Selexipag: A Selective Prostacyclin Receptor Agonist that Does Not Affect Rat Gastric Function

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ABSTRACT

Selexipag [2-{4-[{(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}-N-(methylsulfonyl)acetamide] is an orally available prostacyclin (PGI2) receptor (IP receptor) agonist that is chemically distinct from PGI2, and is in clinical development for the treatment of pulmonary arterial hypertension. Selexipag is highly selective for the human IP receptor in vitro, whereas analogs of PGI2 can activate prostaglandin receptors other than the IP receptor. The goal of this study was to determine the impact of selectivity for the IP receptor on gastric function by measuring 1) contraction of rat gastric fundus ex vivo and 2) the rates of gastric emptying and intestinal transport in response to selexipag in comparison with other PGI2 analogs. The rat gastric fundus expresses mRNA encoding multiple prostanoid receptors to different levels: prostaglandin E receptor 1 (EP1) > prostaglandin E receptor 3 (EP3), IP receptor > prostaglandin D2 receptor 1, thromboxane receptor. Selexipag and metabolite (4-{(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy}acetic acid (ACT-333679) did not contract gastric fundus at concentrations up to 10^{-3} M. In contrast, the PGI2 analogs iloprost and beraprost evoked concentration-dependent contraction of gastric fundus. Contraction to treprostinil was observed at high concentration (10^{-4} M). Contraction to all PGI2 analogs was mediated via activation of EP3 receptors, although EP1 receptors also contributed to the contraction of gastric fundus to iloprost and beraprost. Antagonism of IP receptors did not affect responses. Oral selexipag did not significantly alter gastric function in vivo, as measured by rates of stomach emptying and intestinal transport, whereas beraprost slowed gastrointestinal transport. The high functional selectivity of selexipag and ACT-333679 for the IP receptor precludes a stimulatory action on gastric smooth muscle and may help minimize gastric side effects such as nausea and vomiting.

Introduction

An imbalance in activities between vasodilator [nitric oxide, prostacyclin (PGI2)] and vasoconstrictor (thromboxane, endothelin) pathways contributes to the pathogenesis of pulmonary arterial hypertension (PAH) (Tuder et al., 1999). Restoration of PGI2 function is one strategy in the effective management of the disease, and analogs of PGI2 provide therapeutic benefits in PAH. Activation of the IP (IP receptor) receptor by PGI2 analogs leads to vasodilation and reduced proliferation of vascular smooth muscle cells (Gomberg-Maitland and Olschewski, 2008). Administration of these drugs, however, can be painful and inconvenient for the patient (Humbert et al., 1999, Sitbon et al., 2002). In addition, gastric side effects, including abdominal cramping, gastric pain, nausea, vomiting, and diarrhea, are common in patients treated with PGI2 analogs (Widlitz et al., 2007). These compounds are not selective for the IP receptor (Kimiyama et al., 1997; Narumiya et al., 1999; Abramovitz et al., 2000), and gastric irritation may result from activation of other prostanoid receptors that are expressed in the gastrointestinal tract (Dey et al., 2006). Binding assays demonstrate that both iloprost and beraprost have high affinity for EP1 and EP3 receptors (Kimiyama et al., 1997; Abramovitz et al., 2000). These receptor subtypes mediate contraction of rat gastric smooth muscle and may contribute to the gastric side effects of PGI2 analogs.
gastrointestinal function as evidenced by contraction to the EP2/EP1 receptor agonist sulprostone (Bennett et al., 1980). Furthermore, contraction of rat fundal strips (Dong et al., 1986) and guinea pig ileum (Lawrence et al., 1992) to PGI2 analogs has been reported.

Structural derivatives of 4,5-diphenyloxazole are potent agonists at IP receptors and were developed as potential antithrombotic agents (Meanwell et al., 1994). Selexipag 2-[4-[(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy]-[4-(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy]-n-(methylsulfonyl)aceticamide; previously known as NS-304 and ACT-293987 is a diphenyl pyrazine derivative (Kuwano et al., 2007) and is an orally available IP receptor agonist that is in clinical development for the treatment of PAH. Selexipag is chemically distinct from analogs of PGI2. In vitro, selexipag has high selectivity for the human IP receptor over other prostanoid receptors (Kuwano et al., 2007). Selexipag is readily hydrolyzed to the active metabolite 4-[5-(6,5-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy]-[4-(5,6-diphenylpyrazin-2-yl)(isopropyl)amino]butoxy]-n-(methylsulfonyl)aceticacid (ACT-333679; also known as MRE-269), which is also a potent and selective agonist at IP receptors. ACT-333679 has at least 130-fold higher affinity for the IP receptor over other prostanoid receptors. The receptor selectivity profile of selexipag and ACT-333679 was derived from radioligand displacement studies using recombinant cells engineered to express human prostanoid receptors. Selectivity of selexipag and ACT-333679 for the IP receptor using functional ex vivo and whole body systems has not been systematically evaluated.

This study sought to determine the impact of selectivity of selexipag and ACT-333679 for the IP receptor on gastric function by measuring 1) contraction of rat gastric fundus ex vivo and 2) gastric emptying and intestinal transport in vivo in response to selexipag and PGI2 analogs. Reactivity of rat gastric fundus was studied because perturbation of the gastrointestinal system by PGI2 analogs is commonly observed in the clinic (Gomberg-Maitland and Olschewski, 2008) and prostaglandins regulate gastric function (Wallace, 2008). The PGI2 analogs iloprost, beraprost, and tretoprostil were studied in comparison with selexipag and its metabolite ACT-333679.

Data presented here demonstrate that selexipag and ACT-333679, unlike analogs of PGI2, do not affect gastric function, which may help minimize off-target side effects in the gastrointestinal system such as nausea and vomiting.

Materials and Methods

Animals. Male Wistar rats (12 weeks) were obtained from the Biotechnology and Animal Breeding Division at Harlan (Fullinsdorf, Switzerland). All rats were housed in climate-controlled conditions with a 12-h light-dark cycle and had free access to normal pellet rat chow and drinking water in accordance with local guidelines (Basel-Landschaft Cantonal Veterinary Office).

Measurement of Receptor mRNA Expression. Expression levels of mRNA encoding prostanoid receptors in the rat gastric fundus were first measured to establish that this tissue expressed multiple prostanoid receptor subtypes. Rats were euthanized by CO2 asphyxiation, and their stomachs were removed. Total RNA was isolated from gastric fundus without mucosa (30-kg samples) using the RNeasy fibrous tissue kit according to the manufacturer's protocol (QIAGEN GmbH, Hilden, Germany). Remaining genomic DNA was digested by using the DNase free kit (Ambion, Austin, TX). The quantity of RNA was analyzed with a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and RNA quality was assessed with an Agilent Technologies (Santa Clara, CA) Bioanalyzer 2100. Total RNA was reverse-transcribed by using the high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA). QPCR was performed on the ABI 7500 machine by using TaqMan primers. The following TaqMan primers and probes from Applied Biosystems were developed as potential antithrombotic agents (Indianapolis, IN) were used for QPCR: DP-Rn00824628_m1, EP1-Rn0143271_s1, EP3-Rn0121735_m1, IP-Rn01764022_m1, and 18s rRNA assay number 4310983E (Applied Biosystems). TBXAR2 forward ggtggagatgatggttcagc, reverse gcaagctgctgcaggatgaag, and probe 77 ggtggtgg of the Universal Probe Library system from Roche Diagnostics were used. Results were calculated by using a modified ΔΔt method. This method allows comparison between gene expression values for different genes based on an identical linear scale. A value of 1 is defined as no expression.

Rat Isolated Gastric Fundal Strips. After euthanasia, fundal strips were prepared from rats according to the method described by Vane (1957). In brief, the stomach was excised, and the mucosa was carefully removed. Longitudinal incisions were performed along the length of the fundus to produce strips (13 mm) that were then suspended in tissue baths (10 ml) containing Krebs-Henseleit buffer of the following composition: 115 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 1.5 mM KH2PO4, 2.5 mM CaCl2; 25 mM NaHCO3, and 10 mM glucose. Bathing solution was maintained at 37°C and aerated with 95% O2/5% CO2, pH 7.4. A resting force of 2 g (19.6 mN) was applied to the strips, which were then allowed to stabilize for 45 min before the start of the experiment. Changes in force generation were measured with an isometric force recorder (EMKA Technologies Inc., Paris, France). The viability of each strip was determined by exposure to carbachol (10-5 M), and subsequent responses were expressed as a percentage of this reference contraction. Fundal strips were exposed to either drug vehicle or receptor antagonists for 30 min before obtaining cumulative concentration-response curves to agonists. The choice and concentrations of receptor antagonists were based on published data. The following receptor antagonists were used: (2E)-3-(3',4'-dichlorobiphenyl-2-yl)-(N-2-thienylsulfonyl)acrylamide (DBTSA) (EP3 receptor; Gallant et al., 2002, Kuwano et al., 2008), 8-chloro-dibenzo[b,f] [1,4]oxazepine-10-(11H)-carboxy-(2-acetyl)hydrazide (SC19220) (EP1 receptor; Bennett et al., 1980), 8-chloro-2-[3-[[2-furanylmethyl]thio]-1-oxopropyl]hydrazide, dibenz[b,f][1,4]oxazepine-10(11H)-carboxylic acid hydrazide (SC51322) (EP1 receptor; McCormick et al., 2010), (4Z)-7-[(1R,2R,3S,5S)-5-[[1,1'-biphenyl]-4-ylmethoxy]-3-hydroxy-2-1-piperidiny]cyclopentyl-4-heptanoic acid (GR32191) (IP receptor; Lumley et al., 1989), 4,5-dihydroxy-N-[4-(4-[(1-methylethoxy)phenyl]methyl)phenyl]-1H-imidazol-2-amine (CAY10441) (IP receptor; Jones et al., 2006), 9,15-dihydroxy-11-fluoro-15-(2,3-dihydro-1H-inden-2-yl)-16, 17,18,19,20-pentanor-prosta-5,13E-dien-1-ol acid (AL8810) (FP receptor; Behm et al., 2009), and 3-[2-(cyclohexyl-2-hydroxyethyl)aminol]-2,5-dioxo-1-phenylmethy]-4-imazolidinedeptanionic acid (BW264C) (DP receptor; Giles et al., 1989).

Rat Isolated Pulmonary Artery. Relaxation of rat pulmonary artery was measured to confirm the pharmacological activity of selexipag, ACT-333679, and iloprost. The main pulmonary artery and two side branches were isolated, and rings were prepared. Care was taken to avoid damage to the endothelium. Rings of pulmonary artery were suspended in tissue baths (10 ml) containing Krebs-Henseleit buffer. Bathing solution was maintained at 37°C and aerated with 95% O2/5% CO2, pH 7.4. A resting force of 0.5 g (4.9 mN) was applied to the vessels. Endothelial function was tested by measuring the ability of acetylcholine (10-5 M) to relax arterial rings contracted with phenylephrine (10-4 M). Rings of pulmonary artery were then contracted with 10-5 M prostaglandin F2α (PGF2α). When the developed force had stabilized, cumulative concentration-relaxation curves to selexipag, ACT-333679, and iloprost were obtained. The interval between additions of higher concentrations of compounds to the baths was determined by the time required for the response to reach plateau. Arterial rings were exposed to CAY10441 (10-6 M) for 15 min before contraction with PGF2α, in experiments
that measured the effect of IP receptor antagonism on relaxation to iloprost.

**Measurement of Gastric Emptying and Intestinal Transport.** The effects of selexipag and beraprost on gastrointestinal function in vivo were tested. Rats were fasted overnight. Selexipag, beraprost, or drug vehicle were administered by oral gavage, and a charcoal meal (1 ml/rat, 5% carbon powder suspended in 10% arabic gum) was given 1 h later. Rats were sacrificed after 30 min, their abdomens were opened, and stomachs and intestines were isolated. The effects of the compounds on intestinal transport were determined by measuring the distance that the charcoal meal had traveled along the gastrointestinal tract and was expressed as a percentage of the total distance from the pylorus to the caecum. Stomach weights were measured in the same animals as an index of gastric emptying. Validation of the gastrointestinal transport model was confirmed by using the inhibitor atropine (3 mg/kg/ml i.p.) and the stimulant neostigmine (30 mg/kg/10 ml p.o.).

**Materials.** Selexipag, ACT-333679, and DBTSA were synthesized by Nippon Shinyaku Co. Ltd (Kyoto, Japan). Iloprost, beraprost, treprostinil, AL8810, CAY10441, SC19220, SC51322, and sulprostone were obtained from Cayman Chemical (Ann Arbor, MI). Acetylcholine, atropine, BWA868C, carbamyl choline chloride (carbachol), GR32191B, neostigmine, phenylephrine, and PGF2α were purchased from Sigma-Aldrich (St Louis, MO).

**Statistical Evaluation of Results.** Contraction of rat fundal strips is expressed as a percentage of the reference contraction to carbachol (10⁻⁵ M). Results are presented as mean ± S.E.M. In some experiments, the S.E.M. values are smaller than the data symbol. n values refer to the number of animals. pEC50 values are defined as the negative logarithm of the concentration of agonist that evokes half-maximal response. The functional inhibitory potency (pD2 value) for DBTSA was calculated according to the method of van Rossum (1963) and was defined as the negative logarithm of the concentration causing a 50% reduction in the maximum force generated by sulprostone: pD2 = pD1 + log(X - 1), where pD1 is the negative logarithm of the concentration of DBTSA, and X is the ratio of maximal contraction to sulprostone in the absence and presence of DBTSA. The effects of receptor antagonists on contraction of gastric fundus to analogs of PGI2 were quantified by comparing calculated areas under curves: control versus treated, from Sigma-Aldrich (St Louis, MO).

**Table 1**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Antagonist</th>
<th>pEC50 Control</th>
<th>pEC50 Treated</th>
<th>Emax Control</th>
<th>Emax Treated</th>
<th>Control vs. Treated Difference in AUC</th>
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<tr>
<td>EP1</td>
<td>DBTSA (10⁻⁵ M)</td>
<td>7.5 ± 0.1</td>
<td>6.8 ± 0.1*</td>
<td>78.0 ± 4.1</td>
<td>52.0 ± 4.3**</td>
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<td>6</td>
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<tr>
<td>EP3</td>
<td>SC19220 (3 × 10⁻⁵ M)</td>
<td>7.4 ± 0.2</td>
<td>6.8 ± 0.1*</td>
<td>80.1 ± 3.3</td>
<td>83.3 ± 4.4</td>
<td>N.S.</td>
<td>6</td>
</tr>
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N.S., not significant; AUC, area under curve.

**Results**

**Prostanoid Receptor mRNA Expression and Function.** Initial experiments using reverse transcription-QPCR established expression of mRNA encoding multiple prostanoid receptors in rat gastric fundus. A differential expression pattern for receptor mRNA was recorded (Fig. 1). The highest levels of mRNA encoded the EP1 receptor, although mRNA for both the IP and EP3 receptors was also expressed. TP and DP receptor mRNA expression was detectable but to an almost negligible degree.

To test the functionality of EP1 and EP3 receptors, the response of isolated gastric fundal strips to the EP1/EP3 receptor agonist sulprostone was measured. Sulprostone contracted rat gastric fundus in a concentration-dependent manner (10⁻¹⁰ to 10⁻⁵ M). Contraction to sulprostone was significantly inhibited by DBTSA (10⁻⁵ M) (Table 1). The calculated pD2 value for DBTSA was 4.7 ± 0.1 (n = 6). In addition, the EP1 receptor antagonist SC19220 (3 × 10⁻⁶ M) caused a significant rightward displacement of the concentration-contraction curve to sulprostone (Table 1).

**IP Receptor Selectivity and Gastric Fundus.** The impact of selectivity for the IP receptor on gastric responsiveness was first studied by measuring contraction of rat gastric fundus to selexipag and ACT-333679 in comparison with analogs of PGI2. The IP receptor-selective compounds selexipag and ACT-333679 did not contract rat fundal strips above baseline force, even at concentrations up to 10⁻³ M (Fig. 2). Indeed, both compounds evoked weak relaxation of baseline force in both the absence and presence of the IP receptor antagonist CAY10441 (3 × 10⁻⁶ M) (Fig. 3). Selexipag and ACT-333679 were shown to be pharmacologically active in rat because both compounds evoked concentration-dependent relaxation of precontracted rat pulmonary artery (pEC50 values of 5.38 ± 0.03 and 5.61 ± 0.06, respectively; n = 6). Likewise, iloprost relaxed rat pulmonary artery (control pEC50 value of 6.6 ± 0.1; n = 4). Relaxation to iloprost was significantly inhibited by CAY10441 (10⁻⁶ M) (areas under curves: control versus treated, P < 0.001; n = 4).

In contrast to the findings with selexipag and ACT-333679, the nonselective PGI2 analogs iloprost and beraprost induced concentration-dependent contraction of rat fundal strips (Fig. 4). In addition, treprostinil evoked a small contraction of gastric fundus at the highest concentration tested (10⁻⁴ M). The contraction to iloprost and beraprost was significantly inhibited by the EP3 receptor antagonist DBTSA (10⁻⁵ M) (iloprost, P < 0.01; beraprost, P < 0.05; Fig. 4, A and B). Contraction to the highest concentration of treprostinil tested was also significantly inhibited by DBTSA (P < 0.05; Fig. 4C). The IP receptor CAY10441 did not significantly alter contraction of rat fundal strips to the PGI2 analogs (Fig. 4, D–F). The role of EP1 receptors in the contraction to PGI2 analogs was investigated by using the antagonist SC19220. Contraction to iloprost and beraprost, but not treprostinil, was significantly inhibited by SC19220 (3 × 10⁻⁶ M) (Fig. 4, G–I). The role of EP1 receptors in the contraction to PGI2 analogs was investigated by using the antagonist SC19220. Contraction to iloprost and beraprost, but not treprostinil,
was inhibited by SC19220, as indicated by significant reductions in the areas under the curves (iloprost control versus treated, \( P < 0.05 \); beraprost control versus treated, \( P < 0.05 \)) (Fig. 5, A and D). The inhibitory effect of SC19220 on contraction to iloprost and beraprost was confirmed by using the congener SC51322 (10^{-6} M) (areas under curves: iloprost control versus treated, \( P < 0.001, n = 5 \); beraprost control versus treated, \( P < 0.05, n = 5 \)). Antagonism of TP receptors and DP receptors using GR32191B and BWA868C, respectively, did not significantly inhibit contraction of rat fundal strips to PGI2 analogs (areas under curves: control versus treated, \( P > 0.05, n = 4 \)).

**Gastrointestinal Activity in Rats.** The effects of selexipag and beraprost were compared on the rates of gastric emptying and intestinal transport. Doses of selexipag and beraprost were chosen to attain concentrations in the stomach that reflect the highest concentrations used in contractility studies ex vivo. Calculation of doses for in vivo experiments was based on a rat stomach volume of 2.5 ml. Preliminary experiments validated the charcoal assay for measurement of intestinal transport using standard reference drugs. The inhibitor of acetylcholinesterase neostigmine accelerated gastrointestinal transport (122 \( \pm \) 4% versus control 100%; \( n = 10–13; \( P < 0.01 \)), whereas the nonselective muscarinic receptor antagonist atropine inhibited gastrointestinal transport (78 \( \pm \) 5% versus control 100%; \( n = 10–13; \( P > 0.05 \), \( n = 6 \)).
Selexipag did not retard gastric emptying or intestinal transport at 1 or 3 mg/kg (Fig. 6). In addition, beraprost at a dose of 0.03 mg/kg had no significant effect on emptying of the stomach or rate of intestinal transport compared with vehicle control. A higher dose of beraprost (0.1 mg/kg), however, significantly slowed gastric emptying and intestinal transport ($P < 0.05$; Fig. 6).

**Discussion**

The results of this study demonstrate that selexipag can be distinguished from analogs of PGI$_2$ in terms of gastric reactivity. Selexipag and metabolite ACT-333679 do not contract rat gastric fundus ex vivo, and selexipag does not retard gastric emptying or intestinal transport at pharmacologically relevant doses. In contrast, analogs of PGI$_2$ contract gastric fundus via activation of EP$_3$ and EP$_1$ receptors, and beraprost slows gastric function. Contraction of rat fundus may disturb the regulated and coordinated waves of contraction in the stomach, leading to dysregulation of food mixing and gastric emptying (Pal et al., 2007). The pronounced contraction of gastric smooth muscle to analogs of PGI$_2$ reported here may help explain the abdominal pain and cramping associated with use of these compounds in the clinic. In addition, perturbation of gastric function in vivo observed in
response to beraprost is consistent with the emetic characteristic of this class of compound. Selexipag, on the other hand, lacks contractile efficacy and does not disrupt gastric function in this model, suggesting that compounds with high selectivity for the IP receptor may offer improved gastric tolerability over nonselective PGI2 analogs.

The rat gastric fundus was chosen as the test model in this study because prostaglandins regulate gastric function (Wallace, 2008), and perturbation of the gastrointestinal system by analogs of PGI2 is commonly observed in the clinic. The suitability of gastric fundus for studying selectivity of IP receptor agonists over other prostanoid receptors was established in initial experiments that measured the expression of mRNA encoding multiple prostanoid receptors. In addition, the predominant EP1 and EP3 receptors were shown to be functional as measured by contraction of gastric fundus to the EP1/EP3 receptor agonist sulprostone. Both DBTSA and SC19220 effectively inhibited fundal contraction to sulprostone, supporting the involvement of EP3 and EP1 receptors.

Displacement binding studies using recombinant cell expression systems demonstrate that the IP receptor agonists selexipag and ACT-333679 are highly selective for the human IP receptor (Kuwano et al., 2007). For example, the Kᵢ value for ACT-333679 at the IP receptor is 130-fold lower than that at other prostanoid receptors. The affinity values for selexipag and ACT-333679 at the rat IP receptor are 2.1 × 10⁻⁷ and 2.2 × 10⁻⁷ M, respectively, and both compounds would be expected to activate IP receptors at the concentrations used in the current study. Selexipag or ACT-333679 did not induce contraction of the rat gastric fundus above baseline, demonstrating that smooth muscle IP receptors do not play a direct role in contraction of rat fundus. The possibility that activation of the IP receptor could inhibit an excitatory effect of these compounds in the gastric fundus can be excluded given that a contractile response to selexipag or ACT-333679 was not revealed in the presence of the IP receptor antagonist CAY10441 (Jones et al., 2006). In addition, it seems unlikely that conversion of selexipag to metabolite ACT-333679 occurred over the duration of the experiment, given that responses to both compounds were similar, and the mucosa was removed, negating any mucosal hydrolytic activity (Fukuhara et al., 1996). The absence of significant responses to selexipag and ACT-333679 in rat gastric fundus did not extend to other tissues in this species. Pharmacological activity of both compounds was confirmed by measuring concentration-dependent relaxation of the rat pulmonary artery precontracted with PGF₂α. The calculated pEC₅₀ value for relaxation is in good agreement with previous findings (Kuwano et al., 2008). The lack of efficacy of selexipag and ACT-333679 on gastric reactivity relates directly to the high degree of IP receptor selectivity of these compounds. The PGI₂ analog cicaprost, which is more selective than other PGI₂ analogs for the IP receptor (Abramovitz et al., 2000), also fails to contract rat gastrointestinal smooth muscle at submicromolar concentrations (Qian and Jones, 1995).

The analogs of PGI₂ tested in this study contracted rat gastric fundus. Iloprost and beraprost evoked robust contractile responses, whereas weak contraction to treprostinil was observed at the highest concentration tested. Maximal responses to PGI₂ analogs were inferior to that evoked by the EP₁/EP₃ receptor agonist sulprostone, supporting the previous findings of Dong et al. (1986). Contraction to all PGI₂ analogs tested here was sensitive to antagonism of EP₃ receptors. These data are consistent with findings in the EP3(-/-) mouse model where contraction of fundal strips to PGI₂ is abolished (Okada et al., 2000). Iloprost and beraprost both bind EP₁ receptors with high affinity (Abramovitz et al., 2000, Kuwano et al., 2007) and might be expected to activate EP₁ receptors at concentrations used in this study. Affinity data for treprostinil at non-IP receptors are not available, but the observation that treprostinil contracted fundus at a high concentration via activation of EP₁ receptors suggests that this PGI₂ analog is also not selective for the IP receptor.

A role for the EP₁ receptor subtype in the contractile response to PGI₂ analogs was also considered given that the level of EP₁ receptor mRNA is highly expressed in gastric fundus, and iloprost has high affinity for this receptor subtype (equal Kᵢ values at EP₁ and IP receptors) (Abramovitz et al., 2000). Indeed, the IP₁ receptor antagonist SC19220 caused a statistically significant inhibition of contraction to both iloprost and beraprost. These data are consistent with the findings of Bennett et al. (1980).

DBTSA may also antagonize TP and DP receptors at the concentration used in this study (Gallant et al., 2002). The possibility that antagonism of TP receptors contributed to the effect of DBTSA is unlikely, however, because the selective TP receptor antagonist GR32191B did not significantly inhibit contraction. Likewise, antagonism of DP receptors by DBTSA can be dismissed because the DP receptor antagonist BWA8686C did not influence contraction to any of the PGI₂ analogs tested. Furthermore, although contractile FP receptors may be present in gastric fundus (Dong et al., 1986), the selective FP receptor antagonist AL8810 did not significantly affect contraction to any of the PGI₂ analogs tested.

The second goal of this study was to establish the impact of selectivity for the IP receptor on gastric performance in vivo. To this end, the effects of selexipag and beraprost on the rates of gastric emptying and intestinal transport were compared. Doses of selexipag and beraprost were chosen to attain concentrations in the stomach that reflect the high concentrations studied using the isolated gastric fundus. In addition, the doses chosen effectively lower pulmonary arterial pressure and reduce ventricular hypertrophy in a rat model of PAH (Kuwano et al., 2008). Selexipag at doses of 1 and 3 mg/kg in the stomach that are equivalent to 2 × 10⁻⁴ and 6 × 10⁻⁴ M, respectively, had no effect on the rate of gastric emptying or intestinal transport. These data are consistent with ex vivo data showing that selexipag does not contract gastric fundus even at 10⁻³ M. Beraprost at 0.1 mg/kg, equivalent to a contractile concentration of 2.7 × 10⁻⁵ M, significantly reduced the rates of stomach emptying and intestinal transport. A lower dose of beraprost (0.03 mg/kg), which is equivalent to 7 × 10⁻⁶ M, did not impede gastric function despite displaying contractile efficacy ex vivo. Taken together, these data suggest that robust contraction of gastric smooth muscle is required to disturb gastric physiology in vivo.

Normal gastric emptying requires the coordinated and rhythmic contraction of the stomach (Pal et al., 2007). Disruption of gastric smooth muscle activity underlies nausea and vomiting (Holmes et al., 2009). Thus, contraction of gastrointestinal smooth muscle, as observed in this study, has important implications for the oral tolerability of a compound. Several lines of evidence support a role for EP₁ recep-
tors in the perturbation of gastric function. The EP_3 receptor agonist sulprostone evokes emesis and diarrhea in the ferret (Kan et al., 2002). Iloprost also induces emesis in the ferret, whereas the selective IP receptor agonist cicaprost has no effect (Kan et al., 2002). Moreover, in the present study, iloprost evoked EP_3 receptor-dependent contraction of rat fundus, which may disturb the regulated and coordinated waves of contraction in the stomach, leading to dysregulation of food mixing and gastric emptying (Pal et al., 2007). It is noteworthy that gastric muscular dysrhythmias are initiated by EP_3 receptor agonists in the mouse (Forrest et al., 2009).

Observations on emesis cannot be performed in rodents (Holmes et al., 2009), but delayed gastric emptying and increased chewing and swallowing are observed in the rat in response to substances that would be expected to be emetic in other species such as the ferret (Andrews and Horn, 2006). Delayed gastric emptying in the rat is considered as a surrogate marker for vomiting (Bradner and Schurig, 1981). The finding in the present study that beraprost contracted gastric fundus via activation of EP_3 receptors and slowed gastric emptying is consistent with the emetic properties of the drug in the clinic.

In conclusion, the results of this study demonstrate that the selective IP receptor agonist selexipag can be distinguished from PGI_2 analogs in terms of gastric reactivity in the rat. Selexipag does not disrupt gastric function and offers potential for improved gastric tolerability over nonselective PGI_2 analogs currently used in the clinic for the treatment of PAH.

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References


