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Lack of haemodynamic effects of nitric oxide on post-capillary pulmonary hypertension induced by acute sino-aortic denervation

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1 The aims of the present experiments were to define a new experimental model of pulmonary hypertension induced by a post-capillary mechanism and to assess the haemodynamic effects of nitric oxide on post-capillary pulmonary hypertension.

2 Cardiopulmonary variables of 28 male beagle dogs, anaesthetized with chloralose, 16 spontaneous breathing and 12 with assisted ventilation, were studied before and after sino-aortic denervation (SAD). The haemodynamic effects of inhaled nitric oxide (25 p.p.m., 10 min), N^{ω}-nitro-L-arginine methyl ester (20 mg kg^{-1}, i.v.), urapidil (0.5 mg kg^{-1}, i.v.) and propranolol (300 μg kg^{-1}, i.v.) were studied after SAD.

3 SAD induced an acute and transient pulmonary hypertension, more marked in spontaneous breathing dogs. This pulmonary hypertension involved a post-capillary mechanism, secondary to the left ventricular haemodynamic effects of the acute increase of left ventricular after-load induced by systemic hypertension. In fact, the increase of mean pulmonary arterial pressure after SAD and the decrease of this parameter after urapidil or propranolol were strongly correlated with the variations of pulmonary capillary wedge pressure. Furthermore, no significant change in pulmonary vascular resistances was found after SAD or administration of α or β-adrenoceptor antagonists.

4 Inhaled nitric oxide did not reverse pulmonary hypertension induced by SAD. N^{ω}-nitro-L-arginine methyl ester had no significant haemodynamic effect on pulmonary circulation.

5 In conclusion, the lack of effect of inhaled nitric oxide and nitric oxide synthase inhibitor on pulmonary circulation parameters after SAD suggests that endothelium-derived nitric oxide is not involved in the mechanisms leading to post-capillary pulmonary hypertension.

Keywords: Sino-aortic denervation; post-capillary pulmonary hypertension; nitric oxide; N^{ω}-nitro-L-arginine methyl ester

Introduction

The prognosis and treatment of pulmonary arterial hypertension depend on its aetiology. Haemodynamic investigations have defined two types of pulmonary hypertension: post-capillary, due to left heart disorders or mitral diseases, and pre-capillary, secondary to chronic pulmonary disease, pulmonary embolism or congenital heart disease. Recently, it was demonstrated that endothelium-derived nitric oxide contributes to basal pulmonary and systemic vascular resistances through its smooth muscle relaxant properties (Ignarro *et al*., 1987; Palmer *et al*., 1987). Furthermore, altered nitric oxide production has been found in some cases of pulmonary hypertension. Inhaled nitric oxide, a selective pulmonary vasodilator (Pepke-Zaba *et al*., 1991), was shown to reverse hypoxic pulmonary vasoconstriction in various animal models (Frostell *et al*., 1991; Pinson *et al*., 1993; Romand *et al*., 1994a). Inhaled nitric oxide relaxes both large and small arteries but its rapid inactivation by haemoglobin limits its efficacy as a pulmonary venous dilator (Roos *et al*., 1994; Tod *et al*., 1995). Thus, inhalation of nitric oxide gas could be used in the treatment of pre-capillary pulmonary hypertension. Effective protocols have been developed for persistent pulmonary hypertension of the newborn (Kinsella *et al*., 1992; Roberts *et al*., 1992), pulmonary hypertension following surgical corrections of congenital heart disease (Sellden *et al*., 1993) or acute respiratory distress syndrome (Rossaint *et al*., 1993). However the role of endothelium-derived nitric oxide and the effects of inhaled nitric oxide gas on post-capillary pulmonary hypertension remain unknown. In isolated lungs, it was recently shown that inhaled nitric oxide was effective in relaxing the small veins but had no effect on the large veins (Roos *et al*., 1994; Tod *et al*., 1995). In sheep, passive left atrial hypertension induced a pulmonary vasoconstriction and this increase of pulmonary vascular resistance could be reversed by inhaled nitric oxide (Hermo *et al*., 1994). In man, the effects of inhalation of nitric oxide on pulmonary capillary wedge pressure and left ventricular function remain controversial and could be different in normal control subjects and in patients with heart failure.

The aims of the present experiments were (1) to define a new experimental model of pulmonary hypertension induced by a post-capillary mechanism and (2) to assess the haemodynamic effects of nitric oxide on post-capillary pulmonary hypertension in dogs. We previously found that acute sino-aortic denervation induced an increase of left ventricular after-load due to a severe systemic hypertension (Montastruc & Montastruc, 1981). This model of neurogenic hypertension is associated with an increase of plasma catecholamines. α and β-adrenoceptor antagonists are able to reduce the rise in blood pressure. In the present study, we have investigated the haemodynamic effects on pulmonary circulation of acute sino-aortic denervation. We also investigated the haemodynamic effects of nitric oxide gas and endothelium-derived nitric oxide by use of N^{ω}-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor.

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Methods

Twenty eight male beagle dogs (mean weight: 16±1 kg) were anaesthetized with α-chloralose (80 mg kg^{-1}, i.v.). Because the best mode of ventilation to obtain a post-capillary pulmonary hypertension was not known, we conducted the experiments in two groups of dogs with spontaneous or assisted ventilation. After intubation of the trachea, 16 were left on spontaneous respiration in atmospheric air with additional oxygen and 12 had their lungs ventilated (Pump Ideal Palmer; respiratory rate, 12 breaths per min; tidal volume, 17 ml kg^{-1}). In all cases, the oxygen output was adjusted to maintain mixed venous blood oxygen saturation in the normal range. Temperature was maintained constant at 37–38°C. The experiments were conducted in accordance with the guiding principles in the care and use of animals of the Council of the American Physiological Society.

A thermistor-tipped Swan Ganz catheter (Catheter Oximetric) was inserted via the right external jugular vein and positioned by means of pressure monitoring in a branch of the pulmonary artery for measurements of pulmonary arterial pressure (PAP), pulmonary capillary wedge pressure (PCWP), right atrial pressure (RAP) and mixed venous blood oxygen saturation. A polyethylene catheter was placed in the abdominal aorta via the left femoral artery for systemic arterial pressure (SAP) measurements and arterial blood sampling.

Pulmonary and systemic vascular pressures were measured by transducers (Honeywell) and a computer system (IBM PC). The zero reference was leveled at midchest, and vascular pressures were measured at end expiration. Heart rate (HR) was determined from a continuously monitored electrocardiographic lead. Cardiac output was measured by thermodilution with injections of 10 ml of 0.9% sodium chloride at 0°C and was calculated as the mean of three determinations. Arterial pH, P_{O_2}, P_{CO_2} and concentrations of [HCO^-_3] were measured immediately after drawing the samples with an automated analyser.

The pulmonary pressure gradient (mmHg) was calculated from the formula: mean pulmonary arterial pressure-pulmonary capillary wedge pressure. The pulmonary vascular resistances (PVR) were calculated from the following: PVR = pulmonary pressure gradient divided by cardiac output. The systemic pressure gradient (mmHg) was calculated from the formula: mean arterial pressure-right arterial pressure. The systemic vascular resistances (SVR) were determined from the following: SVR = systemic pressure gradient divided by cardiac output.

After ensuring steady-state conditions and recording haemodynamic determinations at baseline, acute sino-aortic denervation was performed, as previously described (Montastruc & Montastruc, 1981). Briefly, by bilateral neck incisions, the carotid arteries and sinuses were isolated, and the carotid bifurcations with the carotid sinus nerves were sectioned. Then the aortic depressor nerves were sectioned with the vagus in the cervical region.

In 4 dogs left on spontaneous respiration the effects of sino-aortic denervation were studied for 30 min after the end of the intervention. In a group of 6 dogs, 3 left on spontaneous respiration and 3 with assisted ventilation, the haemodynamic effects of inhaled nitric oxide (25 p.p.m., 10 min) were studied before and immediately after sino-aortic denervation (Channick *et al*., 1994; Romand *et al*., 1994b). After denervation, the haemodynamic effects of intravenous N^{ω}-nitro-L-arginine methyl ester (20 mg kg^{-1}), a nitric oxide synthase inhibitor, urapidil (0.5 mg kg^{-1}), an α_1-adrenoceptor antagonist, and propranolol (300 μg kg^{-1}), a non-selective β-adrenoceptor antagonist, were studied in three groups of 6 dogs, 3 left on spontaneous respiration and 3 with assisted ventilation. The effects of each drug were measured 10 min after the onset of their administration.

Drugs

N^{ω}-nitro-L-arginine methyl ester was purchased from Sigma Chemical Company (Germany). Chloralose was obtained from Prolabo (France). Propranolol (Avlocardyl, ICI) and urapidil (Eupressyl, BYK) were used as the clinically available preparations. Nitric oxide was released from a tank containing nitric oxide in nitrogen at a concentration of 300 p.p.m. (AGA SA, France).

Statistical analysis

Results are expressed as mean±s.e.mean. Statistical comparisons were made by Student's paired *t* test or Rank Wilcoxon test. Linear regression was tested between changes in mean pulmonary arterial pressure and capillary wedge pressure. A P value <0.05 was considered as significant.

Results

Effects of acute sino-aortic denervation

In the systemic circulation, acute sino-aortic denervation induced an acute and marked increase of systolic and diastolic arterial pressure, associated with a rise in both heart rate and SVR, without significant change in cardiac output (Table 1).

In the pulmonary circulation, acute sino-aortic denervation induced an increase in pulmonary arterial pressure. However, some differences occurred between spontaneous breathing dogs and dogs with assisted ventilation (Table 1). In spontaneous breathing dogs, sino-aortic denervation induced an acute and marked pulmonary hypertension with a significant increase of systolic and diastolic pulmonary arterial pressure, resulting from an important rise in pulmonary capillary wedge pressure. In contrast, in dogs with assisted ventilation the slight increase of pulmonary arterial pressure and pulmonary capillary wedge pressure induced by sino-aortic denervation did not reach the level of significance.

Table 1 Haemodynamic effects of acute sino-aortic denervation in dogs in spontaneous respiration or assisted ventilation

	Spontaneous ventilation (n = 12)		*Assisted ventilation (n = 12)*	
	Before SAD	*After SAD*	*Before SAD*	*After SAD*
Heart rate (beats min^{-1})	133±16	199±11*	156±10	214±12*
Mean systemic arterial pressure (mmHg)	144±8	234±13*	143±5	213±8*
Systolic pulmonary arterial pressure (mmHg)	27±3	39±4*	32±2	34±3
Diastolic pulmonary arterial pressure (mmHg)	7±1	21±3*	6±1	9±2
Mean pulmonary arterial pressure (mmHg)	14±2	28±4*	16±1	18±2
Pulmonary capillary wedge pressure (mmHg)	5±1	18±3*	5±1	8±2
Right atrial pressure (mmHg)	3.5±1.4	5.0±2.3	2.3±1.0	4.0±1.3
Cardiac output (l min^{-1})	4.3±0.6	4.6±0.6	4.9±0.9	4.5±0.7
Sytemic vascular resistance (Wu)	36±6	54±8*	29±2	52±7*
Pulmonary vascular resistance (Wu)	2.1±0.2	2.2±0.5	2.2±0.3	2.5±0.6
Mixed venous blood oxygen saturation (%)	80±2	79±3	83±3	82±4

SAD, acute sino-aortic denervation. *$P<0.05$ before *vs* after SAD. Mean values±s.e.mean, $n=24$.

In spite of this marked increase of pulmonary arterial pressure after acute sino-aortic denervation, PVR did not significantly increase in spontaneous breathing dogs, as well as in dogs with assisted ventilation. Thus, the mechanism responsible for pulmonary hypertension after sino-aortic denervation involved only the increase of pulmonary capillary wedge pressure. This post-capillary pulmonary hypertension mechanism was confirmed by the strong linear relationship between the increase of mean pulmonary arterial pressure and the rise in pulmonary capillary wedge pressure after acute sino-aortic denervation ($r=0.89$, $P<0.0001$).

This pulmonary arterial hypertension appeared immediately after the bilateral sino-aortic denervation but was transient. In fact, in four dogs left on spontaneous respiration, the increase of systolic, diastolic and mean arterial pulmonary pressures, as well as pulmonary capillary wedge pressure, became non-significant 30 min after sino-aortic denervation (Figure 1). The systemic arterial hypertension appeared immediately after denervation, tended also to decrease 30 min after denervation but systolic, diastolic and mean arterial systemic pressures remained increased. In contrast, the rise in heart rate, appeared immediately after denervation, remained unchanged during the follow-up (Figure 2). For these reasons, the drug study was carried out immediately after sino-aortic denervation.

The study of mixed venous blood oxygen saturation demonstrated the absence of hypoxia before or after acute sino-aortic denervation in spontaneous breathing dogs and in dogs with assisted ventilation (Table 1). The study of arterial blood gases confirmed the absence of any significant hypoxia in spontaneous breathing dogs before (PaO_2: 89 ± 5 mmHg, SaO_2: $96\pm1\%$) or after denervation (PaO_2: 82 ± 7 mmHG,

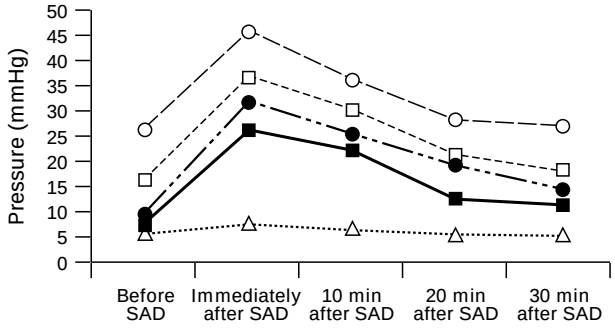

Figure 1 Time course for changes in pulmonary haemodynamic parameters after sino-aortic denervation (SAD) in dogs with spontaneous respiration. (○) systolic pulmonary arterial pressure; (□) mean pulmonary arterial pressure; (●) diastolic pulmonary arterial pressure; (■) pulmonary capillary wedge pressure; (△) right atrial pressure. Each point represents mean of 4 dogs (error bars omitted for clarity).

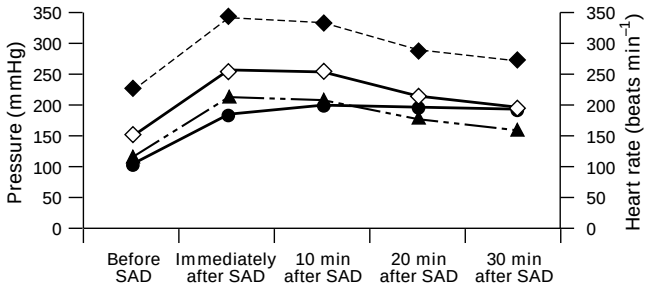

Figure 2 Time course for changes in systemic haemodynamic parameters after sino-aortic denervation (SAD) in dogs with spontaneous respiration. (●) Heart rate; (◆- - - -◆) systolic systemic arterial pressure; (▲) diastolic systemic arterial pressure; (◇——————◇) mean systemic arterial pressure. Each point represents mean of 4 dogs (error bars omitted for clarity).

Table 2 Haemodynamic effects of inhaled nitric oxide (25 p.p.m 10 min) before and after acute sino-aortic denervation

	Before SAD		*After SAD*	
	Without NO	*With NO*	*Without NO*	*With NO*
Heart rate (beats min^{-1})	160±11	171±11	217±10*	208±8*
Mean systemic arterial pressure (mmHg)	147±6	149±2	208±17*	194±10*
Mean pulmonary arterial pressure (mmHg)	13±1	13±1	23±5*	22±5*
Pulmonary capillary wedge pressure (mmHg)	2±1	2±1	10±4*	8±3*
Right atrial pressure (mmHg)	0.5±1.8	0.1±0.6	2.5±1.6	2.0±1.1
Cardiac output (l min^{-1})	5.4±0.6	5.2±0.6	5.4±1.3	5.3±1.2
Systemic vascular resistance (Wu)	28±3	30±4	45±10*	43±10*
Pulmonary vascular resistance (Wu)	2.0±0.3	2.1±0.3	3.2±0.9	3.4±0.8
Mixed venous blood oxygen saturation (%)	81±2	79±4	79±5	73±5

SAD, acute sino-aortic denervation; NO, nitric oxide; *$P<0.05$ before *vs* after SAD. Mean values±s.e.mean, $n=6$ (3 dogs with spontaneous respiration and 3 dogs with assisted ventilation).

Table 3 Haemodynamic effects of N$^{\omega}$-nitro-L-arginine methyl ester (20 mg kg^{-1}, i.v.) after acute sino-aortic denervation

		After SAD	
	Before SAD	*Before L-NAME*	*After L-NAME*
Heart rate (beats min^{-1})	155±12	194±19*	182±18
Mean systemic arterial pressure (mmHg)	140±10	220±11*	250±10
Mean pulmonary arterial pressure (mmHg)	14±1	20±2*	19±4
Pulmonary capillary wedge pressure (mmHg)	6±1	11±2*	13±2
Right atrial pressure (mmHg)	4.4±1.2	6.2±0.6	4.5±0.3
Cardiac output (l min^{-1})	4.5±0.6	3.9±0.4	2.4±0.2
Systemic vascular resistance (Wu)	30±4	57±7*	104±4^{+}
Pulmonary vascular resistance (Wu)	1.9±0.3	2.0±0.4	2.3±0.8
Mixed venous blood oxygen saturation (%)	82±3	79±5	76±4

L-NAME, N$^{\omega}$-nitro-L-arginine methyl ester, *$P<0.05$ before *vs* after SAD; $^{+}P<0.05$ before *vs* after L-NAME. Mean values±s.e.mean, $n=6$ (3 dogs with spontaneous respiration and 3 dogs with assisted ventilation).

Table 4 Haemodynamic effects of urapidil (0.5 mg kg^{-1},i.v.) after acute sino-aortic denervation

		After SAD	
	Before SAD	*Before urapidil*	*After urapidil*
Heart rate (beats min^{-1})	138±15	210±13*	202±14
Mean systemic arterial pressure (mmHg)	140±11	236±12*	161±15$^{\diamond}$
Mean pulmonary arterial pressure (mmHg)	15±3	24±4*	16±2$^{\diamond}$
Pulmonary capillary wedge pressure (mmHg)	5±2	16±4*	7±2$^{\diamond}$
Right atrial pressure (mmHg)	3.4±1.3	5.2±1.1	2.0±0.6$^{\diamond}$
Cardiac output (l min^{-1})	3.9±0.6	4.1±0.4	4.6±0.3
Systemic vascular resistance (Wu)	33±6	60±8*	35±4$^{\diamond}$
Pulmonary vascular resistance (Wu)	2.2±0.3	1.9±0.2	2.1±0.4
Mixed venous blood oxygen saturation (%)	82±4	83±3	82±5

SAD, acute sino-aortic denervation; *$P<0.05$ before *vs* after SAD, $^{\diamond}P<0.05$ before *vs* after urapidil. Mean values±s.e.mean, $n=6$ (3 dogs with spontaneous respiration and 3 dogs with assisted ventilation).

Table 5 Haemodynamic effects of propranolol (300 μg kg^{-1}, i.v.) after acute sino-aortic denervation

		After SAD	
	Before SAD	*Before propranolol*	*After propranolol*
Heart rate (beats min^{-1})	150±14	227±11*	152±5$^{\diamond}$
Mean systemic arterial pressure (mmHg)	144±9	220±14*	147±18$^{\diamond}$
Mean pulmonary arterial pressure (mmHg)	15±3	25±4*	17±2$^{\diamond}$
Pulmonary capillary wedge pressure (mmHg)	3±2	10±3*	6±2
Right atrial pressure (mmHg)	2.5±1.8	2.7±1.5	3.2±1.5
Cardiac output (l min^{-1})	5.4±0.9	5.9±1.0	4.0±0.9
Systemic vascular resistance (Wu)	29±4	39±5*	43±14
Pulmonary vascular resistance (Wu)	2.1±0.4	2.9±0.8	3.5±1.3
Mixed venous blood oxygen saturation (%)	81±4	79±5	78±5

SAD, acute sino-aortic denervation; *$P<0.05$ before *vs* after SAD, $^{\diamond}P<0.05$ before *vs* after propranolol. Mean values±s.e.mean, $n=6$ (3 dogs with spontaneous respiration and 3 dogs with assisted ventilation).

Sa_{O_2}:93±3%), as well as in dogs with assisted ventilation before (Pa_{O_2}: 194±10 mmHg, Sa_{O_2}: 100±2%) or after denervation (Pa_{O_2}: 185±9 mmHg, Sa_{O_2}: 99±9%). Nevertheless, dogs left on spontaneous respiration showed a mild acidosis after acute sino-aortic denervation (pH: 7.33±0.02 and 7.23±0.02, before and after denervation, respectively), resulting from a rise of Pa_{CO_2} (48±2 and 57±2 mmHg, before and after denervation, respectively) without significant change of HCO^-_3 (25±2 and 24±1 mmol, before and after denervation, respectively). In contrast, in dogs with assisted ventilation pH (7.34±0.03 and 7.32±0.02, before and after denervation, respectively) and Pa_{CO_2} (37±3 and 42±3 mmHg, before and after denervation, respectively) remained in the normal range.

Effects of nitric oxide gas and nitric oxide synthase inhibitor

In a group of six dogs, three with spontaneous respiration and three with assisted ventilation, inhaled nitric oxide (25 p.p.m.) administered for 10 min had no significant effect before and immediately after acute sino-aortic denervation on systemic or pulmonary pressures and did not significantly change pulmonary capillary wedge pressure and cardiac output (Table 2). In particular, nitric oxide gas did not reverse pulmonary hypertension induced by sino-aortic denervation.

In a second group of six dogs, three with spontaneous respiration and three with assisted ventilation, i.v. N^{ω}-nitro-L-arginine methyl ester (20 mg kg^{-1}), administered immediately after acute sino-aortic denervation, induced a slight increase of systemic arterial pressure and a non-significant decrease of cardiac output resulting in an increase of systemic vascular resistance. N^{ω}-nitro-L-arginine methyl ester had no significant haemodynamic effects on post-capillary pulmonary hypertension induced by sino-aortic denervation (Table 3).

Effects of urapidil and propranolol

In a third group of six dogs, three with spontaneous respiration and three with assisted ventilation, i.v. urapidil (0.5 mg kg^{-1}) significantly decreased systemic and pulmonary hypertension induced by acute sino-aortic denervation, without significant variation of heart rate and cardiac output (Table 4). This decrease of pulmonary arterial pressure was induced by a significant drop of pulmonary capillary wedge pressure whereas pulmonary vascular resistance did not change.

In a fourth group of six dogs, three with spontaneous respiration and three with assisted ventilation, intravenous propranolol (300 μg kg^{-1}) significantly decreased systemic and pulmonary hypertension induced by acute sino-aortic denervation. Heart rate decreased simultaneously (Table 5). Propranolol tended to decrease pulmonary capillary wedge pressure in spite of non-significant diminution of cardiac output.

The variations in mean pulmonary arterial pressure after urapidil or propranolol were strongly related to the decrease of pulmonary capillary wedge pressure induced by these drugs ($r=0.82$, $P=0.0069$).

Discussion

Sino-aortic denervation induces an acute and transient pulmonary hypertension, more important in spontaneous breathing dogs than in dogs with assisted ventilation. This pulmonary hypertension involves a post-capillary mechanism. In fact, the increase of mean pulmonary arterial pressure after sino-aortic denervation and the decrease of this parameter after urapidil or propranolol are strongly correlated with the variations of pulmonary capillary wedge pressure. Furthermore, no significant PVR change was found after acute sino-

aortic denervation or administration of α- or β-adrenoceptor antagonists. Thus, this post-capillary pulmonary hypertension does not modify pulmonary vascular reactivity in dogs, whereas a passive left atrial hypertension can induce pulmonary arterial vasoconstriction (Hermo *et al*., 1994).

The marked increase of pulmonary capillary wedge pressure after acute sino-aortic denervation is secondary to the left ventricular haemodynamic effects of the acute increase of left ventricular after-load induced by systemic hypertension.

In spontaneous breathing dogs, the respiratory acidosis participates, probably via these heart effects, to the increase of pulmonary capillary wedge pressure leading to pulmonary hypertension. The effects of this slight acidosis on pulmonary vascular reactivity remain uncertain. In fact, in dogs there are controversial data regarding the effects of metabolic acidosis on pulmonary vascular reactivity. In intact dogs exposed to hypoxia, lactic acid infusion at controlled $PaCO_2$ resulted in reduction of the hypoxia-induced increase of pulmonary arterial pressure and PVR (Malik & Kidd, 1973). In awake dogs, mild acidosis produced by an infusion of HCl did not affect hypoxic pulmonary vasoconstriction (Thilenius & Derenzo, 1972). In anaesthetized dogs, the increment in PVR per unit decrement in arterial pH, induced by short-term acid infusions, was similar during ambient air breathing and acute hypoxia (Bergofsky *et al*., 1962). In excised dog lobe preparations, it appeared that hypoxic pulmonary vasoconstriction was invariant over the pH range of 7–7.3 (Lloyd, 1966). More recently, in intact dogs, it was demonstrated that increasing the blood concentration of H^+ by HCl infusion enhanced hypoxic pulmonary vasoconstriction (Lejeune *et al*., 1990). In cats, Viles & Shepherd (1968) demonstrated a pH-independent vasodilating effect of CO_2 on the pulmonary vasculature. In conclusion, metabolic acidosis probably potentiates hypoxia-induced increase of pulmonary arterial pressure or PVR, but raising the $PaCO_2$ has no pulmonary vasoconstrictor effect. Thus, in the absence of hypoxia in dogs after acute sino-aortic denervation, a hypoxia-induced increase of pulmonary arterial pressure can be eliminated and the slight respiratory acidosis does not increase PVR.

In spite of the existence of α- and β-adrenoceptors in pulmonary arteries, acute sino-aortic denervation, which induces an acute rise of catecholamine plasma levels (Montastruc & Montastruc, 1981), does not increase pulmonary vascular resistance. Thus, whereas mechanisms of acute hypoxic change in pulmonary vascular reactivity can involve the adrenergic system (Fishman, 1976; Archer *et al*., 1989), acute sino-aortic denervation fails to modify pulmonary vascular resistance.

A putative limitation of the model of acute sino-aortic denervation is the consequence of the section of the vagus with the aortic-depressor nerves. In fact, parasympathetic nerves have been found in pulmonary arteries of some animal species. Furthermore, the administration of acetylcholine can induce a pulmonary arterial vasodilatation (Hyman *et al*., 1989) and nitric oxide is involved in vagally mediated pulmonary vasodilatation (McMahon & Kadowitz, 1992). Thus it is possible that sectioning of the vagus can explain the lack of change in pulmonary vascular reactivity after sino-aortic denervation in spite of the marked increase of plasma catecholamines. In the systemic circulation, sectioning the vagus during acute sino-aortic denervation had no significant pressure effects but could slightly increase the tachycardia induced by sectioning the depressor nerves.

α- and β-adrenoceptor antagonists reduce pulmonary arterial hypertension induced by acute sino-aortic denervation in spite of their opposite effects on pulmonary vascular reactivity. In fact, pulmonary vascular resistances do not decrease after their administration. The decrease of pulmonary hypertension observed after the administration of α- or β-adrenoceptor antagonists is not due to a change in pulmonary arterial reactivity. This change of pulmonary pressure is only related to the decrease of pulmonary capillary wedge pressure induced by the correction of systemic hypertension by these drugs. Urapidil decreases peripheral vascular resistances by blocking postsynaptic α_1-adrenoceptors and reduces the central sympathetic tone. Propranolol decreases arterial hypertension by blocking pre-synaptic β-adrenoceptors and reduces the central sympathetic tone.

The present results show that inhaled nitric oxide does not reverse systemic and pulmonary hypertension induced by sino-aortic denervation. The absence of haemodynamic effects on systemic circulation of inhaled nitric oxide in our model is expected. In fact, when nitric oxide is inhaled, it reaches the pulmonary vascular smooth muscle through diffusion from alveolar spaces, causing vascular relaxation. As nitric oxide is rapidly inactivated in blood by oxy-haemoglobin, its vasodilator effect is restricted to the pulmonary vasculature without any systemic vasodilator effects (Frostell *et al*., 1991; Pinson *et al*., 1993). However, the absence of a haemodynamic effect on the pulmonary circulation of inhaled nitric oxide is more surprising. In fact, experiments in various animal models of pulmonary hypertension have shown that inhaled nitric oxide improved pulmonary haemodynamics and gas exchange. Thus inhaled nitric oxide was effective in causing pulmonary vasodilatation during hypoxic pulmonary vasoconstriction (Frostell *et al*., 1991; Pinson *et al*., 1993; Channick *et al*., 1994; Romand *et al*., 1994a), heparin-protamine vasoconstriction (Fratacci *et al*., 1991), group B streptococcal sepsis (Berger *et al*., 1993), persistent pulmonary hypertension in newborn lambs (Zayek *et al*., 1993) and acute lung injury (Kavanagh *et al*., 1994; Romand *et al*., 1994b). In clinical studies, inhaled nitric oxide has been shown to decrease effectively pulmonary arterial pressure in severe pulmonary hypertension following surgical corrections of congenital heart diseases (Sellden *et al*., 1993) and in persistent pulmonary hypertension of the newborn (Kinsella *et al*., 1992; Roberts *et al*., 1992). In end stage heart failure, in patients referred for cardiac transplantation with pre-capillary pulmonary hypertension and high pulmonary arterial resistances, inhalation of nitric oxide decreased mean pulmonary arterial pressure and pulmonary arterial resistance and did not change systemic arterial pressure, systemic arterial resistance or cardiac output (Pornin *et al*., 1994; Semigran *et al*., 1994). Thus, inhaled nitric oxide is an effective treatment in some cases of pre-capillary pulmonary hypertension.

In our model, the acute pulmonary hypertension involves only a post-capillary mechanism; thus, in the absence of any effect on left ventricular loading conditions, inhaled nitric oxide cannot reverse pulmonary hypertension. In isolated lungs from lambs, it was recently demonstrated that inhaled nitric oxide relaxed both large and small arteries but had no effect on small and large veins during hypoxia (Tod *et al*., 1995). Furthermore, inhaled nitric oxide relaxed endothelin-1-constricted veins in rat isolated lungs only when perfused by a blood-free solution (Roos *et al*., 1994). Thus, inhaled nitric oxide was inactivated by haemoglobin in blood-perfused lungs before reaching the large pulmonary veins. The lack of effect of a nitric oxide synthase inhibitor on pulmonary circulation parameters after acute sino-aortic denervation suggests that endothelium-derived nitric oxide does not interfere with post-capillary pulmonary hypertension. In contrast, the rise of systemic vascular resistance observed after intravenous N^{ω}-nitro-L-arginine methyl ester demonstrates that endothelium-derived nitric oxide acts as a counter-regulatory mechanism to attenuate systemic vasoconstriction induced by sino-aortic denervation. In man, the effects of nitric oxide on left ventricular functions and pulmonary capillary wedge pressure remain controversial. In patients with congestive heart failure, in spite of a decrease of pulmonary vascular resistances (Adatia *et al*., 1995), inhaled nitric oxide increased left ventricular end diastolic pressure (Moreno *et al*., 1994) and pulmonary capillary wedge pressure (Semigran *et al*., 1994), without altering left ventricular contractility or relaxation (Moreno *et al*., 1994). However, inhibition of nitric oxide synthase potentiated the inotropic response to stimulation of β-adrenoceptors, suggesting that nitric oxide could contribute to left ventricular dysfunction (Hare *et al*., 1994).

In summary, sino-aortic denervation in dogs is a new model of acute post-capillary pulmonary hypertension. The absence of effect of inhaled nitric oxide and a nitric oxide synthase inhibitor on pulmonary circulation parameters after sino-aortic denervation demonstrates that endothelium-derived nitric oxide is not involved in the mechanisms leading to post-capillary pulmonary hypertension.

References

ADATIA, I., PERRY, S., LANDZBERG, M., MOORE, P., THOMSON, J.E. & WESSEL, D.L. (1995). Inhaled nitric oxide and haemodynamic evaluation of patients with pulmonary hypertension before transplantation. *J. Am. Coll. Cardiol.*, **25,** 1656–1664.

ARCHER, S.L., MCMURTRY, I.F. & WEIR, E.K. (1989). Mechanisms of acute hypoxic and hyperoxic changes in pulmonary vascular reactivity. In *Pulmonary Vascular Physiology and Pathophysiology*. ed. Weirt, E.K. & Reeves, J.T. pp. 241–290. New York: Marcel Dekker Inc.

BERGER, J.I., GIBSON, R.L., REDDING, G.J., STANDAERT, T.A., CLARKE, W.R. & TRUOG, W.E. (1993). Effect of inhaled nitric oxide during group B streptococcal sepsis in piglets. *Am. Rev. Respir. Dis.*, **147,** 1080–1086

BERGOFSKY, E.H., LEHR, D.E. & FISHMAN, A.P. (1962). The effect of changes in hydrogen ion concentration on the pulmonary circulation. *J. Clin. Invest.*, **41,** 1492–1502.

CHANNICK, R.N., NEWHART, J.W., JOHNSON, F.W. & MOSER, K.M. (1994). Inhaled nitric oxide reverses hypoxic pulmonary vasoconstriction in dogs. A practical nitric oxide delivery and monitoring system. *Chest*, **105,** 1842–1847.

FISHMAN, A.P. (1976). Hypoxia on the pulmonary circulation. How and where it acts. *Circ. Res.*, **38,** 221–231.

FRATACCI, M.D., FROSTELL, C.G., CHEN, T.Y., WAIN, J.C., ROBINSON, D.R. & ZAPOL, W.M. (1991). Inhaled nitric oxide: a selective pulmonary vasodilator of heparin-protamine vasoconstriction in sheep. *Anesthesiology*, **75,** 990–999.

FROSTELL, C.G., FRATACCI, M.D., WAIN, J.C., JONES, R. & ZAPOL, W.M. (1991). Inhaled nitric oxide: a selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation*, **83,** 2038–2047.

HARE, J.M., LOH, E., CREAGER, M.A. & COLUCCI, W.S. (1994). Nitric oxide inhibits the contractile response to beta-adrenergic stimulation in humans with left ventricular dysfunction. *Circulation*, **90,** I-193 (Abst. 1035).

HERMO, C.J., KOIZUMI, T. & NEWMAN, J.H. (1994). Effect of inhaled nitric oxide on pulmonary vascular resistance during left atrial hypertension in sheep. *Circulation*, **90,** I-308 (Abstr. 1655).

HYMAN, A.L., HOWARD, L. & DEMPSEZY, C.W. (1989). Autonomic control of the pulmonary circulation. In *Pulmonary Vascular Physiology and Pathophysiology*. ed. Weirt, E.K. & Reeves, J.T. pp. 291–324. New York: Marcel Dekker Inc.

IGNARRO, L.J., BUGA, G.M., WOOD, K.S., BYRNS, R.E. & CHAUDHURI, G. (1987). Endothelin-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. U.S.A.*, **84,** 9265–9269.

KAVANAGH, B.P., MOUCHAWAR, A., GOLDSMITH, J. & PEARL, R.G. (1994). Effects of inhaled NO and inhibition of endogenous NO synthesis in oxidant-induced acute lung injury. *J. Appl. Physiol.*, **76,** 1324–1329.

KINSELLA, J.P., NEISH, S.R., SHAFFER, E. & ABMAN, S.H. (1992). Low-dose inhalational nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet*, **340,** 819–820.

LEJEUNE, P., BRIMIOULLE, S., LEEMAN, M., HALLEMANS, R., MELOT, C. & NAEJE, R. (1990). Enhancement of hypoxic pulmonary vasconstriction by metabolic acidosis in dogs. *Anesthesiology*, **73,** 256–264.

LLOYD, T.C. (1966). Influence of blood pH on hypoxic pulmonary vasoconstriction. *J. Appl. Physiol.*, **21,** 358–364.

MALIK, A.B. & KIDD, B.S.L. (1973). Independent effects of changes in H^+ and CO_2 concentrations on hypoxic pulmonary vasoconstriction. *J. Appl. Physiol.*, **34,** 318–323.

MCMAHON, T.J., & KADOWITZ, P.J. (1992). Methylene blue inhibits neurogenic cholinergic vasodilator responses in the pulmonary vascular bed of the cat. *Am. J. Physiol.*, **263,** L573–L584.

MONTASTRUC, J.L. & MONTASTRUC, P. (1981). Antihypertensive action of bromocriptine in neurogenic hypertensive dogs. *Arch. Int. Pharmacodyn.*, **252,** 210–218.

MORENO, P.R., KUSHWAHA, S.S., FUSTER, V., COCKRILL, B.A., KACMARECH, R., ZAPOL, W.M., FIFER, M.A., DEC, G.W. & SEMIGRAN, M.J. (1994). Direct myocardial effects of inhaled nitric oxide in heart failure. *Circulation*, **90,** I-609 (Abst. 3284).

PALMER, R.M., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327,** 524–526.

PEPKE-ZABA, J., HIGENBOTTAM, T.W., DINH-XUAN, A.T., STONE, D. & WALLWORK, J. (1991). Inhaled nitric oxide as a cause of selective pulmonary vasodilation in pulmonary hypertension. *Lancet*, **338,** 1173–1174.

PINSON, U., LOPEZ, F.A., HEIDELMEYER, C.F., ROSSAINT, R. & FALKE, K.J. (1993). Inhaled nitric oxide reverses hypoxic pulmonary vasoconstriction without impairing pulmonary gas exchange. *J. Appl. Physiol.*, **74,** 1287–1292.

PORNIN, M., GUINVARCH, A., GUILLEMAIN, R., CHEVALIER, P., RAHMAN, W., MEROUE, S., COISNE, D., ALLAL, J. & GUIZE, L. (1994). Haemodynamic effects of inhaled nitric oxide in pulmonary hypertension in congestive heart failure: a preliminary study. *Eur. Heart J.*, **15,** 524S (Abstr. 2874).

ROBERTS, J.D., POLANER, D.M., LANG, P. & ZAPOL, W.M. (1992). Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet*, **340,** 818–819.

ROMAND, J.A., PINSKY, M.R., FIRESTONE, L., ZAR, H.A. & LANCASTER, J.R. (1994a). Inhaled nitric oxide partially reverses hypoxic pulmonary vasoconstriction in dog. *J. Appl. Physiol.*, **76,** 1350–1355.

ROMAND, J.A., PINSKY, M.R., FIRESTONE, L., ZAR, H.A. & LANCASTER, J.R. (1994b). Effect of inhaled nitric oxide on pulmonary hemodynamics after acute lung injury in dogs. *J. Appl. Physiol.*, **76,** 1356–1362.

ROOS, C.M., RICH, G.F., UNCLES, D.R., DAUGHERTY, M.O. & FRANK, D.U. (1994). Sites of vasodilation by inhaled nitric oxide vs. sodium nitroprusside in endothelium-constricted isolated rats lungs. *J. Appl. Physiol.*, **77,** 51–57.

ROSSAINT, R., FALKE, K.J., LOPEZ, F., SLAMA, K., PISON, U. & ZAPOL, W.M. (1993). Inhaled nitric oxide for the adult respiratory distress syndrome. *N. Engl. J. Med.*, **328,** 399–405.

SELLDEN, H., WINBERG, P., GUSTAFSSON, L.E., LUNDELL, B., BÖÖK, K. & FROSTELL, C.G. (1993). Inhalation of nitric oxide-reduced pulmonary hypertension after cardiac surgery in a 3.2-Kg infant. *Anesthesiology*, **78,** 577–580.

SEMIGRAN, M.J., COCKRILL, B.A., KACMAREK, R., THOMPSON, B.T., ZAPOL, W.M., DEC, G.W. & FIFER, M.A. (1994). Haemodynamic effects of inhaled nitric oxide in heart failure. *J. Am. Coll. Cardiol.*, **24,** 982–988.

THILENIUS, O.G. & DERENZO, C. (1972). Effects of acutely induced changes in arterial pH on pulmonary vascular resistance during normoxia and hypoxia in awake dogs. *Clin. Sci.*, **42,** 277–287.

TOD, M.L., O'DONNELL, D.C. & GORDON, J.B. (1995). Sites of inhaled NO-induced vasodilation during hypoxia and U-46619 infusion in isolated lamb lungs. *Am. J. Physiol.*, **268,** H1422–H1427.

VILES, P.H. & SHEPHERD, J.T. (1968). Relationship between pH, PO_2, and PCO_2 on the pulmonary vascular bed of the cat. *Am. J. Physiol.*, **215,** 1170–1176.

ZAYEK, M., CLEVELAND, D. & MORIN, F.C. (1993). Treatment of persistent pulmonary hypertension in the newborn lamb by inhaled nitric oxide. *J. Pediatr.*, **122,** 743–750.

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