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Training does not affect the alteration in pulmonary artery vasoreactivity in pulmonary hypertensive rats

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Abstract

This study examined the effects of training on intrinsic vasorelaxation and vasoconstriction properties of pulmonary hypertensive rat arteries. Fifty seven male Wistar rats were randomly assigned to 4 groups: normotensive sedentary ($n=14$), normotensive trained ($n=15$), pulmonary hypertensive sedentary ($n=15$) and pulmonary hypertensive trained ($n=13$). Pulmonary hypertension was obtained using a chronic hypoxia exposure model. Endothelium-dependent vasorelaxation to acetylcholine (10^{-8}–10^{-4} M), endothelium-independent vasorelaxation to sodium nitro-prusside (10^{-8}–10^{-4} M), and vasoconstriction to epinephrine (10^{-9}–10^{-4} M) and endothelin-1 (10^{-12}–10^{-7} M) were assessed on isolated rings of large pulmonary arteries. Alterations in endothelium-dependent and -independent vasorelaxation properties as well as enhanced vasoconstrictor responses were obtained in pulmonary hypertensive rats. Chronic exercise did not affect those pulmonary vasoreactivity alterations. A predominant effect of chronic hypoxia over training seems to be partially responsible for this phenomenon, probably through impairment in nitric oxide bioavailability and vascular smooth muscle sensitivity.

Keywords: Pulmonary hypertension; Chronic exercise; Vasorelaxation; Vasoconstriction

1. Introduction

Chronic hypoxia exposure has been widely used in the comprehensive approach of the pathophysiology of pulmonary hypertension. There are two important pathological features that contribute to the increase in pulmonary artery pressure, which are alterations in pulmonary vascular structure and changes in pulmonary vascular function (Jeffery and Wanstall, 2001). In both human and animal, one major mechanism related to the pathophysiology of pulmonary hypertension is endothelial dysfunction within the pulmonary vasculature (Dinh-Xuan et al., 1991; Loscalzo, 1992). Diminished nitric oxide bioavailability appears to play a critical role in this dysfunction (Eddahibi et al., 1992; Madden et al., 1995; Sasaki et al., 2004). Impairment in endothelium-independent vasorelaxation in pulmonary hypertension is controversial but could also play a significant role. When compared to normotensive controls, pulmonary hypertensive rats exhibit a selective impairment of soluble guanylate cyclase leading in turn to an attenuation of sodium nitro-prusside-induced relaxation (Crawley et al., 1992). The endothelins are a family of highly potent vasoconstrictor peptides (Inoue et al., 1989). Endothelin-1 represents one of the most important vasoconstrictor and is also implicated in the pathophysiology of pulmonary hypertension (Galié et al., 2004). Pulmonary hypertension obtained by chronic hypoxia exposure is associated with increased circulating levels of endothelin-1 (Elton et al., 1992; Bialecki et al., 1998) and endothelin receptor antagonists have been shown to prevent the development of pulmonary hypertension in such experimental conditions (Eddahibi et al., 1995). However, controversial results have been reported on effect of endothelin-1 on vasoreactivity of isolated pulmonary arteries from pulmonary hypertensive and normotensive animals (MacLean et al., 1995; Bialecki et al., 1998; MacLean and McCulloch, 1998; Lal et al., 1999; Deuchar et al., 2002).

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Considerable evidence documents vascular endothelium as an important site of functional adaptations to exercise training in both coronary (Laughlin and McAllister, 1992; Oltman et al., 1995) and peripheral (Chen and Li, 1993; Delp and Laughlin, 1997) circulations. Exercise training has been shown to improve endothelium-mediated vasorelaxation through enhanced nitric oxide pathway. Increased endothelial nitric oxide synthase gene expression in aortic endothelial cells (Delp and Laughlin, 1997; Deuchar et al., 2002) and nitric oxide synthase protein levels in whole peripheral arteries (Deuchar et al., 2002; Hassoun et al., 2004) of various animal models suggest that improved nitric oxide production contributes to improve endothelial vasomotor function consecutive to training. Effect of training on vasodilator function of vascular endothelial cells from the pulmonary circulation is much more controversial. Indeed, improved pulmonary endothelium-dependent vasorelaxation has been demonstrated following exercise training in various animal models (Chen and Li, 1993; Johnson et al., 2000, 2001) while others did not report any specific adaptations (Mitani et al., 1999; Johnson and Laughlin, 2000). Training-induced adaptations of endothelial cell function concern also intrinsic vasoconstrictor property changes since reduced endothelin-1 sensitivity of porcine coronary arteries (Jones et al., 1999) has been reported after training.

Exercise training is today largely used in the therapeutic approach of numerous cardio-respiratory diseases including pulmonary hypertension (Rogers and Howard, 1992; Podolsky and Haber, 1993; Mador et al., 2004). Recent studies have established that the training state limits the development of pulmonary hypertension in acute or chronic hypoxia exposure models in animals (Kashimura and Sakai, 1991; Véras-Silva et al., 1997; Henderson et al., 2001). However, whether training can modulate alteration in endothelium function classically reported in several experimental models of pulmonary hypertension is surprisingly unknown.

The present study was therefore specifically designed to assess the effect training on endothelial and smooth muscle function of rat isolated pulmonary arteries in a chronic hypoxic model of pulmonary hypertension. We hypothesised that, in pulmonary hypertensive rats, training would limit alteration in vasorelaxation properties and reduced vasoconstrictor potency to endothelin-1.

2. Materials and methods

2.1. Chronic hypoxic rat model of pulmonary hypertension

Four-month aged male Wistar rats (Harlan laboratories, Gannat, Puy de Dôme, France) weighing 300–350 g at the start of the experiment were randomly assigned to one of the following groups: pulmonary hypertensive trained ($n=14$), pulmonary hypertensive sedentary ($n=15$), normotensive trained ($n=14$) and normotensive sedentary ($n=15$). Pulmonary hypertension was obtained using a chronic hypoxic exposure model, as previously described and validated in our laboratory (Melin et al., 2002). Briefly, hypobaric environments were obtained by using steel chambers fitted with a clear plastic glass door to illuminate and observe the animals. Hypobaric hypoxia was obtained by using a specific vacuum pump (Becker Mot63, Rambouillet, France). In each chamber, barometric pressure, humidity and temperature conditions were continuously estimated by using electronic sensors. All rats were maintained for 5 weeks in their own environment, at a barometric pressure of 760 mm Hg ($PIO_2 \approx 159$ mm Hg, altitude ≈ 80 m) for normotensive sedentary and trained rats or of 475 mm Hg ($PIO_2 \approx 90$ mm Hg, altitude ≈ 4000 m) for pulmonary hypertensive sedentary and trained rats. All pulmonary hypertensive and normotensive rats were kept in the same room with the same 12 : 12 h light–dark cycle. Rats chow and tap water were provided ad libitum. Room temperatures were maintained at ≈21 °C using air conditioning. Pulmonary hypertension was assessed by measuring the ratio of right ventricular / total ventricular weight. This constitutes a reliable index of pulmonary hypertension in rats (Hunter et al., 1974). Rats were anesthetized with intraperitoneal ketamine HC1 (50 to 75 mg/kg) and xylazine (10 to 15 mg/kg) and hearts were quickly removed, trimmed of pericardium, visible fat and blood vessels. The right ventricular free wall was carefully dissected. Left ventricles were opened and rinsed. Right ventricular free wall and left ventricular plus septum were blotted lightly and weighed on a precise analytical scale (Sartorius BP 160 P, Göttingen, Germany). All procedures were performed in agreement with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publications No. 85-23, revised 1996) and with the approval of the French Ministry of Agriculture.

2.2. Training

Training sessions were conducted in normotensive and pulmonary hypertensive trained rats during the 5-week environmental exposure. In order to supervise training intensities with precision, maximal aerobic velocity was evaluated for each rat before the study period in normoxia ($PIO_2 \approx 159$ mm Hg) and hypoxia ($PIO_2 \approx 90$ mm Hg). Maximal aerobic velocities were also evaluated during the 3rd week of exposure in living environments only in order to adapt training intensities. Both maximal aerobic velocities obtained in normoxia and hypoxia were estimated using a driven wheel during a continuous and progressive maximal exercise test. Under normoxia, the driven wheel was set at a speed of 10 m/min for 2–3 min, after which the speed was increased by 4 m/min every 90 s until 85–90% of the expected maximal aerobic velocity was reached. Then the speed was increased by 0.5–1 m/min every 60 s until maximal effort. Maximal aerobic velocity in hypoxia was evaluated using the same protocol, but with a starting speed of 7 m/min. Training lasted 5 weeks and was conducted at the same relative intensity for both groups (i.e. 80% of maximal aerobic velocity in normoxia for normotensive trained and 80% of maximal aerobic velocity in hypoxia for pulmonary hypertensive trained). Training sessions lasted about 20 min the first week and reached 60 min in the last week. Rats trained

at the same PIO_2 they were subjected to during normal living conditions. Maximal aerobic velocities were evaluated on the 5th week in normoxia only. In order to get an insight into the effect of the training program, muscle samples were taken from the soleus, frozen in liquid nitrogen and stored at −80 °C until processed for citrate synthase activity as described by Srere (1969).

2.3. Isolated pulmonary arteries

Rats were anesthetized with intraperitoneal ketamine HC1 and xylazine as previously described and heart and lungs were removed and placed in cold Krebs–Henseleit bicarbonate buffer. Extralobar right and left branches of pulmonary artery were cleared of all visible connective tissues and cut into rings of 2 mm in length (internal diameter: 0.9–1.2 mm). All rings were mounted onto stainless steel supports and submerged in a 5 ml organ chamber containing Krebs–Henseleit bicarbonate buffer at 37 °C, continuously bubbled with 95% O_2 to maintain a partial oxygen pressure of 670–680 mm Hg and 5% CO_2 to maintain a pH of 7.4 in the incubation bath. The rings were connected to an isometric force transducer (EMKA technologies, EMKA Paris, France), linked to an amplifier (EMKA technologies, EMKA Paris, France) and a computerized acquisition system, to record changes in isometric force. Each ring was equilibrated for 60 min at a resting tension of 1.5 *g*. After the equilibration period, test concentrations of norepinephrine (10^{-6} M) and acetylcholine (10^{-6} M) were added to the bath to ensure viability and endothelial integrity. All rings were then washed until tension returned to baseline levels. To assess global contractile response (nonreceptor-dependant), contraction induced by KCl (80 mM) was measured. In some rings, contractile responses to activation of mediated-receptor contractions were assessed by cumulative addition of epinephrine (10^{-9} to 10^{-4} M) and endothelin-1 (10^{-12} to 10^{-7} M) to the vessel bath. In other rings, endothelium-dependent as well as -independent relaxation was examined. Each vessel ring was pre-contracted with epinephrine (10^{-6} M). This concentration of epinephrine was chosen as it induced a contraction similar to the maximal contraction induced by KCl (80 mM). After the pre-constriction reached a plateau, the relaxant response to cumulative concentrations of acetylcholine (10^{-8} to 10^{-4} M) and then sodium nitro-prusside (10^{-8} to 10^{-4} M) was assessed. Vessel rings were rinsed to stable resting tension levels between each drug intervention.

2.4. Drugs and solutions

The composition of the Krebs-bicarbonate buffer (pH 7.4) was as follows (in mM): NaCl 118, $NaHCO_3$ 25, KCl 4.8, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, and glucose 11. All drugs were dissolved in distilled water and concentrations were expressed as final molar concentration in Krebs–Henseleit solution of the bath. All biochemicals were obtained in the highest purity available from Sigma (St. Quentin-Fallavier, France).

2.5. Statistical analyses

Responses to activation of mediated-receptor contractions were expressed as the developed change in tension (in *g*) from baseline resting tension and as percent in maximal response induced by KCl (80 mM). Relaxation was expressed as percent relaxation from pre-contracted tension. pEC_{50} values were calculated by computer interpolation from individual cumulative concentration–response curves for all drugs except endothelin-1 as a plateau was not observed for some groups at the highest dose used in the present experiment (10^{-7} M). Data are expressed as mean±S.E.M. of *n* experiments with segments from different arteries. The effects of pulmonary hypertension and training on maximal aerobic velocity as well as concentrations within each drug treatment were assessed by a two-way analysis of variance with repeated measures. Because interaction between the main factors was significant in each case, a two-way analysis of variance examined independently of time or concentration the effect of pulmonary hypertension and training, followed by post hoc tests of Fisher's Protected Least Significant Difference when appropriated. The same analysis was also applied to citrate synthase and pEC_{50} data. $P<0.05$ was considered statistically significant.

3. Results

3.1. Indexes of exercise training and pulmonary hypertension

Evidence of a training effect was provided by maximal aerobic velocity as well as citrate synthase data. Although no differences existed at the beginning of the study between groups for maximal aerobic velocity (pre-test: normotensive sedentary=36.0±0.7, pulmonary hypertensive sedentary=37.3±0.9, normotensive trained=36.3±0.6, pulmonary hypertensive trained=36.3±0.7 m/min), the latter increased significantly in normotensive and pulmonary hypertensive trained only (post-test: normotensive sedentary=35.7±0.5, pulmonary hypertensive sedentary=36.6±0.6, normotensive trained=44.3±1.3,

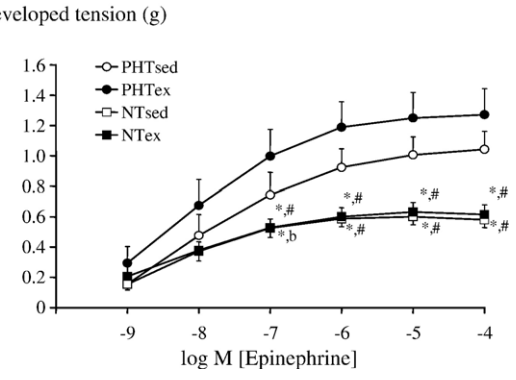

Fig. 1. Vasoconstrictor responses to cumulative concentrations of epinephrine in pulmonary arteries from pulmonary hypertensive trained ($n=7$) and untrained ($n=6$) rats as well as normotensive trained ($n=8$) and untrained ($n=8$) rats. Values are mean±S.E.M. PHTex: pulmonary hypertensive trained rats, PHTsed: pulmonary hypertensive sedentary rats, NTex: normotensive trained rats, NTsed: normotensive sedentary rats. *: $P<0.05$ vs. PHTsed; #: $P<0.05$ vs. PHTex.

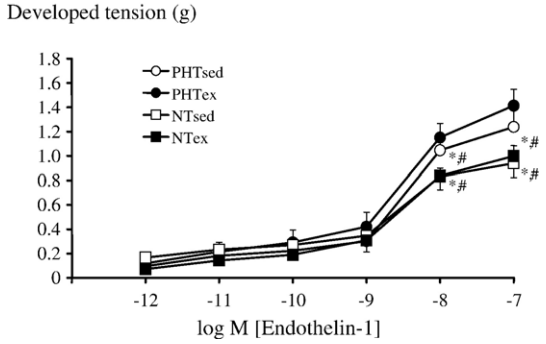

Fig. 2. Vasoconstrictor responses to cumulative concentrations of endothelin-1 in pulmonary arteries from pulmonary hypertensive trained ($n=10$) and untrained ($n=13$) rats as well as normotensive trained ($n=12$) and untrained ($n=10$) rats. Values are mean±S.E.M. PHTex: pulmonary hypertensive trained rats, PHTsed: pulmonary hypertensive sedentary rats, NTex: normotensive trained rats, NTsed: normotensive sedentary rats. *: $P<0.05$ vs. PHTsed; #: $P<0.05$ vs. PHTex.

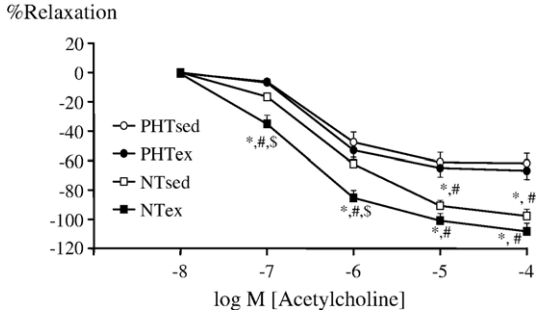

Fig. 3. Vasorelaxation responses to cumulative concentrations of acethylcholine in pulmonary arteries from pulmonary hypertensive trained ($n=8$) and untrained ($n=9$) rats as well as normotensive trained ($n=10$) and untrained ($n=8$) rats. Values are mean±S.E.M. PHTex: pulmonary hypertensive trained rats, PHTsed: pulmonary hypertensive sedentary rats, NTex: normotensive trained rats, NTsed: normotensive sedentary rats. *: $P<0.05$ vs. NTsed; #: $P<0.05$ vs. PHTex, $: $P<0.05$ vs. PHTsed.

pulmonary hypertensive trained=46.2±1.0 m/min; $P<0.001$ trained vs. sedentary rats). Training resulted also in a higher citrate synthase activity of the soleus muscle in pulmonary hypertensive and normotensive trained rats when compared with their sedentary counterparts (normotensive sedentary=35.2±4.7, pulmonary hypertensive sedentary=37.4±3.4, normotensive trained=62.4±7.9, pulmonary hypertensive trained=64.8±8.1 IU/g wet weight; $P<0.01$ trained vs. sedentary rats). It was of note to highlight that for both variables similar increases were obtained in the two trained groups. In addition, the right ventricular to total ventricular weight ratio was higher in pulmonary hypertensive than normotensive groups (normotensive sedentary=0.202±0.009, pulmonary hypertensive sedentary=0.265±0.008, normotensive trained=0.199±0.006, pulmonary hypertensive trained=0.250±0.007, $P<0.01$ pulmonary hypertensive vs. normotensive rats), indicating a significant degree of pulmonary hypertension in the former.

3.2. Contractile responses

Both epinephrine and endothelin-1 produced in all groups a concentration-dependent contraction. Figs. 1 and 2 show that agonist-induced contractions were greater in pulmonary hypertensive than normotensive rats, differences being significant for concentrations $\geq 10^{-7}$ M for epinephrine and $\geq 10^{-8}$ M for endothelin-1. For both vasoactive drugs, maximal contraction was also higher in pulmonary hypertensive than normotensive rats. Sensitivity to epinephrine did not differ between groups (Table 1). Moreover, training did not affect the vasoconstrictor responses to epinephrine and endothelin-1 in either pulmonary hypertensive or normotensive rats (Figs. 1 and 2; Table 1). Similar results were obtained regarding nonreceptor-mediated contraction induced by KCl. Indeed, KCl-induced contraction was significantly higher ($P<0.001$) in pulmonary hypertensive than normotensive rats (normotensive sedentary=0.62±0.03, normotensive trained=0.67±0.04, pulmonary hypertensive sedentary=0.88±0.05, pulmonary hypertensive trained=0.90±0.05 g). KCl-induced contraction was not modified by training. Whatever drug concentration, no differences existed anymore between all groups for endothelin-1 and epinephrine-induced constrictions when data were expressed as percent of maximal response to KCl (Table 1, maximal response only).

3.3. Relaxation responses

Acetylcholine resulted in a concentration-dependent relaxation from epinephrine-induced pre-constriction in pulmonary arteries from all groups. In normotensive rats, training induced a greater relaxation at each acetylcholine concentration $<10^{-5}$ M (Fig. 3) and enhanced sensitivity to acetylcholine, as

Table 1
Sensitivity and maximal response to vasoactive agents in pulmonary arteries from sedentary and trained normotensive and pulmonary hypertensive rats

	Epinephrine			Endothelin-1		Acetylcholine		Sodium nitro-prusside	
	pEC_{50}	Max tension (g)	% of KCl-induced contraction	Max tension (g)	% of KCl-induced contraction	pEC_{50}	Max relaxation (%)	pEC_{50}	Max relaxation (%)
NTsed	8.24±0.23	0.62±0.05*,#	120±8	0.94±0.11*,#	152±8	6.21±0.05	−97.7±4.7*,#	7.52±0.09a	−106.2±3.7*,#
NTex	8.79±0.46	0.64±0.06*,#	119±10	0.99±0.08*,#	144±5	6.58±0.07*,#,$	−108.4±5.8*,#	8.33±0.20*,#,$	−107.3±4.5*,#
PHTsed	8.14±0.16	1.04±0.12	114±6	1.24±0.14	146±5	6.30±0.07	−61.8±6.7	7.04±0.07	−82.8±3.1
PHTex	8.07±0.35	1.12±0.08	117±6	1.37±0.05	143±5	6.34±0.03	−66.9±5.9	7.24±0.14	−89.7±5.7

Values are mean±S.E.M.; NTsed: normotensive sedentary rats, NTex: normotensive trained rats, PHTsed: pulmonary hypertensive sedentary rats, PHTex: pulmonary hypertensive trained rats. Sensitivity is expressed as negative log of molar concentration. Max tension: maximal developed tension to epinephrine or endothelin-1; Max relaxation: maximal relaxation response expressed as percent relaxation from pre-contracted rings to epinephrine (10^{-6} M). *: $P<0.05$ vs. PHTsed, #: $P<0.05$ vs. PHTex, $: $P<0.05$ vs. NTsed.

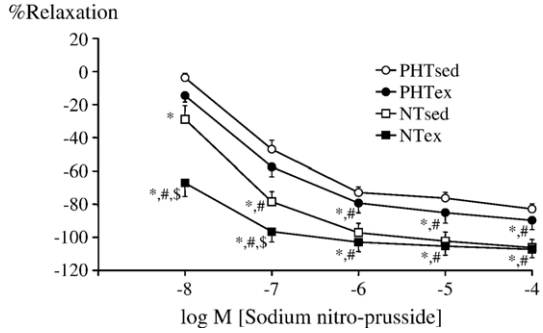

Fig. 4. Vasorelaxation responses to cumulative concentrations of sodium nitroprusside in pulmonary arteries from pulmonary hypertensive trained ($n=6$) and untrained ($n=6$) rats as well as normotensive trained ($n=7$) and untrained ($n=6$) rats. Values are mean±S.E.M. PHTex: pulmonary hypertensive trained rats, PHTsed: pulmonary hypertensive sedentary rats, NTex: normotensive trained rats, NTsed: normotensive sedentary rats. *: $P<0.05$ vs. NTsed; #: $P<0.05$ vs. PHTex, $: $P<0.05$ vs. PHTsed.

indicated by a lower pEC_{50} in normotensive sedentary when compared to normotensive trained (Table 1). Maximal response to acetylcholine was however not affected by training in those groups. Acetylcholine-induced relaxation was markedly attenuated in pulmonary hypertensive rats. Pulmonary arteries from the two pulmonary hypertensive groups displayed a decreased sensitivity to acetylcholine and lower maximal relaxation potency (Table 1). Training did not affect however this alteration in endothelium-relaxation properties as similar cumulative concentration–response curves were obtained between pulmonary hypertensive trained and pulmonary hypertensive sedentary (Fig. 3). Relaxation to sodium nitro-prusside was enhanced as a result of training in normotensive rats (Fig. 4). Sensitivity to sodium nitro-prusside was increased in normotensive trained when compared to normotensive sedentary while the maximal response did not differ between the two groups (Table 1). Direct smooth muscle relaxation mediated by sodium nitro-prusside was also altered in pulmonary hypertensive rats. Pulmonary arteries in those groups demonstrated a lower relaxation to sodium nitro-prusside throughout the concentration–response curve, a lower maximal response and a decreased sensitivity. As for acetylcholine, the training state did not affect the alteration in endothelium-independent relaxation of pulmonary hypertensive rats (Fig. 4; Table 1).

4. Discussion

The major result from the present study was that exercise training did not affect the alteration in pulmonary artery vascular reactivity in pulmonary hypertensive rats. Especially, the training-induced improvement in pulmonary artery vasorelaxation classically obtained in normotensive rats was not observed in pulmonary hypertensive rats.

4.1. Pulmonary artery vasoreactivity in pulmonary hypertensive rats

This study reports a compromised endothelium-dependent vasomotor function in pulmonary artery from pulmonary hypertensive sedentary rats. This is in accordance with numerous previous studies which have shown that the vasodilatory response to acetylcholine is diminished in experimental animal models of pulmonary hypertension (Msadden et al., 1994; Sasaki et al., 2004) or in patients with chronic obstructive lung disease (Dinh-Xuan et al., 1991). Diminished nitric oxide bioavailability has been shown to play a critical role in this endothelium-dependent relaxation dysfunction. Indeed, long-term oral treatment with L-arginine improved endothelium-dependent relaxation of isolated pulmonary artery rings from monocrotaline-induced pulmonary hypertension (Sasaki et al., 2004). Similarly, in vitro perfusion of L-arginine restored the endothelium-dependent vasodilatory response in perfused lung models from monocrotaline-treated and hypoxic rats (Eddahibi et al., 1992; Madden et al., 1995). Another possible mechanism for impaired synthesis or release of nitric oxide in pulmonary hypertension is a direct depression of nitric oxide synthase by hypoxia (Rengasamy and Johns, 1991).

In the present study, direct smooth muscle relaxation mediated by sodium nitro-prusside was also altered in pulmonary hypertensive sedentary rats. Pulmonary arteries in this group demonstrated a lower relaxation to sodium nitro-prusside throughout the concentration–response curve, a lower maximal response and a decreased sensitivity (Table 1). Unaltered or depressed endothelium-independent vasorelaxation has been reported in isolated pulmonary arteries from various hypertensive models (Rodman et al., 1990; Dinh-Xuan et al., 1991; Crawley et al., 1992; Lal et al., 1999). Our results are however consistent with previous data showing impairment in vasorelaxation to sodium nitro-prusside in pulmonary arteries from chronic hypoxic rats (Rodman et al., 1990; Crawley et al., 1992), patients with chronic obstructive pulmonary disease (Dinh-Xuan et al., 1991) and rats with pulmonary hypertension (Ashmore et al., 1991). Nitric oxide acts in part through stimulation of soluble guanylate cyclase to convert guanylate triphosphate to cyclic guanylate monophosphate (Schmidt and Walter, 1994). Crawley et al. (1992) reported that chronic exposure to hypoxia resulted in a selective impairment of soluble guanylate cyclase in rat pulmonary arteries, leading to an attenuation of both acetylcholine- and sodium nitro-prusside-induced cyclic guanylate monophosphate accumulation and relaxation. In addition, Hassoun et al. (2004) demonstrated that hypoxia decreases soluble guanylate cyclase expression in cultured pulmonary artery smooth muscle cells and suggested that in hypoxic vascular smooth muscle, decreased cyclic guanylate monophosphate synthesis may limit the vasodilator response to nitric oxide. In this context, we can reasonably postulate that a similar mechanism, involving impairment in cyclic guanylate monophosphate-mediated relaxation of pulmonary arteries, is responsible for our findings in pulmonary hypertensive sedentary rats. These observations in concert strongly suggest that impairment of relaxation in pulmonary artery from pulmonary hypertensive sedentary rats involves mechanisms acting via soluble guanlylate cyclase.

Our study reports also that agonist-induced contractions to epinephrine and endothelin-1 were greater in pulmonary hypertensive than normotensive rats. For both vasoactive drugs,

maximal contraction was also higher in pulmonary hypertensive than normotensive rats although sensitivity did not differ (Figs. 1 and 2; Table 1). These results are consistent with Deuchar et al. (2002) in rabbits and MacLean et al. (1995) and MacLean and McCulloch (1998) in rats who also reported that pulmonary hypertension potentiated the maximum response to endothelin-1. However, they disagree with other studies which have reported a reduced sensitivity and maximal response to endothelin-1 in pulmonary arteries from chronic hypoxic rats (Bialecki et al., 1998; Lal et al., 1999). Mechanisms involved in the potentiated response to vasoconstrictor agents in pulmonary hypertensive rats cannot be elucidated from the present study. It is of note to highlight that no differences existed anymore between all groups regarding vasoconstrictor responses to cumulative concentrations of either endothelin-1 or epinephrine when data were expressed as percent of maximal response to KCl (Table 1). Therefore, the enhanced maximal contraction capability in our pulmonary hypertensive rats could be viewed only as a result of specific vascular remodeling due to chronic hypoxia exposure leading to smooth muscle hypertrophia, as previously reported (Meyrick and Reid, 1978; Vender, 1994). However, we cannot exclude that our results could also be related to an alteration in vasorelaxation properties. Indeed, the potentiation of the maximal response to phenylephrine in pulmonary artery rings from rats exposed chronically to hypoxia has been presented to be consistent with impairment in vasorelaxation mechanisms. Using a similar pulmonary hypertension model (i.e. chronic hypoxia exposure) as in our study, Adnot et al. (1991) previously reported that in pulmonary hypertensive rats, the pulmonary vasoconstrictor response to endothelin-1 was greater than in normotensive animals but was no longer potentiated by nitric oxide synthesis inhibitors. Eddahibi et al. (1992) demonstrated that treatment with L-arginine restored the vasodilatory response to acetylcholine but also normalized the vasoconstrictor response to endothelin-1 in lungs from chronically hypoxic rats.

4.2. Effect of training on pulmonary artery vasoreactivity in normotensive rats

Improved endothelium-dependent vasorelaxation of isolated pulmonary artery rings was established in normotensive trained when compared to normotensive sedentary rats. Although enhancement in nitric oxide-mediated vasorelaxation following exercise training has been widely observed for systemic vessels from healthy or pathophysiological animal models (Chen et al., 1996; Delp and Laughlin, 1997; Tatchum-Talom et al., 2000), controversial results have been reported on the pulmonary circulation. Our results are consistent with data obtained in healthy rabbits (Chen and Li, 1993) but differ from others obtained in rats (Mitani et al., 1999). Acetylcholine-induced relaxation has been reported by the same team increased (Johnson et al., 2001) or unchanged (Johnson and Laughlin, 2000) following training in healthy miniature swine, and increased in exercise-trained miniature swine after coronary artery occlusion (Johnson et al., 2000). In their study, Johnson et al. (2001) reported that improvement in endothelium-dependent vasorelaxation was associated with increase in endothelial nitric oxide synthase protein in pulmonary artery tissues. In the present study, direct smooth muscle relaxation mediated by sodium nitro-prusside was also affected by training in normotensive rats which contrasts with previous studies on isolated pulmonary arteries from different species (Chen and Li, 1993; Mitani et al., 1999; Johnson et al., 2001). Discrepancies between our results and others from the literature cannot be established but could be related to the training program which in the present study was individualized and the intensity of which was particularly high (i.e. 80% of maximal aerobic velocity 5 days a week for 5 weeks). Nevertheless, our results suggest an impact of training on pulmonary vascular smooth muscle sensitivity to nitric oxide.

The contractile responses to receptor-mediated contraction did not differ between pulmonary arteries from normotensive sedentary and normotensive trained rats. Very few studies investigated the pulmonary vasoconstrictor response to chronic exercise. Our results are however in accordance with those of Johnson and Laughlin (2000) who reported similar sensitivity and maximal response to norepinephrine in isolated pulmonary artery of healthy exercise-trained and sedentary pigs. Jones et al. (1999) reported however a reduced sensitivity of coronary arteries to endothelin-1 in healthy male pigs consecutive to training.

4.3. Effect of training on pulmonary artery vasoreactivity in pulmonary hypertensive rats

The major yet unexpected results from the present study were that training did not affect the alteration in pulmonary artery vascular reactivity in pulmonary hypertensive rats. Irrespective of drugs, similar concentration–response curves were obtained between pulmonary hypertensive sedentary and pulmonary hypertensive trained rats. To the best of our knowledge, no previous study examined vasomotor function in isolated pulmonary arteries from pulmonary hypertensive animals subjected to exercise training.

The first salient feature was that the training-induced improvement in pulmonary artery vasorelaxation (endothelium-dependent and -independent) observed in normotensive rats was not demonstrated in their pulmonary hypertensive counterparts. Training has been shown to improve endothelium-dependent relaxation in systemic conduit vessels from spontaneously systemic hypertensive animal models (Chen et al., 1996) as a result of increase in endothelial nitric oxide synthase (Graham and Rush, 2004). Training-induced increase in blood flow augments nitric oxide synthase gene expression, probably signalled by increased shear stress (Green et al., 1996). Differences in vascular structure between aorta and pulmonary artery preclude any comparison between these studies and ours but we could speculate that large conduit vessels from the systemic and pulmonary circulation may react differently to training. Mechanical stress and hemodynamic forces, probably stronger during each exercise session in aorta than pulmonary artery, could constitute

a potential source of explanation for this. Pulmonary artery represents indeed a conduit vessel with lower resistance and higher compliance than aorta. Taken together, our results imply a predominant effect of chronic hypoxia exposure over training in one or more of the major determinants of nitric oxide bioavailability in the pulmonary vascular wall as well as on vascular smooth muscle sensitivity to nitric oxide. In the pulmonary vascular beds of normotensive rats, the concentration of endogenous L-arginine in endothelial cells is sufficient to prevent rate limiting of the first step in conversion of L-arginine to nitric oxide. As previously mentioned in that discussion, this is apparently not the case in pulmonary hypertensive rats and it may be postulated that deficit in L-arginine in our experimental model was such pronounced that training could not have exerted its influence. In this context, it is interesting to note that recent findings from our laboratory have shown in isolated ring aorta from chronically hypoxic trained rats that endothelium-dependent vasorelaxation, initially depressed, was restored to the level of that obtained by normoxic trained rats after acute L-arginine supplementation (Reboul et al., 2005). The same analysis can be done regarding the loss of endothelium-independent vasorelaxation in pulmonary hypertensive trained rats through a predominant effect of hypoxia on soluble guanylate cyclase. Finally, experimental data indicated that pulmonary endothelial nitric oxide synthase expression in vivo is up-regulated by shear stress and increased pulmonary blood flow (Black et al., 1998). Pulmonary hypertensive trained probably exhibited a higher pulmonary shear stress than pulmonary hypertensive sedentary rats due to chronic exercise bouts and consequently an up-regulation of endothelial nitric oxide synthase expression would have been logically expected. However, we cannot exclude also the possibility that lack of effect of training in pulmonary hypertensive rats is related to a direct depression of endothelial nitric oxide synthase by hypoxia as previously mentioned (Rengasamy and Johns, 1991).

Endothelin-1 has been shown to be implicated in the pathophysiology of pulmonary hypertension (Galié et al., 2004). In the context of the limitation of pulmonary hypertension by exercise training (Kashimura and Sakai, 1991; Véras-Silva et al., 1997; Henderson et al., 2001), a decrease in smooth muscle responsiveness to endothelin-1 would have been expected in pulmonary hypertensive trained compared to pulmonary hypertensive sedentary rats. In our ex vivo model, that was not the case whatever the mode of expression of the results. In in vivo conditions, other mechanisms could however be implied and might involve changes in the balance between vasorelaxant and vasoconstrictor agents. In this context, it is of note however to highlight that Maeda et al. (2001) have shown that training resulted in decreased endothelin-1 plasma levels in humans.

To conclude, using a chronic hypoxia model of pulmonary hypertension, our results show that training does not affect the alteration in vasomotor function in pulmonary arteries from pulmonary hypertensive rats. Especially, loss of both endothelium-dependent and -independent relaxation properties were obtained in pulmonary hypertensive rats whatever their training status. A predominant effect of chronic hypoxia exposure over training seems to be partially responsible for this, probably through impairment in nitric oxide bioavailability and vascular smooth muscle sensitivity.

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