Nonpeptide Endothelin Receptor Antagonists. X. Inhibition of Endothelin-1- and Hypoxia-Induced Pulmonary Pressor Responses in the Guinea Pig by the Endothelin Receptor Antagonist, SB 217242

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ABSTRACT
This study investigated the effects of the nonpeptide endothelin (ET) receptor antagonist, SB 217242, against ET-1-induced pulmonary pressor responses and in a model of hypoxia-induced pulmonary hypertension in the guinea pig. In guinea pig isolated pulmonary artery rings, SB 217242 (3–300 nM) produced a concentration-dependent inhibition of ET-1-induced contractions, with a pA2 of 8.1. SB 217242 (1 or 3 mg/kg i.v.) elicited a dose-related inhibition of ET-1-induced increases in pulmonary artery and airway insufflation pressure responses in anesthetized guinea pigs. Chronic exposure to hypoxia (9% O2 for 0–14 days) produced a time-dependent increase in mean pulmonary artery pressure. After a 10-day exposure to hypoxia there was about a 100% elevation in pulmonary artery pressure, and right ventricular mass and plasma irET levels increased 3-fold compared with normoxic animals. SB 217242, administered by continuous intraperitoneal infusion via mini osmotic pump (0.36, 3.6 or 10.8 mg/day), significantly reduced (by about 50%) hypoxia-induced pulmonary artery pressure increases at all three doses used. The hypoxia-induced right ventricular hypertrophy was significantly attenuated by the 3.6 and 10.8 mg/day doses. Based on hematocrit, hemoglobin and red blood cell counts, SB 217242 did not affect the normal physiological erythropoietic response to hypoxia. There were no appreciable differences in the maximum contractile effects of ET-1 or the potency of SB 217242 (pKB values, 8.3 and 8.0, respectively) versus ET-1-induced responses in isolated pulmonary arteries from hypoxic versus normoxic guinea pigs. However, there was a marked reduction in endothelium-dependent relaxation of precontracted pulmonary artery isolated from hypoxic compared with normoxic animals. The results of the present study provide further preclinical evidence for a pathophysiological role of ET-1 and the potential therapeutic utility of ET receptor antagonists, such as SB 217242, in pulmonary hypertension.

Pulmonary hypertension is a major complication of chronic hypoxia that may result from chronic obstructive pulmonary disease, congestive heart failure, respiratory distress syndrome, cystic fibrosis and hypoventilation syndrome (Zapol and Snider, 1977; Cody et al., 1992; MacNee, 1994). Right ventricular hypertrophy, increased vascular resistance and enhanced vascular remodeling, including hyperplasia (increase in myocardial and smooth muscle cell number), hypertrophy (increase in muscle cell size) and muscle extension (appearance of new smooth muscle in previously less muscularized arterioles), are hallmarks of chronic hypoxia in the pulmonary circulation, which combine to produce an anatomic resistance to flow (Reid, 1979). Several animal models of hypoxia-induced pulmonary hypertension have been developed, primarily in the rat, and these have been used to investigate the mechanisms underlying the vascular constriction and remodeling components of the disease (Grover et al., 1963; Rabinovitch et al., 1979; Thompson et al., 1989). In our laboratory, a model of hypoxia-induced pulmonary hypertension in an optimized environment for the guinea pig has been developed and characterized (Bochnowicz et al., 1997).

ET-1, originally identified in 1988 (Yanagisawa et al., 1988), is a member of a family of 21-amino-acid peptides, which includes ET-2 (two amino acid substitution from ET-1) and ET-3 (six amino acid substitution) (Inoue et al., 1989; Masaki et al., 1992). The ETs produce an array of effects in many biological systems, which are mediated via two G protein-coupled receptors, designated ETA and ETB (Masaki et al., 1992; Sakurai et al., 1996).

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ABBREVIATIONS: ET-1, endothelin-1; ET-2, endothelin-2; ET-3, endothelin-3; ETA, endothelin A receptor; ETB, endothelin B receptor; SB 217242, (+)-(1S,2R,3S)-3-[2-(2-hydroxyethyl-1-yloxy)-4-methoxyphenyl]-1-(3,4-methylenedioxyphenyI)-5-(prop-1-yloxy)indane-2-carboxylic acid; ANOVA, analysis of variance; PLSD, protected least significant difference; irET, immunoreactive ET.
In the lung ET-1 has been implicated in the pathophysiology of several diseases, including pulmonary hypertension (Stewart et al., 1991; Giaid et al., 1993; Hay et al., 1993a, b; Hay and Goldie, 1995; Michael and Markowitz, 1996). The postulated relationship to pulmonary hypertension is based primarily on the following observations: 1) ET-1 is a potent constrictor of mammalian pulmonary blood vessels, including human pulmonary artery and vein (Brink et al., 1991; Hay et al., 1993a, b); 2) ET-1 is a mitogen for human pulmonary artery smooth muscle cells via an ET receptor-mediated mechanism (Zamora et al., 1993); 3) there are numerous reports of increased plasma levels and enhanced expression of ET in individuals with pulmonary hypertension (Cernacek and Stewart, 1989; Stewart et al., 1991; Yoshibayashi et al., 1991; Allen et al., 1993; Giaid et al., 1993). In addition, in rat models of pulmonary hypertension, ET receptor antagonists have attenuated the characteristic functional and morphological changes (Bonvallet et al., 1994; Chen et al., 1995; DiCarlo et al., 1995; Oparil et al., 1995).

During the past few years several peptide and nonpeptide ET receptor antagonists have been identified (Warner et al., 1996). SB 217242 is a recently identified nonpeptide ET receptor antagonist (Ohlstein et al., 1993). In addition, the compound has good oral bioavailability (67% in the rat after intraduodenal infusion) and, thus, has the appropriate pharmacodynamic profile to be a useful tool to assess the potential pathophysiologic role of ET-1 in diseases at the preclinical level. The purpose of the present study was to characterize the effects of SB 217242 on exogenous ET-1-induced pressor responses in the pulmonary vasculature and in a chronic hypoxia-induced model of pulmonary hypertension in the guinea pig.

Methods

Animals. Male Hartley guinea pigs (Charles River, Portage, MI; weight range, 600–800 g) were randomly selected and assigned to one of seven groups: 1) normoxic, no treatment, n = 6; 2) hypoxic, vehicle, n = 7; 3) hypoxic, SB 217242, 0.36 mg/day, n = 7; 4) hypoxic, SB 217242, 3.6 mg/day, n = 5; 5) hypoxic, vehicle, n = 5; and 6) normoxic, SB 217242, 10.8 mg/day, n = 6. In the text, animals exposed to conditions of hypoxia (9% O2) or normoxia (18% O2) are referred to as “hypoxic” or “normoxic” guinea pigs. Similarly, pulmonary artery preparations from the two sets of animals are indicated in some places in the text as “hypoxic” or “normoxic” tissues or preparations.

Guinea pig isolated pulmonary artery studies. The pulmonary artery was dissected from lungs of male Hartley guinea pigs and positioned around a 21 G blunted syringe needle. Each tissue was cleaned of adherent material and then cut into four 2-mm-wide rings. Individual rings were placed in 10-mL organ baths containing modified Krebs-Henseleit solution gassed with 95% O2, 5% CO2 and maintained at 37°C; the pH was 7.4. The composition of the Krebs-Henseleit solution was (mM): NaCl, 113.0; KCl, 4.8; CaCl2, 2.5; KH2PO4, 1.2; MgSO4, 1.2; NaHCO3, 25.0; and glucose, 5.5. Preparations were connected via stainless steel hooks and silk suture to Grass FT03C force-displacement transducers and mechanical responses were recorded isometrically by MP100 WS/ACKnowledge data acquisition system (BIOPAC Systems, Goleta, CA) run on Macintosh computers. Tissues were equilibrated under approximately 1.5 g resting load, based on previous preliminary data (Hay et al., 1993b), for at least 1 hr before the start of each experiment; during this period, preparations were washed every 15 min with fresh Krebs-Henseleit solution. After the equilibration period, tissues were precontracted with 100 mM KCl. The tissues were then rinsed every 15 min for about 1 hr to return the level of tone to base-line values. The preparations were then left for at least 30 min before the start of the experiment. ET-1 concentration-response curves were obtained by its cumulative addition to the organ bath in half-log increments (Van Rossum, 1963). Each drug concentration was left in contact with the preparation until the response reached a plateau before addition of the subsequent agonist concentration. At the end of the experiment, tissues were exposed again to 100 mM KCl, which served as a reference contraction for data analysis. Paired tissues were exposed to SB 217242 (3–300 nM) or saline vehicle for 30 min before ET-1 cumulative concentration-response curves were initiated. Only one agonist concentration-response curve was generated per tissue.

In some experiments the endothelium-dependent relaxation induced by carbachol was compared in pulmonary arteries isolated from normoxic and hypoxic animals. For these studies, pulmonary artery rings with intact endothelium were contracted with 100 mM KCl, and after plateau of this response, tissues were exposed to cumulative additions of carbachol, administered in half-log increments. After completion of carbachol concentration-response curves, each tissue was given papaverine (100 μM), which produces the maximum relaxation and was used as the reference response for data analysis.

Osmotic pump implantation. On day 0, animals were placed individually into a 6-liter chamber and anesthetized with isoflurane gas for intraperitoneal implantation of mini osmotic pumps, model 2 ML2 (Alzet, Palo Alto, CA), after surgical procedures outlined in the Alzet technical information manual. Sterile conditions were maintained to reduce the incidence of infection associated with surgery, and surgical instruments were sterilized between procedures on each animal with a hot bead sterilizer (Inotech Biosystems, Lansing, MI). The drug was solubilized in sterile solution and osmotic pumps were filled with either sterile water or SB 217242 immediately before surgery. The implantation site was shaved and cleaned first with alcohol and Betadine solution, and the animals were placed in a head box to maintain anesthesia. A midline incision, 1 to 2 cm long, was made in the lower abdomen posterior to the rib cage. The musculoperitoneal layer was carefully pulled up to avoid damage to the bowel, and the layer directly beneath the incision of the cutaneous layer was incised. A filled osmotic pump was then inserted, delivery portal first, into the peritoneal cavity. The musculoperitoneal layer was closed with a running 3–0 silk suture, and the cutaneous incision closed with two to four wound clips. Animals were removed from the head box and returned to room air to recover (for 10 min). Computer-readable tags (BioMedic Data Systems, Maywood, NJ) were injected subcutaneously into the scuff of the neck to allow for accurate identification and to monitor weight and behavioral changes of the individual animals throughout the experiment. Animals were allowed to recover for a minimum of 1 hr, then placed into the hypoxic chamber. SB 217242 or vehicle is released from the osmotic pumps immediately after implantation and begins operating at a constant rate, 5.0 μ/hr, within 4 to 6 hr, for a maximum duration of 14 days.

Hypoxia chamber. The chambers were designed by us to provide an optimized environment for hypoxic exposure of guinea pigs (Bochnowicz et al., 1997) and custom made (Mitchell Plastics, Norton, OH) to meet USDA and SmithKline Beecham requirements in accordance with the Animal Welfare Act. Animals were housed for various times, up to a maximum of 14 days, in sealed 212-liter acrylic chambers. The hypoxia chamber was designed to house eight animals, exceeding the USDA requirement of 103 square inches of flooring/animal weighing more than 350 g, and totaling a floor area of 864 square inches. The chamber was designed with two swing-down, sealed doors on the lower front panel to allow access to the pans for cleaning on a daily basis without changing the entire cage, thus reducing the time of exposure to a normoxic environment.
Chambers were cleaned and disinfected every third day while the animals were being weighed. Hypoxia (9% O₂) was produced by mixing equal flow rates of compressed air and nitrogen gas. Gases were circulated in the chamber with a fan (12 V DC, 3.5 inch square), and gas samples were taken twice daily with a Fyrite O₂ and CO₂ analyzer (Bacarach, Pittsburgh, PA). On returning the animals to the chamber, a 90-min equilibration period occurred before 9% O₂ was reached inside the chamber. Food and water were provided ad libitum. Special feeders and a continuous watering system were constructed to eliminate typical spilling and emptying of water bottles and food cups. Note, that “normoxic” animals were not placed in these chambers. Previous studies indicated that there was no difference between the physiological parameters in normoxic chamber-housed versus conventionally housed guinea pigs (data not shown).

**Acute ET-1-induced pulmonary pressure and bronchoconstrictor responses.** Male Hartley guinea pigs (600–800 g) were anesthetized with sodium pentobarbital (40 mg/kg i.p.), and both external jugular veins, the carotid artery and the trachea were cannulated for drug administration, blood and airway pressure monitoring and ventilation. The pulmonary artery was cannulated and pressure was measured as outlined below. Bilateral vagotomies were performed to minimize neural reflex influences. The animals were paralyzed with pancuronium bromide (0.1 mg/kg i.v.) and ventilated to minimize neural reflex influences. The animals were being weighed. Hypoxia (9% O₂) was produced by mixing equal flow rates of compressed air and nitrogen gas. Gases were circulated in the chamber with a fan (12 V DC, 3.5 inch square), and gas samples were taken twice daily with a Fyrite O₂ and CO₂ analyzer (Bacarach, Pittsburgh, PA). On returning the animals to the chamber, a 90-min equilibration period occurred before 9% O₂ was reached inside the chamber. Food and water were provided ad libitum. Special feeders and a continuous watering system were constructed to eliminate typical spilling and emptying of water bottles and food cups. Note, that “normoxic” animals were not placed in these chambers. Previous studies indicated that there was no difference between the physiological parameters in normoxic chamber-housed versus conventionally housed guinea pigs (data not shown).

### Data analysis

**Data analysis.** All data were reported as mean ± S.E.M. For the results of the *in vivo* studies, statistical analyses were performed using a factorial measures ANOVA; significance was determined with the Fisher’s PLSD at 95% or a 99% level of confidence (Statview II, Abacus Concepts Inc., Berkeley, CA). For *in vitro* contraction studies, agonist-induced responses for each tissue were expressed as a percentage of the reference carbachol (10 μM)-induced contraction obtained at the end of the experiment (“postcarbachol”). Geometric mean EC₅₀ values (pD₂ values) were calculated from linear regression analyses of data. Where appropriate, antagonist potencies were calculated and expressed as pKB, pA₂; pKB = −log [antagonist]/ t - 1, where t is the ratio of agonist concentration required to elicit 50% of the maximal contraction in the presence of the antagonist compared with that in its absence and pA₂ = −log of the antagonist dissociation constant. Results for control and treated tissues were analyzed for differences in both the EC₅₀ values and the maximum contractile responses. Statistical analysis was conducted by ANOVA or two-tailed Student’s *t* test for paired samples where appropriate with a probability value less than .05 regarded as significant.

### Drugs

The following drugs were used: ET-1 (human, porcine) was obtained from Peninsula Laboratories (Belmont, CA). Carbachol and papaverine was purchased from Sigma Chemical Co. (St. Louis, MO). SB 217242 was synthesized by colleagues in the Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals (King of Prussia, PA).

### Results

**Guinea pig isolated pulmonary artery studies.** In pulmonary artery rings isolated from normoxic guinea pigs, ET-1 (0.1 nM to 1 μM) potently produced concentration-related contractions with a pD₂ of 8.31 ± 0.03 (n = 5). The maximum response elicited by ET-1 (0.3 μM) represented approximately 75% of that induced by 100 mM KCl (fig. 1). SB 217242 (3–300 nM) produced a concentration-dependent inhibition of ET-1-induced contractions, reflected by marked shifts to the right in agonist concentration-response curves; Schild plot analysis of the data revealed a pA₂ of 8.1, with a slope that was not significantly different from 1, which indicated competitive antagonism. SB 217242 did not have an effect on the response induced by ET-1 or KCl, the reference contraction. (P = .64, ANOVA, fig. 1).

There were no differences in the potency of ET-1 in pulmonary arteries from normoxic and hypoxic guinea pigs (10-day exposure, 9% O₂) pD₂, 8.04 (normoxic) and 8.09 (hypoxic),
However, there was a small decrease (P = .049) in the maximum response produced by ET-1 in pulmonary artery preparations from normoxic (76.5 ± 4.0%) versus hypoxic (60.2 ± 5.7%) guinea pigs (data expressed as % of KCl (100 mM)-induced maximum contraction; n = 5; fig. 2A). In addition, the potency of SB 217242 (3 μM) for inhibition of ET-1-induced contractions was slightly different in tissues taken from normoxic and hypoxic animals, with respective pKᵦ values of 7.9 and 8.3, P = .048; n = 5) (fig. 2A). In contrast, the endothelium-dependent relaxation elicited by carbachol was markedly reduced in pulmonary artery from normoxic versus hypoxic guinea pigs (P = .013; fig. 2B). The maximum relaxation induced by 1 μM carbachol (% 100 μM papaverine) was: normoxic tissues, 57.6 ± 8.9% (n = 5); hypoxic tissues, 20.3 ± 6.7%, n = 5. Note, the maximal contractions induced by KCl (100 mM) (P = .76) and maximal relaxation elicited by papaverine (100 μM) (P = .17) were similar in normoxic and hypoxic tissues.

Exogenous ET-1 in anesthetized guinea pigs. In anesthetized, paralyzed and ventilated guinea pigs, ET-1 (0.01–3 μg/kg i.v.) produced dose-related increases in pulmonary artery pressure (fig. 3A) and airway insufflation pressure (fig. 3B); the maximum response for both parameters occurred at a dose of 1 μg/kg i.v. The maximum increase in pulmonary vascular pressure was 11.6 ± 1.6 mm Hg (n = 5), which represented a 77% increase from resting mean pulmonary artery pressure. Base-line pulmonary artery pressure, measured immediately before the first dose of ET-1, was not different when comparing vehicle-treated (15 ± 0.8 mm Hg; n = 5). However, there was a small decrease (P = .049) in the maximum response produced by ET-1 in pulmonary artery preparations from normoxic (76.5 ± 4.0%) versus hypoxic (60.2 ± 5.7%) guinea pigs (data expressed as % of KCl (100 mM)-induced maximum contraction; n = 5; fig. 2A). In addition, the potency of SB 217242 (3 μM) for inhibition of ET-1-induced contractions was slightly different in tissues taken from normoxic and hypoxic animals, with respective pKᵦ values of 7.9 and 8.3, P = .048; n = 5) (fig. 2A). In contrast, the endothelium-dependent relaxation elicited by carbachol was markedly reduced in pulmonary artery from normoxic versus hypoxic guinea pigs (P = .013; fig. 2B). The maximum relaxation induced by 1 μM carbachol (% 100 μM papaverine) was: normoxic tissues, 57.6 ± 8.9% (n = 5); hypoxic tissues, 20.3 ± 6.7%, n = 5. Note, the maximal contractions induced by KCl (100 mM) (P = .76) and maximal relaxation elicited by papaverine (100 μM) (P = .17) were similar in normoxic and hypoxic tissues.

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n = 5) or SB 217242 (1 or 3 mg/kg i.v.)-treated guinea pigs (16 + 0.9 and 16.4 ± 0.4 mm Hg, respectively; n = 5) (fig. 3A).

Vehicle or SB 217242 did not alter the base-line airway insufflation pressure (data not shown). ET-1 (1 µg/kg i.v.) produced a maximal increase in airway insufflation pressure of 15.8 ± 3.2 cm H2O which represented approximately a 200% increase from base line.

Pretreatment with SB 217242 (1 or 3 mg/kg i.v. 5 min before agonist administration) produced a dose-related inhibition of ET-1-induced increases in pulmonary artery pressure and airway insufflation pressure responses, manifest by significant rightward shifts in the dose-response curves for each parameter (fig. 3, A and B).

Chronic hypoxia studies. Chronic exposure to hypoxia (9% O2 for 0–14 days) produced a time-dependent increase in mean pulmonary artery pressure which reached statistical significance on the 7th day of hypoxia when compared with day 0 (fig. 4; ANOVA, Fisher’s PLSD; n = 4–6). The pulmonary artery pressure approximately doubled from 14.3 ± 0.9 and 13.4 ± 0.4 mm Hg on day 0 in normoxic untreated animals and normoxic vehicle-treated animals, respectively, to a maximum of 26.8 ± 0.6 mm Hg on day 10 in hypoxic, vehicle-treated animals (fig. 4). Based on this finding, the 10-day time point was selected for the drug comparator studies. In addition, in vehicle-treated/hypoxia-exposed guinea pigs, right ventricular mass, expressed as a percentage of body weight, was significantly greater when compared with normoxic animals (fig. 5). However, histological analysis (hematoxylin and eosin and elastin staining) revealed no obvious evidence of pulmonary vascular remodeling in the various regions of the guinea pig lung that were examined.

Administration of SB 217242 (0.36, 3.6 or 10.8 mg/day) for 10 days, by continuous infusion via mini osmotic pump, resulted in a significant reduction (about 50%) in hypoxia-induced pulmonary artery pressure increases, measured on day 10, at all three doses used (ANOVA, Fisher’s PLSD, n = 5–7, fig. 5). The hypoxia-induced right ventricular hypertrophy also was abrogated significantly by the 3.6 and 10.8 mg/day doses, but not by the 0.36 mg/day dose (ANOVA, Fisher’s PLSD, n = 4–6; fig. 6).

Immunoreactive ET concentrations in plasma were significantly increased (approximately 3-fold) in 10-day hypoxic guinea pigs (35.4 ± 7.7 pg/ml; n = 6) when compared with normoxic animals (12.7 ± 1.8 pg/ml; n = 4; P < .05, ANOVA, Fisher’s PLSD) (table 1). ET-1 levels in hypoxic animals which were treated with SB 217242 at the 0.36 mg/day dose (9.5 ± 4.6 pg/ml; n = 6) and the 3.6 mg/day dose (5.1 ± 0.9 pg/ml; n = 5) were significantly lower than those of hypoxic vehicle-treated guinea pigs (P < .005, respectively; ANOVA, Fisher’s PLSD) and no different from concentrations in normoxic animals (P > .05, ANOVA) (table 1). Plasma levels in
Effects of SB 217242 on irET plasma concentrations in normal and hypoxic guinea pigs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>irET (pg/ml)</th>
<th>n</th>
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<tbody>
<tr>
<td>Normoxic, vehicle</td>
<td>12.8 ± 1.8</td>
<td>4</td>
</tr>
<tr>
<td>Hypoxic, vehicle</td>
<td>35.4 ± 7.7*</td>
<td>6</td>
</tr>
<tr>
<td>Hypoxic, SB 217242, 0.36 mg/day</td>
<td>9.5 ± 4.6**</td>
<td>6</td>
</tr>
<tr>
<td>Hypoxic, SB 217242, 3.6 mg/day</td>
<td>5.1 ± 1.0**</td>
<td>5</td>
</tr>
<tr>
<td>Hypoxic, SB 217242, 10.8 mg/day</td>
<td>40.6 ± 7.1*</td>
<td>8</td>
</tr>
<tr>
<td>Normoxic, SB 217242, 10.8 mg/day</td>
<td>9.5 ± 3.5</td>
<td>8</td>
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* SB 217242 or vehicle was delivered intraperitoneally by mini osmotic pump for 10 days. Results are expressed as mean ± S.E.M.

P < .05 ANOVA, Fisher's PLSD, compared to normoxic.

** P < .02 ANOVA, Fisher's PLSD, compared to hypoxic vehicle.

Effects of SB 217242 on irET plasma concentrations in normal and hypoxic guinea pigs

Plasma concentrations of SB 217242 were not detectable (i.e., less than 10 ng/ml) in the 0.36 mg/day treatment group or the vehicle-treatment group. In the 3.6 mg/day hypoxia treatment group, plasma concentrations were 57.8 ± 44.4 ng/ml. In the 10.8 mg/day hypoxia group, the plasma concentration of SB 217242 was 270.6 ± 49.6 ng/ml, which was slightly greater than the 149.3 ± 28.2 ng/ml (P = .207, not significant) found in the corresponding normoxic 10.8 mg/day treatment group.

The 10-day hypoxia in vehicle-treated guinea pigs resulted in significant increases in hematocrit (39%), hemoglobin (30%) and red blood cell concentrations (27%) in whole blood compared with corresponding normoxic animals (P < .05; ANOVA and Fisher's PLSD; table 2). In hypoxic animals treated with any of the three doses of SB 217242, hematological parameters were not significantly different from vehicle-treated hypoxic animals (P > .05; table 2). In addition, hematocrit, hemoglobin and red blood cell concentrations in normoxic guinea pigs treated with the highest dose of SB 217242 (10.8 mg/day) were not different from normoxic animals (P > .05; table 2).

Discussion

The major findings of the present study are: 1) exposure of guinea pigs to hypoxia (9% O2, 10 days) produced an increase in pulmonary artery pressure, right ventricular hypertrophy and an elevation in irET plasma levels; 2) SB 217242, a potent and selective nonpeptide ET receptor antagonist, inhibited exogenous ET-1-induced elevation in pulmonary artery pressure and airway insufflation pressure, and the hypoxia-induced increases in pulmonary artery pressure, right ventricular hypertrophy and irET plasma levels; 3) hypoxia had little or no influence on the sensitivity of isolated pulmonary artery preparations to ET-1 and the potency of SB 217242 against ET-1-induced contractions, but caused a marked reduction in the endothelium-dependent relaxation compared with that observed in tissues obtained from normoxic guinea pigs; and 4) the normal hematological response to chronic hypoxia (i.e., increased hematocrit and hemoglobin resulting from increased red blood cell synthesis and release) were not affected by SB 217242.

The results of the present study clearly describe the efficacy of the nonpeptide endothelin receptor antagonist, SB 217242, against hypoxia-induced pulmonary hypertension and right ventricular hypertrophy in the guinea pig. The present protocol has been previously well described as an optimal, animal-friendly and relevant model of hypoxia that consistently produces pulmonary vascular and right ventricular pathological sequelae which mimic the changes seen in pulmonary hypertension resulting from hypoxia produced in a variety of clinical conditions (Bochnowicz et al., 1997). The endothelin receptor antagonist, SB 217242, is an orally bioavailable compound which preferentially antagonizes ETA receptors (K<sub>I</sub> = 1.1 nM) compared with ET<sub>B</sub> receptors (K<sub>I</sub> = 111 nM) (Ohslett et al., 1996).

Two seven-transmembrane-spanning G protein-coupled ET receptors, termed ETA and ETB, have been cloned with human tissue (Arai et al., 1993; Hosoda et al., 1992). ET-1 and ET-2 have significantly higher affinity than ET-3 for ET<sub>A</sub> receptors. ET<sub>B</sub> receptors, which recognize the identical carboxy-terminal ends of the ETs, bind all three ligands with similar affinity (Sakurai et al., 1990; Arai et al., 1990; Masaki et al., 1992). Both receptor subtypes are present in mammalian lung, and their activation has been demonstrated to produce many effects in this system including bronchoconstriction, vascular smooth muscle contraction, microvascular permeability, mucus secretion, smooth muscle and fibroblast proliferation, inflammatory cell activation and modulation of neurotransmission (Hay et al., 1993a; Hay and Goldie, 1995; Michael and Markewitz, 1996). In guinea pig pulmonary artery, contractions induced by ET-1 were sensitive to BQ-25, and were proposed to be mediated by ET<sub>A</sub> receptor activation (Hay et al., 1993b). This was confirmed in the present study, in which SB 217242 produced a concentration-dependent antagonism of ET-1-induced contractions.

The ET<sub>B</sub> receptor has been termed a "clearance receptor" based initially on studies in the rat where ET<sub>B</sub> receptors appear to mediate lung clearance of ETs (Fukuroda et al., 1994; Sato et al., 1995). Further evidence supporting this
postulate includes the finding of a doubling of plasma ET-1 levels in humans treated with the combined ET_{A}/ET_{B} receptor antagonist, bosentan (Kiowski et al., 1995). Although some controversy exists concerning ET plasma levels in pulmonary hypertension and hypoxia, the overall evidence points to an increase in ET synthesis and release in relevant clinical conditions and animal models (Michael and Markewitz, 1996). The plasma concentrations of irET in the present study were elevated (about 3-fold) with chronic 10-day hypoxic exposure in vehicle-treated animals when compared with normoxic animals. In the two lower-dose SB 217242-treated animals, irET concentrations were significantly lower than corresponding vehicle-treated animals and not different from normoxic animals. This finding might suggest that increased plasma ET levels were the result and not the cause of hypoxic pulmonary hypertension. Plasma levels of irET in hypoxic guinea pigs treated with the highest dose of SB 217242 were nearly the same as corresponding vehicle-treated hypoxic animals. A plausible explanation might be a reduction in clearance of ET associated with antagonism of the ET_{B} receptor subtype at the highest dose of SB 217242. The demonstration that plasma levels in normoxic animals treated with the highest dose of SB 217242 were no different from normoxic animals suggests that SB 217242 does not, per se, raise base-line irET plasma concentrations. These findings suggest that plasma concentrations of ET in normoxic animals are in an equilibrium of formation and catabolism by peptidase activity and/or excretion. When hypoxia exists, plasma ET levels increase because of increased release from the endothelium. Although SB 217242 is significantly more selective for the ET_{A} subtype (100-fold vs. ET_{B}), at higher doses, antagonism of the ET_{B} receptor (the so-called “clearance receptor”) may occur. Although this receptor may not be important when ET release is low (i.e., normoxic states), it may play a more important role in hypoxic states in which excessive ET release may saturate catabolic peptidase activity which is usually able to handle normal ET levels to maintain its equilibrium.

The present finding of a reduced relaxant effect of carbachol in preconstricted pulmonary artery from hypoxic guinea pigs compared with normoxic animals further highlights the potential pathophysiological significance associated with hypoxia. Nitric oxide, the molecular species which mediates the endothelial-dependent relaxant effects of various vasorelaxants, including carbachol, may play a role in both ET synthesis and activity (Ryan et al., 1993; Kourembanas et al., 1993; Markewitz et al., 1995). Patients with primary pulmonary hypertension have decreased expression of constitutive nitric oxide synthase which may contribute to enhanced ET expression (Giaid et al., 1993; Giaid and Saleh, 1995). The physiological effects of ET-1 are modulated by nitric oxide through: 1) decreasing ET-1 release from endothelial cells (Kourembanas et al., 1993), 2) attenuating ET_{A} receptor affinity and 3) blunting ET-1-induced increases in intracellular calcium (Goligorsky et al., 1994). Therefore, decreased nitric oxide synthetic capacity, demonstrated by the present in vitro results, which indicate decreased endothelium-dependent relaxation, could lead to enhanced ET formation and release. A loss of endothelium-dependent relaxant activity in a perfused lung preparation has been demonstrated in rats exposed to hypoxia (Adnot et al., 1991) and in porcine pulmonary artery, but not vein, exposed in vitro to acute hypoxia (Félotou et al., 1995). Maximum contractile activity to ET-1 in pulmonary artery from hypoxic guinea pigs was similar to that in preparations from normoxic animals. Furthermore, the sensitivity (pD_{2} values) of tissues from normoxic or hypoxic animals were not significantly different, which suggests no appreciable ET_{A} receptor desensitization, and the lack of difference in KCl-induced maximal contractions in normoxic versus hypoxic animals, which suggests no difference in muscular potential for contraction. In addition, there appeared to be a very small difference (P = .48 based on pK_{B} values) in in vitro potency of SB 217242 in pulmonary artery from hypoxic versus normoxic animals.

A role for endothelin in hypoxia-induced pulmonary vascular and right heart remodeling has been suggested by studies in which the ET_{A} receptor antagonist, BQ-123, was shown to prevent and reverse these changes in the rat (Bonvalliet et al., 1994; Chen et al., 1995; DiCarlo et al., 1995; Oparil et al., 1995). The reduction, but not total prevention, in the present study with SB 217242 may result from differences in causative factors in a particular species, subtle differences in the models or intrinsic differences in the compounds used. In addition, chronic hypoxic exposure in rats resulted in increased ET-1 synthesis and enhanced ET_{A} and ET_{B} receptor mRNA, which correlated with increased pulmonary artery pressure and right ventricular hypertrophy (Elton et al., 1992; Li et al., 1994a, b). Several groups have shown an increase in circulating levels of ET-1 in patients with pulmonary hypertension (Cernacek and Stewart, 1989; Stewart et al., 1991; Yoshiyashii et al., 1991; Allen et al., 1993). Whether a true “cause and effect relationship” exists between endothelin and enhanced pulmonary vasoreactivity and vascular remodeling resulting from hypoxia is not known. However, all of this evidence clearly implicates a consistent association.

The lack of the effect of SB 217242 on the hypoxia-induced increases in hematocrit and hemoglobin has two major implications: 1) that pharmacological antagonism of ET receptors by SB 217242 would not inhibit important erythropoietic survival mechanisms and, therefore, not contribute to tissue pathology resulting from hypoxic insult or high-altitude exposure, and 2) that ET receptors do not modulate hypoxia-induced erythropoietin release from the kidney.

In summary, the present results in a guinea pig model of hypoxia-induced pulmonary hypertension provide additional evidence supporting a significant role of ET in the pathophysiology of pulmonary hypertension and the potential utility of potent and selective ET receptor antagonists, such as SB 217242, in the treatment of this condition, by attenuating its two major features, the increase in pulmonary artery pressure and the morphological changes in the heart.

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References


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