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# Nitric oxide (NO) modulation of PAF-induced cardiopulmonary action: interaction between NO synthase and cyclo-oxygenase-2 pathways

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**1** To further investigate into the mechanisms of PAF-induced cardiopulmonary actions, we examined the effects of the nitric oxide synthase (NOS) inhibitor L-N$^{\omega}$-nitro-L-arginine (L-NNA), of the specific cyclooxygenase-2 (COX-2) inhibitor NS 398, and of the combined presence of both COX and NOS inhibitors on the PAF responses in the heart lung preparation of guinea-pig (HLP).

**2** In HLPs perfused with homologous blood, dose-response curves for the haemodynamic and bronchial effects of PAF (1–32 ng) were carried out in the absence or presence of L-NNA (200 $\mu$M). L-NNA caused an increase in the resting pulmonary arterial pressure (PAP) without affecting the other basal values, and strongly potentiated the bronchoconstriction and pulmonary hypertension elicited by PAF. An enhancement of the PAF-induced actions on right atrial pressure (RAP) and cardiac output (CO) was also observed. All the effects of L-NNA were antagonized by L-arginine (2 mM).

**3** The presence of L-NNA in the perfusing blood of HLPs failed to affect the pulmonary hypertensive and bronchoconstrictor responses induced by the thromboxane $A_2$ mimetic U46619 (0.05–1.6 $\mu$g), 5-hydroxytryptamine (0.1–1.6 $\mu$g), and histamine (0.1–1.6 $\mu$g), thus suggesting that these PAF secondary mediators are not responsible for the hyper-responsiveness to PAF induced by L-NNA.

**4** Blocking COX-2 pathway with NS 398 (15–30 $\mu$M) did not alter the cardiopulmonary resting variables. However, a reduction of the PAF-mediated pulmonary hypertension, but not of bronchoconstriction, was observed. When L-NNA was added to the perfusing medium of HLPs pre-treated with NS 398 or with indomethacin (15 $\mu$M), the basal PAP values were enhanced. However, in the combined presence of COX and NOS inhibitors, only a slight increase in the hypertensive responses to the highest doses of PAF was observed, whereas the PAF mediated actions at bronchial and cardiac level were unaffected.

**5** This study indicates that (i) the cardiopulmonary actions induced by PAF are specifically modulated by endogenous NO through the NOS pathway, and (ii) COX-2 isoform is involved in the pulmonary hypertensive, but not bronchoconstrictor, effects of PAF. Furthermore, an interaction between PAF stimulated COX, particularly COX-2, and NOS pathways appears to take a functional role at both bronchial and cardiovascular level.

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**Keywords:** Platelet activating factor; nitric oxide; cyclo-oxygenase-2; thromboxane $A_2$; L-NNA; NS 398; pulmonary hypertension; bronchoconstriction; heart-lung preparation; guinea-pig

**Abbreviations:** ANOVA, analysis of variance; CO, cardiac output; COX-1, cyclo-oxygenase-1; COX-2, cyclo-oxygenase-2; HLP, heart lung preparation; L-NNA, L-N$^{\omega}$-nitro-L-arginine; NO, nitric oxide; NS-398, N-(2-cyclohexyloxy-4-nitrophenyl)-methanesulphonamide; PAP, pulmonary arterial pressure; RAP, right atrial pressure; $TxA_2$, thromboxane $A_2$

## Introduction

The platelet-activating factor (PAF), the phospholipid mediator generated in inflammatory and allergic reactions, exerts important effects at both the cardiovascular and pulmonary levels. Intravenous administration of PAF has been shown to induce systemic hypotension, pulmonary vascular hypertension and bronchoconstriction (Braquet *et al*., 1987; Goldstein *et al*., 1991). It is also well established that pulmonary functions are modulated by nitric oxide (NO)–the endogenous gas released from both vascular endothelium (Ignarro *et al*., 1987; Palmer *et al*., 1987) and airway epithelium (Folkerts & Nijkamp, 1998) – which contributes to the maintenance of vascular and bronchial tone.

Athough the effects and mechanisms of PAF-induced bronchoconstriction have been extensively studied (Iwama *et al*., 1988; Smith *et al*., 1988) and the bronchodilator action of both endogenous (Gillespie & Sheng, 1988; Buga *et al*., 1989) and exogenous (Dupuy *et al*., 1992; Albertini & Clement, 1995) NO has been well demonstrated, to the best of our knowledge, the functional role of the endogenously produced NO on the bronchoconstriction by PAF has not

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been examined. Moreover, controversial data have been reported on the role of endogenous NO in PAF-induced effects at cardiovascular level, depending on the experimental model, the district and species examined. In rats, endothelially released NO appeared to contribute to the vasodilator action of PAF in *in vitro* preparations (Moritoki *et al*., 1992; Kamata *et al*., 1996), but not to the Paf-induced systemic hypotension in *in vivo* experiments (Yoshikawa *et al*., 1997). Conversely, a contribution of NO to the systemic hypotension induced by PAF has been demonstrated in dogs *in vivo*, but with opposite results concerning the involvement of NO in pulmonary hypertension (Noguchi *et al*., 1996; Wang *et al*., 1998). Therefore, the first aim of our work was to examine the role of endogenous NO on PAF-induced cardiopulmonary actions in the blood-perfused heart-lung preparation of guinea-pig (HLP), which may be considered an intermediate situation between *in vitro* and *in vivo* experimental models, and makes it possible to simultaneously assess bronchial, pulmonary vascular and cardiac parameters.

In this preparation (Argiolas *et al*., 1995), like in different experimental models and species (Heffner *et al*., 1983; Hu & Man, 1991; Mentz *et al*., 1992; Uhlig *et al*., 1994). PAF-induced effects have been ascribed to the relative contribution of various secondary mediators from platelets, such as histamine, 5-hydroxytryptamine (5-HT), thromboxane A2 ($TxA_2$), and from lung tissues, particularly arachidonic acid (AA) metabolites through cyclo-oxygenase (COX) and lipoxygenase (LOX) pathways. We have already suggested that the release of COX products plays a greater role in PAF-induced pulmonary hypertension than in bronchoconstriction. At the moment, two isoforms of COX have been identified (Vane *et al*., 1994; Kurumbail *et al*., 1996; Mitchell & Warner, 1999), namely the constitutive form (COX-1), which is associated with cellular homeostasis, and the inducible form (COX-2). The latter is expressed in a variety of tissues, including human pulmonary epithelial cells (Mitchell *et al*., 1994) and rat lungs (Uhlig *et al*., 1996), in response to various stimuli (DeWitt & Smith, 1995). Since the expression of COX-2 has also been recently found in response to PAF in experiments on cell cultures from various tissues (Alvi *et al*., 1999; Bazan *et al*., 1997; Serou *et al*., 1999), a further purpose of this study was to verify whether the COX-2 isoform contributes to PAF-induced cardiopulmonary actions.

Finally, several studies have given evidence that COX and NO synthase (NOS) pathways, when stimulated by agents like lipopolysaccharides (LPS), cytokines, calcium ionophore, and arachidonic acid, interact with each other. But the results obtained so far are contrasting and the functional effects of this interaction have not been fully elucidated. The synthesis of endogenous NO through NOS pathway enhanced COX activity and prostaglandins production both in *in vivo* (Sautebin *et al*., 1995) and in cell culture models (Salvemini *et al*., 1993; Tetsuka *et al*., 1994; Davidge *et al*., 1995; Watkins *et al*., 1997). Conversely, the inhibition of COX-2 induction and activity by NO has also been shown in LPS-activated rat macrophages (Swierkosz *et al*., 1995). Contrasting data have also been reported as far as the cross-talk of COX on NOS pathway is concerned. The inhibition of the COX pathway had no effect on NOS activity in rat macrophages (Swierkosz *et al*., 1995), whereas it allowed the NOS pathway to reveal itself in canine saphenous vein (Illiano *et al*., 1996). Therefore, in order to add some insight into the mechanisms and the functional effect of this phenomenon, the potential interaction between COX, presumably COX-2, and NOS pathways was investigated in response to stimulation by PAF in our experimental model.

Some of these results have been published in abstract form (del Basso *et al*., 1999).

## Methods

### *Heart lung preparation of guinea-pig*

The experimental procedure and the techniques to measure the haemodynamic and bronchial parameters have been described in detail previously (Argiolas *et al*., 1995) and will be given only briefly here. Blood, obtained through a carotid cannula from donor Hartley Duncan guinea-pigs (700–800 g) anaesthetized with ethyl urethane (1.5 g $kg^{-1}$ i.p.), was collected in the presence of heparin (7.5 I.U. $ml^{-1}$). HLPs were performed on male guinea-pigs (350–400 g), anaesthetized with 1.5 g $kg^{-1}$ i.p. ethyl urethane, under artificial ventilation with 5% $CO_2$ in air (miniature Starling pump, frequency 50 $min^{-1}$; stroke 6 ml). A Starling peripheral resistance (60 mmHg), an electromagnetic flowmeter probe and a collecting thermoregulated reservoir containing 50 ml of blood, were inserted in the extracorporeal system. The peripheral resistance was connected with the aortic cannula and the reservoir with the venous cannula. Right and left atrial pressures (RAP and LAP), pulmonary arterial (PAP), left ventricular (LVP) and aortic (AoP) pressures were recorded by means of Statham electromanometers on a Grass multichannel polygraph (mod.7), together with the cardiac output (CO; measured with a Skalar MDL 1401 electromagnetic flowmeter), heart rate (HR) and ventilation overflow (Basile bronchospasm transducer, mod 7020). Signals of haemodynamic variables were transmitted to an MS DOS computer *via* an analog-digital converter, and then analysed. Pulmonary vascular resistance (PVR) was calculated by the computer according to the following formula: $PVR = (PAP-LAP) \cdot CO^{-1}$. Blood gases and pH determinations were made using a Radiometer blood gas analyzer (ABL 30).

### *Experimental protocol*

PAF, U 46619, histamine, and 5-hydroxytryptamine (5-HT) containing solutions (10–100 $\mu$l) were administered by bolus injection into the venous cannula at least 15 min after stable values of all parameters were recorded. Each dose increment was initiated on return of parameters to pre-injection or to stable values and, in any case, a period of at least 15 min was allowed to elapse between each dose increment. When dose-response curves were performed, only one dose-response relationship was tested in each animal for each different treatment, unless otherwise stated. Inhibitors were added to the perfusing blood after the surgical procedure was over and allowed to circulate at least for 20 min before PAF was administered.

### *Drugs*

All chemicals used were of analytical grade. PAF (L-$\alpha$-phosphatidylcholine,$\beta$-acetyl-$\gamma$-O-hexadecyl), U 46619 (9,11-dideooxy-11$\alpha$,9$\alpha$-epoxy-methanoprostaglandin F2$\alpha$), hista-

mine dihydrochloride, 5-hydroxytryptamine creatinine sulphate, indomethacin, L-arginine, L-NNA ($N^{\omega}$-nitro-L-arginine) were obtained from Sigma Chemical Co (St. Louis, MO, U.S.A.). NS 398 (N-(2-cyclohexyloxy-4-nitrophenyl)-methanesulphonamide) was obtained by Calbiochem (Inalco Spa, Milano, Italy).

PAF was dissolved to a concentration of 1 mg $ml^{-1}$ in 0.9% saline and stored frozen. Working solutions were prepared from the stock solution and were diluted on a daily basis with saline solution. NS 398 was dissolved in small volume of dimethylsulphoxide (DMSO). Indomethacin was dissolved in a small amount of absolute ethanol and sodium bicarbonate (150 mM). They were then diluted further with physiological solution as appropriate; the final concentration of DMSO or of ethanol, respectively, never exceeded 0.01% (v $v^{-1}$) in the perfusing blood. 5-HT was dissolved in 0.1% ascorbic acid and kept at $+4^{\circ}C$. All the other drugs were dissolved in distilled water.

### *Data analysis*

Data are expressed as means ± standard error of the means and *n* indicates the number of experiments in each group. The differences between the PAF, U 46619, histamine, and 5-HT dose-response curves in the absence and presence of NOS, COX-1 and COX-2 inhibitors were made by repeated measures ANOVA with Bonferroni-Dunn's procedure for multiple comparison, calculated by a Macintosh LC630 computer using the data analysis package Stat View (Abacus Concepts, Inc., Berkeley, CA, U.S.A., 1992). A $P$ value $<0.05$ was considered to be significant, unless differently requested by the statistical analysis.

### *Ethics*

The doses of anaesthetics were consistent with those normally used in the laboratory practice and we followed the principles set forth in the Directive of the Council of the European Communities (86/609/EEC) on animal care and use.

## Results

### *Effects of the NO-synthase inhibitor L-NNA on PAF-induced cardiopulmonary responses in HLPs*

As already reported (Argiolas *et al*., 1995), bolus injections of PAF in the perfusing blood of control HLPs caused major effects at bronchial and pulmonary vascular levels. At the cardiac level, a slight increase in right atrial filling pressure and a decrease in CO were obtained, particularly evident after administration of the highest doses of PAF (Figure 1).

To verify the possible involvement of endogenous NO in PAF cardiopulmonary actions, experiments were carried out by adding the NOS inhibitor L-NNA (200 $\mu$M) to the perfusing blood of HLPs in the absence, or 5 min after administration, of the substrate for NOS, L-arginine (20 mM). As shown in Table 1, the presence of L-NNA in the perfusing blood, but not when combined with L-arginine, significantly increased PAP and PVR basal values in comparison to control HLPs, without affecting the bronchial and the other haemodynamic resting values. The effects of L-NNA and of the combined presence of L-NNA and L-arginine on the cardiopulmonary responses to PAF are summarized in Figure 1. In particular, as far as the bronchoconstriction produced by PAF is concerned, the presence of L-NNA potentiated the ventilation overflow responses, which were significantly higher than those of the control ($P<0.001$) and L-NNA + L-arginine ($P<0.005$) groups. Moreover, as in the case of bronchoconstriction, L-NNA significantly enhanced PAF-induced pulmonary hypertension, and the dose-response curves for both PAP and the calculated PVR were significantly higher than the responses of the control and L-NNA + L-arginine groups ($P<0.001$ for both groups). The potentiating effects of L-NNA on PAF-induced broncho- and pulmonary vascular constrictions were more evident at the lowest than at the highest doses of PAF. Indeed, a strong hypertension and bronchoconstriction occurred even after subliminal doses of PAF (1 – 2 ng), i.e. at doses which did not induce by themselves any functional response in control preparations. Some changes in PAF-induced cardiac responses were also observed. Namely, the increase in RAP and the reduction of CO produced by PAF were significantly potentiated by L-NNA as compared to the response of controls ($P<0.001$ for all comparisons). Conversely, the action of PAF on LAP, LVP, heart rate and AoP (data not shown) were unaffected. In HLPs perfused with blood containing L-arginine, further addition of L-NNA did not induce any hyper-responsiveness to PAF, and the dose-response curves for the bronchial and haemodynamic variables overlapped those of the control group ($P>0.05$ for all variables).

### *Effects of L-NNA on the cardiopulmonary effects induced by U 46619, 5-hydroxytryptamine and histamine*

To investigate whether secondary mediator(s) produced by PAF may be responsible for the potentiation by L-NNA of PAF cardiopulmonary actions, dose-response curves to the exogenous administration of several substances which may be involved in vasoconstrictor and bronchoconstrictor PAF-induced actions (Argiolas *et al*., 1995) were carried out in the presence and absence of the NOS inhibitor.

As shown in Figure 2, increasing doses of the $TxA_2$ receptor agonist, U 46619, caused dose-dependent bronchoconstriction and pulmonary hypertension, with a slight decrease in CO in control HLPs. The presence of L-NNA in the perfusing blood significantly enhanced ($P<0.05$) the resting PAP values, which were higher ($16.8 \pm 0.8$ $cmH_2O$; $n=6$) than those of controls ($14.1 \pm 0.3$ $cmH_2O$; $n=6$). However, the responses elicited by U46619 on PAP and PVR, as well as those on the bronchial and cardiac variables were unaffected by the NOS inhibitor ($P>0.05$ for all variables in comparison with control group), except for a significant potentiation of the action on CO ($P<0.01$).

In the same way as U 46619, histamine ($n=4$) and 5-HT ($n=4$) produced dose-dependent bronchoconstrictor and pulmonary hypertensive responses in HLPs (Figure 3). The addition of L-NNA to the perfusing blood enhanced the resting PAP values in both experimental groups ($18.1 \pm 0.5$ $cmH_2O$ *versus* $13.6 \pm 1.2$ $cmH_2O$ of control group for histamine; $21.6 \pm 1.0$ $cmH_2O$ *versus* $16.1 \pm 1.2$ $cmH_2O$ of control group for 5-HT; $P<0.05$ for

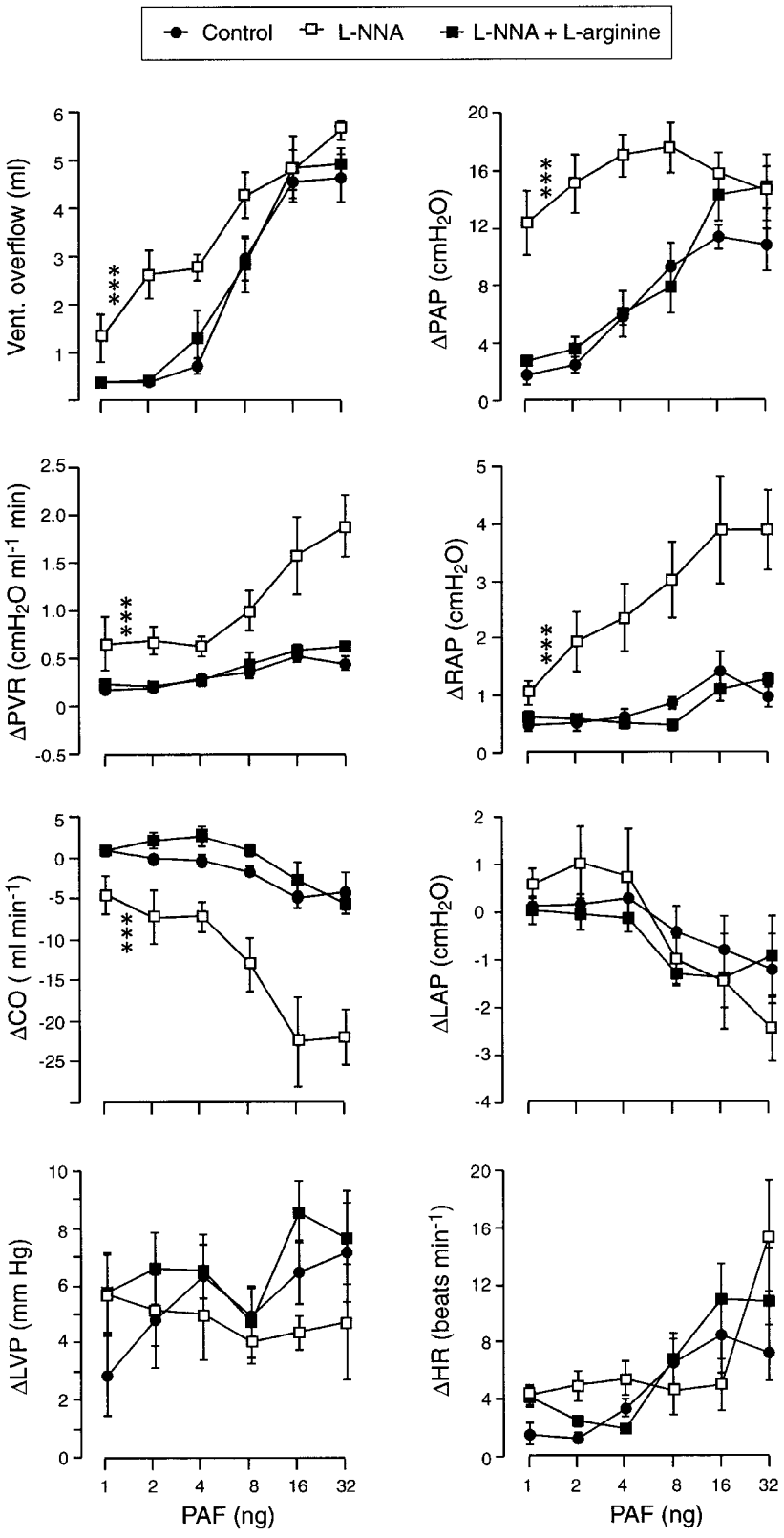


**Figure 1** Effects of 200 $\mu$M L-NNA ($n=7$) and of L-NNA + 20 mM L-arginine ($n=6$) on the responses of the bronchial and haemodynamic variables to increasing doses of PAF in heart lung preparations of guinea-pig. Vent. overflow = ventilation overflow: PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; RAP = right atrial pressure; CO = cardiac output; LAP = left atrial pressure; LVP = left ventricular pressure; HR = heart rate. Each point and bar represents the mean ± s.e.mean. Significance was assessed using ANOVA for repeated measure with Bonferroni's difference test: $***P<0.001$ in comparison with control group ($n=7$).

both substances by ANOVA). Nevertheless, the presence of the NOS inhibitor failed to significantly affect the cardiovascular and bronchial responses induced by histamine or 5-HT ($P>0.05$ for all comparisons).

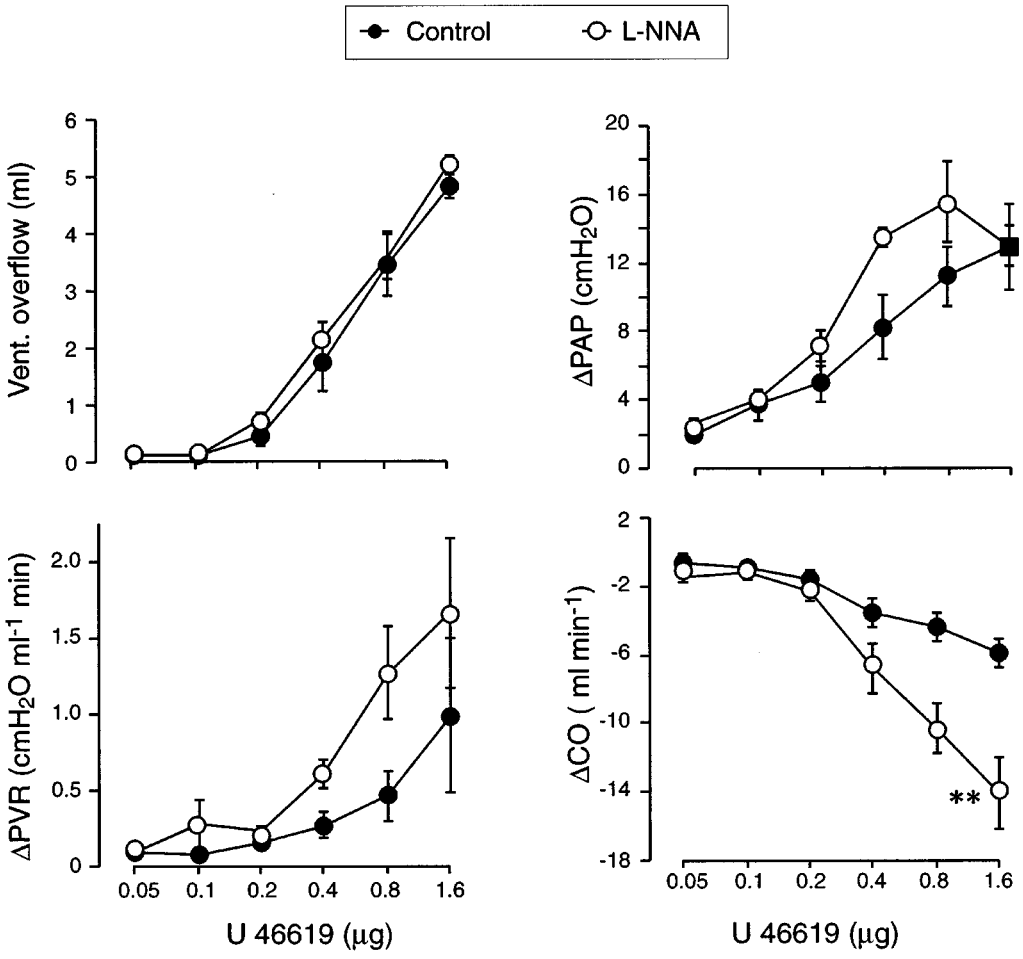


**Figure 2** The effect of L-NNA on the bronchial (Vent. overflow). pulmonary arterial pressure (PAP), pulmonary vascular resistance (PVR) and cardiac output (CO) dose-response curves evoked by U 46619 in heart lung preparations of guinea-pig. Each point and bar represents the mean±s.e.mean of six experiments. ANOVA for repeated measure with Bonferroni's difference test: $^{**}P<0.01$ *versus* control group.

**Table 1** Basal values for bronchial and haemodynamic variables of guinea-pig heart-lung preparations perfused with autologous blood

| | *Controls* | *L-NNA* | *L-NNA + L-arginine* | *Indo* | *Indo + L-NNA* | *NS 398* | *NS 398 + L-NNA* |
|---|---|---|---|---|---|---|---|
| Number of experiments | 7 | 7 | 6 | 7 | 6 | 7 | 7 |
| Ventilation overflow (ml) | 0.28±0.01 | 0.24±0.02 | 0.23±0.01 | 0.23±0.01 | 0.24±0.01 | 0.28±0.01 | 0.24±0.02 |
| Cardiac output (ml $min^{-1}$) | 44.2±2.0 | 44.7±3.2 | 44.3±2.8 | 47.4±1.4 | 41.9±3.3 | 53.7±2.2 | 49.7±2.4 |
| Heart rate (beat $min^{-1}$) | 223±5 | 25±10 | 232±8 | 247±5 | 227±8 | 261±12 | 236±13 |
| Aortic pressure (mmHg) | 78.2±3.2 | 72.9±1.9 | 72.5±1.5 | 72.5±2.3 | 69.9±1.9 | 77.9±1.4 | 78.9±1.9 |
| Pulmonary arterial pressure ($cmH_2O$) | 14.2±0.5 | 19.3±0.9 *** | 14.1±0.8 | 13.7±0.6 | 19.6±0.7 *** | 15.0±0.5 | 18.1±0.7 ** |
| Pulmonary resistance (cm $H_2O$ min $ml^{-1}$) | 0.27±0.01 | 0.39±0.03 ** | 0.24±0.02 | 0.23±0.01 | 0.39±0.04 *** | 0.22±0.01 | 0.34±0.02 ** |
| Right atrial pressure ($cmH_2O$) | 2.5±0.3 | 2.6±0.3 | 3.0±0.2 | 2.9±0.4 | 2.7±0.2 | 2.8±0.5 | 2.5±0.4 |
| Left atrial pressure ($cmH_2O$) | 3.0±0.4 | 2.8±0.3 | 3.7±0.4 | 3.8±0.4 | 3.9±0.5 | 3.9±0.3 | 3.7±0.1 |
| Left ventricular pressure (mmHg) | 57.4±4.0 | 55.4±1.9 | 55.3±1.5 | 55.0±3.2 | 55.8±5.4 | 49.3±1.8 | 47.3±2.2 |
| Blood $PCO_2$ (mmHg) | 40.4±0.6 | 40.0±0.6 | 39.8±1.0 | 40.9±1.2 | 39.9±1.5 | 40.2±0.5 | 39.6±0.5 |
| Blood $PO_2$ (mmHg) | 155.6±3.4 | 158.6±1.4 | 159.9±3.3 | 152.0±2.1 | 157.7±0.4 | 150.5±2.4 | 148.6±2.6 |
| Blood pH | 7.3±0.01 | 7.3±0.02 | 7.3±0.02 | 7.3±0.02 | 7.3±0.02 | 7.4±0.02 | 7.3±0.02 |

$^{***}P<0.0001$ as compared with its own control group (ANOVA). $^{**}P<0.005$.

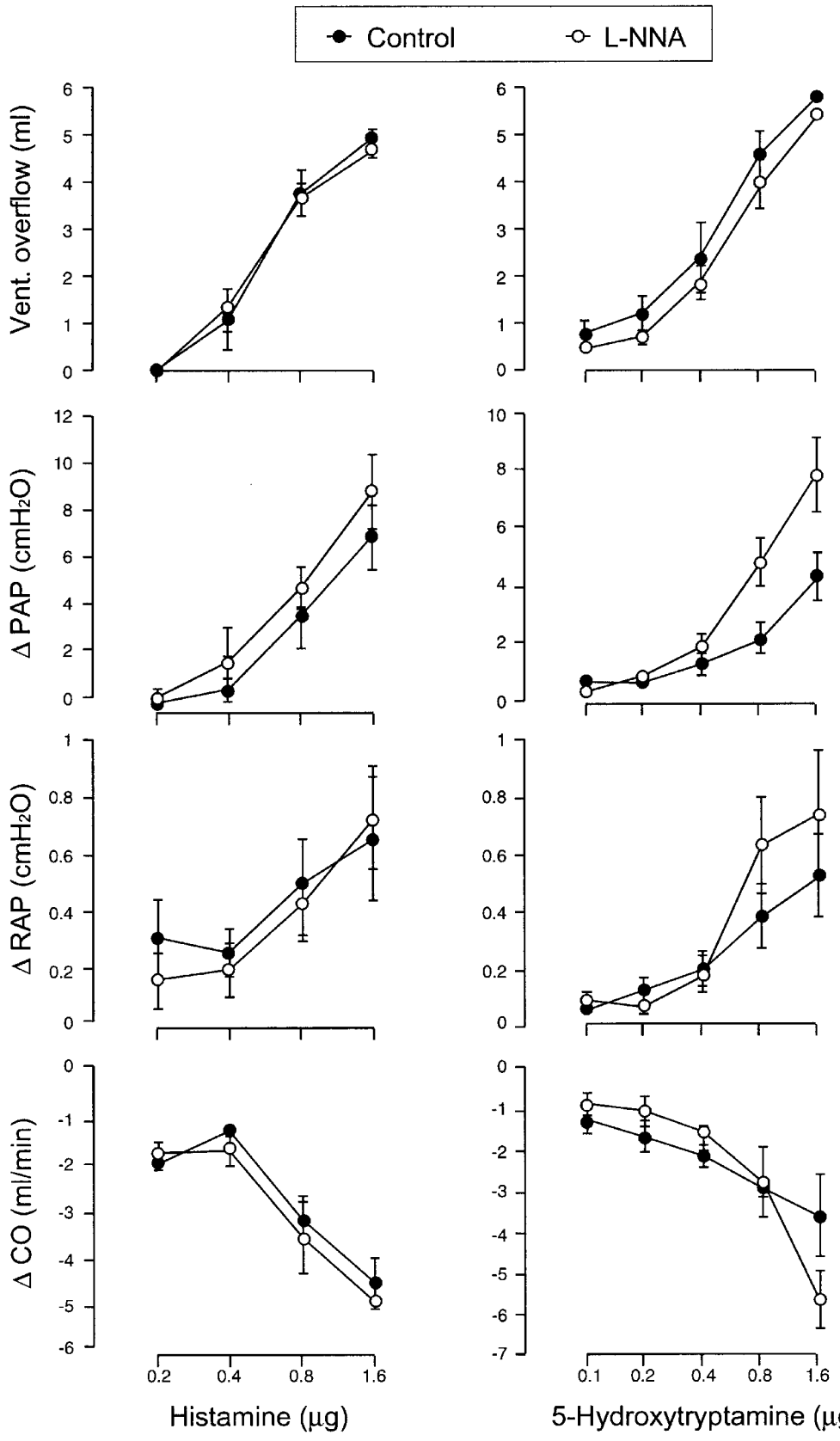


**Figure 3** Effects of L-NNA on the bronchial (Vent. overflow), pulmonary vascular pressure (PAP), right atrial pressure (RAP) and cardiac output (CO) dose-response curves to Histamine (left panel) and 5-Hydroxytryptamine (right panel) in heart lung preparations of guinea-pig. Each point and bar represents the mean ± s.e.mean of four experiments. ANOVA for repeated measure with Bonferroni's difference test: $P > 0.05$ *versus* control group for all comparisons.

*Effects of the specific COX-2 inhibitor, NS 398, on PAF-induced cardiopulmonary responses*

The involvement of the COX-2 pathway in the PAF-induced cardiopulmonary responses was assessed by adding the specific inhibitor NS 398 (15–30 $\mu$M) to the perfusing blood of HLPs. The presence of NS 398 did not affect the bronchial or haemodynamic basal values of HLPs (Table 1). However, as shown by a typical tracing (Figure 4), in the presence of the COX-2 inhibitor the pulmonary hypertensive, but not the bronchoconstrictor, effects of PAF were strongly reduced in comparison to control preparations. Accordingly, the dose-response curves to PAF for PAP and PVR were significantly shifted to the right by the presence of NS 398 in comparison to controls ($P<0.001$ for $\Delta$PAP and $P<0.005$ for $\Delta$PVR), whereas the bronchoconstrictor and the cardiac effects of PAF were unaffected (Figure 5). A reduction of PAF action on RAP was also observed in the presence of NS 398 ($P<0.005$ *versus* controls), probably attributable to the reduced pulmonary hypertension. These data are in agreement with data already obtained in HLPs utilizing the unspecific COX inhibitor indomethacin (Argiolas *et al*., 1995).

*Effects of the combined presence of NS 398 or indomethacin and L-NNA on PAF-induced cardiopulmonary responses*

Further experiments were carried out adding L-NNA to HLPs perfused with blood pretreated with NS 398 (15–30 $\mu$M) or with indomethacin (15 $\mu$M) to verify the possible interaction between PAF stimulated COX, particularly COX-2, and NOS pathways.

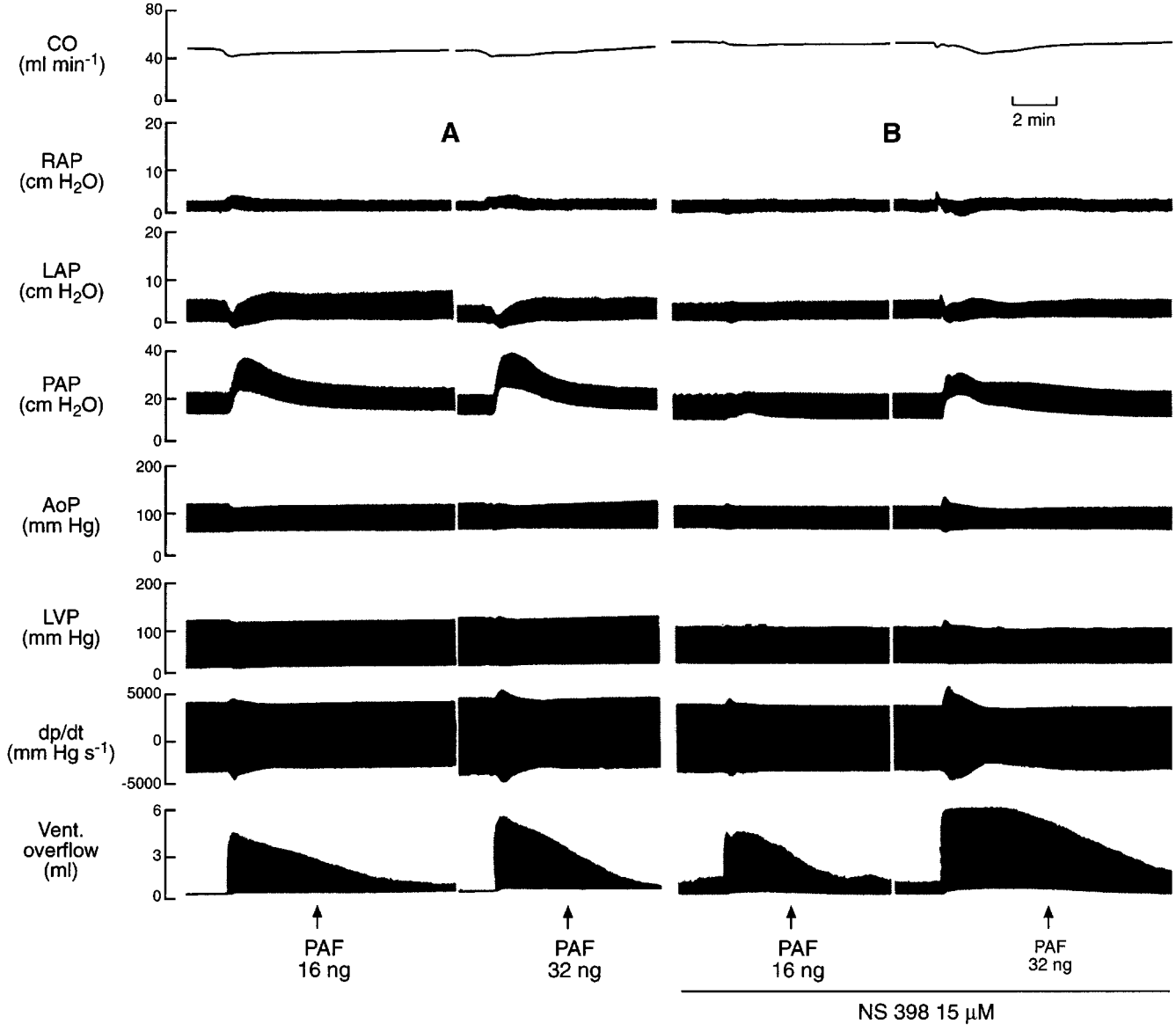


**Figure 4** Two representative original tracings showing the effects of the presence of NS 398 (B) on the bronchial and haemodynamic responses induced by high doses of PAF on the heart lung preparation of guinea-pig. (A) Control preparation. CO = cardiac output; RAP = right atrial pressure; LAP = left atrial pressure; PAP = pulmonary arterial pressure; AoP = aortic pressure; LVP = left ventricular pressure; dp/dt = first derivative of LVP; Vent. overflow = resistance to air inflation.

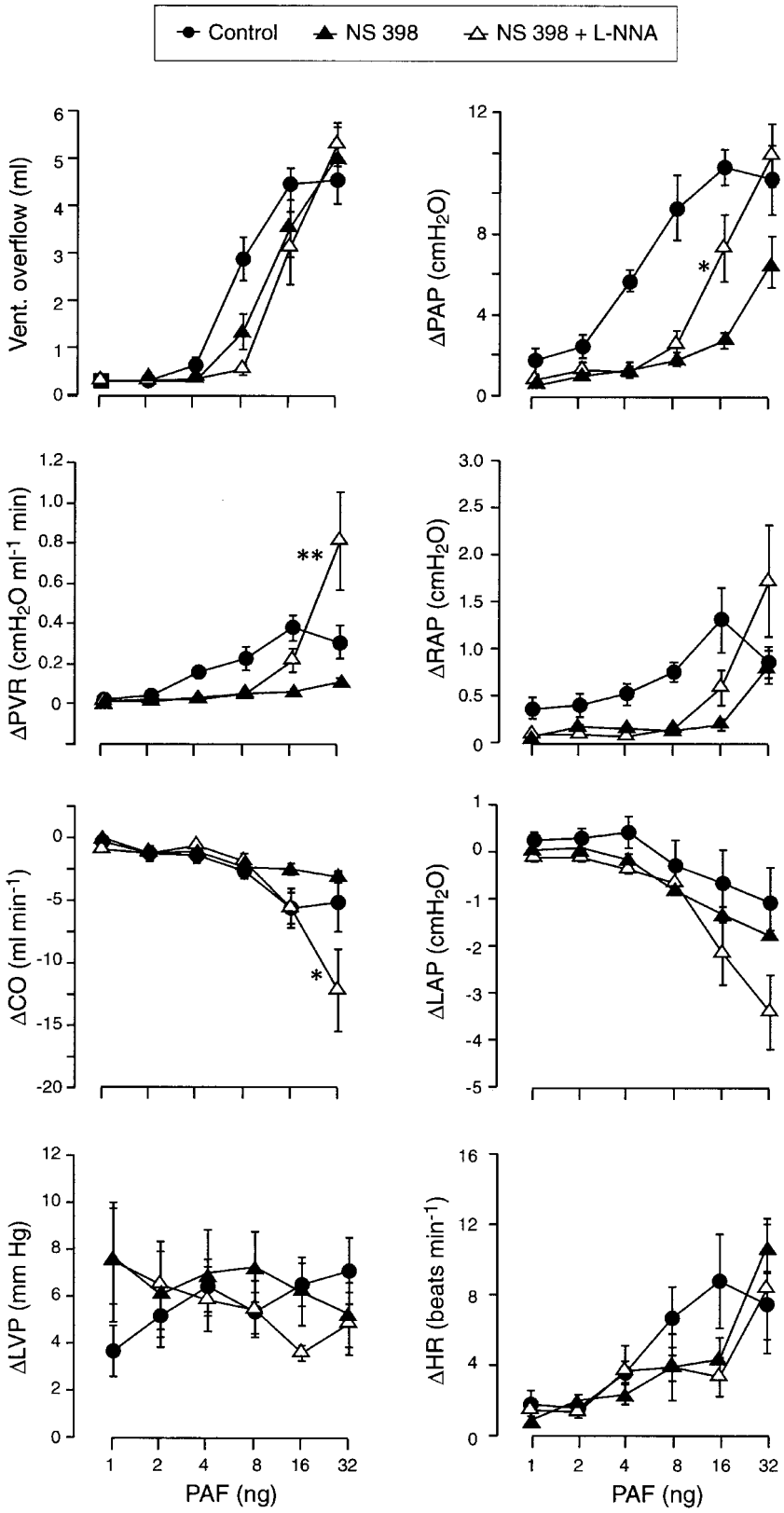


**Figure 5** The effect of L-NNA on the dose response curves for the bronchial and haemodynamic responses induced by PAF in heart lung preparations of guinea-pig perfused with blood containing NS 398 (15–30 $\mu$M). Controls are the same as those in Figure 2. Vent. overflow = ventilation overflow; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; RAP = right atrial pressure; CO = cardiac output; LAP = left atrial pressure; LVP = left ventricular pressure; HR = heart rate. Each point and bar represents the mean ± s.e.mean of seven experiments. ANOVA for repeated measure with Bonferroni's difference test: $^{**}P<0.01$ and $^{*}P<0.05$ when NS 398 + L-NNA is compared with NS 398 alone.

The presence of indomethacin, as above reported for NS 398, did not significantly affect any of the haemodynamic and bronchial baseline values, but the further addition of L-NNA produced a significant enhancement of PAP and PVR resting values in both groups (Table 1).

As summarized in Figures 5 and 6, after the blockade of COX-2 by NS 398 or of COX 1/2 by indomethacin, respectively, the addition of L-NNA to the perfusing blood did not cause any significant hyper-responsiveness of the bronchial responses to PAF. Indeed, the dose-response curves to PAF for ventilation overflow in the combined presence of the COX and NOS inhibitors overlapped those of HLPs containing the COX inhibitor alone ($P>0.05$ for all comparisons). Conversely, a slight but significant enhancement of PAF induced pulmonary hypertension – particularly evident at the highest doses – was observed in the combined presence of L-NNA and NS-398 ($P<0.05$ and $P<0.01$ for ΔPAP and ΔPVR, respectively, in comparison with NS 398 alone) or indomethacin ($P<0.01$ for ΔPAP and $P<0.05$ for ΔPVR, respectively, in comparison with indomethacin alone). In the presence of NS 398 or indomethacin, the addition of L-NNA did not modify the responses induced by PAF on all other haemodynamic variables, except for a slight potentiation of CO reduction ($P<0.05$), observed in HLPs perfused with NS 398 + L-NNA when compared with NS-398 alone.

## Discussion

As already described (Argiolas *et al*., 1995), the administration of PAF in HLPs caused major effects at the pulmonary level, inducing bronchoconstriction and pulmonary hypertension, with slight changes in RAP and CO. In the present experiments, both the bronchial and pulmonary vascular responses to PAF were strongly potentiated by the presence of the NOS inhibitor, L-NNA. This hyper-responsiveness to PAF was particularly evident at far lower doses, which were without any effect in control preparations. Moreover, an enhancement of the PAF-induced increase in RAP and a severe reduction of CO were also observed. Since L-NNA failed to potentiate the PAF-induced responses when a high concentration of L-arginine, the substrate for NO synthase,

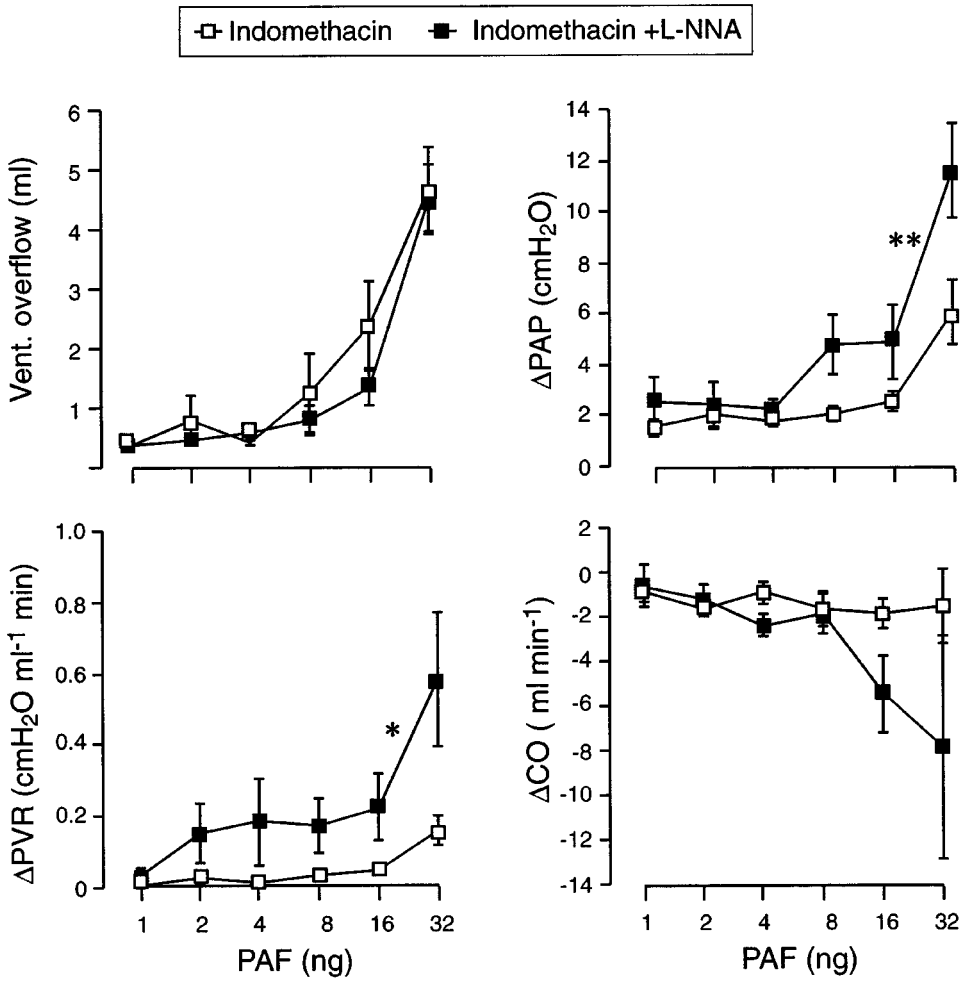


**Figure 6** The effect of L-NNA on the dose response curves for the bronchial and haemodynamic responses induced by PAF in heart lung preparations of guinea-pig perfused with blood containing indomethacin (15 $\mu$M). Vent. overflow = ventilation overflow; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; CO = cardiac output. Each point and bar represents the mean ± s.e.mean. ANOVA for repeated measure with Bonferroni's difference test: $^{**}P<0.01$ and $^{*}P<0.05$ when indomethacin + L-NNA ($n=6$) is compared with indomethacin alone ($n=7$).

was simultaneously present in the blood, our data suggest that NO – endogeneously generated through the L-arginine: NO pathway – acts as a counter-regulatory mechanism to attenuate not only the bronchoconstrictor and pulmonary hypertensive, but also the cardiac actions induced by PAF.

As far as the bronchoconstriction by PAF is concerned, to our knowledge, no data have been so far reported on the role of endogenous NO. Therefore, our results indicating that NO – probably produced by the guinea-pig bronchial epithelium – modulates PAF induced bronchoconstriction may add some functional evidence to the various experimental approaches indicating NO as a suppressor of airway responsiveness (Folkerts & Nijkamp, 1998). Moreover, as mentioned in the Introduction, although it has been well demonstrated that exogenous NO is able to reverse PAF-induced pulmonary hypertension in pigs and dogs (Albertini & Clement, 1994; Sherif *et al*., 1996; Yamada *et al*., 1998), the involvement of endogenous NO remains still uncertain and discrepant results have been reported. Pretreatment with L-NAME did not affect PAF-dependent pulmonary hypertension in pigs (Albertini & Clement, 1994) and yielded controversial results in dogs. Indeed, in the latter species, PAF-induced pulmonary hypertension has been shown to be potentiated by L-NAME in a recent work of Wang *et al*. (1998), whereas it was unaffected by the same NOS inhibitor in previous experiments by Noguchi *et al*. (1996). In the latter work, however, an increase in the baseline PAP value after L-NAME was observed. Our present results on HLPs may give a reasonable explanation for these contrasting data. Somehow in agreement with both the results obtained in dogs, the block of NOS in our experiments caused not only a significant increase in PAP basal value, but also a potentiation of the pulmonary hypertension by PAF, which was especially evident after subliminal and low doses. Thus, the contrasting results in dogs may be ascribed, in addition to the different experimental conditions, also to the fact that the effect of L-NAME on pulmonary hypertension was verified by Noguchi *et al*. (1996) only after a single, namely the highest, dose of PAF, where the potentiating effect is less evident. Moreover, the data on PAF actions in HLPs may add some insight into the species-specificity of the experimental animal models utilized to investigate pulmonary hypertension. Indeed, the increase in PAP and PVR evoked by PAF in our experimental model is accompanied by a strong rise of RAP without changes in LAP or LVP. This indicates the involvement of a pre-capillary mechanism, which can lead to dysfunction of the right heart, followed by a decrease in CO. Conversely, PAF-induced pulmonary hypertension in cats and dogs caused a slight decrease (Bellan *et al*., 1992) or was without any effect on RAP (Noguchi *et al*., 1996), respectively. Therefore, our data substantiate the assumption that endogenous, as well as exogenous (Semigran *et al*., 1994), NO plays an important role in pre-capillary pulmonary hypertension, whereas it does not appear to be involved in the mechanisms leading to post-capillary pulmonary hypertension, as observed in dogs after sino-atrial denervation (Galinier *et al*., 1997). This suggests that the PAF-induced hypertension in the guinea-pig may represent a good experimental model to study the role of NO in pulmonary hypertension.

In previous experiments on HLPs (Argiolas *et al*., 1995), we have demonstrated that the release of secondary mediators from platelets – probably histamine, 5-HT, $TxA_2$ – plays an important role in both the bronchoconstriction and pulmonary hypertension induced by PAF. Therefore, we decided to verify whether these secondary mediators may be responsible for the endogenous release of NO, as reported for $TxA_2$ in isolated perfused rat kidneys (Zhang & Sassard, 1993; Ziyyat *et al*., 1996). The results, showing that the bronchial and pulmonary vascular actions induced by the exogenous administration of these substances were unaffected by L-NNA, seem to indicate that they are not involved in NO production by PAF. However, the involvement of $TxA_2$ on NO release at the cardiac level may not be excluded, since a potentiation by L-NNA of the reduction of CO induced by U 46619 was observed. Moreover, these experiments showing that L-NNA caused a significant increase in the resting PAP basal value in all groups – as in the PAF experiments – but did not potentiate the pulmonary hypertensive effects, may indicate that PAF hyper-responsiveness induced by L-NNA cannot be ascribed to the unspecific block of NO basal release by the vascular endothelium. Therefore, it may be speculated that the production of NO is induced by the direct stimulation of PAF receptors, as reported in rat mesenteric arterial bed *in vitro* (Kamata *et al*., 1996), or by PAF-produced secondary mediators different from those examined.

Besides the platelet derived secondary mediators, several experimental studies performed on various species (Laurindo *et al*., 1989; Yamanaka *et al*., 1992; Argiolas *et al*., 1995) have consistently demonstrated that the pulmonary hypertensive effect of PAF is also due to products of the COX pathway from lung tissues. Conversely, COX products appear of minor relevance in PAF-induced bronchoconstriction, which has been ascribed to both a direct effect of PAF and an indirect mechanism also mediated by AA metabolites from the LOX pathway (Iwama *et al*., 1988). The current data now extend these studies by showing that PAF-induced pulmonary hypertension is reduced by the COX-2 specific inhibitor NS 398, in a fashion analogous to indomethacin. This strongly suggests that COX-2 is activated or upregulated in response to PAF receptors located on the vessels to produce the metabolites involved in PAF-induced hypertension. Moreover, PAF has been confirmed not to stimulate the COX-2 isoform to produce bronchoconstrictor prostanoids from the bronchial tissue of guinea-pig, unlike the result reported for LPS in the rat (Uhlig *et al*., 1996).

In any case, the above discussed data appears to clearly indicate that both COX – COX-2 in particular – and NOS pathways are involved in PAF cardiovascular actions. Moreover, the results obtained in the presence of both NS 398 and L-NNA encourage us to suggest the existence of a functional interaction between these PAF-stimulated pathways. As a point of fact, after the blockade of COX, particularly of the COX-2 pathway, the addition of L-NNA failed to potentiate the bronchial and cardiac effects of the phospholipid mediator. This appears quite interesting considering that, as mentioned before, COX-2 does not seem to be involved in PAF-induced bronchoconstriction in our model, whereas the latter data allow us to hypothesize that the activation/expression of this enzyme by PAF may be of relevance for NO production by airways. As far as PAF-induced pulmonary hypertension is concerned, a slight enhancement of PAP responses by L-NNA, especially evident after the highest doses, was still observed in the presence of

COX inhibitors. However, as shown in Figure 7, the potentiation by L-NNA of the PAF-induced pulmonary hypertension was lower in the presence of NS 398 than in its absence, which suggests an effect of COX on the NOS pathway at the pulmonary vascular, as at the bronchial, level. To ascribe the reduced enhancement of PAF-induced pulmonary hypertension by L-NNA in the presence of NS 398 to an effect of NOS on COX activity may also be a matter of speculation. Anyway, our data from HLPs provide evidence that PAF-stimulated COX and NOS activities interact with each other, as reported for different stimulating agents *in vitro* (Tsai *et al*., 1994; Mollace *et al*., 1998; Watkins *et al*., 1997) and *in vivo* (Sautebin *et al*., 1995).

Finally, an interesting speculation may still be made taking into account the effects of COX and NOS inhibitors on the resting PAP values of HLPs. Indeed, unlike what happens with PAF-induced pulmonary hypertensive responses, the blockade of COX-1/2 did not affect the increase in the PAP resting values induced by L-NNA. Therefore, the interaction between COX and NOS pathways does not appear to have any functional role in the basal production of NO by vascular endothelial cells. This may lead us to hypothesize that PAF induces NO production through the activation/expression of the inducible NOS form, which has been shown to interact with the COX-2 isoform (Watkins *et al*., 1997).

In conclusion, our results indicate that the endogenous production of NO through the L-arginine-NO pathway plays an important modulatory role not only on the bronchoconstriction and pulmonary vascular hypertension produced by PAF, but also on the cardiac effects probably resulting from the pulmonary precapillary hypertensive action of the phospholipid mediator. Moreover, the activation/expression of the COX-2 pathway by lung tissue after PAF stimulation appears to be responsible for the release of the secondary mediators involved in the vascular, but not the bronchial effect of PAF. Nevertheless, an interaction between PAF-stimulated COX-2 and NOS pathways may be functionally relevant to PAF actions not only at the vascular but also at the bronchial level.

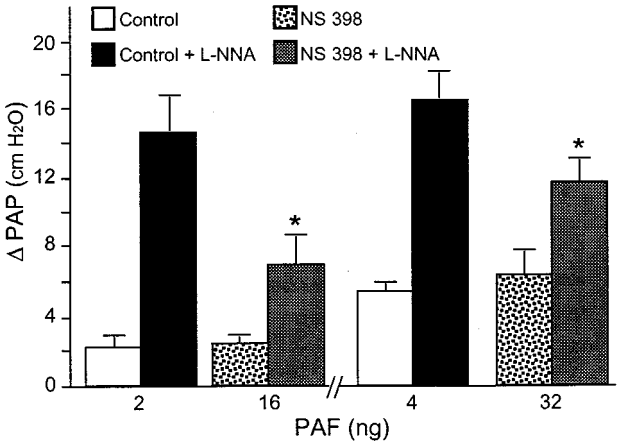


**Figure 7** The effect of L-NNA on the pulmonary hypertensive responses induced by PAF in heart lung preparations of guinea-pig in the absence or presence of NS 398 (data from Figure 5). At doses of PAF producing equipotent hypertensive responses in control and NS 398 treated group, the potentiation by L-NNA was higher in control than in NS 398 preparations. Each colums represents the mean ± s.e.mean of seven experiments. ANOVA: $*P<0.05$ when NS 398 + L-NNA is compared with Control + L-NNA.

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