

Selective reduction of PVR by inhalation of a cGMP analogue in a porcine model of pulmonary hypertension

CHARLES A. LAWSON, ARTHUR J. SMERLING, YOSHIFUMI NAKA, DANIEL BURKHOFF, MARC L. DICKSTEIN, DAVID M. STERN, AND DAVID J. PINSKY

Departments of Anesthesiology, Physiology, and Medicine, Columbia University College of Physicians and Surgeons, New York, New York 10032

Lawson, Charles A., Arthur J. Smerling, Yoshifumi Naka, Daniel Burkhoff, Marc L. Dickstein, David M. Stern, and David J. Pinsky. Selective reduction of PVR by inhalation of a cGMP analogue in a porcine model of pulmonary hypertension. *Am. J. Physiol.* 268 (*Heart Circ. Physiol.* 37): H2056–H2062, 1995.—Selective reduction of pulmonary vascular resistance (PVR) remains a therapeutic goal for the treatment of pulmonary hypertension, but current therapeutic options remain limited. Although the gas nitric oxide (NO) selectively dilates the pulmonary vascular bed, it requires special equipment for administration, has a short biologic half-life, and is potentially toxic. We hypothesized that stimulation of the NO pathway at the level of its second messenger, guanosine 3',5'-cyclic monophosphate (cGMP), by targeted pulmonary delivery of a membrane-permeable nonhydrolyzable cGMP analogue would cause selective pulmonary vasodilation. Pulmonary hypertension was induced in 21 pigs by the intravenous infusion of a thromboxane A₂ analogue (9,11-dideoxy-9 α ,11 α -epoxymethanoprostaglandin F_{2 α}). Inhaled 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP) lowered PVR in a time- and dose-dependent manner, with maximal effect achieved after 20 min. Compared with physiological saline control, 8-BrcGMP inhalation (3.0 μ g/kg) lowered PVR by 25 \pm 3% ($P < 0.01$), whereas there was no significant decline in systemic vascular resistance (4 \pm 6%); mean pulmonary arterial pressure declined 13 \pm 3% ($P < 0.01$), whereas there was little change in mean arterial pressure; cardiac output increased 10 \pm 4% ($P < 0.05$). PVR did not decrease after inhalation of noncyclic 8-bromoguanosine 5'-monophosphate, indicating that stimulation of the NO-cGMP pathway beyond the level of NO results in pulmonary vasodilation independent of stimulation of purinergic receptors. Inhaled 8-BrcGMP had no deleterious effect on load-independent measures of ventricular contractility, as shown by left ventricular pressure-volume loops generated at different preloads. Because selective pulmonary vasodilation was not observed after intravenous administration of 8-BrcGMP, these studies demonstrate that targeted delivery of a cGMP analogue by inhalation can selectively reduce PVR.

pulmonary vascular resistance; guanosine 3',5'-cyclic monophosphate; nitric oxide

PULMONARY HYPERTENSION is associated with significant morbidity and mortality, yet therapeutic options remain limited, because agents that lower pulmonary vascular resistance (PVR) also lower systemic vascular resistance (SVR) (18). Inhalation of nitric oxide (NO) gas has been shown to selectively lower PVR in pulmonary hypertension (12, 20), but there are concerns about the formation of toxic products from the reaction of NO with oxygen (2, 5), logistic difficulties associated with delivery of a gas, the necessity of constant administration for continued effect because of the short biologic half-life of NO

(12, 24), and potential chromosomal effects (2). Because NO exerts its vasodilating effects by increasing the guanosine 3',5'-cyclic monophosphate (cGMP) content in vascular smooth muscle (15, 16), we hypothesized that stimulation of the NO pathway with a nonhydrolyzable membrane-permeable analogue of cGMP, 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP) (14–16), administered by inhalation, would confer relative pulmonary selectivity and circumvent the difficulties and potential toxicities associated with the administration of NO. These studies used a porcine model of pulmonary hypertension to demonstrate the potential therapeutic usefulness of this approach.

METHODS

This experimental protocol was approved by the Columbia University Institutional Animal Care and Use Committee. Female swine (Hampshire breed, 32–48 kg) were premedicated intramuscularly with ketamine (20 mg/kg), acepromazine (0.4 mg/kg), and glycopyrrolate (0.0075 mg/kg), intubated, anesthetized with isoflurane (constant exhaled level maintained at 1.3%) and 100% oxygen to eliminate ventilation-perfusion mismatch contribution to shunt fraction (27), and paralyzed throughout the experiment with a curare infusion (9 mg/h). The electrocardiogram was monitored (model 2000, Datascope, Paramus, NJ), and ventilation was controlled with an anesthesia ventilator (model 7000, Ohmeda, Madison, WI) attached to an anesthesia machine (Ohmeda VMC, BOC, W. Yorkshire, UK). Respiratory gases and airway pressures were monitored with an Ohmeda analyzer (model 5250, Ohmeda, Louisville, CO). An arterial catheter was inserted percutaneously into the femoral artery, and the right external jugular vein was exposed via cutdown. Insertion of an 8.5F introducer sheath (Arrow, Reading, PA) into the external jugular vein was followed by placement of a 7.5F pulmonary artery thermomodulation catheter (Baxter Edwards Critical Care, Irvine, CA), which was advanced to the pulmonary artery with hemodynamic monitoring. Systemic arterial, pulmonary arterial, central venous (CVP), and pulmonary capillary wedge pressures (PCWP) were measured (Abbott, North Chicago, IL) at right atrial level and displayed on monitors (model 2000, Datascope, Paramus, NJ). Animal temperature was measured continuously by rectal probe and maintained by radiant heating lamps. Cardiac outputs (CO) were calculated using the thermodilution technique employing a CO computer (Baxter Edwards Critical Care). Arterial and mixed venous blood gas measurements for pH, PCO₂ (mmHg), PO₂ (mmHg), and hemoglobin oxygen saturation were performed on a calibrated arterial blood gas analyzer (Nova Biomedical, Waltham, MA). Hematocrit was determined using a capillary microcentrifuge.

Hemodynamics were recorded at end expiration at baseline and every 10–15 min thereafter and included measurements of heart rate (HR, beats/min), CVP (mmHg), PCWP (mmHg), mean arterial and mean pulmonary arterial pressures (MAP and MPAP, mmHg), and thermodilution CO (l/min). PVR and SVR were calculated as described below. Three serial measure-

ments of CO using iced saline injection were averaged for each time point and were performed at the same point in the respiratory cycle for each experiment. When a stable baseline PVR was demonstrated, pulmonary hypertension was induced by continuous intravenous infusion of the thromboxane A₂ analogue 9,11-dideoxy-9 α ,11 α -epoxymethanoprostaglandin F_{2 α} (Sigma Chemical, St. Louis, MO) (10) at 0.07–0.11 $\mu\text{g}\cdot\text{kg}^{-1}\text{min}^{-1}$. After stable measurements of PVR in the hypertensive state were achieved, physiological saline (0.9% sodium chloride) was administered by inhalation in the following manner: 0.2-ml aliquots were aerosolized using oxygen to create droplets that were delivered directly into the trachea during the inspiratory phase of each respiratory cycle until a total of 5 ml was given. This was followed by ≥ 1 h of observation. After consistent measurements of PVR were obtained, 8-BrcGMP (0.03–300 $\mu\text{g}/\text{kg}$, Sigma Chemical) was dissolved in a 5-ml volume of physiological saline and administered by inhalation in a manner identical to that used for the physiological saline control. Hemodynamic data were recorded at 10- to 15-min intervals. When no hemodynamic response was noted after 2 h of administration of the lowest dose of 8-BrcGMP (0.03 $\mu\text{g}/\text{kg}$), a higher dose was administered to establish a dose response. In separate experiments, 8-bromoguanosine 5'-monophosphate (8-BrGMP, 300 $\mu\text{g}/\text{kg}$; Sigma) dissolved in 5 ml of physiological saline was similarly administered by inhalation. After a 2-h observation period, 8-BrcGMP (300 $\mu\text{g}/\text{kg}$ of 8-BrcGMP dissolved in 5 ml of physiological saline) was injected intravenously as a bolus, and hemodynamic measurements were recorded every 10–15 min.

To assess whether inhaled 8-BrcGMP may depress myocardial contractility (6, 11, 26), experiments were performed with or without the induction of pulmonary hypertension using the same thromboxane A₂ analogue. Left ventricular (LV) contractile state was assessed in these animals by measuring the end-systolic pressure-volume relationship (ESPVR), with conductance used as an index of ventricular volume (3, 25). A 7-Fr conductance catheter (10 pole, Webster Labs, Baldwin Park, CA) was introduced into the carotid artery, and the tip was positioned in the LV apex under fluoroscopic guidance. The abdominal inferior vena cava was exposed, and venous return was impeded as needed with a snare. LV pressure was measured using a Statham strain gauge connected to the end lumen in the conductance catheter. Data were digitized (200-Hz sampling rate) on an IBM-compatible computer and analyzed off-line with custom-designed software. Pressure-volume loops were obtained at different preloads during brief periods of inferior vena caval occlusion, and end-systolic pressures (P_{es}) and volumes (V_{es}) were identified in the standard fashion (3). The slope (E_{es}) and volume axis intercept (V_0) were calculated using linear regression analysis of V_{es} against P_{es} : $P_{\text{es}} = E_{\text{es}}(V_{\text{es}} - V_0)$. Inhaled 8-BrcGMP (30 $\mu\text{g}/\text{kg}$) was administered as described above, and ESPVR was measured every 15 min for 2 h. To gauge the effect of a known negative inotrope (28), an intravenous bolus of esmolol (1 mg/kg) was given after the 2-h period, and the same measurements were obtained.

CVP, PCWP, MAP, MPAP, CO, and arterial and mixed venous blood gases were measured. PVR ($\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}$) and SVR ($\text{dyn}\cdot\text{s}\cdot\text{cm}^{-5}$) were calculated as follows: $\text{PVR} = 80 \cdot (\text{MPAP} - \text{PCWP})/\text{CO}$; $\text{SVR} = 80 \cdot (\text{MAP} - \text{CVP})/\text{CO}$. Pulmonary shunt fraction was determined using the following formula: $Q_s/Q_t = (\text{CcO}_2 - \text{CaO}_2)/(\text{CcO}_2 - \text{C}\bar{\text{v}}\text{O}_2)$, where Q_s is pulmonary shunt flow, Q_t is total flow across the pulmonary vascular bed, CcO_2 is pulmonary capillary O₂ content, CaO_2 is arterial O₂ content, and $\text{C}\bar{\text{v}}\text{O}_2$ is mixed venous O₂ content (27). Data were analyzed using Student's *t*-test for paired or unpaired data, as indicated. Dose-response data were analyzed by analysis of variance, with the Bonferroni/Dunnnett post hoc comparison of individual

means used to test for significant differences. Data are expressed as means \pm SE, with $P < 0.05$ considered statistically significant.

RESULTS

Pulmonary hypertension was established in a porcine model by use of intravenous infusion of a potent vasoconstrictor, which is an analogue of thromboxane A₂ (9,11-dideoxy-9 α ,11 α -epoxymethanoprostaglandin F_{2 α}) (10). Compared with baseline, after thromboxane treatment PVR increased from 102 ± 7 to $378 \pm 8 \text{ dyn}\cdot\text{s}\cdot\text{cm}^{-5}$ ($P < 0.005$) and SVR increased from 825 ± 67 to $1,117 \pm 31 \text{ dyn}\cdot\text{s}\cdot\text{cm}^{-5}$ ($P < 0.05$). In addition, MPAP increased from 15 ± 1 to $33 \pm 2 \text{ mmHg}$ ($P < 0.005$), MAP increased from 69 ± 5 to $85 \pm 5 \text{ mmHg}$ ($P < 0.05$), and CO remained unchanged (7 ± 1 vs. $6 \pm 0.3 \text{ l/min}$, $P = \text{NS}$; Fig. 1).

After pulmonary hypertension was established, inhalation of the membrane-permeable cGMP analogue 8-BrcGMP (14–16) caused a significant decline in MPAP, CVP, and PVR (data for individual animals are shown in Table 1 and Fig. 2). In contrast, SVR did not decrease significantly, and MAP remained unchanged after inhalation of 8-BrcGMP.

To establish the optimal effective dose of inhaled 8-BrcGMP, a dose-response relationship was constructed using doses ranging from 0.03 to 300 $\mu\text{g}/\text{kg}$. The maximal decrease in PVR occurred at an inhaled dose of 3 $\mu\text{g}/\text{kg}$ ($-24.5 \pm 2.7\%$ for 8-BrcGMP vs. inhaled physiological saline, $P < 0.01$), with no further decrease seen at higher doses (Fig. 3B). The lowering of PVR after inhaled 8-BrcGMP was reflected in a significant decline in MPAP ($-12.7 \pm 2.8\%$), also occurring at 3 $\mu\text{g}/\text{kg}$ (Fig. 3A). MAP was not significantly different at any given dose (Fig. 3C). In addition to its beneficial effects on PVR and MPAP, CO was significantly increased after inhalation of doses of 0.3 and 3.0 $\mu\text{g}/\text{kg}$ (Fig. 3E). Diminished PVR was greatest 15–29 min

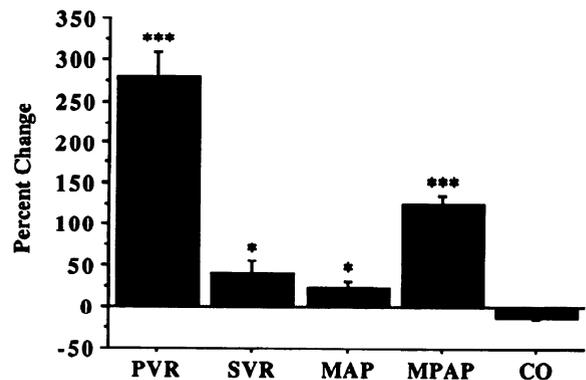


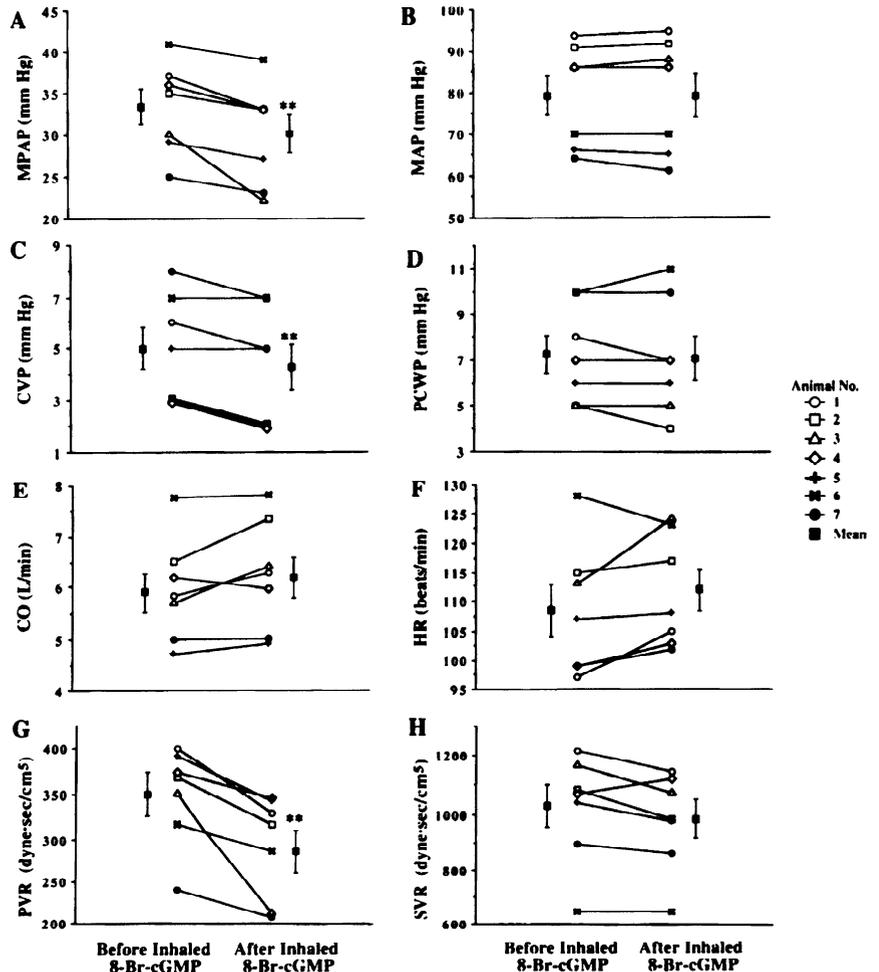
Fig. 1. Establishment of pulmonary hypertension. Thromboxane A₂ analogue 9,11-dideoxy-9 α ,11 α -epoxymethanoprostaglandin F_{2 α} was infused intravenously at 0.07–0.11 $\mu\text{g}\cdot\text{kg}^{-1}\text{min}^{-1}$ to obtain a mean pulmonary arterial pressure (MPAP) of $\sim 30 \text{ mmHg}$. Bar graphs represent percent change (mean \pm SE, $n = 7$) during thromboxane analogue infusion compared with baseline values for pulmonary vascular resistance (PVR), systemic vascular resistance (SVR), mean arterial pressure (MAP), MPAP, and cardiac output (CO). Significantly different from baseline: * $P < 0.05$; *** $P < 0.005$. Hemodynamic data are presented in Table 1 and Fig. 2.

Table 1. Hemodynamic data before and after 8-BrcGMP inhalation

	Animal No.													
	1		2		3		4*		5		6†		7	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
HR, beats/min	97	105	115	117	113	124	99	103	107	108	128	123	99	102
MAP, mmHg	94	95	91	92	86	88	86	86	66	65	70	70	64	61
MPAP, mmHg	37	33	35	33	30	22	36	33	29	27	41	39	25	23
CVP, mmHg	6	5	3	2	3	2	3	2	5	5	7	7	8	7
PCWP, mmHg	8	7	5	4	5	5	7	7	6	6	10	11	10	10
CO, l/min	5.8	6.3	6.5	7.3	5.7	6.4	6.2	6.0	4.7	4.9	7.8	7.8	5.0	5.0
PVR, dyn·s·cm ⁻⁵	400	330	369	318	351	213	374	347	391	343	318	287	240	208
SVR, dyn·s·cm ⁻⁵	1,214	1,143	1,083	986	1,165	1,075	1,071	1,120	1,038	980	646	646	896	864
pH _a	7.48	7.47	7.54	7.51	7.46	7.47	7.45	7.51	7.55	7.54			7.57	7.50
PaCO ₂ , mmHg	40	39	38	38	39	45	46	39	34	42			29	30
PaO ₂ , mmHg	441	386	575	497	488	458	485	422	487	552			556	605
SaO ₂	100	100	100	100	100	100	100	100	100	100			100	100
pH _v	7.46	7.46	7.49	7.47	7.44	7.44			7.50	7.50			7.52	7.50
PvCO ₂ , mmHg	52	42	45	48	44	51			43	40			32	35
PvO ₂ , mmHg	47	50	43	43	50	62			37	38			136	103
SvO ₂	84	87	82	81	86	92			76	77			99	99
Q _s /Q _t	0.17	0.22	0.07	0.11	0.14	0.21			0.11	0.07			0.20	0.11

Dose of 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP) was 300 µg/kg. HR, heart rate; MAP, MPAP, CVP, and PCWP, mean arterial, mean pulmonary arterial, central venous, and pulmonary capillary wedge pressures, respectively; CO, cardiac output; PVR and SVR, pulmonary and systemic vascular resistances; pH_a, PaCO₂, PaO₂, and SaO₂, arterial pH, PCO₂, PO₂, and hemoglobin saturation; pH_v, PvCO₂, PvO₂, and SvO₂, mixed venous pH, PCO₂, PO₂, and hemoglobin saturation; Q_s/Q_t, shunt fraction. *Mixed venous blood gas data not available. †Blood gas analysis not available.

Fig. 2. Effect of inhaled 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP, 300 µg/kg) on MPAP (A), MAP (B), central venous pressure (CVP, C), pulmonary capillary wedge pressure (PCWP, D), CO (E), heart rate (HR, F), PVR (G), and SVR (H) during thromboxane analogue infusion for each animal shown in Table 1. **Significantly different from pretreatment, $P < 0.01$.



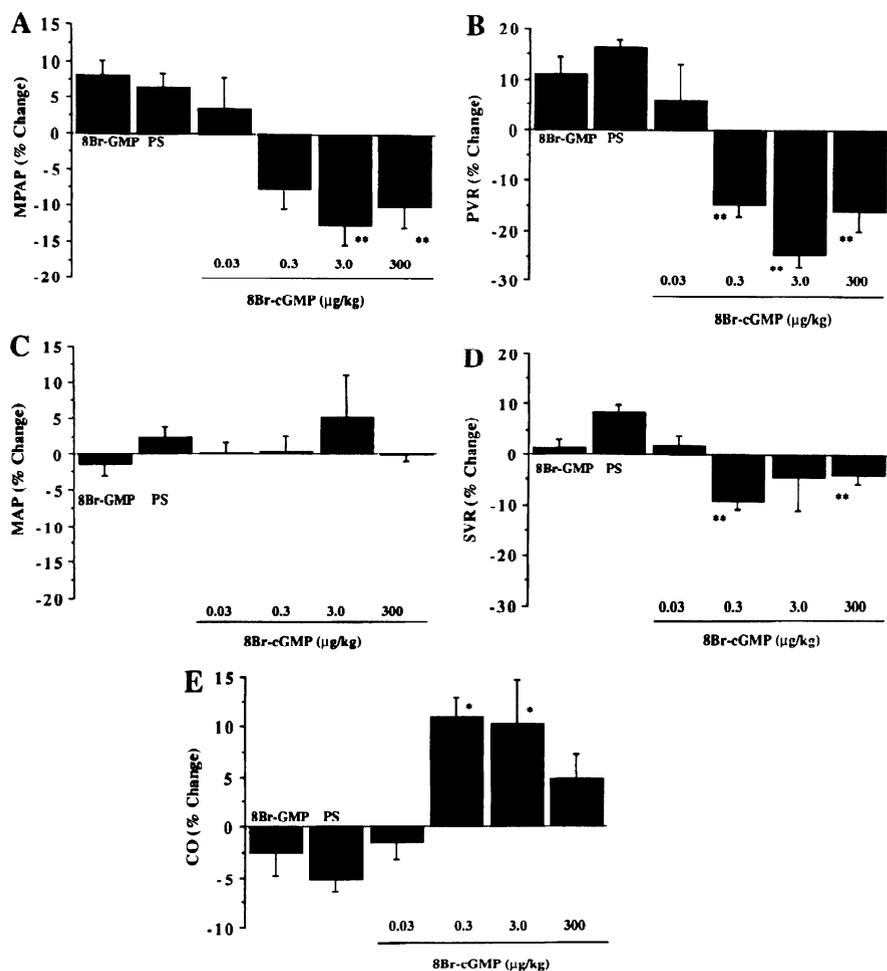


Fig. 3. Maximal changes in MPAP (A), PVR (B), MAP (C), SVR (D), and CO (E) after administration of different doses of 8-BrGMP (0.03 µg/kg, *n* = 3; 0.3 µg/kg, *n* = 3; 3.0 µg/kg, *n* = 3; 300 µg/kg, *n* = 7) and 8-bromoguanosine 5'-monophosphate (8-BrGMP, *n* = 5), all dissolved in 5 ml of physiological saline (PS), and PS (*n* = 5) by inhalation. Primary data from 300 µg/kg dose are presented in Fig. 2. **Significantly different from PS control, *P* < 0.01. *Significantly different from PS control, *P* < 0.05.

after 8-BrGMP inhalation (Fig. 4A). Thereafter, PVR steadily rose to pretreatment values.

To exclude a role for activation of purinergic receptors in the lung (8), inhalation of the noncyclic purine nucleotide analogue 8-BrGMP was given by inhalation. In contrast to inhaled 8-BrGMP (300 µg/kg), inhaled 8-BrGMP (300 µg/kg) failed to lower PVR and had no significant hemodynamic effects in comparison with inhaled physiological saline (Fig. 3).

To determine whether the pulmonary vasodilator effects of inhaled 8-BrGMP caused increased pulmonary shunting of venous blood, arterial and venous blood gas measurements were obtained and pulmonary shunt fractions calculated (Table 1) (27). There was no change in pulmonary shunt fraction after 8-BrGMP inhalation (0.138 ± 0.023 before vs. 0.144 ± 0.030 after, *P* = NS). There was also no difference in pulmonary shunt fraction before and after 8-BrGMP inhalation (0.127 ± 0.018 before vs. 0.123 ± 0.023 after, *P* = NS).

To investigate whether the mode of delivery was responsible for the pulmonary selectivity, identical doses of 8-BrGMP (300 µg/kg) were given by the intravenous or the inhaled route. PVR before and after intravenous delivery was 464 ± 54 and 328 ± 27 dyn·s·cm⁻⁵, respectively (*P* < 0.05). SVR before and after intravenous delivery was $1,556 \pm 142$ and $1,247 \pm 92$ dyn·s·cm⁻⁵, respectively (*P* < 0.05). In contrast, when

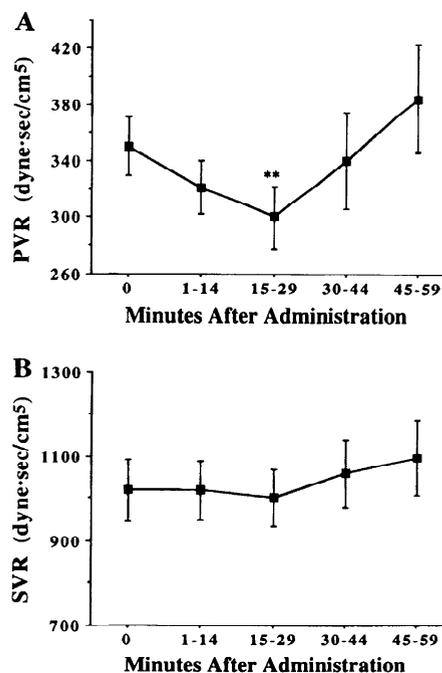


Fig. 4. Effect of inhaled 8-BrGMP (300 µg/kg) on PVR (A) and SVR (B) over time during thromboxane analogue infusion. Each point represents mean change observed at indicated time after 8-BrGMP inhalation. **Significantly different from pretreatment, *P* < 0.01.

8-BrCGMP was inhaled, only the PVR decreased significantly compared with pretreatment values (Fig. 2). These data are plotted as percent change in vascular resistance in Fig. 5, which illustrates that the mode of delivery is responsible for the selective reduction of PVR.

Because 8-BrCGMP has been reported to depress myocardial contractility (26), the effect of inhaled 8-BrCGMP on cardiac contractility was investigated ($n = 3$) using LV conductance as an index of ventricular volume, with simultaneous measurement of intraventricular pressure. ESPVR, a load-independent measure of cardiac contractility (3, 25), was determined by varying preload by controlling blood return via the inferior vena cava. In control pigs not treated with thromboxane, ESPVR was calculated before treatment (Fig. 6A) and remained unchanged after inhalation of an effective pulmonary vasodilator dose of 8-BrCGMP (30 $\mu\text{g}/\text{kg}$; Fig. 6B). Similarly, there was no change in ESPVR after inhaled 8-BrCGMP after establishment of pulmonary hypertension by infusion of the thromboxane A_2 analogue (data not shown). This is in contrast to the depression of myocardial contractility observed after intravenous administration of a known negative inotrope (esmolol, 1 mg/kg; Fig. 6C) (28).

DISCUSSION

Primary and secondary pulmonary hypertension are associated with high morbidity and mortality (18). Although many therapeutic agents have been tried in an attempt to lower the elevated PVR associated with these conditions, no agent has been found that is both simple to administer and consistently effective (18, 22, 23, 30). The efficacy of most agents is limited by nonselective vasodilation, whereby SVR is lowered to a similar or greater degree than PVR, occasionally causing a precipitous drop in blood pressure resulting in patient death (18, 19, 23, 30). A drug is considered to be clinically effective in pulmonary hypertension if it can lower PVR more than it lowers SVR, i.e., is relatively selective for the pulmonary vasculature (18). Because the major clinical impact of chronic pulmonary hypertension stems from flow limitation, a goal of drug therapy is reduction of pulmonary arterial pressures and calculated PVR, with normalization of CO (23).

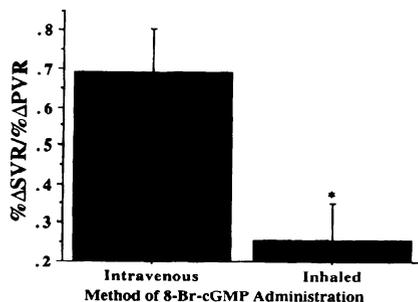


Fig. 5. Selective effect of targeted delivery of 8-BrCGMP on PVR and SVR. 8-BrCGMP (300 $\mu\text{g}/\text{kg}$) was dissolved in 5 ml of physiological saline and administered by inhalation ($n = 7$; primary data in Fig. 2) or intravenous injection ($n = 5$). * Δ PVR significantly different from Δ SVR, $P < 0.05$.

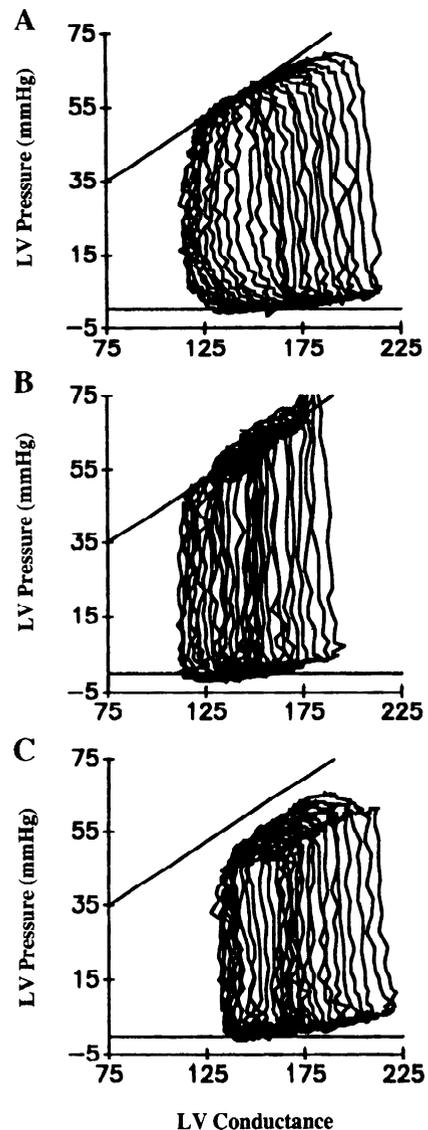


Fig. 6. Left ventricular (LV) contractile function assessed using pressure-volume loops at various preloads. Conductance reflects ventricular volume. A: pressure-volume loops in untreated animal. Systolic function is described by tangent to pressure-volume curves. This line is used as reference in B and C. B: pressure-volume loops 30 min after inhalation of 8-BrCGMP (30 $\mu\text{g}/\text{kg}$) in 5 ml of physiological saline delivered as a mist. There was no decline in ventricular contractility. C: pressure-volume loops within 5 min after esmolol (1 mg/kg iv). End-systolic pressure-volume relationship demonstrates a negative inotropic effect.

Of the numerous approaches to selectively reduce PVR in pulmonary hypertension, the use of inhaled NO has shown particular promise in recent trials (12, 20). Rationale for the use of NO to treat pulmonary hypertension is based on its ability to stimulate soluble guanylate cyclase found in smooth muscle cells throughout the vasculature, leading to an increase in intracellular cGMP and subsequent vasodilation (13). Because NO binds rapidly to and is inactivated by hemoglobin (1), it preferentially dilates the pulmonary vascular bed when administered by inhalation. Recent clinical studies investigating the adult respiratory distress syndrome demonstrate that this therapy is likely to benefit patients

during continuous administration (24). However, there are practical and theoretical concerns regarding NO administration: as a gas, special delivery equipment is required; its effects are dependent on continuous administration (12, 24); its free radical structure renders it highly reactive, producing toxic metabolites such as peroxynitrite (2, 5); and it is possibly carcinogenic (associated with a positive Ames test for mutagenicity) (2). However, there is no evidence that clinically effective doses of NO have produced significant lung injury or methemoglobinemia. We hypothesized that administration of a nonhydrolyzable membrane-permeable analogue of cGMP, 8-BrcGMP (14–16), might have beneficial pulmonary-vasodilating effects similar to NO, although there are as yet no data documenting the safety of long-term 8-BrcGMP administration.

In isolated lung models, 8-BrcGMP administered intravenously effectively reduced hypoxia-induced pulmonary vasoconstriction (1). Although others have given 8-BrcGMP intravenously (9), this method of administration should not cause selective dilation of the pulmonary vasculature, because cGMP is a ubiquitous messenger, occurring in many tissues (14–16). Inhalation of aerosolized prostacyclin has been reported to cause a selective decrease in pulmonary arterial pressure and PVR when compared with intravenous injection (31) and may be effective in the adult respiratory distress syndrome (29). We hypothesized that 8-BrcGMP administered by inhalation would target pulmonary vascular smooth muscle and thereby be relatively selective in reducing PVR.

To test this hypothesis, a model of acute pulmonary hypertension was established using the thromboxane A₂ analogue 9,11-dideoxy-9 α ,11 α -epoxymethanoprostaglandin F_{2 α} (10) given by continuous infusion to induce pulmonary hypertension. Even though this is a model of uniform pulmonary vasoconstriction with no parenchymal lung disease, it may be relevant to clinical pulmonary hypertension, because thromboxane is believed to play a role in diseases as varied as scleroderma, systemic lupus erythematosus, cirrhosis of the liver, and pulmonary emboli (4, 7, 17, 21). Others have shown that endothelium-derived relaxing factor (NO) has a significant role in blunting the pulmonary response to vasoconstrictors such as thromboxane (10), making this model suitable to test the effects of a cGMP analogue. In addition, this model was well suited to assess pharmacological intervention because of the stability of hemodynamic variables achieved during constant infusion of this compound.

Our studies demonstrated that 8-BrcGMP administered by inhalation causes a significant dose-dependent decline in MPAP and PVR. These changes were accompanied by an increase in CO. This, in addition to the significant decrease in CVP, is evidence that 8-BrcGMP inhalation results in unloading of the right ventricle, a goal of pulmonary vasodilator therapy (23). The failure of PVR and pulmonary arterial pressure to return to baseline (pre-thromboxane analogue) values may be due to limited ability to counteract the potent pulmonary vasoconstrictor used in these experiments. This does not necessarily preclude therapeutic efficacy, however, be-

cause clinical benefits have been reported without complete normalization of PVR in treated pulmonary hypertensive patients (23).

One theoretical benefit of inhaled vasodilators such as NO is preferential vasodilation in areas of the lung where delivery is greatest, i.e., the ventilated portions of the lung. To assess the location of compound delivery in our experiments, methylene blue was administered by inhalation in an identical fashion to 8-BrcGMP. In these experiments, noncollapsed alveoli demonstrated histological evidence of methylene blue staining, indicating that the test compounds administered by inhalation reached ventilated alveoli. This is consistent with the shunt fraction data we obtained showing that inhaled 8-BrcGMP had no significant effect on shunt fraction, suggesting that it does not increase perfusion to poorly ventilated alveoli. Together, these data suggest that 8-BrcGMP administered via inhalation is preferentially delivered to the ventilated portions of the lungs.

To further establish that relative pulmonary selectivity was due to directed delivery of 8-BrcGMP into the lungs rather than a tissue-specific property of the compound, comparisons between intravenous and inhaled administration were made. When identical doses of 8-BrcGMP were given, only the inhaled route selectively decreased PVR compared with SVR. This selective pulmonary vasodilation resulting from 8-BrcGMP inhalation is not limited to the thromboxane analogue model. Preliminary studies using a porcine model of hypoxia-induced pulmonary hypertension show similar selective reductions of PVR after inhalation of 8-BrcGMP (Lawson and Pinsky, unpublished observation). Taken together, these data show that pulmonary selectivity is neither compound nor model dependent, suggesting again that local delivery confers relative pulmonary selectivity.

Because purinergic cell surface receptors are widely distributed in the vasculature and binding of purine nucleotides to these surface receptors may cause vasodilation (8), it was necessary to determine whether purinergic receptor-mediated vasodilation occurred in our model of pulmonary hypertension. 8-BrGMP, the noncyclic analogue of 8-BrcGMP, was administered by inhalation for this purpose. This noncyclic compound had no effect in decreasing PVR when given at an inhaled dose (300 μ g/kg) at which 8-BrcGMP demonstrates a clear-cut decline in PVR. This finding suggests that the ability of 8-BrcGMP to reduce PVR is related to its actions as a second-messenger cyclic nucleotide rather than due to purinergic receptor activation.

It has been suggested that stimulation of the NO pathway may result in depression of myocardial contractility (6, 11, 26), which would be of clinical concern in patients with compromised ventricular function. Depression of myocardial contractility has been ascribed to NO production (6, 11), and 8-BrcGMP itself has been shown to exert a moderate negative inotropic effect on isolated ferret cardiac muscle (26), so it was important to measure the effect of inhaled 8-BrcGMP on load-independent measures of myocardial contractility. Be-

cause load-independent measures of right ventricular performance are difficult to obtain because of the geometry of the right ventricle (32), in these studies we used pressure-volume loops to construct load-independent measures of LV function after inhalation of 8-BrCGMP. Under normotensive and thromboxane analogue-induced hypertensive conditions, inhalation of 8-BrCGMP at an effective pulmonary vasodilating dose had no effect on LV ESPVR, suggesting no adverse effect on myocardial contractility.

In conclusion, inhalation of 8-BrCGMP causes selective pulmonary vasodilation in a porcine model of pulmonary hypertension, an effect that is likely mediated by its actions as a second-messenger cyclic nucleotide. The combination of an agent that stimulates the NO-cGMP pathway with a directed method of delivery (such as inhalation) suggests a broad range of pharmacological possibilities for the treatment of pulmonary hypertension.

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Address for reprint requests: C. A. Lawson/D. J. Pinsky, College of Physicians and Surgeons, Columbia University, P & S 11-518, 630 W. 168th St., New York, NY 10032.

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