ELSEVIER

European Journal of Pharmacology 317 (1996) 335–342

ejp

Nitric oxide regulation of TP receptor-mediated pulmonary vasoconstriction in the anesthetized, open-chest rat

Jean-Pierre Valentin, Anne-Marie Bessac, Myriam Maffre, Gareth W. John *

Centre de Recherche Pierre Fabre, Division of Cardiovascular Diseases, 17 Avenue Jean Moulin, 81106 Castres, France

Received 4 September 1996; accepted 10 September 1996

Abstract

We investigated the influence of endothelial nitric oxide (NO) on the pulmonary pressor activity of the stable thromboxane A_2 analogue, U-46619 (9,11-dideoxy-9α-(methanoepoxy) prostaglandin $F_{2\alpha}$), in anesthetized open-chest Sprague-Dawley rats ($n = 6–9$ per group). NO synthase inhibition, as obtained by N^{ω}-nitro-L-arginine methyl ester (L-NAME; 0.63 mg/kg i.v. + 20 mg/kg/h), induced sustained systemic hypertension (mean maximal increase, Δ, in mean systemic arterial pressure = 38 ± 6 mmHg; $P < 0.05$ vs. vehicle) associated with slight bradycardia (Δ heart rate = −42 ± 8 beats/min; $P < 0.05$ vs. vehicle) and delayed- (> 30 min) onset pulmonary hypertension (Δ mean pulmonary arterial pressure = 10 ± 3.4 mmHg; $P < 0.05$ vs. vehicle). In separate experiments, when mean systemic arterial pressure was maximally increased by L-NAME, the difference between mean pulmonary arterial pressure and mean left atrial pressure was greater in L-NAME-treated rats (41 ± 16% compared to 10 ± 1% in the vehicle group; $P < 0.05$), strongly suggesting that spontaneously released NO modulated pulmonary vascular resistance. L-Arginine at a dose which reduced by ~50% the L-NAME-associated systemic hypertension did not alter the late rise in mean pulmonary arterial pressure (Δ mean pulmonary arterial pressure = 12 ± 4 mmHg; P = NS vs. L-NAME alone). U-46619, elicited rapid, dose-dependent, and transient increases in mean pulmonary arterial pressure (Δ = 8.8 ± 2.0 and 21.2 ± 1.9 mmHg at 1.25 and 20 μg/kg i.v. respectively; both $P < 0.01$ vs. vehicle). U-46619 (1.25 μg/kg)-induced increases in mean pulmonary arterial pressure were fully antagonized by the thromboxane A_2/prostanoid (TP) receptor antagonist, SQ 29,548 ([1*S*-[1α,2α(5*Z*),3α,4α]]-7-[3-[[2-[(phenyl-amino)-carbonyl] hydrazino] methyl]-7-oxabicyclo [2.2.1]hept-2-yl]-5-heptenoic acid) (0.63 mg/kg i.v. + 0.63 mg/kg/h). Injection of U-46619 (1.25 μg/kg), 15 min after L-NAME administration, evoked a 24.7 ± 0.9 mmHg increase in mean pulmonary arterial pressure ($P < 0.01$ vs. U-46619 in control rats), which was (i) greater than that produced by a 16-fold higher dose of U-46619 alone, (ii) fully antagonized by SQ 29,548, (iii) significantly attenuated during coadministration of L-NAME and L-arginine (10 mg/kg i.v. + 160 mg/kg/h; Δ mean pulmonary arterial pressure = 14.6 ± 4.3 mmHg; $P < 0.05$ vs. U-46619 following L-NAME alone and P = NS vs. U-46619 in control rats). These results indicate that, under normal circumstances, pulmonary vasomotor tone is regulated by spontaneously released NO. Moreover, pulmonary vascular NO attenuates TP receptor-mediated pressor responses, strongly suggesting that in addition to mediating pulmonary vasoconstriction, TP receptor activation also concomitantly releases NO within the pulmonary vasculature.

Keywords: Pulmonary hypertension; Nitric oxide (NO); TP receptor; U-46619; N^{ω}-Nitro-L-arginine methyl ester (L-NAME); SQ 29,548

1. Introduction

Endothelial nitric oxide (NO) is a powerful vasodilator which regulates pulmonary vascular resistance in animals and man, and acts as a physiological brake against vasoconstriction (Barnes and Liu, 1995; Moncada and Higgs, 1993). It has been suspected that disturbance of NO synthase activity may be of key importance in the pathophysiology of pulmonary vascular diseases (Peacock, 1995). In this regard, it was recently demonstrated that the pulmonary endothelial expression of NO synthase is indeed reduced in patients with pulmonary hypertension (Giaid and Saleh, 1995), leading to reduced NO availability and subsequent vasoconstriction. Inhalation of NO gas selectively lowers pulmonary vascular resistance (Pepke-Zaba et al., 1991), indicating that the hypertensive pulmonary vasculature is still responsive to NO.

Thromboxane A_2 is a major product of arachidonic acid metabolism in the lung that stimulates contraction of vascular smooth muscle via activation of TP receptors (Barnes and Liu, 1995; Coleman et al., 1994). An important role of thromboxane A_2 in the development and/or maintainance of pulmonary hypertension has been pro-

* Corresponding author. Tel.: (33-5) 6371-4223; Fax: (33-5) 6371-4363.

posed by the observation of elevated levels of the stable thromboxane A_2 metabolite, thromboxane B_2 (Rubin, 1995). In vitro evidence suggested that contraction of rat aortic rings with the stable thromboxane A_2 analogue, U-46619 (9,11-dideoxy-9α-(methanoepoxy) prostaglandin $F_{2\alpha}$), was attenuated in the presence of endothelium due to the release of an endothelium derived relaxing factor (probably NO; Folger et al., 1991). Consequently, if reduced NO synthase activity and NO production eventually lead to enhanced TP receptor-mediated pulmonary vasoconstriction, then this may have clinical relevance. However, it is not known at the present time whether endothelial NO attenuates TP receptor-mediated pulmonary hypertension in vivo.

We therefore investigated the influence of endothelial NO on the pulmonary pressor responses evoked by the stable thromboxane A_2 analogue, U-46619, in anesthetized, open-chest rats, by employing the NO synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME), an agent which has previously been shown to inhibit pulmonary endothelial NO synthase (Fineman et al., 1992; McMahon et al., 1991; Nishiwaki et al., 1992).

2. Materials and methods

In accordance with French law and the local ethical committee guidelines for animal research, male Sprague-Dawley rats (300–400 g, OFA, Iffa-Credo, France) were anesthetized with sodium pentobarbital (60 mg/kg i.p.; Sanofi Laboratories, France) and placed on a heated table to maintain rectal temperature at 37 ± 0.5°C. They were prepared for acute experimentation as described previously (Bertolino et al., 1995a,b). Briefly, animals underwent tracheotomy and were mechanically ventilated. Catheters were inserted into a femoral vein and artery for infusing fluids and drugs, sampling blood, and continuous measurement of arterial pressure via a Statham P10EZ pressure transducer connected to a Gould amplifier and a computerized data acquisition system (Acq*Knowledge*, BIOPAC Systems, Goleta, CA, USA). A left thoracotomy was performed through the third intercostal space. The pulmonary artery was exposed and a curved 19-gauge needle, connected to a Silastic tube (Dow Corning, Midland, MI, USA), was inserted near the bifurcation of the artery from the ventricle. The Silastic catheter was secured to the exposed muscle layer of the animal, then the thorax was closed. Pulmonary arterial pressure was recorded as described for the systemic arterial pressure. Experiments were started 15–30 min after completion of surgical procedures.

2.1. Effect of NO synthase inhibition and of a thromboxane A_2 analogue on systemic and pulmonary hemodynamic parameters

Rats received an intravenous injection of L-NAME (0.63 mg/kg + 20 mg/kg per h; $n = 8$) or the vehicle (NaCl, 0.9%; $n = 23$). The 23 rats receiving the vehicle of L-NAME were divided into 3 subgroups. They received either the thromboxane A_2 analogue, U-46619, at one of the following doses (1.25 or 20 μg/kg; $n = 8$ and 7, respectively) or its vehicle (Na_2CO_3, 2 mM; $n = 8$). U-46619 or its vehicle was injected 15 min after initiation of the infusion of the L-NAME vehicle.

To determine whether spontaneously released NO modulated pulmonary vascular resistance, mean pulmonary arterial and mean left atrial pressures were simultaneously recorded and the difference, an index of pulmonary vascular tone, determined in the presence and absence of L-NAME in a separate series of experiments. Left atrial pressure was recorded continuously by means of a catheter placed in the left atrium via the apex. Six rats received L-NAME (0.63 mg/kg + 20 mg/kg per h) whereas 6 others received a vehicle infusion over a 15 min period at which time the experiment was ended.

Table 1
Hemodynamic values measured at baseline and prior to U-46619 or vehicle administration

Treatment	*n*	BW (g)	MSAP (mmHg)			MPAP (mmHg)			HR (beats/min)		
			Baseline	Before U-46619/vehicle	Δ	Baseline	Before U-46619/vehicle	Δ	Baseline	Before U-46619/vehicle	Δ
Vehicle	23	360 ± 9	98 ± 6 ^{NS}	92 ± 4	−6.1 ± 4.9	18.9 ± 0.7 ^{NS}	18.6 ± 0.7	−0.3 ± 0.4	362 ± 16 ^{NS}	347 ± 14	−15 ± 6
L-NAME	14	350 ± 7	106 ± 6 ^{a}	144 ± 8	37.8 ± 5.6 ^{b}	17.4 ± 0.3 ^{NS}	16.7 ± 0.7	−0.7 ± 0.7	388 ± 14 ^{a}	345 ± 12	−42 ± 8 ^{b}
SQ 29,548	14	344 ± 5	102 ± 5 ^{NS}	94 ± 5	−8.0 ± 4.5 ^{c}	19.7 ± 0.7 ^{NS}	19.7 ± 0.6	0.0 ± 0.4	369 ± 14 ^{NS}	353 ± 10	−16 ± 11 ^{c}
L-NAME + SQ 29,548	12	338 ± 7	111 ± 5 ^{a}	142 ± 8	31.5 ± 7.3 ^{b,d}	16.9 ± 0.5 ^{NS}	17.0 ± 0.7	0.1 ± 0.7	388 ± 13 ^{a}	342 ± 13	−46 ± 12 ^{b,d}
L-NAME + L-arginine	17	359 ± 8	97 ± 5 ^{a}	117 ± 7	20.4 ± 4.2 ^{b,d}	21.1 ± 1.1 ^{a}	22.6 ± 1.1	1.5 ± 0.4 ^{b,c,d}	366 ± 13 ^{NS}	349 ± 12	−16 ± 11 ^{c,e}

Values are mean ± S.E.M. of measurements obtained at baseline and 15 min after initiation of one of the treatments indicated in the first column (i.e., before injection of either U-46619 or its vehicle). *n*, number of rats; BW, body weight; MPAP, mean pulmonary arterial pressure; MSAP, mean systemic arterial pressure; HR, heart rate. ^{a} $P < 0.005$ baseline vs. before U-46619/vehicle. ^{b} $P < 0.05$ vs. vehicle; ^{c} $P < 0.05$ vs. L-NAME; ^{d} $P < 0.05$ vs. SQ 29,548; ^{e} $P < 0.05$ vs. L-NAME + SQ 29,548. ^{NS} Not significant. All other comparisons did not reach statistical significance.

2.2. Influence of L-NAME on the responses to U-46619

Six rats received U-46619 at the dose of 1.25 μg/kg i.v., 15 min after initiation of L-NAME infusion. U-46619 was administered when L-NAME-induced changes in mean systemic arterial pressure and heart rate were maximal, at which time mean pulmonary arterial pressure was consistently not significantly affected.

2.3. Influence of TP receptor blockade on the responses to U-46619, L-NAME alone or in combination

Twenty-six rats received the TP receptor antagonist SQ 29,548 (0.63 mg/kg i.v. + 0.63 mg/kg per h) and were divided into 4 subgroups. Six rats received U-46619 (1.25 μg/kg i.v.) whereas 8 received its vehicle, 15 min after initiation of the infusion of SQ 29,548. In 2 additional groups, L-NAME (0.63 mg/kg + 20 mg/kg per h) was coadministered with SQ 29,548; these rats received either the thromboxane A_2 analogue U-46619 (1.25 μg/kg i.v.; $n = 6$) or its vehicle ($n = 6$) 15 min after initiation of the infusion of SQ 29,548 and L-NAME. When L-NAME and SQ 29,548 were infused simultaneously the infusion rate was reduced to 20 μl/min in order to maintain the volume administered constant.

2.4. Influence of L-arginine administration on the responses to L-NAME alone or in association with U-46619

L-Arginine (10 mg/kg i.v. + 160 mg/kg per h) was coadministered with L-NAME (0.63 mg/kg i.v. + 20 mg/kg per h) in 17 rats (both at a rate of 20 μl/min); these rats received either U-46619 (1.25 μg/kg i.v.; $n = 9$) or its vehicle ($n = 8$) 15 min after initiation of the infusion of L-arginine and L-NAME.

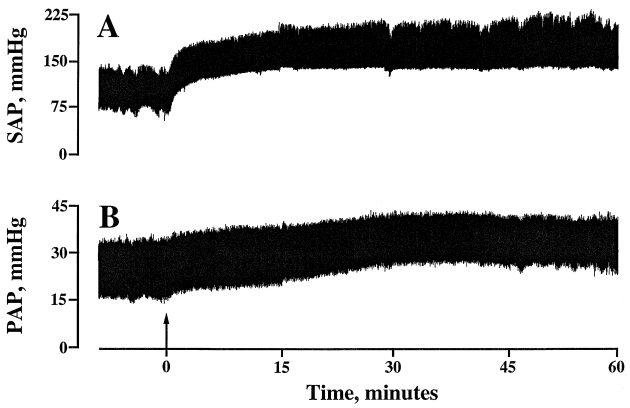

Fig. 1. Typical recordings of systemic (SAP; A) and pulmonary (PAP; B) arterial pressures following nitric oxide synthase inhibition as obtained by injection of 0.63 mg/kg of L-NAME over 2 min, starting at 0 min as indicated by the arrow, followed by a constant infusion of 20 mg/kg per h at the rate of 40 μl/min over 1 h. L-NAME induced rapid, marked, and sustained systemic hypertension accompanied by delayed onset pulmonary hypertension.

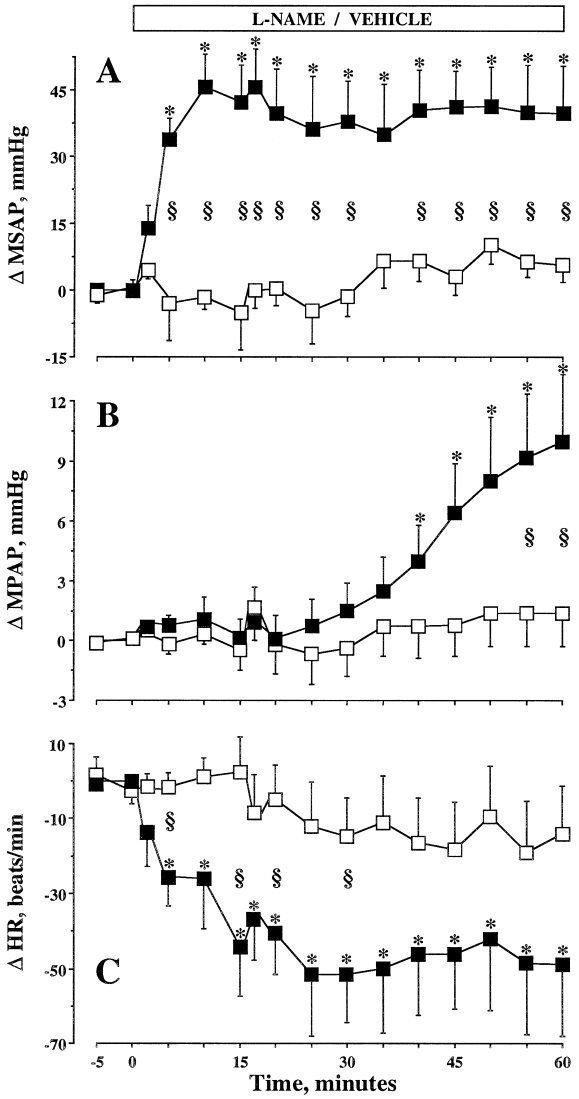

Fig. 2. Time-course of the effects of nitric oxide (NO) synthase inhibition on mean absolute changes in mean systemic (ΔMSAP; A) and pulmonary arterial pressures (ΔMPAP; B), as well as heart rate (ΔHR; C) in open-chest anesthetized rats. NO synthase inhibition was obtained by injection of 0.63 mg/kg of L-NAME over 2 min followed by a constant infusion of 20 mg/kg per h (filled squares; $n = 8$). No significant changes in mean systemic arterial pressure, mean pulmonary arterial pressure and heart rate were detected in time control, vehicle-infused rats (NaCl, 0.9% as a 1 ml/kg solution over 2 min followed by a constant infusion at a rate of 40 μl/min over 1 h; open squares; $n = 8$). * $P < 0.05$ vs. baseline; § $P < 0.05$ between groups.

2.5. Drugs and solutions

SQ 29,548 ([1*S*-[1α,2α(5*Z*),3α,4α]]-7-[3-[[2-[(phenyl-amino)-carbonyl] hydrazino] methyl]-7-oxabicyclo [2.2.1]hept-2-yl]-5-heptenoic acid) and U-46619 (9,11-dideoxy-9α-(methanoepoxy) prostaglandin $F_{2\alpha}$) purchased from Cayman (Ann Arbor, MI, USA) were dissolved in Na_2CO_3 (2 mM). They were maintained on ice after dissolution and injected in μg/kg base weight. U-46619 was administered as a 1 ml/kg solution over 2 min

whereas SQ 29,548 was injected as a 1 ml/kg solution over 2 min followed by a constant infusion at a rate of 40 μl/min for 1 h. N^{ω}-Nitro-L-arginine methyl ester hydrochloride and L-arginine hydrochloride (both from Sigma, St. Louis, MO, USA) were dissolved in sterile NaCl, 0.9%, and injected in μg/kg base weight as 1 ml/kg solutions over 2 min followed by a constant infusion at a rate of 40 μl/min (except when specified otherwise) for 1 h.

2.6. Calculations and statistical analysis

Data are expressed as mean absolute (or relative) maximal changes (Δ) ± S.E.M. One-way analysis of variance followed by the Dunnett test were used to assess significance among and between groups (StatView, Abacus Concepts, Berkeley, CA, USA). $P = 0.05$ was considered the minimum level of significance.

3. Results

3.1. Effect of NO synthase inhibition on mean systemic arterial pressure, mean pulmonary arterial pressure, left atrial pressure and heart rate

A typical recording of systemic (A) and pulmonary (B) arterial pressure following NO synthase inhibition is presented in Fig. 1. NO synthase inhibition was associated with a rapid (within 5 min), marked (Δmean systemic arterial pressure = 38 ± 6 mmHg; $P < 0.05$ vs. vehicle) and sustained increase in mean systemic arterial pressure which was accompanied by a slight bradycardia (Δheart rate = −42 ± 8 beats/min; $P < 0.05$ vs. vehicle) reaching a plateau within 15 min (Table 1; Fig. 2). Mean pulmonary arterial pressure increased progressively after 30 min attaining a maximum of 27.7 ± 3.2 mmHg (Δmean pulmonary arterial pressure = 10 ± 3.4 mmHg; $P < 0.05$ vs. vehicle group; Table 2; Figs. 1 and 2) at the end of the experiment. No significant changes in mean systemic arterial pressure, mean pulmonary arterial pressure, or heart rate were detected in control, vehicle-infused rats (Tables 1 and 2; Fig. 2).

Table 3
Baseline and maximal hemodynamic values measured after L-NAME or vehicle administration

Treatment	Vehicle ($n = 6$)		L-NAME ($n = 6$)	
	Baseline	Absolute change	Baseline	Absolute change
BW (g)	381 ± 2		367 ± 6	
MSAP (mmHg)	60.5 ± 2.4	6.7 ± 1.9	67.8 ± 4.6	47.5 ± 6.5 [a]
MPAP (mmHg)	15.9 ± 1.0	1.4 ± 0.2	16.1 ± 1.1	5.4 ± 2.0 [a]
MLAP (mmHg)	3.6 ± 0.5	0.2 ± 0.1	3.3 ± 0.1	0.5 ± 0.1 [a]
(MPAP − MLAP) (mmHg)	12.3 ± 0.8	1.2 ± 0.2	12.8 ± 1.1	4.9 ± 2.0 [a]

Values are mean ± S.E.M. *n*, number of rats; BW, body weight; MSAP, mean systemic arterial presure; MPAP, mean pulmonary arterial pressure; MLAP, mean left atrial pressure. [a] $P < 0.05$ vs. vehicle group. Baseline values were measured at 0 min following 15 min stabilisation. Maximal values were measured 5–15 min after initiation of L-NAME or its vehicle.

To determine whether spontaneously released NO modulated pulmonary vascular resistance, we simultaneously recorded mean pulmonary arterial and mean left atrial pressures and determined the difference as an index of pulmonary vascular tone in the presence and absence of L-NAME in a separate series of experiments. The results are presented in Table 3. Mean systemic arterial pressure was lower in these animals compared to that reported in the first set of experiments, probably due to the heavier instrumentation (Tables 1 and 3). L-NAME elicited comparable increases in mean systemic arterial pressure to those reported in the previous experiments (48 ± 6 mmHg; $P < 0.05$ vs. vehicle) whereas mean pulmonary arterial pressure increased by 35 ± 12% ($P < 0.05$ vs. vehicle) within 5–15 min. Furthermore, mean left atrial pressure as well as the difference between mean pulmonary arterial and mean left atrial pressures were significantly greater in L-NAME-compared to vehicle-treated animals (15 ± 2 vs. 5 ± 2% and 41 ± 16 vs. 10 ± 1% for mean left atrial pressure and the difference in L-NAME- and vehicle-treated rats, respectively; both $P < 0.05$ between groups) (Table 3). A tendency for mean systemic and pulmonary arterial pressures to increase more rapidly compared to the first set of experiments was also noted following L-NAME.

Table 2
Mean basal and maximal pulmonary arterial pressure values

Treatment	*n*	BW (g)	Mean pulmonary arterial pressure		
			Basal (mmHg)	Maximal (mmHg)	% Change
Vehicle	8	361 ± 15	20.5 ± 0.9 [NS]	21.9 ± 1.9	7 ± 8
L-NAME	8	349 ± 8	17.7 ± 0.3 [a]	27.7 ± 3.2	58 ± 20 [b,c]
SQ 29,548	8	346 ± 8	20.4 ± 1.1 [NS]	23.0 ± 1.4	14 ± 8
L-NAME + SQ 29,548	6	335 ± 10	16.1 ± 0.3 [a]	30.6 ± 4.3	90 ± 27 [b,c]
L-NAME + L-arginine	8	363 ± 12	19.4 ± 1.6 [a]	31.4 ± 4.0	68 ± 24 [b,c]

Values are mean ± S.E.M. *n*, number of rats; BW, body weight. [a] $P < 0.05$ basal vs. maximal. [b] $P < 0.05$ vs. vehicle; [c] $P < 0.05$ SQ 29,548. [NS] Not significant. All other comparisons did not reach statistical significance. Baseline values were measured at 0 min following 15 min stabilisation. Maximal values were measured 50–60 min after initiation of the treatments indicated in the first column.

3.2. Effect of the thromboxane A_2 analogue, U-46619, on mean systemic arterial pressure, mean pulmonary arterial pressure and heart rate

Following intravenous injection of U-46619, mean pulmonary arterial pressure increased promptly, within 2–3 min (Δ mean pulmonary arterial pressure = 8.8 ± 2.0 and 21.2 ± 1.9 mmHg at 1.25 and 20 μg/kg, respectively; both $P < 0.01$ vs. vehicle group and $P < 0.05$ between doses; Fig. 3), then progressively returned to preinjection values. Systemic hypertension developed at the low dose, whereas hypotension occurred at the high dose (Fig. 3). No significant change in heart rate was detected at either dose (data not shown). We next determined whether pulmonary pressor responses evoked by U-46619 were influenced by NO.

3.3. Influence of NO synthase inhibition on the responses to U-46619

Preinjection values (pre-vehicle or U-46619) for mean systemic arterial pressure, mean pulmonary arterial pressure and heart rate are presented in Table 1. Mean systemic arterial pressure was significantly higher and heart rate lower in L-NAME-treated animals compared to vehicle-infused animals whereas mean pulmonary arterial pressure was not significantly affected. Injection of U-46619 (1.25 μg/kg) 15 min after L-NAME administration resulted in a 24.7 ± 0.9 mmHg increase in mean pulmonary arterial pressure ($P < 0.01$ vs. U-46619 in control rats) associated with a 64.4 ± 16.1 mmHg decrease in mean systemic arterial pressure from a basal value of 143 ± 14

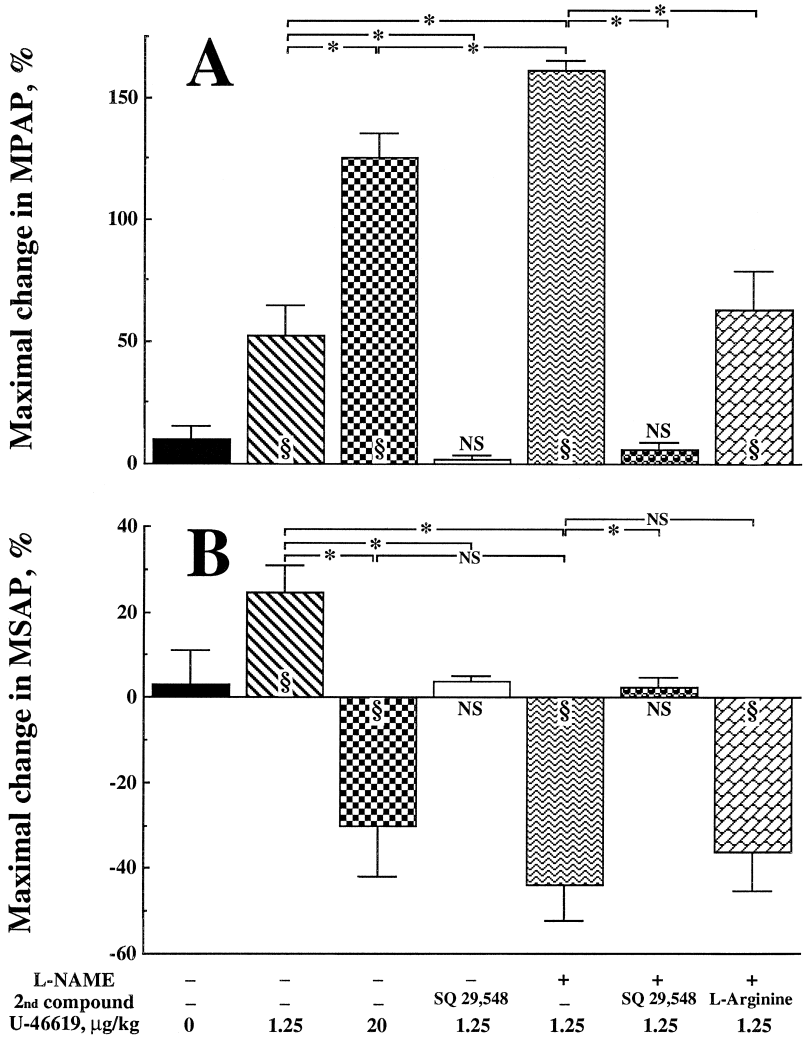

Fig. 3. Influence of TP receptor blockade and L-arginine on the responses to U-46619 alone or during nitric oxide synthase inhibition as obtained by L-NAME (0.63 mg/kg bolus + 20 mg/kg per h). The TP receptor antagonist SQ 29,548 was administered at the dose of 0.63 mg/kg bolus + 0.63 mg/kg per h. L-Arginine was administered at the dose of 10 mg/kg bolus + 160 mg/kg per h. Values are mean maximal absolute changes in mean pulmonary (ΔMPAP; A) and systemic arterial pressures (ΔMSAP; B) observed during the 5 min following U-46619 (1.25 or 20 μg/kg) or its vehicle (Na_2CO_3, 2 mM). Administration of SQ 29,548 abolished the U-46619 (1.25 μg/kg)-induced changes in both mean pulmonary arterial pressure and mean systemic arterial pressure either in the presence or absence of L-NAME. Injection of U-46619, during coadministration of L-NAME and L-arginine, resulted in a significant attenuation of the rise in mean pulmonary arterial pressure associated with a similar reduction in mean systemic arterial pressure. § $P < 0.05$ vs. vehicle-infused rats; * $P < 0.05$ between groups; NS, not significant between groups or vs. vehicle-infused rats.

mmHg (Fig. 3). The effect of U-46619 (1.25 μg/kg) on mean pulmonary arterial pressure during NO synthase inhibition was significantly greater than that produced by a 16-fold higher dose of U-46619 administered alone (i.e., 20 μg/kg; Δmean pulmonary arterial pressure = 21.2 ± 1.9 mmHg). We next investigated whether the pulmonary pressor responses induced by U-46619 alone or during NO synthase inhibition were mediated by TP receptors.

3.4. Influence of TP receptor blockade on the responses to U-46619, L-NAME alone or in combination

SQ 29,548 was devoid of any significant effect on mean systemic arterial pressure, mean pulmonary arterial pressure, and heart rate compared to vehicle-infused animals (Tables 1 and 2). As depicted in Fig. 3, the U-46619 (1.25 μg/kg)-induced increases in mean pulmonary and systemic arterial pressures were fully antagonized by SQ 29,548. Treatment with SQ 29,548, at a dose which prevented these responses to U-46619, had no effect on L-NAME-induced systemic and pulmonary hypertension as well as bradycardia (Tables 1 and 2). Furthermore, administration of SQ 29,548 during L-NAME infusion abolished U-46619-induced increases in mean pulmonary arterial pressure and decrease in mean systemic arterial pressure (Fig. 3). To verify that the effects of L-NAME were mediated by NO synthase inhibition, we determined whether they could be reversed by L-arginine.

3.5. Influence of L-arginine administration on the responses to L-NAME alone or in association with U-46619

L-Arginine infusion at a dose which reduced by approximately 50% the L-NAME-induced systemic hypertension blocked the bradycardia but did not alter the rise in mean pulmonary arterial pressure (Tables 1 and 2). Following coadministration of L-NAME and L-arginine, U-46619 (1.25 μg/kg) evoked significantly lower maximal increases in mean pulmonary arterial pressure ($\Delta = 14.6 \pm 4.3$ mmHg; $P < 0.05$ vs. U-46619 during L-NAME infusion in the absence of L-arginine and P = NS vs. U-46619 in control rats) associated with a similar reduction in mean systemic arterial pressure ($\Delta = 41.3 \pm 14.5$ mmHg from a basal value of 115 ± 9 mmHg; Fig. 3).

4. Discussion

We investigated the influence of endothelial NO on the pulmonary pressor responses evoked by TP receptor activation in anesthetized open-chest rats using the NO synthase inhibitor, L-NAME. The stable thromboxane A_2 analogue, U-46619, elicited rapid, dose-dependent, and transient increases in mean pulmonary arterial pressure. When NO synthase was inhibited, TP receptor-mediated pulmonary hypertension was (a) substantially potentiated, (b) reversed by L-arginine, and (c) antagonized by the selective TP receptor antagonist, SQ 29,548. The results indicate that pulmonary vascular NO attenuates TP receptor-mediated pressor responses, strongly suggesting that in addition to mediating pulmonary vasoconstriction, TP receptor activation simultaneously releases NO within the pulmonary vasculature.

4.1. Pulmonary and systemic hemodynamic effects resulting from NO synthase inhibition

NO synthase inhibition as obtained by L-NAME, produced rapid and sustained increases in mean systemic arterial pressure, associated with parallel but slight bradycardia, whereas mean pulmonary arterial pressure was significantly raised only after a delay of over 30 min. Increased mean systemic arterial pressure (Chyu et al., 1992; Gardiner et al., 1990; Rees et al., 1989; Stamler et al., 1994) and mean pulmonary arterial pressure (Fineman et al., 1992; McMahon et al., 1991; Wiklund et al., 1990) following NO synthase inhibition have been described previously. Systemic NO synthase is rapidly inhibited by L-NAME since mean systemic arterial pressure rose within a few minutes after L-NAME administration, in agreement with previous reports, suggesting that spontaneous NO release regulates systemic arterial pressure (Rees et al., 1989). The reduction in heart rate has been attributed to a reflex phenomenon rather than a direct effect of the NO synthase inhibitors (Gardiner et al., 1990; Rees et al., 1989). NO synthase inhibition-induced delayed-onset pulmonary hypertension, occurring more than 30 min after mean systemic arterial pressure was maximally increased, has not previously been reported. In contrast, McMahon et al. (1991) found that in closed-chest cats, the peak increase in lobar arterial pressure occurred earlier than the maximal rise in mean systemic arterial pressure following NO synthase inhibition. Although we did not measure cardiac output in our experiments, it is highly likely that it was reduced during NO synthase inhibition, as expected from the increases in blood pressure and decreases in heart rate, and as reported in the rat by Wang et al. (1992, 1995). In these studies, carried out in rats, NO synthase inhibition evoked 15–50% increases in mean systemic arterial pressure, associated with substantial (30–50%) reductions in cardiac output (Wang et al., 1992, 1995). This fact combined with the increase in the difference between mean pulmonary arterial pressure and mean left atrial pressure we observed at a time when mean systemic arterial pressure was maximally increased, heart rate and cardiac output decreased, and mean pulmonary arterial pressure only slightly or not detectably increased, can therefore be interpreted as an early rise in pulmonary vascular resistance secondary to inhibition of basal/tonic NO release (McGregor and Sniderman, 1985). Our results therefore suggest that spontaneous NO release is involved in regulating pulmonary vascular resistance in anesthetized open-chest

rats, in agreement with previous studies performed in humans (Cooper et al., 1996), newborn lambs (Fineman et al., 1992), cats (McMahon et al., 1991) and dogs (Perrella et al., 1991). However, Xue et al. (1994) could not detect endothelial NO synthase immunostaining in rat small pulmonary resistance vessels but did detect NO synthase immunostaining in medium-sized vessels, which could be speculated to play a role in the downstream regulation of pulmonary vascular resistance. Furthermore, Hasunuma et al. (1991) failed to demonstrate an involvement of basal/tonic NO release in the maintenance of the low pulmonary vascular tone in the isolated perfused rat lung, but this could be explained by the limited sensitivity of the experimental model employed. Despite the fact that pulmonary vascular resistance is acutely modulated by the basal release of NO, mean pulmonary arterial pressure increased substantially and in a delayed manner. It could be postulated that the pulmonary supply and/or availability of L-arginine within the pulmonary vasculature may be greater than that in the systemic vasculature and therefore requires a larger amount of L-NAME in order to be fully inhibited thus explaining the slow and progressive rise in mean pulmonary arterial pressure. The delayed increase in mean pulmonary arterial pressure could also be explained by differential tissue sensitivity to NO synthase inhibition. In this regard, Lahera et al. (1994) demonstrated a greater sensitivity to NO synthase inhibition in the renal compared to the systemic vascular beds, whereas Gardiner et al. (1990) detected the following order of sensitivity to NO synthase inhibition in rats: hindquarter > mesenteric > renal. Further studies are clearly required to resolve this issue. Alternatively, the pulmonary hypertension caused by NO synthase inhibition may be, at least in part, an indirect consequence of the systemic effect of L-NAME. This is supported by the observation of a lack of reversibility of the delayed rise in mean pulmonary arterial pressure by L-arginine. Whatever the reason for the delayed increase in mean pulmonary arterial pressure following NO synthase inhibition, our findings clearly support a role for spontaneously released NO in the regulation of pulmonary vascular resistance in the rat.

4.2. Pulmonary and systemic hemodynamic effects resulting from TP receptor activation

U-46619 induced dose-dependent pulmonary hypertension and systemic hypo- or hypertension depending upon the dose, as reported by us (Bertolino et al., 1995a,b) and others (Carrithers et al., 1994; Kaye et al., 1995; Nossaman et al., 1992). Furthermore the large increase in the difference between mean pulmonary arterial pressure and mean left atrial pressure, exclusively due to the increase in mean pulmonary arterial pressure, we observed (data not shown) confirms that TP receptor activation regulates pulmonary vascular resistance as previously reported in man by Frostell et al. (1991). The silent TP receptor antagonist, SQ 29,548 (Ogletree et al., 1985; Bertolino et al., 1995b), abolished both the systemic and pulmonary pressor responses, indicating that the effects of U-46619 were specifically mediated through activation of TP receptors.

4.3. Influence of NO synthase inhibition on the responses to TP receptor activation

U-46619 was administered when L-NAME-induced changes in mean systemic arterial pressure and heart rate were maximal, at which time mean pulmonary arterial pressure was not significantly affected; therefore changes in pulmonary responsiveness cannot be fully attributed to alterations in baseline conditions. NO synthase inhibition potentiated the pulmonary hypertension resulting from TP receptor activation. In these circumstances, the pulmonary hypertensive activity of U-46619 (1.25 μg/kg) was greater than that induced by a 16-fold higher dose (i.e., 20 μg/kg) administered alone, thus suggesting that in addition to mediating pulmonary vasoconstriction, TP receptor activation elicited NO release within the pulmonary vasculature. Further evidence that L-NAME inhibited agonist (U-46619)-stimulated pulmonary vascular NO release derives from the reversibility experiments with L-arginine. Our results are in agreement with those of Nishiwaki et al. (1992) in chronically instrumented conscious dogs and of Fineman et al. (1992) in intact newborn lambs. Based on these results, it is therefore conceivable that TP receptor activation, as obtained by U-46619, locally released NO, which may act directly on the adjacent vascular smooth muscle cells to counteract the pulmonary vasoconstriction by providing an opposing vasodilatory stimulus. Furthermore, L-NAME and methylene blue have been shown to enhance the vasoconstrictor responses to phenylephrine in human and bovine pulmonary vascular rings (Dinh-Xuan et al., 1991; Ignarro et al., 1987) as well as to angiotensin II in isolated rat lungs (Hasunuma et al., 1991) but not in the intact cat lungs (Hyman et al., 1989). Endogenously released NO may therefore act to modulate the final response to a variety of pulmonary vasoconstrictor agents. Thus the major finding of the present investigation is that TP receptor activation, in addition to producing vasoconstriction, appears to simultaneously release NO within the pulmonary vasculature. This is the first time to our knowledge that these effects of TP receptor activation have been reported in vivo, thereby confirming and extending the in vitro observations of Folger et al. (1991) in rat aortic rings. Moreover, our findings may have clinical significance.

4.4. Conclusion

Acute NO synthase inhibition produced marked quick-onset systemic but delayed-onset pulmonary hypertension. When NO synthase was inhibited, TP receptor-mediated pulmonary hypertension was (a) substantially potentiated,

(b) reversed by L-arginine, and (c) antagonized by the selective TP receptor antagonist, SQ 29,548. The present findings indicate that, under normal circumstances, pulmonary vasomotor tone is regulated by spontaneously released NO. Moreover, pulmonary vascular NO attenuates TP receptor-mediated pressor responses, strongly suggesting that in addition to mediating pulmonary vasoconstriction, TP receptor activation also concomitantly releases NO within the rat pulmonary vasculature in vivo.

References

Barnes, P.J. and S.-F. Liu, 1995, Regulation of pulmonary vascular tone, Pharmacol. Rev. 47, 87.

Bertolino, F., J.-P. Valentin, M. Maffre, A.M. Bessac and G.W. John, 1995a, TxA_2 receptor activation elicits organ-specific increases in microvascular permeability in the rat, Am. J. Physiol. 268, R366.

Bertolino, F., J.-P. Valentin, M. Maffre, A.M. Bessac, F. Grelac, J. Maclouf, S. Lévy-Toledano, A. Delhon, F.C. Colpaert and G.W. John, 1995b, Intrinsic activity of the non-prostanoid thromboxane A_2 receptor antagonist, daltroban (BM 13,505), in human platelets in vitro and in the vasculature of the rat in vivo, Br. J. Pharmacol. 115, 210.

Carrithers, J.A., D. Brown, F. Liu and J.A. Orr, 1994, Thromboxane A_2 mimetic U-46619 induces systemic and pulmonary hypertension and delayed tachypnea in the goat, J. Appl. Physiol. 77, 1466.

Chyu, K.-Y., P.H. Guth and G. Ross, 1992, Effect of N^{ω}-nitro-L-arginine methyl ester on arterial pressure and on vasodilator and vasoconstrictor responses: influence of initial vascular tone, Eur. J. Pharmacol. 212, 159.

Coleman, R.A., W.L. Smith and S. Narumiya, 1994, International union of pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes, Pharmacol. Rev. 46, 205.

Cooper, C.J., M.J. Landzberg, T.J. Anderson, F. Charbonneau, M.A. Creager, P. Ganz and A.P. Selwyn, 1996, Role of nitric oxide in the local regulation of pulmonary vascular resistance in humans, Circulation 93, 266.

Dinh-Xuan, A.T., T.W. Higenbottam, C.A. Clelland, J. Pepke-Zaba, G. Cremona, A.Y. Butt, S.R. Large, F.C. Wells and J. Wallwork, 1991, Impairment of endothelium-dependent pulmonary artery relaxation in chronic obstructive lung disease, New Engl. J. Med. 324, 1539.

Fineman, J.R., R. Chang and S.J. Soifer, 1992, EDRF inhibition augments pulmonary hypertension in intact newborn lambs, Am. J. Physiol. 262, H1365.

Folger, W.H., D. Lawson, C.S. Wilcox and J.L. Mehta, 1991, Response of rat thoracic aortic rings to thromboxane mimetic U-46,619: roles of endothelium-derived relaxing factor and thromboxane A_2 release, J. Pharmacol. Exp. Ther. 258, 669.

Frostell, C., M.-D. Fatacci, J.C. Wain, R. Jones and W.M. Zapol, 1991, Inhaled nitric oxide. A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction, Circulation 83, 2038.

Gardiner, S.M.A., A.M. Compton, T. Bennet, R.M.J. Palmer and S. Moncada, 1990, Control of regional blood flow by endothelium-derived nitric oxide, Hypertension 15, 486.

Giaid, A., and D. Saleh, 1995, Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension, New Engl. J. Med. 333, 214.

Hasunuma, K., T. Yamaguchi, D.M. Rodman, R.F. O'Brien and I.F. McMurty, 1991, Effects of inhibitors of EDRF and EDHF on vasoreactivity of perfused rat lungs, Am. J. Physiol. 260, L97.

Hyman, A.L., P.J. Kadowitz and H.L. Lippton, 1989, Methylene blue selectively inhibits pulmonary vasodilator responses in cats, J. Appl. Physiol. 66, 1513.

Ignarro, L.J., R.E. Byrns and K.S. Wood, 1987, Endothelium-dependent modulation of cGMP levels and intrinsic smooth muscle tone in isolated bovine intrapulmonary artery and vein, Circ. Res. 60, 82.

Kaye, A.D., B.D. Nossaman, I.N. Ibrahim, B.J. DeWitt, C.-J. Feng and P.J. Kadowitz, 1995, Analysis of thromboxane receptor-mediated responses in the feline pulmonary vascular bed, Crit. Care Med. 23, 164.

Lahera, V., M.G. Salom, F. Miranda-Guardiola, S. Moncada and J.C. Romero, 1994, Effects of N^{G}-nitro-L-arginine methyl ester on renal function and blood pressure, Am. J. Physiol. 267, L120.

McGregor, M. and A. Sniderman, 1985, On pulmonary vascular resistance: the need for more precise definition, Am. J. Cardiol. 55, 217.

McMahon T.J., J.S. Hood, J.A. Bellan and P.J. Kadowitz, 1991, N^{ω}-nitro-L-arginine methyl ester selectively inhibits pulmonary vasodilator responses to acetylcholine and bradykinin, J. Appl. Physiol. 71, 2026.

Moncada, S. and A. Higgs, 1993, The L-arginine-nitric oxide pathway, New Engl. J. Med. 329, 2002.

Nishiwaki, K., D.P. Nylan, P. Rock, P.M. Desai, W.P. Peterson, C.G. Pribble and P.A. Murray, 1992, N^{ω}-Nitro-L-arginine and pulmonary vascular pressure-flow relationship in conscious dogs, Am. J. Physiol. 262, H1331.

Nossaman, B.D., T.J. McMahon, M.S. Ragheb, I.N. Ibrahim, C.R. Babycos, J.S. Hood and P.J. Kadowitz, 1992, Blockade of thromboxane/endoperoxide receptor-mediated responses in the pulmonary vascular bed of the cat by sulotroban, Eur. J. Pharmacol. 213, 1.

Ogletree, M.L., D.N. Harris, R. Greenberg, M.F. Haslanger and M. Nakane, 1985, Pharmacological actions of SQ 29,548, a novel selective thromboxane antagonist, J. Pharmacol. Exp. Ther. 234, 435.

Peacock, A.J., 1995, Treatment of pulmonary hypertension, Drugs Today 31, 201.

Pepke-Zaba, J., T. Higenbottam, A. Dinh-Xuan, D. Stone and J. Wallwork, 1991, Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension, Lancet 338, 1173.

Perrella, M.A., F.L. Hildebrand Jr., K.B. Margulies and J.C. Burnett Jr., 1991, Endothelium-derived relaxing factor in regulation of basal cardiopulmonary and renal function, Am. J. Physiol. 261, R323.

Rees, D.D., R.M.J. Palmer and S. Moncada, 1989, Role of endothelium-derived nitric oxide in the regulation of blood pressure, Proc. Natl. Acad. Sci. USA 86, 3375.

Rubin, L.J., 1995, Pathology and pathophysiology of primary pulmonary hypertension, Am. J. Cardiol. 75, 51A.

Stamler, J.S., E. Loh, M.-A. Roddy, K.E. Currie and M.A. Creager, 1994, Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans, Circulation 89, 2035.

Wang, Y.-X., I. Gavras, T. Wierzba, B. Lammek and H. Gavras, 1992, Inhibition of nitric oxide, bradykinin, and prostaglandins in normal rats. Hypertension 19 (Suppl. 2), 255.

Wang, Y.-X., S.L. Lim and C.C.Y. Pang, 1995, Increase by N^{G}-nitro-L-arginine methyl ester (L-NAME) of resistance to venous return in rats, Br. J. Pharmacol. 114, 1454.

Wiklund, N.P., M.G. Persson, L.E. Gustafsson, S. Moncada and P. Hedqvist, 1990, Modulatory role of endogenous nitric oxide in pulmonary circulation in vivo, Eur. J. Pharmacol. 185, 123.

Xue, C., A. Rengasamy, T.D. Le Gras, P.A. Koberna, G.C. Dailey and R.A. Johns, 1994, Distribution of NOS in normoxic vs. hypoxic rat lung: upregulation of NOS by chronic hypoxia, Am. J. Physiol. 267, L667.