction of tegaserod with 5-HT$_{2B}$ receptors has yet to be determined. Considering that the *in vitro* binding affinity of tegaserod for the human 5-HT$_{2B}$ and 5-HT$_4$ receptor subtypes is identical (i.e. 4 nM), and that the maximum steady-state plasma concentration is approximately 9 nM after oral administration of a 6 mg tablet in man (Appel-Dingemanse *et al*., 2001), it can be assumed that 5-HT$_{2B}$ receptors are likely to be occupied by tegaserod clinically. The functional significance of this 5-HT$_{2B}$ receptor interaction will be dependent on the nature and degree of a physiological or pathophysiological role for 5-HT at this receptor subtype. Rodent studies implicate activation of 5-HT$_{2B}$ receptors in the development of the enteric nervous system and in the regulation of cardiovascular function (Fiorica-Howells *et al*., 2000; Nebigil & Maroteaux, 2003). In 5-HT$_{2B}$ 'knockout' mice, impaired cardiac ventricular contractility and myofibrillar degeneration is observed, while upon 5-HT$_{2B}$ receptor overexpression cardiac hypertrophy is evident (Nebigil & Maroteaux, 2003), although, to our knowledge, no such studies have investigated the impact of 5-HT$_{2B}$ receptor ablation or overexpression on gastrointestinal structure or function. However, it is evident that mRNA for the 5-HT$_{2B}$ receptor is widely expressed in the human and rodent gastrointestinal tract (Fiorica-Howells *et al*., 2000; Borman *et al*., 2002). In man, 5-HT$_{2B}$ receptor protein is localized in circular and longitudinal muscle layers, and in myenteric neurones of the colon (Borman *et al*., 2002). Furthermore, exogenously applied 5-HT is associated with a 5-HT$_{2B}$ receptor-mediated augmentation of neuronally mediated contraction of human isolated colonic longitudinal smooth muscle (Borman *et al*., 2002), consistent with intestinal prokinetic activity in man. It is conceivable that 5-HT$_{2B}$ receptor antagonism would result in a reduction of gastrointestinal motility and, as has been postulated (Borman *et al*., 2002), an inhibition of visceral hypersensitivity. Potentially, 5-HT$_{2B}$ receptor antagonist, rather than 5-HT$_4$ agonist, activity of tegaserod is responsible for its ability to alleviate abdominal pain and discomfort in patients with IBS. However, if 5-HT$_{2B}$ receptor antagonism reduces gastrointestinal motility in man, this may limit the 5-HT$_4$ receptor-mediated clinical prokinetic activity of tegaserod. The observation, in this study, that tegaserod, in the presence of piboserod, failed to slow colonic transit in comparison to vehicle treatment, was consistent with a lack of evidence indicating a role for the 5-HT$_{2B}$ receptor in guinea-pig colonic function. It remains to be determined how important the 5-HT$_{2B}$ receptor antagonist activity of tegaserod is in humans. It is unfortunate that the development of prucalopride, a 5-HT$_4$ receptor agonist with at least 300-fold selectivity over the 5-HT$_{2B}$ receptor subtype (Briejer *et al*., 2001), was suspended before its clinical prokinetic activity could be compared directly to that of tegaserod. A comparison of the clinical efficacy data for tegaserod and cisapride is uninformative as, in addition to its 5-HT$_4$ receptor agonist activity, cisapride has significant affinity at 5-HT$_3$, 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors (Nemeth & Gullikson, 1989; Prins *et al*., 1997; Theravance Inc., unpublished observations). Future clinical studies, using novel 5-HT$_4$ receptor agonists, should elucidate whether or not a compound with greater 5-HT$_4$ receptor selectivity than tegaserod will be associated with improved efficacy in disorders of reduced gastrointestinal motility.

The technical expertise of Courtney Gee, Mick O'Keefe, Shana Johnson and Feibi Zheng is gratefully appreciated. We also thank Drs Roger Thomas and Sharath Hegde for their comments on the manuscript during its preparation.

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(Received May 28, 2004
Revised June 29, 2004
Accepted July 7, 2004)