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Original article

Measurement of 5-HT$_4$ receptor-mediated esophageal responses by digital sonomicrometry in the anesthetized rat

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Abstract

Introduction: In vitro studies have demonstrated a 5-HT$_4$ receptor-mediated relaxation of the pre-contracted rat esophagus. However, it is unclear whether 5-HT$_4$ receptor agonists affect resting esophageal tone in vivo. The activity of 5-HT and several well-established 5-HT$_4$ receptor agonists (tegaserod, BIMU-8, cisapride, renzapride, and mosapride) was investigated in a novel in vivo model designed to measure esophageal relaxation using the technique of digital sonomicrometry. **Methods:** Miniature piezo-electric crystals were implanted externally in a longitudinal orientation on the distal esophagus of isoflurane-anesthetized, adult male Sprague–Dawley rats. Measurement of the time for transmission of ultrasonic pulses between the implanted crystals provided a continuous recording of inter-crystal distance and hence esophageal muscle length. **Results:** Following cumulative intravenous administration, 5-HT (1–100 μg/kg), tegaserod (1–1000 μg/kg), BIMU-8 (3–3000 μg/kg), renzapride (10–3000 μg/kg), cisapride (30–3000 μg/kg), and mosapride (30–10,000 μg/kg) produced a dose-dependent increase in esophageal inter-crystal distance. The mean ED$_{50}$ values for tegaserod, BIMU-8, renzapride, cisapride, and mosapride were 11, 49, 51, 141, and 1825 μg/kg, respectively. Pre-treatment with the selective 5-HT$_4$ receptor antagonist, piboserod (SB-207266; 1 mg/kg subcutaneously) significantly attenuated the effects of intravenous tegaserod (1–1000 μg/kg). Following cumulative intraduodenal administration (0.03–10 mg/kg), tegaserod and mosapride exhibited a dose-dependent increase in esophageal inter-crystal distance. The doses associated with a 10% increase in muscle length from the resting level were 2.6 and >10 mg/kg for tegaserod and mosapride, respectively. **Discussion:** In conclusion, dose-dependent, 5-HT$_4$ receptor agonist-mediated increases in longitudinal muscle length in the rat esophagus were observed in vivo using the technique of digital sonomicrometry. This in vivo model of esophageal activity may prove useful in evaluating the activity of novel 5-HT$_4$ receptor agonists.

Keywords: 5-HT (5-Hydroxytryptamine, serotonin); 5-HT$_4$ receptor; Cisapride; Esophagus; Mosapride; Rat; Relaxation; Sonomicrometry; Tegaserod

1. Introduction

The neurotransmitter, serotonin (5-hydroxytryptamine; 5-HT) plays an important physiological role, mediating responses as diverse in function as smooth muscle contraction or relaxation, platelet aggregation, and excitation or inhibition of neuronal activity within the central nervous system (De Clerk, van Neuten, & Reneman, 1984; Dhasmana, Zhu, Cruz, & Villalon, 1993; Gershon, Mawe, & Branchek, 1989; Peters, Malone, & Lamber, 1992). The importance of 5-HT in both physiological and pathophysiological processes is suggested by the observed clinical efficacy of therapies which act via serotonergic mechanisms (e.g., selective 5-HT reuptake inhibitors for depression and panic disorder (Boyer & Feighner, 1992), 5-HT$_3$ receptor antagonists for chemotherapy-induced emesis (Marty, 1989) and diarrhea-predominant irritable bowel syndrome (Farthing, 1999), and 5-HT$_1$ receptor agonists for migraine (Humphrey, 1991).

A wealth of data exists indicating that 5-HT plays a pivotal role in gastrointestinal motility in a variety of species, including man. Indeed, 90% of the 5-HT in the body is located in the enterochromaffin cells of the alimentary tract (Kim & Camilleri, 2000). Of the seven distinct families of 5-HT receptors and their additional sub-types (Hoyer & Martin, 1997), 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_3$, and 5-HT$_4$ receptors mediate

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many of the physiological effects of 5-HT in the gastrointestinal tract. Accordingly, in isolated tissues, activation of 5-HT_4 receptors, which are positively coupled to adenylate cyclase, results in relaxation of the smooth muscle of the rat esophagus (Baxter, Craig, & Clarke, 1991; Reeves, Baunce, & Humphrey, 1991), neuronally-mediated contraction of the guinea pig ileum (Eglen, Johnson, Leung, & Wong, 1993) and colon (Elswood, Bunce, & Humphrey, 1991), facilitation of the peristaltic reflex in guinea pig ileum (Craig & Clarke, 1991; Costall, Naylor, & Tuladhar, 1993), enhancement of canine stomach contractility (Prins, van Der Grijn, Lefebvre, & Schuurkes, 2001) and inhibition of the spontaneous contractions of human colonic circular smooth muscle (Hillier, Tam, Bunce, & Grossman, 1994; McClean, Coupar, & Molenaar, 1993; Tam, Hillier, & Bunce, 1994). Additionally, in vivo, 5-HT_4 receptor activation is associated with an increase in gastrointestinal motility (Gullikson et al., 1993). Indeed, the 5-HT_4 receptor agonists, cisapride (Propulsid®), mosapride (Mosid®), and tegaserod (Zelnorm®), have been shown to cause prokinetic activity in the human gastrointestinal tract in healthy subjects and patients with gastrointestinal motility disorders (Camilleri & Von der Ohe, 1994; De Ponti & Tonini, 2001; Lacy & Yu, 2002).

Several well established in vitro models exist for assaying 5-HT_4 receptor agonist potency and efficacy, but the ability to investigate this agonist activity in vivo, in rodents, is generally limited to intestinal transit or gastric emptying models (Droppleman, Gregory, & Alphin, 1980; Hegde et al., 1995; Nagakura, Ito, Kiso, Naitoh, & Miyata, 1997). Implanted strain gauge force transducers have been used successfully in guinea pigs (Inui, Yoshikawa, Nagai, Toshida, & Ito, 2002) to measure real-time gastrointestinal contractility, although due to the technical challenges, are more commonly used in larger species (e.g., dog). While the in vitro rat esophagus preparation (Bieger & Triggle, 1985) has proved useful for characterizing the pharmacology of 5-HT_4 receptor agonists and antagonists (Baxter et al., 1991; Ford, Baxter, Eglen, & Clarke, 1992; Moummi, Yang, & Gullikson, 1992; Reeves et al., 1991; Ronde, Ansanay, Dumuis, Miller, & Bockaert, 1995), to our knowledge, no rodent in vivo model of esophageal activity has been described for determining the potency and efficacy of 5-HT_4 receptor agonists. The objective of this study was to develop a robust and sensitive rodent model to record the esophageal responses to 5-HT_4 receptor activation in real-time, and in so doing, determine whether 5-HT_4 receptor activation affects resting esophageal tone in vivo. The technique of digital sonomicrometry has been traditionally utilized in cardiovascular physiology and pharmacology (Gorman et al., 1996; Mandrek, 1991). The application of this technique to the gastrointestinal field has been reported recently for measuring stomach motility in response to intravenous cholecystokinin (Adelson & Million, 2004). The digital sonomicrometry methodology involves the use of miniature piezo-electric crystals to transmit and receive ultrasonic pulses within tissue. These crystals can be easily implanted and are capable of providing a continuous record of inter-crystal distance and hence muscle length. We hypothesized that digital sonomicrometry would be a sensitive method to record drug and in particular, 5-HT_4 receptor agonist-mediated changes in esophageal muscle activity, in vivo, in the rat.

2. Methods

2.1. Materials

5-Hydroxytryptamine and VIP were purchased from Sigma-Aldrich. Tegaserod and cisapride were purchased from Apin Chemicals and Sequoia Research Products, respectively. BIMU-8, mosapride, renzapride, and piboserod were prepared following published synthetic procedures (Bianchi, Butti, Rossi, Barzaghi, & Marcaria, 1983; Fedouloff et al., 2001; Gaster et al., 1995; Kato et al., 1992, 1991; King et al., 1993; Tapia et al., 1999). 5-Hydroxytryptamine and VIP were formulated in 5% dextrose in distilled water (D5W). VIP was kept on ice until immediately prior to injection. BIMU-8, renzapride, and piboserod were formulated in D5W and the pH adjusted to 5.5. Tegaserod and mosapride were formulated in 10% sulfobutyl ether-beta cyclodextrin (10% SBE-CD) and the pH adjusted to 5.5. Cisapride was formulated in 10% SBE-CD and pH-buffered with citric acid (20 mM) to pH 5.0. Doses were expressed in terms of the free base weights of each compound.

2.2. Animal preparation

Male Sprague–Dawley rats (250–350 g; Harlan) were acclimatized to the colony room (temperature controlled at 21±1 °C and 12:12 h light–dark cycle commencing at 7:00 am) for at least 5 days following arrival. Standard rat diet (Harlan Teklad) and drinking water were available ad libitum. 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.

On the day of the study, the animals were anesthetized with isoflurane (2–3%) in a perspex induction chamber and anesthesia then maintained with isoflurane (2%) via a nose cone for the duration of the study. Rats were placed, in a prone position, 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 by blunt dissection and ligated distally. A micro-renathane cannula (MRE-033) was inserted into the femoral vein to permit intravenous administration of test agents. Next, a midline incision was made in the skin and muscle layers of the mid-abdomen. The stomach was exposed, and in some experiments, an incision was made in the upper duodenum approximately 1 cm from the pyloric sphincter, to permit intraduodenal administration of test agents. A micro-renathane cannula (MRE-40 with a 1 cm silicone rubber tip) was inserted approximately 1.5 cm into the duodenum via the incision and closed with a 6-0 silk suture using a purse-string closure. Finally, two piezo-electric crystals (1 mm diameter; Sonometrics Corp.) were gently glued, in a longitudinal

orientation, to the distal esophagus, approximately 1 cm from the lower esophageal sphincter, using Vetbond tissue adhesive. Efforts were made to ensure that the distance between crystals was similar in each animal (approximately 2.0 mm). The wires connecting the crystals to the measurement device (Sonometrics Corp. TRX series 8) were exteriorized through the abdominal incision site, which was then closed with nylon suture (Ethicon 4-0).

2.3. Experimental procedure

Following surgery, animals were allowed to acclimatize for at least 30 min while baseline esophageal tone stabilized. 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. Additionally, an oscilloscope (LG model# OS-5100RA) was connected in parallel to the measurement device in order to confirm visually the signal fidelity. For the intravenous experiments, vehicle followed by increasing doses of test article (0.3–1 ml/kg over 3 s) were administered cumulatively (half-log increments) via the femoral cannula. For the intraduodenal experiments, vehicle or increasing doses of test article (30–10,000 μg/kg in log increments; 1–10 ml/kg over 10–20 s) was administered via the intraduodenal cannula. Each dose was administered only when an esophageal response to the preceding dose had reached a maximum (typically 4–5 min for the intravenous experiments and 20–30 min for the intraduodenal experiments). At the end of each experiment, vasoactive intestinal peptide (VIP) was administered intravenously; a robust decrease in inter-crystal distance provided confirmation that the tissue and crystal recordings were viable. In a separate study, the selective 5-HT_4 receptor antagonist, piboserod (SB-207266; Sanger, Banner, Smith, & Wardle, 1998) was administered subcutaneously (in the right flank) 10 min prior to intravenous tegaserod administration.

2.4. Statistical analysis

Changes in inter-crystal distance (in mm and as % change from resting level) were averaged for each treatment group. On occasions where a clear maximum response could not be achieved (due to drug solubility limitations), the dose of agonist associated with an increase of 10% from the resting level (on the linear portion of the agonist response curve) was calculated. On all other occasions, using the dose–response relationship for each agent, 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:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10\text{^}((\log ED_{50} - X)\text{*HillSlope})),$$

where X is the logarithm of the agonist dose, and Y is the response, where Bottom and Top are the curve asymptotes. The effect of piboserod on 5-HT_4 agonist-mediated responses was compared to vehicle treatment using a Student's t-test ($p<0.05$ considered statistically significant).

3. Results

3.1. Model development/optimization

Following crystal placement, 30 min typically proved sufficient to establish a stable sonomicrometry recording of inter-crystal distance. No spontaneous changes in esophageal muscle length were observed and preparations remained viable for up to 2 h (the maximum study duration).

Topical application of 5-HT (0.3 μmole, 0.1 ml), but not vehicle, to the region of the esophagus where the crystals were implanted, induced a robust increase in muscle length (data not shown). This method of administering compounds was not pursued further due to the likely inaccuracy involved with administering a consistent volume directly to the desired area. Additionally, there was a concern that physical manipulation of the crystals as a result of the topical application per se could result in variability of the response observed. However, observation of the topically-applied, 5-HT-evoked, responses indicated that there was a significant level of muscle tone on the esophagus in isoflurane-anesthetized rats.

Intravenous administration of VIP (10 μg/kg) at the end of each experiment resulted in a reduction of the inter-crystal distance in the majority of animals. In some rats (<5%), no response to VIP was evident and data from these animals was therefore excluded from analysis. The absence of a VIP-mediated muscle shortening was assumed to reflect sub-optimal placement of crystals with regard to tissue geometry or local tissue damage during surgery.

3.2. Agonist-mediated responses following intravenous administration

5-Hydroxytryptamine (1–100 μg/kg) and the 5-HT_4 receptor agonists, tegaserod (1–1000 μg/kg), BIMU-8 (3–3000 μg/kg), renzapride (10–3000 μg/kg), cisapride (30–3000 μg/kg), and mosapride (30–10,000 μg/kg), but not their respective vehicles (2 ml/kg), evoked dose-dependent increases in inter-crystal distance, following cumulative intravenous dosing (Figs. 1 and 2). Due to observed respiratory distress with 5-HT (>100 μg/kg), a true maximum of the dose–response curve to 5-HT was not reached, and the data were limited. However, it was evident that at tolerated doses (i.e., 1–100 μg/kg), 5-HT was associated with esophageal muscle lengthening. The ED_{50} values (with 95% confidence intervals) for the equivalent response mediated by tegaserod, BIMU-8, renzapride, cisapride, and mosapride were 11 (7–15; $n=4$), 49 (25–96; $n=5$), 51 (33–77; $n=5$), 141 (46–434; $n=3$), and 1825 (236–14,080; $n=5$) μg/kg, respectively. Tegaserod was therefore approximately 4-, 5-,

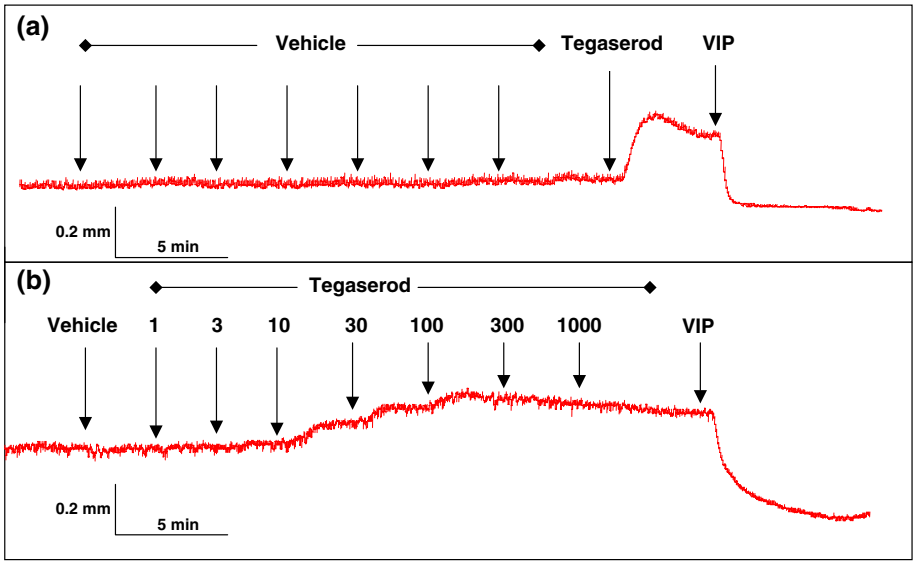

Fig. 1. Representative sonomicrometry recordings of the esophageal relaxation (upward deflection representing increased inter-crystal distance) in response to cumulative intravenous dosing of (a) vehicle (10% SBE-CD; 2 ml/kg) or (b) tegaserod (1–1000 μg/kg). Tegaserod, but not vehicle, elicited a dose-dependent increase in length. Tegaserod (300 μg/kg), administered after the repeated vehicle dosing elicited an increase in length (a). VIP (10 μg/kg) dosed intravenously at the end of the experiment produced a consistent decrease in length.

13-, and 166-fold more potent than BIMU-8, renzapride, cisapride, and mosapride, respectively, following cumulative intravenous dosing.

The mean (±SEM) maximum response evoked by tegaserod, BIMU-8, renzapride, cisapride, and mosapride was 0.19±0.02 mm (n=4), 0.20±0.02 mm (n=5), 0.10±0.01 mm (n=5), 0.18±0.02 mm (n=3), and 0.09±0.02 mm (n=5), respectively. These values corresponded to percentage changes from the pre-dose inter-crystal distance of 9.0±0.7%, 11.0±1.2%, 5.4±0.6%, 8.3±0.9%, and 4.4±1.0% for tegaserod, BIMU-8, renzapride, cisapride, and mosapride, respectively.

Tegaserod (1–1000 μg/kg, n=4) did not produce any significant change in inter-crystal distance when administered 10 min after subcutaneous piboserod (1 mg/kg), a selective 5-HT_4 receptor antagonist (Figs. 3 and 4).

3.3. Agonist-mediated responses following intraduodenal administration

Tegaserod (0.03–10 mg/kg) and mosapride (0.03–10 mg/kg), but not their respective vehicles (2 ml/kg), evoked dose-dependent increases in inter-crystal distances, following cumulative intraduodenal administration (Fig. 5). After reaching a maximum, responses were generally maintained for at least 30 min. As a result of limited solubility, the entire dose–response relationship could not be established for either tegaserod or mosapride, and as a consequence an accurate ED_{50} value could not be determined. The doses associated with an increase in muscle length of 10% from the resting level were 2.6 and >10 mg/kg, for tegaserod and mosapride, respectively. The mean (±SEM) largest change in inter-crystal distance was 0.27±0.09 mm for tegaserod and 0.12±0.04 for mosapride

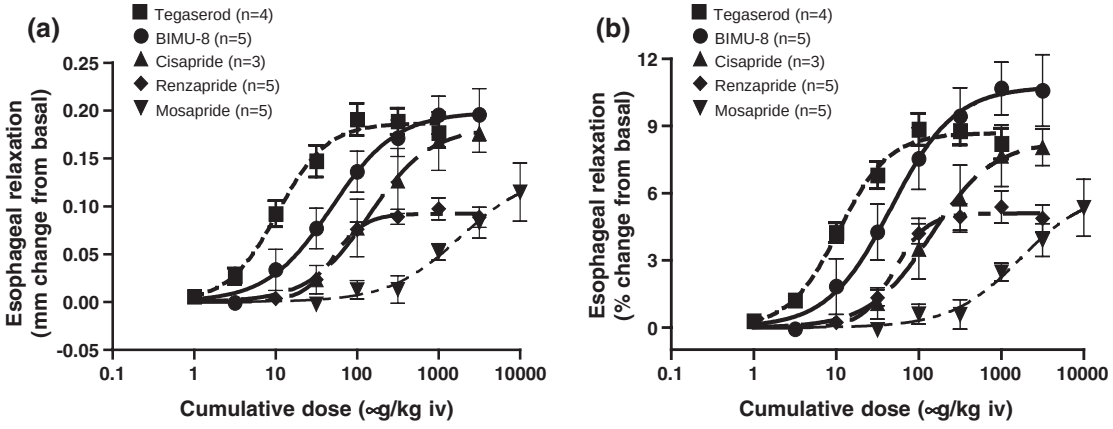

Fig. 2. Dose-dependent increases in muscle length in the rat esophagus following cumulative intravenous administration of tegaserod (■; 1–1000 μg/kg), BIMU-8 (●; 3–3000 μg/kg), cisapride (▲; 30–3000 μg/kg), renzapride (♦; 10–3000 μg/kg), and mosapride (▼; 30–10,000 μg/kg). The esophageal response is expressed as (a) the absolute change in inter-crystal (in mm) and (b) the percentage change from the pre-dose baseline.

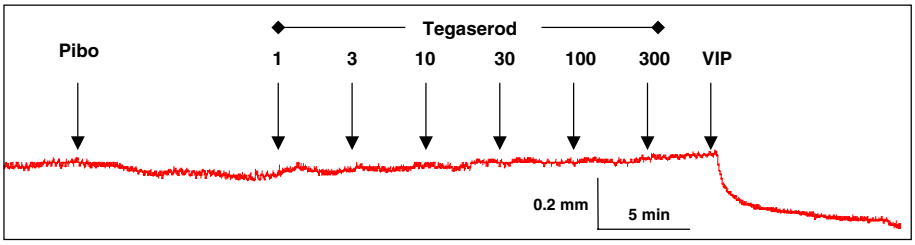

Fig. 3. Representative sonomicrometry recording of the response to cumulative intravenous dosing of tegaserod (1–300 μg/kg) after pre-treatment with piboserod (Pibo; 1 mg/kg) subcutaneously. Tegaserod had no effect on esophageal muscle length following piboserod administration.

(both at 10 mg/kg intraduodenally). These values corresponded to percentage changes from the pre-dose inter-crystal distance of 13.9±4.8% and 6.2±2.2% for tegaserod ($n=4$) and mosapride ($n=4$), respectively.

4. Discussion

Activation of 5-HT_4 receptors in animals and man is associated with an increase in gastrointestinal motility (Barone, Jessen, Colaizzi, & Bierman, 1994; Lacy & Yu, 2002). In humans, the 5-HT_4 receptor agonists, cisapride, mosapride, and tegaserod, have demonstrated clinical efficacy in treating gastrointestinal disorders such as chronic constipation, constipation predominant irritable bowel syndrome and diabetic gastroparesis (De Ponti & Tonini, 2001). Tegaserod was recently approved for the treatment of chronic constipation and constipation-predominant irritable bowel syndrome.

The majority of pre-clinical data suggest that the gastrointestinal prokinetic effects of 5-HT_4 receptor agonists are mediated neurogenically via stimulation of acetylcholine release from enteric neurons (Matsuyama, Sakiyama, Nei, & Tanaka, 1996; Rizzi, Coccini, Manzo, & Tonini, 1992). In contrast, 5-HT_4 receptor-mediated relaxation of the rat esophagus occurs via a direct interaction with 5-HT_4 receptors on smooth muscle (Cohen, Susemichel, Bloomquist, & Robertson, 1994; Reeves, Bunce, Humphrey, & Gunning, 1989; Triggle, Ohia, & Bieger, 1988). The in vitro rat esophagus preparation is a useful tissue for assessing the potency and efficacy of 5-HT_4 receptor agonists. To our knowledge, however, no in vivo rodent model of esophageal activity has been reported for assessing the activity of 5-HT_4 receptor agonists. The technique of digital sonomicrometry, used in the present study, has provided a novel and sensitive method to demonstrate 5-HT_4 receptor-mediated changes in esophageal muscle length in anesthetized rats. The utility of the model was demonstrated for both intravenous and intraduodenal routes of administration of test agents. It is likely that this technique will prove equally applicable to other regions of the gastrointestinal tract, providing a real-time recording of smooth muscle activity. Indeed, its utility has already been demonstrated in measuring stomach motility in the rat (Adelson & Million, 2004). The esophagus of the anesthetized rat is ideally suited as spontaneous activity (characteristic of other regions of the gastrointestinal tract) is absent, ensuring that drug-induced responses are easily visualized and analyzed.

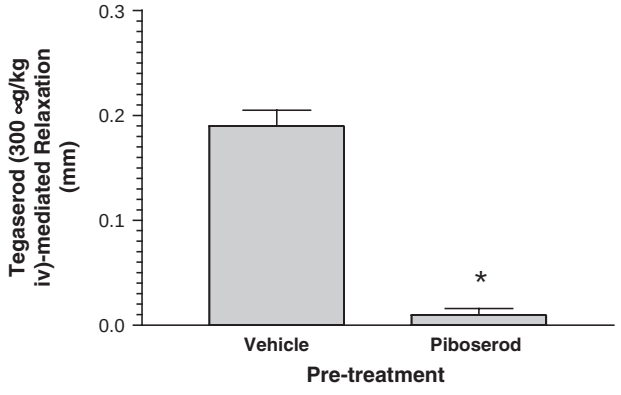

Fig. 4. Effect of pre-treatment with piboserod (1 mg/kg sc; $n=4$) and its vehicle (D5W; 2 ml/kg; $n=3$), on muscle length evoked by intravenous tegaserod (300 μg/kg). ★$p<0.05$ (Student's t-test, compared to the vehicle group).

Vasoactive intestinal peptide elicited a decrease in longitudinal muscle length consistent with contraction. While VIP has been reported to mediate inhibition of both pre-contracted cat esophageal circular smooth muscle (Behar, Guenard, Walsh, & Biancani, 1989) and carbachol-mediated contraction of the isolated rabbit lower esophageal sphincter (Yamashita et al., 1992), there is a report of VIP causing dose-dependent contractions in the body of the opossum esophagus (Rattan, Grady, & Goyal, 1982). In a separate series of experiments (data not shown), crystals were placed in a lateral, rather than longitudinal, orientation and intravenous VIP induced an increase in esophageal muscle length, consistent with relaxation of the circular muscle.

On the basis of the findings of this study, it is concluded that 5-HT_4 receptor activation results in an increase in muscle length, consistent with relaxation of resting esophageal tone in the rat. Tegaserod, BIMU-8, renzapride, cisapride, and mosapride each produced a robust and dose-dependent increase in esophageal inter-crystal distance. Although cisapride and BIMU-8 possess some affinity for 5-HT_3 receptors (Bonhaus et al., 1993; Taniyama et al., 1991), and tegaserod is a potent 5-HT_{2B} receptor antagonist (Beattie et al., 2004), all five agents are potent 5-HT_4 receptor agonists implicating activation of this 5-HT receptor subtype in the "relaxation" response. There is no evidence that 5-HT_2 or 5-HT_3 receptors are involved in 5-HT-induced relaxation of the rat esophagus in vitro. The absence of a relaxation response to tegaserod following pretreatment with piboserod, a highly selective 5-HT_4 receptor antagonist, further supports the conclusion that the response

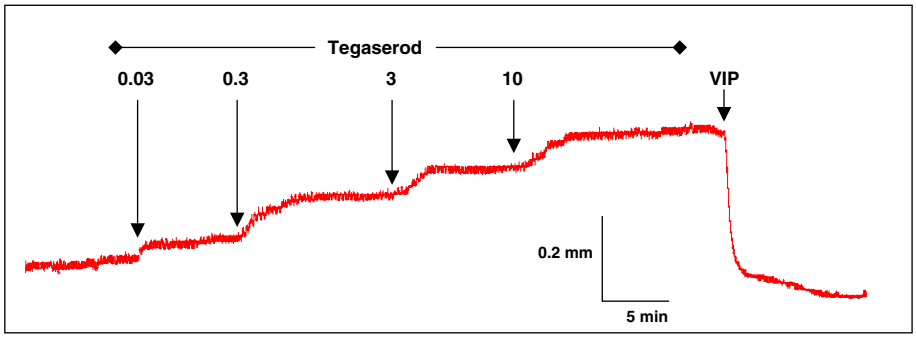

Fig. 5. Representative sonomicrometry recording of the esophageal relaxation in response to cumulative intraduodenal dosing of tegaserod (0.03–10 mg/kg). VIP (10 μg/kg), dosed intravenously at the end of the experiment, was used as a positive control (decrease in muscle length).

observed in this study is predominantly, if not exclusively, 5-HT_4 receptor-mediated.

Of the five 5-HT_4 receptor agonists studied, the rank order of potency in this model following intravenous administration was tegaserod > BIMU-8 ~ renzapride > cisapride > mosapride. Published in vitro rat esophagus data indicates that BIMU-8, renzapride, and cisapride are equipotent and somewhat more potent than mosapride (pEC_{50} values of 7.5, 7.6, 7.4, and 6.7 nM, respectively: Leung et al., 1996; Mine et al., 1997). These are the first reported data for tegaserod in the rat esophagus. The data from the present study also agree with the rank order of potency of tegaserod and cisapride with respect to 5-HT_4 receptor-mediated cAMP accumulation in HEK-293 cells stably transfected with human 5-HT_{4a} cDNA (pEC_{50} values of 8.7 and 7.8 for tegaserod and cisapride, respectively; Pindon et al., 2001). The discrepancy between the absolute agonist potencies in these systems may reflect a difference in 5-HT_4 receptor density or stimulus–response coupling in the rat and human, or differences in access of the compounds to 5-HT_4 receptors in a cell-based assay compared to the esophagus in an intact physiological system.

In vivo data demonstrating activation of the 5-HT_4 receptor and subsequent changes in gastrointestinal motility have been reported in a number of different species and regions of the alimentary tract (Taniyama et al., 2000). Unfortunately, there are a limited number of in vivo investigations in which the effects of multiple 5-HT_4 agonists were compared directly. In a dog model of antral contractility, measured using chronically implanted strain gauges, the rank order of potency with respect to stimulation of contractile activity of the antrum was BIMU-8 > cisapride > mosapride (Mine et al., 1997). These data agree with the observations made with the rat esophagus in the present study. Additionally, in separate reports both renzapride (Gullikson, Virina, Loeffler, & Erwin, 1991) and tegaserod (Nguyen et al., 1997) have been shown to increase transit in the dog as measured using the technique of radioscintigraphy.

No substantial difference was observed in the maximum response of the rat esophagus to tegaserod, BIMU-8, and cisapride following intravenous administration. Renzapride and mosapride, on the other hand, over the doses tested, exhibited a maximum response much lower than the other 5-HT_4 receptor agonists. The partial agonist activity of renzapride and mosapride is entirely consistent with published data (Reeves et al., 1991; Yoshida & Ito, 1994). Tegaserod has also been described as a partial agonist (Appel, Kumle, Hubert, & Duvauchelle, 1997), being associated with a lower intrinsic activity than either BIMU-8 or cisapride in guinea pig isolated colon or ileum (Bucheit et al., 1995; Mine et al., 1997; Vickery et al., 2004). However, the compound appeared to act as a full agonist in the present study, which is consistent with findings at human 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ recombinant splice variants with respect to cAMP accumulation (Pindon et al., 2001). Our group has confirmed that tegaserod has high intrinsic activity at human recombinant 5-HT_4 receptors (Beattie et al., 2004).

In summary, using digital sonomicrometry, dose-dependent 5-HT_4 receptor agonist-mediated relaxation of the endogenous rat esophageal tone was observed in vivo. Following both intravenous and intraduodenal administration, tegaserod and mosapride, 5-HT_4 receptor agonists with clinical efficacy in gastrointestinal motility disorders, produced a dose-dependent and robust relaxation of the esophagus. This esophageal relaxation model should prove useful in evaluating the in vivo potency and efficacy of novel 5-HT_4 receptor agonists and aid in the identification of compounds of potential clinical value in gastrointestinal disease.

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