

Physiologic characterization of endothelin A and B receptor activity in the ovine fetal pulmonary circulation.

D D Ivy, ... , J P Kinsella, S H Abman

J Clin Invest. 1994;**93**(5):2141-2148. <https://doi.org/10.1172/JCI117210>.

Research Article

To determine the potential contribution of endothelin (ET) to modulation of high pulmonary vascular resistance in the normal fetus, we studied the effects of BQ 123, a selective ET-A receptor antagonist, and sarafoxotoxin S6c (SFX), a selective ET-B receptor agonist, in 31 chronically prepared late gestation fetal lambs. Brief intrapulmonary infusions of BQ 123 (0.1-1.0 mcg/min for 10 min) caused sustained increases in left pulmonary artery flow (Qp) without changing main pulmonary artery (MPA) and aortic (Ao) pressures. In contrast, BQ 123 did not change vascular resistance in a regional systemic circulation (the fetal hindlimb). To determine whether big-endothelin-1 (big-ET-1)-induced pulmonary vasoconstriction is mediated by ET-A receptor stimulation, we studied the effects of big-ET-1 with or without pretreatment with BQ 123. BQ 123 (0.5 mcg/min for 10 min) blocked the rise in total pulmonary resistance caused by big-ET-1. CGS 27830 (100 mcg/min for 10 min), an ET-A and -B receptor antagonist, did not change basal tone but blocked big-ET-1-induced pulmonary vasoconstriction. Brief and prolonged intrapulmonary infusion of SFX (0.1 mcg/min for 10 min) increased Qp twofold without changing MPA or Ao pressures. Nitro-L-arginine (L-NA), a selective endothelium-derived nitric oxide (EDNO) antagonist, blocked vasodilation caused by BQ 123 and SFX. We conclude that: (a) BQ 123 causes sustained fetal pulmonary vasodilation, but did not change vascular resistance in the fetal hindlimb; [...]

Find the latest version:

<https://jci.me/117210/pdf>



Physiologic Characterization of Endothelin A and B Receptor Activity in the Ovine Fetal Pulmonary Circulation

D. Dunbar Ivy,* John P. Kinsella,† and Steven H. Abman‡

Divisions of *Cardiology, †Neonatology, and ‡Pulmonary Medicine, Department of Pediatrics, University of Colorado School of Medicine & The Children's Hospital, Denver, Colorado 80218

Abstract

To determine the potential contribution of endothelin (ET) to modulation of high pulmonary vascular resistance in the normal fetus, we studied the effects of BQ 123, a selective ET-A receptor antagonist, and sarafotoxin S6c (SFX), a selective ET-B receptor agonist, in 31 chronically prepared late gestation fetal lambs. Brief intrapulmonary infusions of BQ 123 (0.1–1.0 mcg/min for 10 min) caused sustained increases in left pulmonary artery flow (Qp) without changing main pulmonary artery (MPA) and aortic (Ao) pressures. In contrast, BQ 123 did not change vascular resistance in a regional systemic circulation (the fetal hindlimb). To determine whether big-endothelin-1 (big-ET-1)-induced pulmonary vasoconstriction is mediated by ET-A receptor stimulation, we studied the effects of big-ET-1 with or without pretreatment with BQ 123. BQ 123 (0.5 mcg/min for 10 min) blocked the rise in total pulmonary resistance caused by big-ET-1. CGS 27830 (100 mcg/min for 10 min), an ET-A and -B receptor antagonist, did not change basal tone but blocked big-ET-1-induced pulmonary vasoconstriction. Brief and prolonged intrapulmonary infusion of SFX (0.1 mcg/min for 10 min) increased Qp twofold without changing MPA or Ao pressures. Nitro-L-arginine (L-NA), a selective endothelium-derived nitric oxide (EDNO) antagonist, blocked vasodilation caused by BQ 123 and SFX. We conclude that: (a) BQ 123 causes sustained fetal pulmonary vasodilation, but did not change vascular resistance in the fetal hindlimb; (b) Big-ET-1-induced pulmonary vasoconstriction may be mediated through ET-A receptor stimulation; and (c) ET-B receptor stimulation causes pulmonary vasodilation through EDNO release. These findings support the hypothesis that endothelin may play a role in modulation of high basal pulmonary vascular resistance in the normal fetus. (*J. Clin. Invest.* 1994. 2141–2148.) Key words: Nitric Oxide • BQ 123 • sarafotoxin S6c • CGS 27830 • pulmonary hypertension

Introduction

In utero, the normal fetal lung is characterized by high pulmonary vascular resistance, as pulmonary blood flow accounts for only 8–10% of the combined ventricular output (1). At birth, pulmonary blood flow dramatically increases 8–10-fold with a

progressive fall in pulmonary pressure. Mechanisms responsible for maintenance of high pulmonary vascular resistance in the perinatal pulmonary circulation are incompletely understood. Physical factors, such as the lack of an air-liquid interface or ventilation, low oxygen tension, and perhaps vasoactive mediators, such as leukotrienes, may play a role in the maintenance of high pulmonary vascular tone in utero (2–8). Whether other vasoconstrictor stimuli, such as the newly described peptide endothelin (9), contribute to high pulmonary vascular resistance in the normal fetus is unknown.

Endothelin (ET)¹⁻¹ is the first peptide of the endothelin family to be described (9). The mRNA of preproendothelin-1, the precursor of ET-1, has been localized to small bronchioles and blood vessels in the perinatal lung (10), and immunoreactive ET-1 levels are elevated in normal human umbilical cord blood samples at birth (11). Pharmacologic studies have demonstrated that endothelin has both vasodilator and vasoconstrictor activities (12, 13). During prolonged intrapulmonary infusion, ET-1 vasodilation is transient and followed by systemic and pulmonary hypertension (12). Cassin et al. (13) demonstrated that brief intrapulmonary infusion of ET-1 causes potent fetal vasodilation, but causes vasoconstriction in the ventilated lung with lower pulmonary vascular tone. In addition, big-endothelin-1, the precursor to ET-1, increases fetal pulmonary vascular resistance without even transient vasodilation, suggesting that the effects of endogenous ET-1 production may differ from the effects of pharmacologic infusions of ET-1 (12–14). The physiologic or pathophysiologic roles of ET-1 in the perinatal lung are unknown.

The effects of exogenous ET-1 or big-ET-1 on pulmonary vascular tone may be partially dependent upon stimulation of different ET receptors (15). The cDNA cloning of ET receptors from bovine and rat lung have revealed two distinct receptor subtypes: ET-A and ET-B (16–17). ET-A and ET-B receptors are present in many vascular beds including lung, brain, heart, and kidney (15). The ET-A receptor, which is selective for ET-1 and ET-2, is generally present on vascular smooth muscle cells, and may mediate smooth muscle contraction (18). The ET-B receptor, which is present on endothelial cells and is non-isopeptide selective, may mediate vasodilation (19). However, ET-B receptors have been found in both vascular endothelium and smooth muscle (20). Recent developments of selective receptor agonists and antagonists for endothelin have provided new tools to study the physiologic roles of endothelin (21, 22). BQ 123, a selective endothelin A receptor antagonist (21), sarafotoxin S6c, a selective endothelin B re-

Address all correspondence to D. Dunbar Ivy, M.D., The Children's Hospital, 1056 E. 19th Ave., Department of Cardiology, Denver, CO 80218.

Received for publication 14 June 1993 and in revised form 31 January 1994.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/94/05/2141/08 \$2.00

Volume 93, May 1994, 2141–2148

1. Abbreviations used in this paper: Ao, aorta; EDNO, endothelium-derived nitric oxide; ET, endothelin; L-NA, nitro-L-arginine; LPA, left pulmonary artery; MPA, main pulmonary artery; PVR, pulmonary vascular resistance; Qp, left pulmonary artery flow; SFX, sarafotoxin S6c; TPR, total pulmonary resistance.

ceptor agonist (22), and CGS 27830, an endothelin A and B receptor antagonist 20 times more selective for the endothelin A receptor than the endothelin B receptor (23), have been used in various experimental settings, but their effects in the fetal lung have not been studied.

Therefore, to characterize the physiologic activities of the endothelin A and B receptors in the fetal lung and to study the potential role of ET-1 in modulating fetal pulmonary vascular tone, we performed a series of experiments using the ET-A receptor antagonist, BQ 123, the ET-B receptor agonist, sarafotoxin S6c, and the ET-A and ET-B receptor antagonist, CGS 27830, in the chronically prepared late gestation fetal lamb.

Methods

Surgical preparation. All procedures and protocols were previously reviewed and approved by the Animal Care and Use Committee at the University of Colorado Health Sciences Center (Denver, CO). Mixed breed (Columbia-Rambouillet) pregnant ewes between 125–137 d gestation (term = 147 d) were fasted 24 h before surgery. Ewes were sedated with intravenous pentobarbital sodium (2–4 g) and anesthetized with 1% tetracaine hydrochloride (3 mg) by lumbar puncture. Ewes were kept sedated but breathed spontaneously throughout the surgery. Penicillin 500 mg and streptomycin 1 g were administered to the ewe at surgery. Under sterile conditions, the fetal lamb's left forelimb was delivered through a uterine incision. A skin incision was made under the left forelimb after local infiltration with lidocaine (2–3 ml, 1% solution). Polyvinyl catheters were advanced into the ascending aorta and superior vena cava after insertion into the axillary artery and vein. A left thoracotomy exposed the heart and great vessels. Catheters were inserted into the left pulmonary artery (LPA) main pulmonary artery (MPA) and left atrium (LA) by direct puncture through purse string sutures, as previously described (24). Catheters were guided into position with a 14- or 16-gauge intravenous placement unit (Angiocath; Travenol, Deerfield, IL). These catheters were secured by tightening the purse string suture as the introducer was withdrawn. The LPA catheter was inserted at the bifurcation of the MPA and the ductus arteriosus and guided through the common pulmonary artery into the LPA. The MPA catheter was inserted between the ductus arteriosus and the pulmonic valve. A 6-mm ultrasonic flow probe (Transonics, Ithaca, NY) was placed around the LPA to measure LPA blood flow (Q_p). The uteroplacental circulation was kept intact and the fetuses were gently placed in the uterus, with exposed surfaces bathed in warm towels. Ampicillin 500 mg was added to the amniotic cavity before closure of the hysterotomy. Ampicillin 250 mg was infused daily in the fetus and amniotic cavity during the first 3 d after surgery. Studies were performed after a postoperative recovery time of 48–72 h. In four animals, catheters were placed in the fetal hindlimb pedal artery for pressure measurement, fetal forelimb brachial vein, external iliac artery for infusion, pudendoepigastric vein, and amniotic cavity. An ultrasonic flow probe was placed around the external iliac artery to measure hindlimb flow as described by Wilkening et al. (25).

Physiological measurements. Flow transducer cables were attached to an internally calibrated flowmeter (Transonics) for continuous measurements of Q_p . The absolute values of flows were determined from phasic blood flow signals obtained during baseline periods, as previously described (23, 26, 27). The main pulmonary artery, aortic, and amniotic cavity catheters were connected to a Gould-Statham P23 ID pressure transducer. Pressures were referenced to the amniotic cavity pressure. Calibration of the pressure transducer was performed with a mercury column manometer. Heart rate was determined from the flow meter or phasic pulmonary blood flow tracings. Measurements were continuously recorded on a Gould chart recorder. Calculation of resistances are reported as total pulmonary resistance (TPR; $\text{mmHg} \cdot \text{min} \cdot \text{ml}^{-1} = \text{PAP}/Q_p$) or pulmonary vascular resistance (PVR; $\text{mmHg} \cdot \text{min} \cdot \text{ml}^{-1} = \text{PAP} - \text{LA}/Q_p$). Blood samples for pH, PCO_2 , PO_2 , hemoglobin, and oxygen saturation were drawn from the main pulmonary artery catheter and were measured at 39.5°C with a Radi-

ometer OSM-3 blood gas analyzer and hemoximeter (Radiometer, Copenhagen).

Experimental design. Protocol 1: hemodynamic effects of intrapulmonary and regional systemic (hindlimb) infusion of BQ 123 on the fetus. ($n = 7$ animals, mean gestational age = 129 d). Serial arterial blood gas tensions, hemoglobin, oxygen saturation, and hemodynamic parameters were recorded throughout baseline, drug infusion, and recovery periods. Hemodynamic parameters included phasic and mean Q_p , heart rate, and pressures in the MPA and Ao. After 30 min of baseline hemodynamic measurements, random doses of BQ 123 (0.1, 0.5, and 1.0 mcg/min.) were infused for 10 min in the LPA. Hemodynamic measurements were continuously recorded for 60 min after the infusion. To determine whether these effects were selective for the pulmonary circulation, BQ 123 (0.5 mcg/min for 10 min) was infused into the external iliac artery of the fetal hindlimb ($n = 4$ animals). Serial measurements of hindlimb pedal artery pressure and external iliac flow were recorded for 60 min after infusion.

Protocol 2: effects of BQ 123 infusion on big-ET-1-induced pulmonary vasoconstriction. ($n = 6$ animals, mean gestational age = 130 d). To determine whether the big-ET-1-induced rise in total pulmonary resistance is due to ET-A receptor stimulation, we studied the effects of intrapulmonary infusion of big-ET-1 with and without pretreatment with BQ 123. After 30 min of baseline measurements, big-ET-1 was infused in the LPA (1.5 mcg/min for 10 min). Hemodynamic measurements were continuously recorded for 60 min. Arterial blood gas tensions, hemoglobin, and oxygen saturations were measured before and after drug infusion. After at least 24 h recovery, the study was repeated after pretreatment with BQ 123. BQ 123 (0.5 mcg/min for 10 min) was infused in the LPA, followed by infusion of big-ET-1 (1.5 mcg/min for 10 min; LPA). Hemodynamic measurements were continuously recorded for 60 min. Arterial blood gas tensions, hemoglobin, and oxygen saturations were recorded before and after the infusions. These studies were performed in random order.

Protocol 3: effects of CGS 27830 infusion on big-ET-1-induced pulmonary vasoconstriction. ($n = 7$ animals, mean gestational age = 131 d). To determine whether CGS 27830 alters basal tone in the fetus, we studied the effects of intrapulmonary infusion of CGS 27830. After 30 min of baseline measurements, CGS 27830 was infused in the LPA (100 mcg/min for 10 min). Hemodynamic measurements were continuously recorded for 30 min. Arterial blood gas tensions, hemoglobin, and oxygen saturations were measured before and after drug infusion. To further examine the role of ET-A receptor stimulation, we studied the effects of intrapulmonary infusion of big-ET-1 with and without pretreatment with CGS 27830. After 30 min of baseline measurements, big-ET-1 was infused in the LPA (1.5 mcg/min for 10 min). Hemodynamic measurements were continuously recorded for 60 min. Arterial blood gas tensions, hemoglobin, and oxygen saturations were measured before and after drug infusion. After at least 24 h recovery, the study was repeated after pretreatment with CGS 27830. CGS 27830 (100 mcg/min for 10 min) was infused in the LPA, followed by infusion of big-ET-1 (1.5 mcg/min for 10 min; LPA). Hemodynamic measurements were continuously recorded for 60 min. Arterial blood gas tensions, hemoglobin, and oxygen saturations were recorded before and after the infusions. These studies were performed in random order.

Protocol 4: hemodynamic effects of intrapulmonary infusion of sarafotoxin S6c ($n = 7$ animals, mean gestational age = 130 d). After 30 min of baseline hemodynamic measurements, sarafotoxin S6c (SFX) (0.1 mcg/min for 10 min) was infused in the LPA. Hemodynamic measurements were continuously recorded for 60 min after the infusion. To determine whether SFX caused hypertension with prolonged infusion, SFX (0.025 mcg/min) was infused in the LPA for 2 h ($n = 2$ animals). Hemodynamic measurements were continuously recorded for the entire infusion. Arterial blood gas tensions, hemoglobin, and oxygen saturations were recorded before and after the infusions.

Protocol 5: hemodynamic effects of nitro-L-arginine treatment on fetal pulmonary vasodilation with SFX and BQ 123 infusions. ($n = 4$ animals, mean gestational age = 130 d). To determine whether the vasodilator effects of SFX and BQ 123 were mediated by the release of

endothelium-derived nitric oxide (EDNO), we studied the effects of the EDNO antagonist, nitro-L-arginine (L-NA) treatment on SFX and BQ 123 pulmonary vasodilation. Random doses of BQ 123 (0.5 mcg/min for 10 min) and SFX (0.1 mcg/min for 10 min) were infused in the LPA. After 30 min of baseline hemodynamic measurements, L-NA (5–20 mg) was infused in the LPA. SFX (0.1 mcg/min) or BQ 123 (0.5 mcg/min) were infused for 10 min into the LPA. Hemodynamic measurements were continuously recorded for 60 min. Arterial blood gas tensions, hemoglobin, and oxygen saturations were recorded before and after infusions.

Data analysis. Data are presented as means \pm 1 SE. Statistical analysis was performed with the Statview SE software package (Abacus Concepts, Berkeley, CA). Comparisons were made using a two way analysis of variance for repeated measures (ANOVA) and Fisher's protected least significant difference test. $P < 0.05$ was considered significant.

Results

Protocol 1: hemodynamic effects of intrapulmonary and regional systemic (hindlimb) infusions of BQ 123 on the fetus. BQ 123 caused progressive and sustained increases in Qp without changing MPA or Ao pressure (Fig. 1). As shown, after brief infusion of BQ 123, Qp continued to increase, reaching peak flow at 30 min, and remaining elevated > 60 min after starting the infusion. Baseline values for heart rate (179 ± 6 bpm), pH (7.35 ± 0.01), PCO_2 (51 ± 1 mmHg), PO_2 (17 ± 1 mmHg), hemoglobin (7.0 ± 0.6 g/dl), and oxygen saturation ($57 \pm 2\%$) did not change during the study. Fig. 2 illustrates the hemodynamic effects of different doses of BQ 123. Although BQ 123 at 0.1 mcg/min did not have any hemodynamic effects, BQ 123 (0.5 and 1.0 mcg/min) increased Qp twofold without changing MPA or Ao pressures. BQ 123 decreased

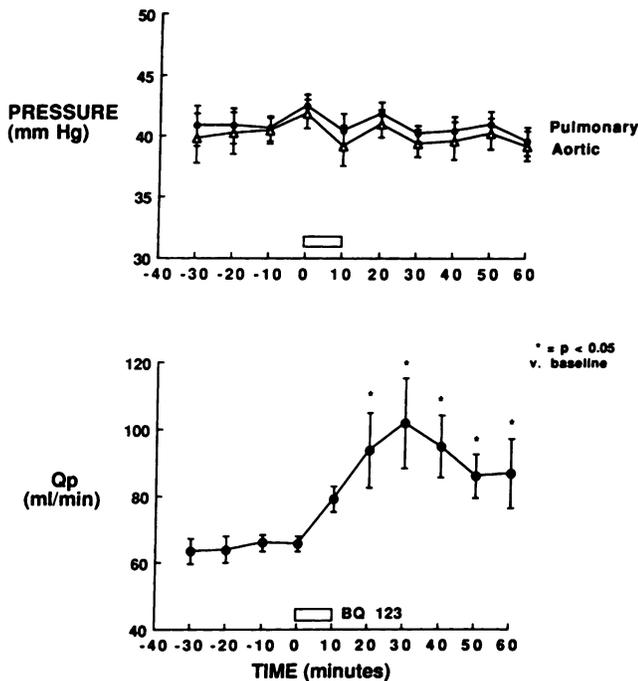


Figure 1. Hemodynamic effects of brief intrapulmonary infusion of BQ 123 at 0.5 mcg/min in late gestation fetal lambs. BQ 123 (0.5 mcg/min for 10 min in the LPA) caused a sustained and progressive increase in Qp without changing main pulmonary artery or aortic pressures.

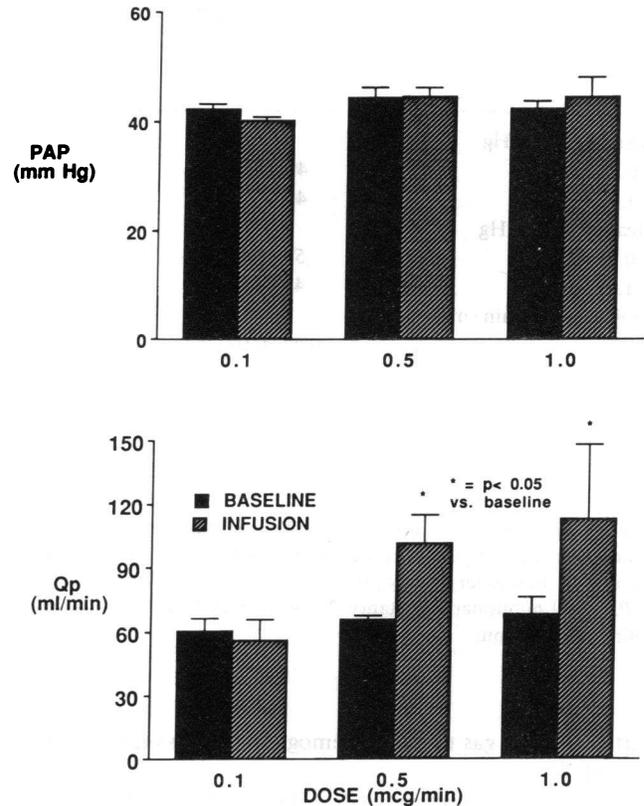


Figure 2. Hemodynamic effects of intrapulmonary infusion of BQ 123 in the late-gestation fetal lamb at 0.1, 0.5, and 1.0 mcg/min. BQ 123 at 0.5 and 1.0 mcg/min caused similar increases in Qp (left pulmonary artery flow) without changing main pulmonary artery pressure (PAP).

total pulmonary resistance by 65 and 77% with the 0.5 and 1.0 mcg/min doses ($P < 0.05$, respectively). In four animals, BQ 123, at higher doses up to 10.0 mcg/min for 10 min, did not cause further increases in Qp. MPA and Ao pressures, heart rate, arterial blood gas tensions, hemoglobin, and oxygen saturation did not change with the doses used in this study (Table I).

BQ 123 (0.5 mcg/min for 10 min) did not change hindlimb pressure or flow. Baseline external iliac flow was 48 ± 14 ml/min with hindlimb pedal artery pressure of 44 ± 3 mmHg. There was no significant change in hindlimb pressure or flow as at 30 min as external iliac flow was 47 ± 15 ml/min and hindlimb pedal artery pressure was 46 ± 4 mmHg. Baseline values for heart rate (152 ± 8 bpm), pH (7.36 ± 0.01), PCO_2 (51 ± 2 mmHg), and PO_2 (20 ± 1 mmHg) did not change during the study.

Protocol 2: effects of BQ 123 infusion on big-ET-1 induced hypertension. Intrapulmonary infusion of big-ET-1 (1.5 mcg/min for 10 min) increased MPA and Ao pressures (Table II). Total pulmonary resistance (TPR) increased from 0.61 ± 0.07 to 0.93 ± 0.13 mmHg \cdot min \cdot ml $^{-1}$ ($P < 0.05$). Pretreatment with BQ 123 (0.5 mcg/min for 10 min) blocked the increase in TPR (Fig. 3). Big-ET-1 increased MPA pressure from 44 ± 2 to 57 ± 2 mmHg at 30 min. Pretreatment with BQ 123, attenuated the rise in MPA pressure (42 ± 2 baseline to 49 ± 2 mmHg) and Qp increased (68 ± 8 to 101 ± 16 ml/min at 60 min). Big-ET-1 decreased heart rate from baseline values, but did not change

Table I. Hemodynamic Effects of Intrapulmonary Infusion of BQ 123 (0.5 and 1.0 mcg/min for 10 min)

	Baseline	Infusion [‡]
Mean AoP, mmHg		
0.5	40±2	40±1
1.0	42±2	44±2
Mean PAP, mmHg		
0.5	51±1	52±1
1.0	43±2	45±3
TPR, mmHg · min · ml ⁻¹		
0.5	0.65±0.02	0.42±0.05*
1.0	0.64±0.09	0.49±0.11*
HR, bpm		
0.5	180±6	181±5
1.0	180±8	185±6

Values are mean±SE. Comparisons between baseline and infusion values were made by repeated-measures ANOVA. *AoP*, aortic pressure; *PAP*, pulmonary artery pressure; *HR*, heart rate, beats per min; *TPR*, total pulmonary resistance. * $P < 0.05$ vs. baseline. † Values 30 min after infusion.

arterial blood gas tensions, hemoglobin, or oxygen saturation (Table II).

Protocol 3: effects of CGS 27830 infusion on big-ET-1 induced hypertension. Intrapulmonary infusion of CGS 27830 did not change basal tone in the fetus (Table III). Intrapulmonary infusion of big-ET-1 (1.5 mcg/min for 10 min) increased MPA and Ao pressures (Table IV). Pulmonary vascular resistance (PVR) increased from $0.64±0.03$ to $1.00±0.11$ mmHg · min · ml⁻¹ ($P < 0.05$). Pretreatment with CGS 27830 (100 mcg/min for 10 min) blocked the increase in PVR (Fig. 4). Big-ET-1 increased MPA pressure from $46±1$ to $62±1$ mmHg at 20 min. Pretreatment with CGS 27830 blocked the rise in MPA pressure ($49±3$ baseline to $50±2$ mmHg) and Qp ($70±6$ to $70±11$) (Table IV). Arterial blood gas tensions, hemoglobin, and heart rate did not change.

Protocol 4: hemodynamic effects of intrapulmonary infusion of sarafotoxin S6c. Brief intrapulmonary infusion of SFX (0.1 mcg/min for 10 min) increased LPA flow from $67±6$ to $168±19$ ml/min at 10 min without changing MPA or Ao pressure (Fig. 5). TPR fell from $0.66±0.06$ to $0.27±0.03$ mmHg · min · ml⁻¹ ($P < 0.05$). In contrast with BQ 123, SFX-induced pulmonary vasodilation was rapid in onset, attained peak flow at 10 min, and rapidly decreased toward baseline shortly after the infusion was stopped (Fig. 5). Arterial blood gas tensions, hemoglobin, and oxygen saturation did not change, but heart rate fell slightly following SFX infusion (Table V).

An increase in Qp was sustained during the 2-h infusion of SFX. Qp increased from $75±5$ to $186±26$ ml/min at 1 h ($P < 0.05$). Qp remained significantly increased at 2 h with flow of $103±18$ ml/min. MPA and Ao pressure did not significantly change during the two hour infusion. Arterial blood gas tensions, hemoglobin, and oxygen saturation did not change during the infusion.

Protocol 5: hemodynamic effects of L-NA treatment on fetal pulmonary vasodilation with sarafotoxin S6c and BQ 123 infusions. L-NA decreased Qp from $70±13$ to $45±12$ ml/min and increased TPR from $0.67±0.12$ to $1.26±0.33$ mmHg · min ·

ml⁻¹ ($P < 0.05$). L-NA pretreatment blocked the rise in Qp from BQ 123 or SFX (Fig. 6). After L-NA infusion, neither SFX nor BQ 123 increased flow. TPR significantly increased following L-NA, and remained elevated after SFX and BQ 123 infusions ($1.88±0.91$ and $1.04±0.17$, respectively). There were no significant differences between arterial blood gas tensions, hemoglobin, or oxygen saturation before and after infusion.

Discussion

We found that BQ 123, a selective ET-A receptor antagonist, caused sustained fetal pulmonary vasodilation. In contrast, BQ 123 did not cause vasodilation in the systemic circulation of the fetal hindlimb, suggesting that vasodilation to BQ 123 is specific to the fetal lung. BQ 123 also blocked big-ET-1 induced pulmonary vasoconstriction, demonstrating that the hemodynamic effects of big-ET-1 hypertension are probably mediated through ET-A receptors. In addition, CGS 27830, an endothelin A and B receptor antagonist, did not change basal tone and blocked big-ET-1-induced hypertension. Stimulation of the ET-B receptor by SFX caused pulmonary vasodilation, even with prolonged intrapulmonary infusion. Finally, L-NA, a selective endothelium-derived nitric oxide antagonist, blocked SFX S6c and BQ 123 pulmonary vasodilation. These findings suggest that in the developing fetal circulation, stimulation of the ET-A receptor most likely mediates vasoconstriction and that ET-B receptor stimulation mediates vasodilation, and that ET-1 contributes to vasoregulation of the normal fetal lung.

Although mechanisms maintaining high pulmonary vascular resistance in the fetus are uncertain, possible explanation for this high resistance include lack of an air liquid interface or ventilation, low oxygen tension (2–7), decreased vasodilators, such as prostacyclin, bradykinin or endothelium-derived nitric oxide or increased vasoconstrictors such as platelet activating factor or leukotrienes (28–37). Endothelin has potent vasoconstrictor properties (9), but its role in the fetal lung is poorly understood.

Table II. Hemodynamic Effects of Intrapulmonary Infusion of Big-ET-1 with and without Pretreatment with BQ 123

	Baseline	Infusion [‡]
Qp, ml/min		
Big-ET	75±7	67±8
BQ/Big-ET	68±8	101±16*
Mean PAP, mmHg		
Big-ET	44±2	57±2*
BQ/Big-ET	42±2	49±2*
Mean AoP, mmHg		
Big-ET	42±1	57±3*
BQ/Big-ET	42±2	49±2*
HR, bpm		
Big-ET	191±6	167±6*
BQ/Big-ET	166±8	169±12

Values are mean±SE. Comparisons between baseline and infusion values were made by repeated-measures ANOVA. *Qp*, left pulmonary artery flow; *PAP*, pulmonary artery pressure; *AoP*, aortic pressure; *HR*, heart rate, beats per min.

* $P < 0.05$ vs. baseline. † Values 30 min after infusion.

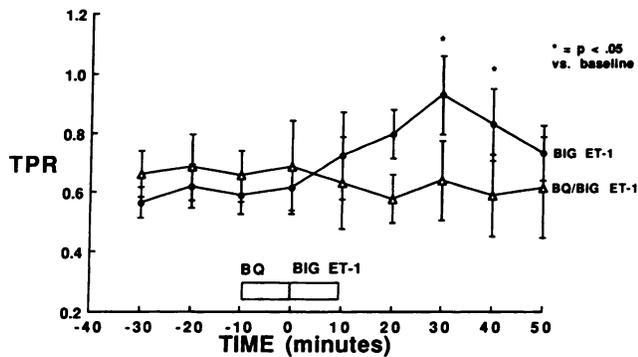


Figure 3. Effects of BQ 123 on big-ET-1-induced pulmonary hypertension. Changes in total pulmonary resistance (TPR) after intrapulmonary infusion of big ET-1 (1.5 mcg/min for 10 min) with and without pretreatment with BQ 123 (BQ/BIG ET-1) (0.5 mcg/min for 10 min). Big ET-1 increased TPR, which is attenuated with BQ 123 pretreatment.

ET-1 has complex hemodynamic effects in the fetal lung. Brief infusion of ET-1 causes potent vasodilation acutely, however with prolonged infusion, hypertension develops (12). Furthermore, ET-1 causes tone-dependent effects in the fetal pulmonary vasculature. ET-1 causes vasodilation in high tone pulmonary vasculature, however, vasoconstriction is seen when the pulmonary vascular bed is ventilated (13). Mechanisms of ET-1 induced vasodilation in the fetus include ATP-sensitive potassium channels, release of EDNO, or release of prostacyclin (38–40).

Several mechanisms potentially explain BQ 123-induced vasodilation. First, the ET-A receptor blockade could inhibit ET-1 effects of vasoconstriction mediated by calcium influx or phosphoinositide hydrolysis (41–44). Second, ET-A receptor blockade could lead to enhanced activation of the ET-B receptor, thus causing vasodilation. ATP-sensitive K^+ channels may also mediate vasodilation to ET-1 (39). Finally, prostacyclin may mediate ET-1 induced vasodilation (40). We speculate that prostacyclin release or K^+ channel activation are not the

Table III. Hemodynamic Effects of Intrapulmonary Infusion of CGS 27830 (100 mcg/min for 10 min)

	Baseline	Infusion
Qp, ml/min	69±4	64±4
Mean PAP, mmHg	49±2	49±3
Mean AoP, mmHg	48±2	47±3
LA, mmHg	3±1	3±1
PVR, mmHg·min·ml ⁻¹	0.67±0.06	0.74±0.05
pH, units	7.36±0.01	7.32±0.01
PCO ₂ , mmHg	51±1	54±2
PO ₂ , mmHg	20±2	16±2
Hgb, g/dl	6.2±.19	6.5±.32
HR, bpm	156±5	156±13

Values are mean±SE. Comparisons between baseline and infusion values were made by repeated-measures ANOVA. Qp, left pulmonary artery flow; PAP, pulmonary artery pressure; AoP, aortic pressure; LA, left atrial pressure; PVR, pulmonary vascular resistance; Hgb, hemoglobin; HR, heart rate, beats per min.

Table IV. Hemodynamic Effects of big-ET-1 with and without Pretreatment with CGS 27830 (100 mcg/min for 10 min)

	Baseline	Infusion [†]
Qp, ml/min		
Big-ET	66±3	60±6
CGS/Big-ET	70±6	70±11
Mean PAP, mmHg		
Big-ET	46±1	62±1*
CGS/Big-ET	49±3	50±2
Mean AoP, mmHg		
Big-ET	45±2	62±1*
CGS/Big-ET	46±4	48±3
LA, mmHg		
Big-ET	4±1	4±1
CGS/Big-ET	3±1	4±1
PVR, mmHg·min·ml ⁻¹		
Big-ET	0.64±0.03	1.00±0.11*
CGS/Big-ET	0.64±0.03	0.61±0.08

Values are mean±SE. Comparisons between baseline and infusion values were made by repeated-measures ANOVA. Qp, left pulmonary artery flow; PAP, pulmonary artery pressure; AoP, aortic pressure; LA, left atrial pressure; PVR, pulmonary vascular resistance. * $P < 0.05$ vs. baseline. [†] Values 20 min after infusion.

major mechanism of BQ 123 vasodilation in the ovine fetal lung as L-NA treatment blocked BQ 123 and SFX pulmonary vasodilation. Interestingly, vasodilation to BQ 123 occurs as a threshold phenomenon. BQ 123 infusions from 0.5 to 10.0 mcg/min for 10 min were not significantly different in the amount of vasodilation produced suggesting that BQ 123-induced vasodilation is not dose dependent.

Unlike ET-1, big-ET-1 causes pulmonary vasoconstriction without vasodilation (14) implying that endogenous production of ET-1 may have different effects than exogenous (pharmacologic) infusions of ET-1. Exogenous ET-1 may stimulate both the ET-A and ET-B receptors, whereas when big-ET-1 is infused directly into the fetal pulmonary circulation, it is converted by endothelin converting enzyme to ET-1 on the abluminal surface. This local production of ET-1 may only stimulate the ET-A receptor leading to vasoconstriction. No signifi-

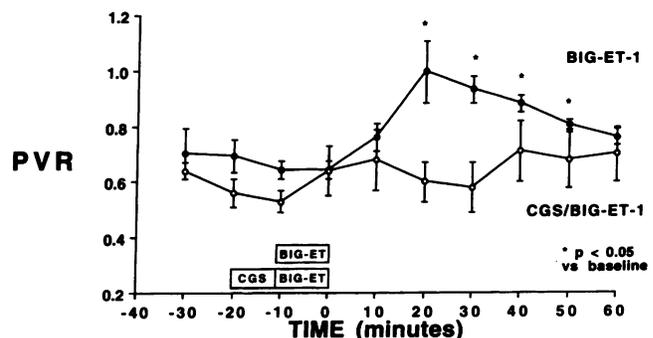


Figure 4. Effects of CGS 27830 on big-ET-1 induced pulmonary hypertension. Changes in PVR after intrapulmonary infusion of big ET-1 (1.5 mcg/min for 10 min) with and without pretreatment with CGS 27830 (CGS/Big-ET) (100 mcg/min for 10 min). Big-ET-1 increased PVR, which is blocked with CGS pretreatment.

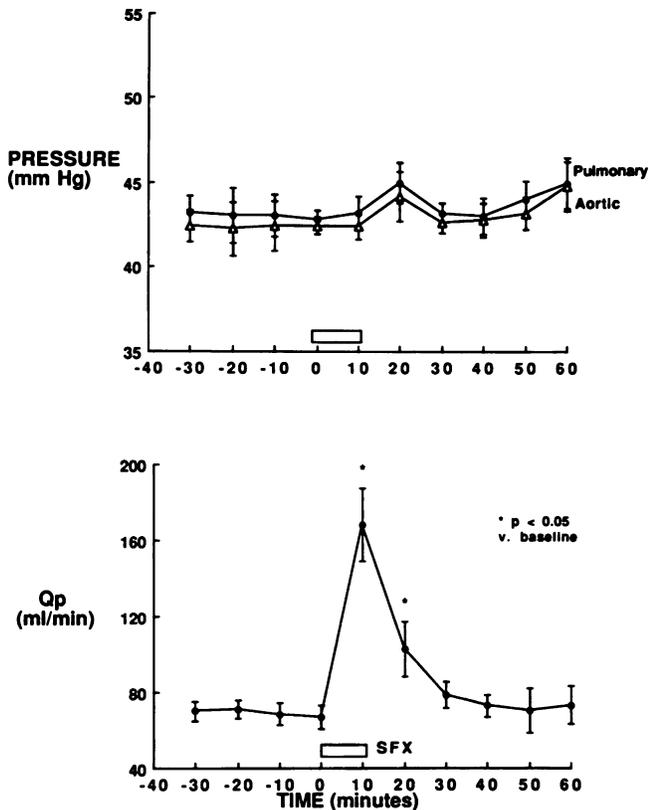


Figure 5. Hemodynamic effects of brief intrapulmonary infusion of SFX, a selective endothelin B receptor agonist. Infusion of SFX (0.1 mcg/min for 10 min) rapidly increased Qp without changing main pulmonary artery or aortic pressures. Qp rapidly decreases to baseline after infusion.

cant ET-B receptor stimulation occurs, therefore, no vasodilation is seen. The lack of ET-B receptor stimulation by big-ET-1 is speculative as ET-B receptor antagonists are not readily available, however Haleen has also suggested the lack of ET-B receptor stimulation by big-ET-1 (45).

The development of selective agonists and antagonists for the ET-A and ET-B receptors have led to improved understanding of their role in vasodilation and vasoconstriction. Ihara has reported a selective ET-A receptor antagonist, BQ

Table V. Hemodynamic Effects of Intrapulmonary Infusion of SFX

	Baseline	Infusion [‡]
pH, units	7.35±0.02	7.33±0.02
Pco ₂ , mmHg	50±1	51±2
Po ₂ , mmHg	20±1	20±1
Hgb, g/dl	7.4±.29	7.7±.22
O ₂ Sat, percent	58±3	56±5
HR, bpm	178±6	161±3*
TPR, mmHg · min · ml ⁻¹	0.66±0.06	0.27±0.03*

Values are mean±SE. Comparisons between baseline and infusion values were made by repeated-measures ANOVA. Hgb, hemoglobin; O₂ Sat, oxygen saturation percent; HR, heart rate, beats per min; TPR, total pulmonary resistance, mmHg · min · ml⁻¹. * P < 0.05 vs. baseline. [‡] Values 10 min after infusion.

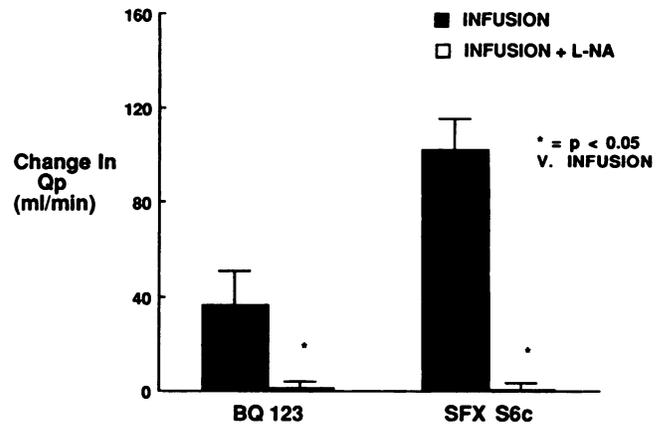


Figure 6. Effects of L-NA, an antagonist of endothelium-derived nitric oxide, on BQ 123 and sarafotoxin S6c (SFX S6c) induced pulmonary vasodilation. Infusions of SFX (0.1 mcg/min) and BQ (0.5 mcg/min) did not increase Qp after L-NA treatment.

123 (21). This cyclic pentapeptide is a potent antagonist of ET-1 induced hypertension in rats and antagonist of ET-1 induced contraction of porcine arteries (21). The ET-A receptor is selective for ET-1 and ET-2 and is believed to be the receptor in vascular smooth muscle mediating vasoconstriction (16). BQ 123 potently and selectively binds to the ET-A receptor (21, 46–47). The ET-B receptor is a nonselective subtype which binds ET-3 > ET-1 (22). CGS 27830 is a nonpeptide antagonist which blocks ET-A and B receptor activity (23). We speculate that BQ 123 but not CGS 27830 causes vasodilation due to concomitant blockade of ET B receptor activity by CGS 27830. The conclusion that big-ET-1 causes vasoconstriction by ET-A receptor stimulation would be strengthened by absence of blockade of big-ET-1 induced hypertension with an ET-B receptor antagonist, however this is not feasible as ET-B receptor antagonists are not readily available. The doses of CGS 27830 used in this study also block ET-B receptor vasodilation by ET-3 (preliminary observations). SFX is a snake venom derivative with high sequence analogy to ET-3, and has been demonstrated to be a selective agonist of the ET-B receptor (22). The exact role of these receptors in the fetal lung is incompletely understood at this time.

The role of the ET-A and ET-B receptors appear to be species as well as site specific. ET-A and ET-B receptors mediate vascular smooth muscle contraction in rabbit jugular vein and rat aorta (48). However, Clozel has shown that the ET-B receptor mediates transient vasodilation followed by vasoconstriction in mesenteric arteries (49). Moreland has shown that stimulation of the ET-B receptor contracts rabbit saphenous vein but not the rabbit carotid artery (50). Furthermore, Cristol et al. (51) have shown that systemic vasoconstriction in the rat is mediated by the ET-A receptor, whereas vasoconstriction in the kidney may be mediated by ET-B receptor stimulation (51). Porcine lung parenchyma has more ET-B receptors than ET-A receptors (52). Our work supports the concept that in the fetal lung, ET-A receptor stimulation most likely leads to vasoconstriction, whereas ET-B receptor stimulation leads to vasodilation.

The clinical significance of endothelin in humans is uncertain. ET-1 levels are increased in the normal newborn umbilical cord at the time of delivery (11, 53). Elevated immunoreac-

tive ET-1 levels have also been found in primary pulmonary hypertension, the Eisenmenger syndrome (54), persistent pulmonary hypertension of the newborn (55), and children with pulmonary hypertension associated with congenital heart disease and bronchopulmonary dysplasia (56). Whether ET-1 plays a role in the modulation of high pulmonary vascular resistance or is simply a marker of pulmonary hypertension is not clear.

In summary, response to ET-1 in the ovine fetal lung is characterized by: (a) BQ 123 causes sustained, selective vasodilation; (b) Big-ET-1-induced pulmonary vasoconstriction is most likely mediated by ET-A receptor stimulation; (c) the ET-B receptor mediates pulmonary vasodilation; (d) BQ 123 or SFX vasodilation is mediated primarily through EDNO. We conclude that the ET-A receptor probably mediates vasoconstriction and that the ET-B receptor mediates vasodilation in the ovine fetal lung. We speculate that endogenous production of ET-1 by vascular endothelium causes primarily vasoconstriction as the ET-B receptor is not significantly activated. We further speculate that ET-1 contributes to the modulation of high pulmonary vascular resistance in the ovine fetal lung.

Acknowledgments

We wish to thank Dr. Randy Webb for providing CGS 27830 for these studies.

This work was supported in part by grants from the National Institutes of Health (H241012 and 46481), the March of Dimes Birth Defects Foundation, the Bugher Physician-Scientist Training Program, and the American Heart Association of Colorado (CF-035-93).

References

- Heyman, M. A., R. K. Creasy, and A. M. Rudolph. 1973. Quantitation of blood flow patterns in the foetal lamb in utero. In *Proceedings of the Sir Joseph Barcroft Symposium: Foetal and Neonatal physiology*. Cambridge Univ. Press, Cambridge, UK. 129-135.
- Cassin, S., G. S. Dawes, J. C. Mott, B. B. Ross, and L. B. Strang. 1964. The vascular resistance of the fetal and newly-ventilated lung of the lamb. *J. Physiol. (Lond.)*. 171:61-79.
- Cassin, S., G. S. Dawes, and B. B. Ross. 1964. Pulmonary blood flow and vascular resistance in immature fetal lambs. *J. Physiol. (Lond.)*. 171:80-89.
- Dawes, G. S., J. C. Mott, J. G. Widdicombe, and D. G. Wyatt. 1953. Changes of the lungs of the newborn lamb. *J. Physiol. (Lond.)*. 121:141-162.
- Enhorning, G., F. H. Adams, and A. Norman. 1966. Effects of lung expansion on the fetal lamb circulation. *Acta Paediatr. Scand.* 55:441-451.
- Morin, F. C., E. A. Egan, W. Ferguson, and C. E. G. Lundgren. 1988. Development of pulmonary vascular response to oxygen. *Am. J. Physiol.* 254 (*Heart Circ. Physiol.* 23): H542-H546.
- Walker, A. M., B. C. Ritchie, T. M. Adamson, and J. E. Maloney. 1988. Effect of changing lung volume on the pulmonary circulation of fetal lambs. *J. Appl. Physiol.* 64:61-67.
- Soifer, S. J., R. D. Loitz, C. Roman, and M. A. Heymann. 1985. Leukotriene end organ antagonists increase pulmonary blood flow in fetal lambs. *Am. J. Physiol.* 249 (*Heart Circ. Physiol.* 18): H570-H576.
- Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto, and T. Masaki. 1988. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature (Lond.)*. 332:411-415.
- MacCumber, M. W., C. A. Ross, B. M. Glaser, and S. H. Snyder. 1989. Endothelin: visualization of mRNAs by in situ hybridization provides evidence for local action. *Proc. Natl. Acad. Sci. USA* 86:7285-7289.
- Nakamura, T., K. Kasai, S. Konuma, T. Emoto, N. Banba, M. Ishikawa, and S. Shimoda. 1990. Immunoreactive endothelin concentrations in maternal and fetal blood. *Life Sci.* 46:1045-1050.
- Chatfield, B. A., I. F. McMurtry, S. L. Hall, and S. H. Abman. 1991. Hemodynamic effects of endothelin-1 in the ovine fetal pulmonary circulation. *Am. J. Physiol.* 261 (*Regulatory Integrative Comp. Physiol.* 30): R182-R187.
- Cassin, S., T. Kristova, T. Davis, P. P. Kadowitz, and G. Gause. 1991. Tone-dependent responses to endothelin in the isolated perfused fetal sheep pulmonary circulation in situ. *J. Appl. Physiol.* 70:1228-1234.
- Jones, O. W., and S. H. Abman. 1992. Hemodynamic Effects of Proendothelin-1 and Phosphoramidon in the Late Gestation Ovine Fetus. *Am. J. Physiol.* In Press.
- Miller, R. C., J. T. Pelton, and J. P. Huggins. 1993. Endothelins-from receptors to medicine. *TIPS (Trends Pharmacol. Sci.)*. 14:54-60.
- Arai, H., S. Hori, I. Aramori, H. Ohkubo, and S. Nakanishi. 1990. Cloning and expression of a cDNA encoding an endothelin receptor. *Nature (Lond.)*. 348:730-732.
- Sakurai, T., M. Yanagisawa, Y. Takuwa, H. Miyazaki, S. Kimura, K. Goto, and T. Masaki. 1990. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature (Lond.)*. 348:732-735.
- Masaki, T., S. Kimura, M. Yanagisawa, and K. Goto. 1991. Molecular and cellular mechanisms of endothelin regulation. Implications for vascular function. *Circulation.* 84:1457-1468.
- Hirata, Y., T. Emori, S. Eguchi, K. Kanno, T. Imai, K. Ohta, and F. Marumo. 1993. Endothelin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells. *J. Clin. Invest.* 91:1367-1373.
- Shetty, S. S., T. Okada, R. L. Webb, D. DelGrande, and R. W. Lappe. 1993. Functionally distinct endothelin B receptors in vascular endothelium and smooth muscle. *Biochem. Biophys. Res. Commun.* 191:459-464.
- Ihara, M., K. Noguchi, T. Saeki, T. Fukuroda, S. Kimura, T. Fukami, K. Ishikawa, M. Nishikibe, and M. Yano. 1992. Biological profiles of highly potent novel endothelin antagonists selective for the ET-A receptor. *Life Sci.* 50:247-255.
- Williams, D. L., K. L. Jones, D. J. Pettibone, E. V. Lis, and B. V. Cline-schmidt. 1991. Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes. *Biochem. Biophys. Res. Commun.* 175:556-561.
- Mugrage, B., J. Moliterni, L. Robinson, R. L. Webb, S. S. Shetty, K. E. Lipson, M. H. Chin, R. Neale, and C. Cioffi. 1993. CGS 27830, a potent nonpeptide endothelin receptor antagonist. *Bioorg. & Medicin. Chem. Lett.* 3:2099-2104.
- Abman, S. H., F. J. Accurso, R. M. Ward, and R. B. Wilkening. 1986. Adaptation of fetal pulmonary blood flow to local infusion of tolazoline. *Pediatr. Res.* 20:1131-1135.
- Wilkening, R. B., D. W. Boyle, and G. Meschia. 1988. Measurement of blood flow and oxygen consumption in the pelvic limb of fetal sheep. *Proc. Soc. Exp. Biol. Med.* 187:498-505.
- Lewis, A. B., M. A. Heyman, and A. M. Rudolph. 1976. Gestational age changes in pulmonary vascular responses in fetal lambs in utero. *Circ. Res.* 39:536-541.
- Lock, J. E., F. Hamilton, H. Luide, F. Coceani, and P. M. Olley. 1980. Direct pulmonary vascular responses in the conscious newborn lamb. *J. Appl. Physiol.* 48:188-196.
- Assali, N. S., G. H. Johnson, C. R. Brinkman, and D. J. Huntsman. 1971. Effects of bradykinin on the fetal pulmonary circulation. *Am. J. Physiol.* 221:1375-1382.
- Campbell, A. G., G. S. Dawes, A. P. Fishman, A. I. Hyman, and A. Perks. 1968. The release of bradykinin-like substance in fetal and newborn lambs. *J. Physiol. (Lond.)*. 195:83-96.
- Davidson, D. 1988. Pulmonary hemodynamics at birth: effects of acute cycloxygenase inhibition in lambs. *J. Appl. Physiol.* 64:1676-1682.
- Heymann, M. A., A. M. Rudolph, A. S. Nies, and K. L. Melmon. 1969. Bradykinin production associated with oxygenation of the fetal lamb. *Circ. Res.* 25:521-534.
- Leffler, C. W., J. R. Hessler, and R. S. Green. 1984. The onset of breathing at birth stimulates pulmonary vascular prostacyclin synthesis. *Pediatr. Res.* 18:938-942.
- Leffler, C. W., J. R. Hessler, and R. S. Green. 1984. Mechanisms of stimulation of prostacyclin synthesis at birth. *Prostaglandins.* 28:877-887.
- Leffler, C. W., T. L. Tyler, and S. Cassin. 1978. Effect of indomethacin on pulmonary vascular response to ventilation of fetal goats. *Am. J. Physiol.* 235 (*Heart Circ. Physiol.* 4): H346-H351.
- Morin, F. C., E. A. Egan, W. Ferguson, and C. E. G. Lundgren. 1988. Indomethacin does not diminish the pulmonary vascular response of the fetus to increased oxygen tension. *Pediatr. Res.* 24:696-700.
- Tod, M. L., K. Yoshimura, and L. R. Rubin. 1991. Indomethacin prevents the ventilation induced decreases in pulmonary vascular resistance of the middle region in fetal lambs. *Pediatr. Res.* 29:449-454.
- Abman, S. H., and K. R. Stenmark. 1992. Changes in lung eicosanoid content during normal and abnormal transition in perinatal lambs. *Am. J. Physiol.* 262 (*Lung Cell. Mol. Physiol.* 6): L214-L222.
- Tod, M. L., and S. Cassin. 1992. Endothelin-1-induced pulmonary arterial dilation is reduced by N-nitro-L-arginine in fetal lambs. *J. Appl. Physiol.* 72:1730-1734.
- Hasunuma, K., D. Rodman, R. F. O'Brien, and I. McMurtry. 1990. Endothelin-1 causes pulmonary vasodilation in rats. *Am. J. Physiol.* 259 (*Heart Circ. Physiol.* 28): H48-H54.
- D'Orleans, P., S. Telemaque, A. Claing, M. Ihara, and M. Yano. 1992. Human big-endothelin-1 and endothelin-1 release prostacyclin via the activation of ET-1 receptors in the rat perfused lung. *Br. J. Pharm.* 103:773-775.

41. Gardiner, S. M., A. M. Compton, T. Bennett, R. M. Palmer, and S. Monocada. 1989. *N*-monomethyl-L-arginine does not inhibit the hindquarters vasodilator action of endothelin-1 in conscious rats. *Eur. J. Pharmacol.* 171:237-240.
42. Minnes, R. K., L. A. MacMillan, J. A. Bellan, M. D. Kerstein, D. B. McNamara, and P. J. Kadowitz. 1989. Analysis of regional responses to endothelin in hindquarters vascular bed of cats. *Am. J. Physiol.* 256 (Heart Circ. Physiol. 25): H598-H602.
43. Sigiura, M., T. Inagami, G. M. Hare, and J. A. Jones. 1989. Endothelin action: inhibition by protein kinase C inhibitor and involvement of phosphoinositols. *Biochem. Biophys. Res. Commun.* 158:170-176.
44. Van Renterghem, C. P. Vigne, J. Barhanin, A. Schmid-Al-Liana, C. Frelin, and M. Lazdunski. 1988. Molecular mechanism of action of the vasoconstrictor peptide endothelin. *Biochem. Biophys. Res. Commun.* 157:977-985.
45. Haleen, S., J. S. Davis, D. M. LaDoucer, and J. A. Keiser. 1993. Why big endothelin-1 lacks a vasodilator response. *J. Cardiovasc. Pharm.* 22(Suppl. 8):S271-S273.
46. Ihara, M., K. Ishikawa, T. Fukuroda, T. Saeki, K. Funabashi, T. Fukami, H. Suda, and M. Yano. 1992. In vitro profile of a highly potent novel endothelin (ET) antagonist BQ-123 selective for the ET-A receptor. *J. Cardiovasc. Pharm.* 20(Suppl. 12):S11-S14.
47. Cioffi, C. L., R. F. Neale, R. H. Jackson, and M. A. Sills. 1992. Characterization of rat lung endothelin receptor subtypes which are coupled to phosphoinositide hydrolysis. *J. Pharmacol. Exp. Ther.* 262:611-618.
48. Sumner, M. S., T. R. Cannon, J. W. Munding, D. G. White, and I. S. Watts. 1992. Endothelin ET-A and ET-B receptors mediate vascular smooth muscle contraction. *Br. J. Pharmacol.* 107:858-860.
49. Clozel, M., G. A. Gray, V. Brue, B. M. Loffler, and R. Osterwalder. 1992. The endothelin ET-B receptor mediates both vasodilation and vasoconstriction in vivo. *Biochem. Biophys. Res. Commun.* 186:867-873.
50. Moreland, S., D. M. McMullen, C. L. Delaney, V. G. Lee, and J. T. Hunt. 1992. Venous muscle contains vasoconstrictor ET-B-like receptors. *Biochem. Biophys. Res. Commun.* 184:100-106.
51. Cristol, J. P., T. Warner, C. Thiemermann, and J. R. Vane. 1993. Mediation via different receptors of the vasoconstrictor effects of endothelins and sarafotoxins in the systemic circulation and renal vasculature of the anaesthetized rat. *Br. J. Pharmacol.* 108:776-779.
52. Nakamichi, K., M. Ihara, M. Kobayashi, T. Saeki, K. Ishikawa, and M. Yano. 1992. Different distribution of endothelin receptor subtypes in pulmonary tissues revealed by the novel selective ligands BQ 123 and [Ala^{1,3,11,15}]ET-1. *Biochem. Biophys. Res. Commun.* 182:144-150.
53. Kojima, T., I. Fukada, M. Takedatsu, Y. Hirata, and Y. Kobayashi. 1992. Circulating levels of ET and ANF during postnatal life. *Acta Paediatr.* 81:676-677.
54. Cacoub, P., R. Dorent, M. Genevieve, P. Nataf, A. Carayon, J. C. Piette, P. Godeau, C. Cabrol, and I. Gandjbakhch. 1993. Endothelin-1 in primary pulmonary hypertension and the Eisenmenger Syndrome. *Am. J. Cardiol.* 71:448-450.
55. Rosenberg, A. A., J. Kennaugh, S. L. Koppenhafer, M. Loomis, and S. H. Abman. 1993. Elevated immunoreactive endothelin-1 levels in infants with persistent pulmonary hypertension of the newborn. *J. Pediatr.* 123:109-114.
56. Allen, S. W., B. A. Chatfield, S. L. Koppenhafer, M. S. Schaffer, R. R. Wolfe, and S. H. Abman. Circulating immunoreactive endothelin-1 levels in children with pulmonary hypertension: association with acute hypoxic pulmonary vasoreactivity. 1993. *Am. Rev. Respir. Dis.* 148:519-522.