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Short communication

The endothelin ET_B receptor antagonist BQ-788 reduces the pulmonary vasodilator effect of endothelin-1 during acute hypoxia in pigs

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Abstract

Pigs were subjected to acute, intermittent hypoxia (fraction of inhaled O_2 0.1, $n = 10$). The increase in mean pulmonary artery pressure during hypoxia was not altered after i.v. administration of the selective endothelin ET_B receptor antagonist BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine) (1 mg). However, the vasodilatory effect of endothelin-1 (25 ng/kg per min) infused into the pulmonary artery during hypoxia was attenuated by BQ-788. The present results suggest that the pulmonary vasodilator effect of exogenously administered endothelin-1 during acute hypoxia is mediated by endothelin ET_B receptors in the pig. Furthermore, endothelin ET_B receptor antagonism with BQ-788 does not influence the pulmonary vascular response to acute hypoxia. © 1997 Elsevier Science B.V.

Keywords: BQ-788; Endothelin; Hypoxia; Pulmonary hypertension

1. Introduction

Increased levels of the potent vasoactive peptide endothelin have been found in pathological conditions associated with pulmonary hypertension (Cernacec and Stewart, 1989). Endothelin-1, the main endothelin isoform found in humans, is produced by the vascular endothelium (Yanagisawa et al., 1988; Inoue et al., 1989). The vascular effects of endothelins are mediated by at least two receptor subtypes: endothelin ET_A receptors, selective for endothelin-1 causing vasoconstriction, and endothelin ET_B receptors, non-selective for the endothelins and causing either vasodilatation (Warner et al., 1989; Masaki et al., 1991) or vasoconstriction (Clozel et al., 1992).

It has been suggested that endogenous endothelin-1 could act as a mediator in the pulmonary vasoconstrictive response evoked by hypoxia (Shirakami et al., 1991). Furthermore, studies on the pulmonary circulation have shown that exogenously administered endothelin-1 can produce vasoconstriction (Horgan et al., 1991) or vasodilatation (Hasunuma et al., 1990). The pulmonary vasodilator effect of endothelin-1 has been demonstrated under conditions with elevated vascular tone, hypoxia or vasoconstrictive agents (Hasunuma et al., 1990; Lippton et al., 1991). In accord, when administered to pigs with increased pulmonary vascular resistance due to hypoxia, infusion of low-dose endothelin-1 causes a vasodilatation in low doses followed by vasoconstriction when the doses are increased (Liska et al., 1995).

To investigate the endothelin ET_B receptor involvement in acute hypoxic pulmonary hypertension we have in this study evaluated the hemodynamic effects of the selective endothelin ET_B receptor antagonist BQ-788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine) (Ishikawa et al., 1994) during normoxia and repeated hypoxia in pigs. In addition, the hemodynamic effects of endothelin-1 infusion in a concentration known to cause pulmonary vasodilatation during acute hypoxia were evaluated in animals receiving BQ-788 and in controls.

2. Material and methods

Pigs (28 ± 1 kg, $n = 10$) were premedicated with ketamine (20 mg/kg i.m.). Following anesthesia with sodium pentobarbital (15 mg/kg i.v.), the pigs were intubated and mechanically ventilated (Engström model 300, LKB, Sweden). Anesthesia was maintained by infusion of fentanyl (10 μg/kg per h), midazolam (100 μg/kg per h) and

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pancuronium bromide (150 μg/kg per h). Inspired ventilatory volumes were adjusted to maintain a pCO_2 of 4.0–5.0 kPa. During normoxia the pigs were ventilated with room air. Hypoxia was induced by a mixture of room air and N_2. The fraction of inhaled O_2 (FiO_2) was kept at 10% during hypoxia (Serve gas monitor, Siemens, Germany). Ringer acetate solution (100–150 ml/h) was administered i.v. and the body temperature was kept at 38°C with a heating pad. Mean systemic artery pressure was measured through a catheter in the left femoral artery. A Swan-Ganz catheter was introduced into the left jugular vein for measurements of mean pulmonary artery pressure, pulmonary capillary wedge pressure and central venous pressure. Hemodynamic measurements were monitored continuously (pressure transducers PVB, Triplus 6023, Germany; pressure monitor Hewlett-Packard 78342 A, Germany) and recorded at each point of measurement (pressure recorder Gould ES 1000, France). Cardiac output was determined in duplicate by thermodilution using a cardiac output computer (COM-2, Baxter, USA). Pulmonary vascular resistance was calculated as ([mean pulmonary artery pressure] − [pulmonary capillary wedge pressure])/[cardiac output], systemic vascular resistance as ([mean systemic artery pressure] − [central venous pressure])/[cardiac output]. Arterial and mixed venous blood samples were obtained simultaneously for measurements of blood gas tension and pH. A bolus dose of BQ-788 (Peninsula Labs., UK) dissolved in 20 ml physiological saline was injected into the left femoral vein. Endothelin-1 (Peninsula Labs., UK) dissolved in sterile water was infused through the Swan-Ganz catheter into the right ventricle.

2.1. Study protocol

Following surgery the pigs were left to rest for 30 min, breathing room air. The pigs were, after a baseline measurement during normoxia, subjected to inhalation of the hypoxic gas mixture during a 15 min period (for details of the model see Liska et al., 1995). Hemodynamic parameters were recorded at the end of this period. Ventilation with room air was thereafter reinstituted. After 30 min rest the animals were randomized to i.v. bolus injection of vehicle only ($n = 5$) or BQ-788 (1 mg, $n = 5$). 30 min after the injections, the hemodynamic parameters were again measured during normoxia and after 15 min of a repeated hypoxic period. Normoxic values were recorded after 30 min followed by a third hypoxic period of 15 min, during which a continuous infusion of endothelin-1 (25 ng/kg per min) was started after 5 min when the mean pulmonary artery pressure had stabilized. Hemodynamic recordings were made after 10 min of endothelin-1 infusion (i.e., 15 min of hypoxia). The duration of hypoxia and the infusion rate of endothelin-1 were chosen based on our previous studies using an identical experimental setup, showing that intrapulmonary infusion of endothelin-1 at 25 ng/kg per min during 10 min cause a marked pulmonary vasodilatory effect during hypoxia (Liska et al., 1995). The dose of BQ-788 was chosen based on experiments performed in rats in vivo (Sargent et al., 1995).

2.2. Statistical analysis

Results are presented as means ± S.E.M. Ordinary or repeated analysis of variance with multiple comparison test

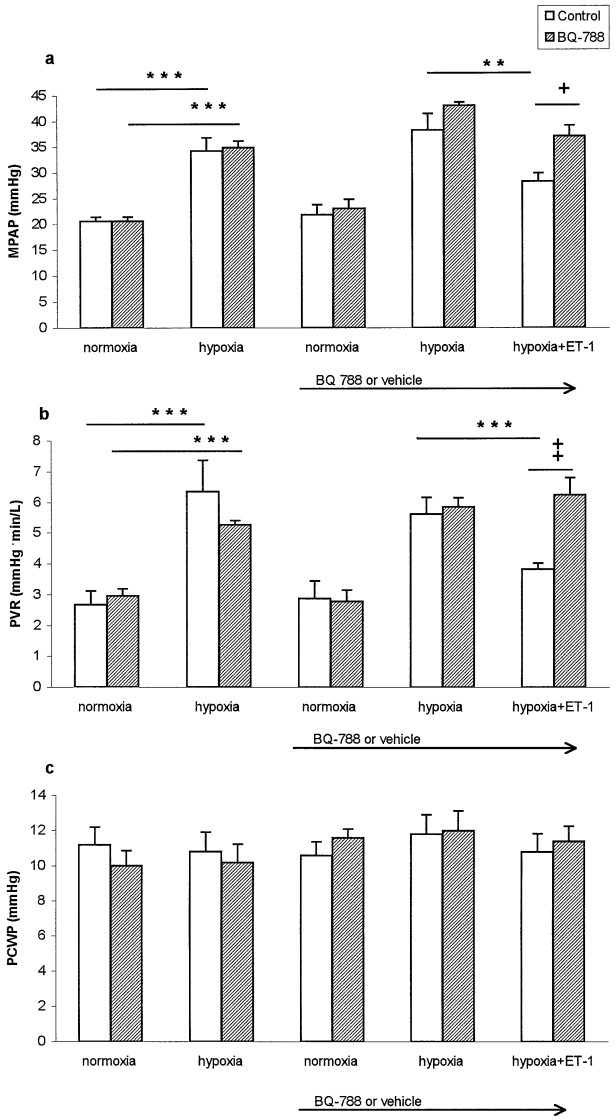

Fig. 1. (a) Mean pulmonary artery pressure (MPAP), (b) pulmonary vascular resistance (PVR) and (c) pulmonary capillary wedge pressure (PCWP) in control animals and animals receiving BQ-788 during basal normoxia, first hypoxic period (i.e. before administration of BQ-788 or vehicle), second normoxic period after BQ-788 or vehicle (arrow), second hypoxia and finally a last hypoxic period during which endothelin-1 (25 ng/kg per min) was infused. Data are presented as means ± S.E.M. ** $P < 0.01$, *** $P < 0.001$, ordinary or repeated analysis of variance with multiple comparison test; values during first hypoxic period compared to basal normoxia; second hypoxic period compared to the third hypoxic period (hypoxia and ET-1). $^{+}$ $P < 0.05$, $^{++}$ $P < 0.01$ Student's *t*-test for unpaired samples during endothelin-1 infusion comparing the control hypoxic group with the group pre-treated with BQ-788.

or the Student *t*-test for unpaired samples were used for statistical evaluation (GraphPad Software, Instat 2.01). $P < 0.05$ was considered significant.

3. Results

Reduction of the FiO_2 to 0.1 decreased the pO_2 from 12.7 ± 0.5 kPa during normoxia to 3.5 ± 0.3 kPa after 15 min of hypoxia. Hypoxia resulted in a reproducible increase in mean pulmonary artery pressure and pulmonary vascular resistance, while the pulmonary capillary wedge pressure did not change (Fig. 1a–c). The hemodynamic parameters returned to baseline values during the intermediate periods of normoxia. Infusion of endothelin-1 (25 ng/kg per min) during the last hypoxic period resulted in a prompt reduction of mean pulmonary artery pressure and pulmonary vascular resistance in the control animals (Fig. 1a,b), while no other effect was detected. Hypoxia evoked a minor decrease in systemic vascular resistance although the mean systemic artery pressure and cardiac output did not change significantly (Fig. 2a–c). Central venous pressure remained unchanged throughout the experiments (not shown). The response was highly reproducible during a second and third period of hypoxia (Figs. 1 and 2).

Administration of BQ-788 (as well as vehicle alone in the control group) had no hemodynamic effect during normoxia (Figs. 1 and 2). Furthermore, during the subsequent period of hypoxia there was no significant change in the response to hypoxia following BQ-788 compared to controls. The last hypoxic period resulted in a prompt increase of mean pulmonary artery pressure and pulmonary vascular resistance of similar magnitude in both groups. However, in contrast to the control group, endothelin-1 infusion (25 ng/kg per min) did not result in a significant reduction of mean pulmonary artery pressure and pulmonary vascular resistance in the animals receiving BQ-788 (Fig. 1a,b). Administration of BQ-788 did not alter the hemodynamic parameters reflecting the systemic circulation during normoxia, hypoxia or endothelin-1 infusion (Fig. 2a–c).

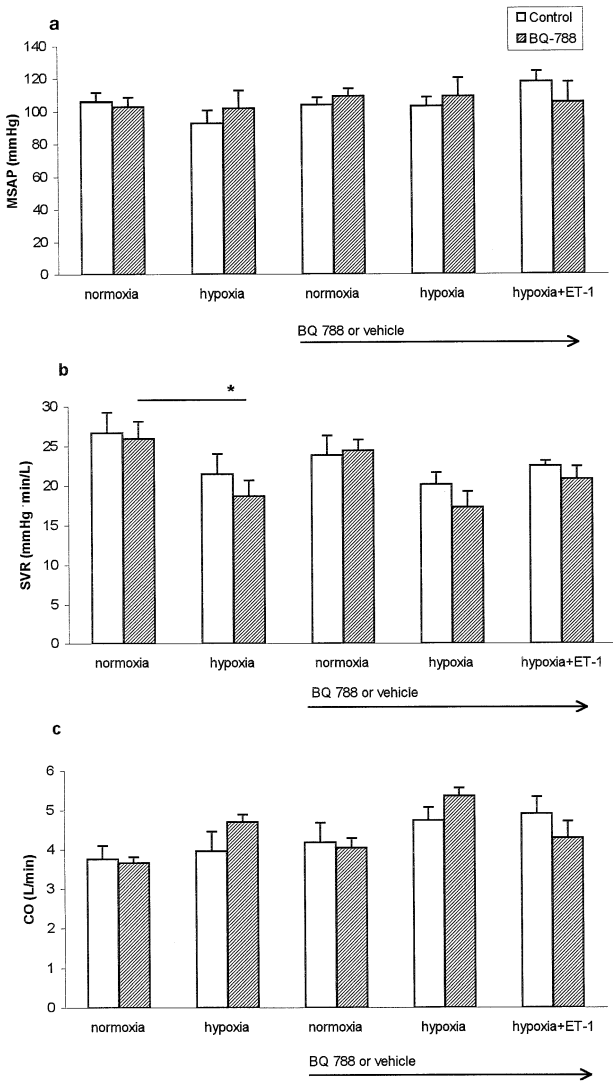

Fig. 2. (a) Mean systemic artery pressure (MSAP), (b) systemic vascular resistance (SVR) and (c) cardiac output (CO) in control animals and animals receiving BQ-788 during basal normoxia, first hypoxic period (i.e. before administration of BQ-788 or vehicle), second normoxic period after BQ-788 or vehicle (arrow), second hypoxia and finally a last hypoxic period during which endothelin-1 (25 ng/kg per min) was infused. Data are presented as means ± S.E.M. * $P < 0.05$, ordinary or repeated analysis of variance with multiple comparison test comparing values during the first hypoxic period to basal normoxia.

4. Discussion

The major finding in this study is that endothelin ET_B receptor antagonism with BQ-788 inhibits the pulmonary vasodilatory effect of endothelin-1 infusion during acute hypoxia in pigs. Furthermore, endothelin ET_B receptor antagonism with BQ-788 did not influence the pulmonary vascular response to acute hypoxia. The present study also confirms our previous results showing that repeated 15 min periods of hypoxia in pigs produce reproducible hemodynamic changes in the pulmonary vascular bed: a consistent increase in mean pulmonary artery pressure and pulmonary vascular resistance (Liska et al., 1995).

Several factors have been described to influence the pulmonary vascular response to exogenous endothelin-1, e.g., pre-existing vascular tone, development of tachyphylaxis and species differences (Cassin et al., 1991; Lippton et al., 1993). Exogenous endothelin-1 in low doses has in animal models been shown to dilate the pulmonary arterial bed during hypoxic pulmonary vasoconstriction (Hasunuma et al., 1990; Deleuze et al., 1992; Liska et al., 1995). Generally, the vascular effects of endothelin are mediated by endothelin ET_A and putative endothelin ET_B receptors located on vascular smooth muscle that mediate vasoconstriction, whereas endothelin ET_B receptors located on the

endothelium induce release of nitric oxide (NO) or prostacyclin leading to vasodilatation (Arai et al., 1990; Sakurai et al., 1990).

Previous studies have suggested that the vasodilatory effect of exogenous endothelin-1 during pulmonary hypertension is mediated by endothelin ET_B receptors. NO inhibition has been found to attenuate the pulmonary vasodilator effect of endothelin-1 injections in the neonatal pig pulmonary vasculature constricted by the thromboxane analogue U-46619 (Perreault and De Martre, 1991). Accordingly, the pulmonary vasodilator effect of exogenous endothelin-1 in the pig seems to present when the pulmonary vascular tone is elevated by other means than hypoxia and dependent on NO production. Furthermore, selective endothelin ET_B receptor agonists produced pulmonary vasodilatation in pulmonary hypertension induced by U-46619 in intact newborn lambs (Wong et al., 1995).

Our study using endothelin ET_B receptor blockade supports that the vasodilatory effect of exogenously administered, low-dose endothelin-1 during acute hypoxia is mediated by endothelin ET_B receptors. Since BQ-788 in the dose used did not influence the development of hypoxic pulmonary vasoconstriction or the removal of the effect by re-institution of oxygen, an unspecific effect of BQ-788 on vascular reactivity seems unlikely.

Earlier studies have suggested that the vasoconstrictor action of endogenous endothelin contributes to hypoxic pulmonary hypertension (Shirakami et al., 1991; Kourembanas et al., 1992). Indeed, recent findings demonstrate that selective endothelin ET_A receptor antagonism reverses the pulmonary hypertensive response to acute and chronic hypoxia in the rat (Oparil et al., 1995; DiCarlo et al., 1995). Using a similar experimental setup as the present study (i.e., acute hypoxic pulmonary hypertension in pigs) we have found an attenuation of the hypoxia-evoked pulmonary hypertension in animals pretreated with the non-selective endothelin receptor antagonist bosentan (Holm et al., 1996), indicating that endogenous endothelin could act as a mediator in this condition also in the pig. The vasoconstrictor effect of endogenous endothelin released during hypoxia as well as high doses of exogenous endothelin-1 could be attributed to activation of predominantly endothelin ET_A receptors located on vascular smooth muscle cells. In accord, our current data demonstrate that endothelin ET_B receptor antagonism using BQ-788 did not per se reduce the pulmonary hypertensive effect of acute hypoxia. The presence of a putative vasocontractile endothelin ET_B receptor located on vascular smooth muscle cell remains to be further investigated in vivo, although in vitro studies of porcine (Zellers et al., 1994) and human pulmonary arteries (Holm and Franco-Cereceda, 1996) have shown no evidence of endothelin ET_B receptor-mediated vasoconstriction.

In conclusion, the present study shows that the endothelin ET_B receptor antagonist BQ-788 attenuates the vasodilator effect of endothelin-1 infusion in the pulmonary circulation during acute hypoxia in pigs. Further studies defining the role of endothelin receptor activation in different pathological conditions associated with pulmonary hypertension may lead to new prospects for treatment of these disorders.

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