British Journal of Pharmacology (1995) 114, 846–850

Interacting roles of nitric oxide and ATP in the pulmonary circulation of the rat

[1]Haroutioun Hasséssian & [2]Geoffrey Burnstock

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT

1 The potentiating effect of N^G-nitro-L-arginine methyl ester, (L-NAME) a nitric oxide synthesis inhibitor, on responses of the rat pulmonary vascular pressure (P_{VP}) to purinoceptor agonists was examined.

2 At a constant flow of 23 ml min^{-1} the P_{VP} was 22.4 ± 2.5 mmHg ($n = 15$), and treatment with 100 μM L-NAME for 15 min was without effect on the P_{VP}. After the tone was raised with 28 nmol 9,11-dideoxy-11α, 9α-epoxymethano-prostaglandin $F_{2\alpha}$ (U-46619), the P_{VP} was 29.4 ± 3.3 mmHg and treatment with 100 μM L-NAME was still without effect on the P_{VP}. It appears that there is a graded release of nitric oxide in response to different levels of steady shear stress and in our experimental model the threshold for detection was not reached under basal conditions.

3 In contrast, when the circulation was challenged with 30 s step, additive increases in flow between 11 and 50 ml min^{-1} ($n = 8$), treatment with 100 μM L-NAME produced a significant ($P < 0.05$) increase in P_{VP} suggesting that changes in flow-derived forces evoke the release of nitric oxide. This was evident for flow rates above 30 ml min^{-1}.

4 In preparations in which tone was raised with U-46619, a dose of 1×10^{-8} mol ATP or 2-meSATP evoked a drop in P_{VP} while α,β-meATP produced an increase in P_{VP} under constant flow of 23 ml min^{-1}. After treatment with 100 μM L-NAME, all three purinoceptor agonists evoked an increase in P_{VP}. The increase in P_{VP} evoked by α,β-meATP was not affected by L-NAME. These results suggest that P_{2Y}-purinoceptor stimulation evokes the release of nitric oxide to produce vasodilatation.

5 Under conditions of constant flow and basal pressure, 100 μM L-NAME significantly ($P < 0.05$) potentiated the increase in P_{VP} evoked by 1×10^{-6} mol ATP, although the increase evoked by 1×10^{-8} mol α,β-meATP, which was of similar magnitude, was not affected. These results indicate that a blockade of evoked nitric oxide release is responsible for the potentiation of the increase in P_{VP} evoked by ATP.

6 This study shows that, while nitric oxide does not appear to be released in the pulmonary circulation of the rat under constant flow conditions, nitric oxide release evoked by purinoceptor agonists attenuates increases in pulmonary vascular pressure.

Keywords: Purinoceptor; nitric oxide; pulmonary circulation; endothelium

Introduction

In the rat, single pulse stimulation of perivascular nerves which supply small intrapulmonary arteries produces excitatory junctional potentials that are mediated by a nonadrenergic, noncholinergic neurotransmitter which is probably adenosine-5'-triphosphate (ATP) (Inoue & Kannan, 1988). Furthermore, ATP is localized in endothelial cells, mast cells as well as erythrocytes, and in response to various stimuli, ATP can be liberated from these sources into the pulmonary circulation (Bergfeld & Forrester, 1992; Ralevic *et al.*, 1992). Hence, the role of ATP in the pulmonary circulation is essentially two fold. ATP released from non-sympathetic neurones evokes excitatory junctional potentials leading to vasoconstriction of pulmonary vessels (Inoue & Kannan, 1988), and purinoceptors mediating vasoconstriction have been demonstrated on pulmonary vascular smooth muscle (Liu *et al.*, 1989; Neely *et al.*, 1991), while activation of purinoceptors on endothelial cells produces vasodilatation (Liu *et al.*, 1989; Neely *et al.*, 1989).

Although the mechanism is not clear, a potentiation of vasoconstriction has been observed in a number of blood vessels following removal of endothelium (Hynes *et al.*, 1988), or after inhibition of nitric oxide synthesis (Vo *et al.*, 1991). In the rat pulmonary vascular bed, we examined whether enhancement of vasoconstriction to purinoceptor agonists occurs as a result of loss of agonist-evoked release of nitric oxide or alternatively, whether nitric oxide is constantly modulating reactivity of pulmonary vascular smooth muscle.

Methods

Isolation and perfusion of pulmonary vascular beds

The experiments were performed on male Wistar rats of 200–300 g weight. The rats were stunned and exsanguinated before the trachea was catheterized, and the lungs removed 'en block', together with the heart, and placed in a Petri dish containing Krebs solution (composition in mM: NaCl 118.0, KCl 5.0, $NaH_2PO_4 \cdot 2H_2O$ 1.2, $NaHCO_3$ 25.5, $MgSO_4 \cdot 7H_2O$ 1.2, D-glucose 5.6, $CaCl_2 \cdot 2H_2O$ 2.5 and 4% bovine serum albumin). The left atrium was removed and a Krebs-filled catheter was fixed inside the main pulmonary artery.

The preparation was transferred to a chamber which was open to room temperature (24.1 ± 0.3°C, $n = 16$), and the preparation was kept moist by gently superfusing with Krebs

[1] Present address: Department of Molecular Physiology and Biophysics, One Baylor Plaza, Baylor College of Medicine, Houston, TX 77030, U.S.A.

[2] Author for correspondence.

solution from a Pasteur pipette. The tracheal catheter was connected to a custom-made respirator (Hammersmith Hospital Engineering Unit), and ventilated with air at a frequency of 1 Hz. The mean tidal volume was 7.6 ± 1.0 ml min^{-1} ($n = 32$) and a mean positive end expiratory pressure of 8.5 ± 0.7 mmHg ($n = 32$) was maintained throughout all experiments. The preparation was perfused with Krebs solution (temperature: 23.4 ± 0.4°C, $n = 16$) which was bubbled with 95% O_2: 5% CO_2 to maintain pH (7.3 ± 0.0, $n = 16$).

Flow-pressure relationship

The tracheal and pulmonary artery catheters were attached to individual pressure transducers (Grass model T4) to monitor airway as well as pulmonary vascular pressure (P_{VP}). The perfusion pressure of the apparatus was measured daily to subtract later from the apparent pressure measured after connecting the preparation and thus determine the real perfusion pressure of the pulmonary circulation. The Masterflex (Cole Parmer Instruments Co., Chicago, Illinois 60648) pump delivered a constant flow.

After the preparation had stabilized, the flow rate was increased to preset levels in a step-wise manner, and the perfusion pressure steps were recorded before reducing the flow by the same steps. The pressure-flow relationship was the same whether the flow was being increased or decreased in a step-wise manner. At this point 700 µl of a N^G-nitro-L-arginine methyl ester (L-NAME) solution was introduced into the 700 ml Krebs reservoir to produce a 100 µM L-NAME solution. After perfusing with L-NAME for 15 min, the flow rate was once again increased to the preset levels as before. Control experiments were conducted in a series of preparations ($n = 3$) where the flow rate was increased to preset levels in a step-wise manner before reducing the flow by the same steps, and repeating this protocol four times. It was found that, in the absence of drug, the pressure-flow relationship did not change with repetition (results not shown).

All measures of P_{VP} were performed 30 s into the step-augmented flow. Flow steps of duration longer than 30 s caused oedema when the flow rate was increased above about 30 ml min^{-1}, therefore we chose 30 s steps in order to examine flow rates that produce P_{VP} substantially higher than 20 mmHg prior to treatment with L-NAME. During constant flow, the changes in vascular perfusion pressure reflect changes in vascular resistance. For a fixed volume perfusion with air, a sustained change in the pulse pressure reflects a change in the compliance of the airways. A rise in the compliance of the airways indicates oedema, and over the duration of the experiments in our study the pulse pressure was constant.

Bolus administration of drugs

With a Hamilton microsyringe, all purinoceptor agonists were injected in a 50 µl volume, through stethoscope tubing, into the Krebs perfusate proximal to the preparation. The drugs were prepared as 1 × 10^{-2} M stock solutions in distilled water and diluted as required. When the response to a drug had attenuated and the P_{VP} had regained its steady level, a further dose was administered. Doses of drugs were given in increasing strength. To avoid any possible desensitization by α,β-meATP, during dose-response analysis, a complete series of ATP doses was injected first, followed by the complete series of 2 meSATP and then by α,β-meATP. When required, the vascular tone was raised with a single bolus injection of 28 nmol 9,11-dideoxy-11α, 9α-epoxymethano-prostaglandin $F_{2\alpha}$ (U-46619) in a 10 µl volume. Such a dose of U-46619 was sufficient to keep the tone raised for the duration of the experiment.

Materials

The salts used to prepare the Krebs solution were purchased from BDH, while adenosine-5'-triphosphate (sodium salt, ATP) and α,β-meATP were purchased from Sigma. The 2-meSATP was obtained from Research Biochemicals Inc. (Natick, MA 01760, U.S.A.). The vasoconstrictor U-46619 was a kind gift from UpJohn (Kalamazoo, Michigan, U.S.A.).

Statistical analysis

Student's paired t test was used when the effect of L-NAME was being examined on a given response. The curves shown in Figure 2 were compared by testing for statistical significance between the difference of regression line slopes in the t statistic. Furthermore, individual points were compared with Student's paired t test and thus both point differences as well as the overall trend were analysed. All values are expressed as means ± s.e.mean, where n represents the number of animals. A probability of $P < 0.05$ was considered significant.

Results

Release of nitric oxide during increasing flow

To determine whether nitric oxide is released in the pulmonary circulation under basal pressure and constant flow conditions, the P_{VP} was measured in the absence and presence of L-NAME. At a constant flow of 23 ml min^{-1}, the P_{VP} was 22.4 ± 2.5 mmHg ($n = 15$). After perfusing with 100 µM L-NAME for 15 min, the P_{VP} was 23.8 ± 3.9 mmHg and not significantly different from before the L-NAME treatment (Figure 1). Similar results were found when treatment with L-NAME was investigated at a higher P_{VP} and constant flow. With 28 nmol U-46619 the perfusion pressure was significantly ($P < 0.05$, $n = 5$) raised to 29.4 ± 3.3 mmHg. Furthermore, 15 min after perfusing with 100 µM L-NAME, the perfusion pressure was 31.8 ± 2.4 mmHg and not significantly different from before the L-NAME treatment.

When the vascular bed was challenged with additive 30 s step increases in flow between 11 and 50 ml min^{-1} ($n = 8$),

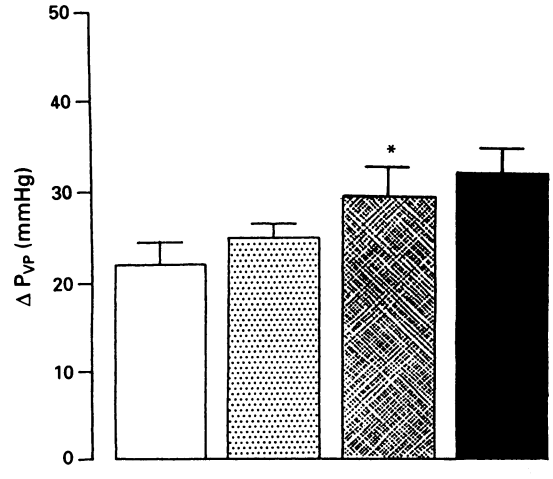

Figure 1 The pulmonary vascular pressure (P_{VP}) measured at a constant flow of 23 ml min^{-1} ($n = 15$), before treatment with any drug (open column), or after treatment with 100 µM N^G-nitro-L-arginine methyl ester (L-NAME) (stippled column), 28 nmol U-46619 alone (shaded column) and 28 nmol U-46619 with 100 µM L-NAME (solid column). The asterisk indicates that the P_{VP} after treatment with U-46619 was significantly ($P < 0.05$, $n = 5$) higher than before treatment with any drug.

the P_{VP} increased proportionately with increasing flow (Figure 2). After perfusion for 15 min with 100 μM L-NAME, similar step increases in flow produced greater increases of P_{VP} (Figure 2). The significantly ($P<0.001$) greater slope of the flow-perfusion pressure curve after L-NAME was introduced represents an increase in vascular resistance.

The vasoconstrictor response to ATP is potentiated by L-NAME

At a constant flow of 23 ml min^{-1} the P_{VP} was 22.2 ± 3.3 mmHg and raised to 28.8 ± 3.8 mmHg ($P<0.05$, $n=5$) by 28 nmol U-46619. In a raised tone preparation, injection of 1×10^{-8} mol ATP or 2-meSATP evoked a vasodilator response while injection of 1×10^{-8} mol α,β-meATP evoked a vasoconstriction (Figure 3). Following treatment with 100 μM L-NAME for 15 min the P_{VP} was 30.8 ± 3.2 mmHg and not significantly changed. Furthermore, injection of ATP or 2-meSATP evoked a vasoconstriction and the vasoconstriction to α,β-meATP was not significantly changed.

In a sample of preparations with basal P_{VP} (24.9 ± 3.2 mmHg, $n=7$) and constant flow (11.5 ± 0.0 ml min^{-1}) injection of 1×10^{-6} mol ATP raised P_{VP} by 4.5 ± 1.0 mmHg which was significantly ($P<0.05$) potentiated 2 fold after treatment with 100 μM L-NAME (Figure 4). Injection of 1×10^{-8} mol α,β-meATP evoked a rise in P_{VP} of 5.6 ± 2.0 mmHg which was not significantly changed in the presence of 100 μM L-NAME (Figure 4).

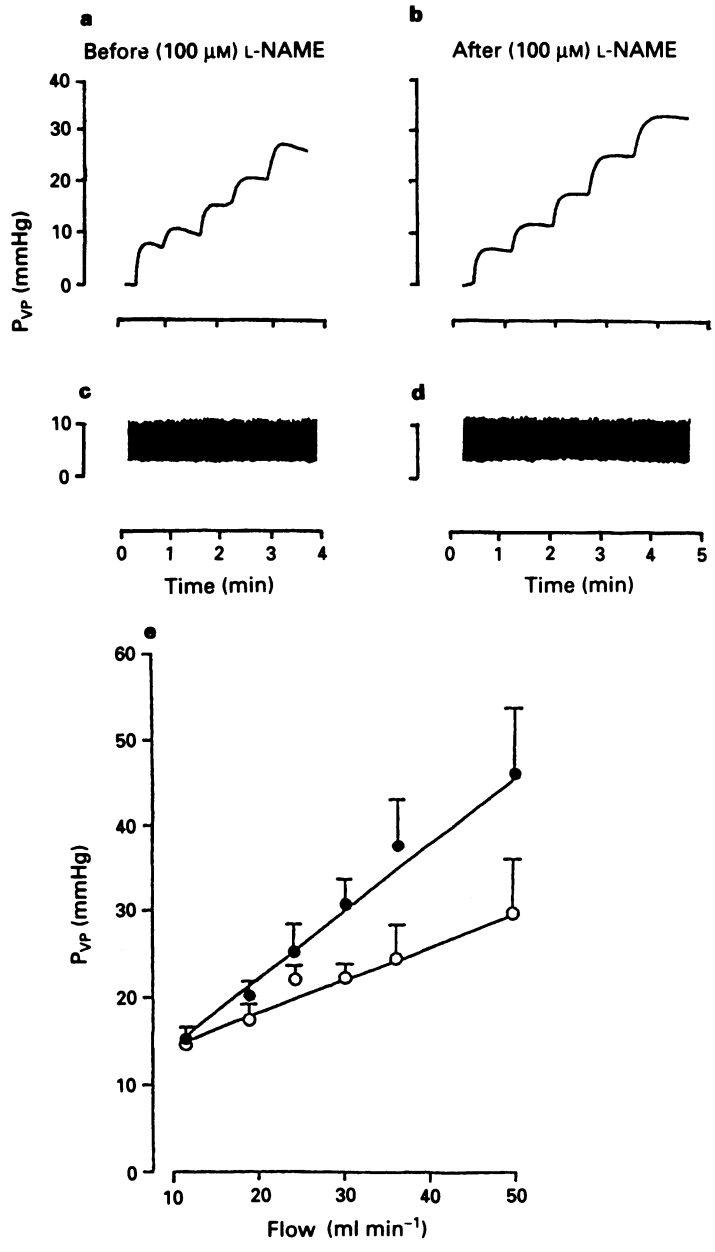

Figure 2 Polygraph traces showing the change in vascular perfusion pressure in (a) and (b) and the airway perfusion pressure in (c) and (d). Step augmentation of the Krebs flow rate (11-20-25-30-35-50 ml min^{-1}) produced corresponding step increases in vascular perfusion pressure without affecting the airways. In the presence of 100 μM N^G-nitro-L-arginine methyl ester (L-NAME) the drop in perfusion pressure after the initial jump at each step has been blocked (b). Furthermore, the pressure jumps are to higher levels with each augmentation of flow rate. (e) The pulmonary vascular pressure (P_{VP}) measured at different flow rates before (○) and after (●) treatment with 100 μM L-NAME for 15 min. The regression line slope of the curve obtained after treatment with L-NAME is significantly ($P<0.001$, $n=8$) greater. Comparison of individual points with Student's paired t test, showed that the perfusion pressure for flow rates of 30 ml min^{-1} and higher is significantly ($P<0.05$ in each case) increased by treatment with 100 μM L-NAME.

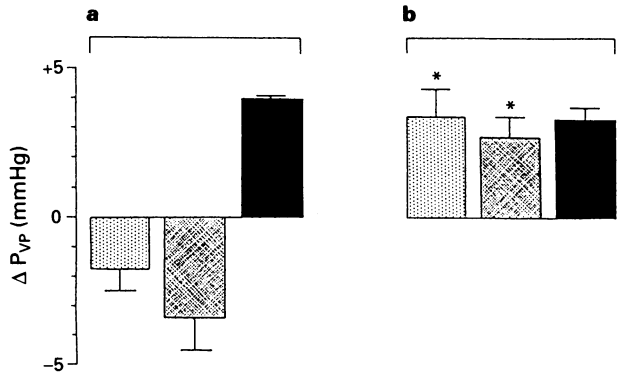

Figure 3 Changes in pulmonary vascular pressure (P_{VP}) evoked by 1×10^{-8} mol of ATP (stippled columns), 2-meSATP (shaded column) or α,β-meATP (solid column), before (a) and after (b) treatment with 100 μM N^G-nitro-L-arginine methyl ester (L-NAME) in preparations in which P_{VP} was raised with U-46619. The asterisks indicate that the response to ATP and 2-meSATP was significantly ($P<0.05$, $n=5$) different after treatment with L-NAME.

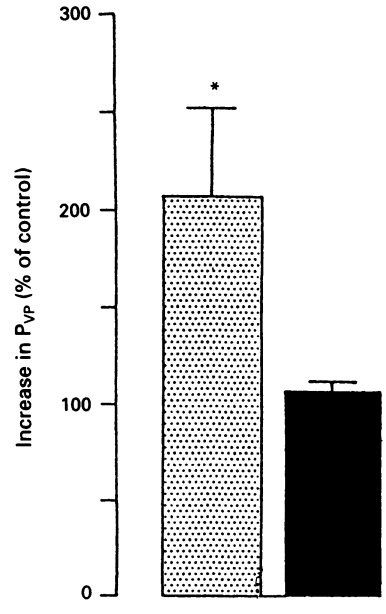

Figure 4 The increase in P_{VP} in response to ATP (stippled column) and α,β-meATP (solid column) at basal P_{VP} with constant flow, compared before and after treatment with 100 μM N^G-nitro-L-arginine methyl ester (L-NAME). The asterisk indicates that the increase in P_{VP} evoked by ATP was significantly ($P<0.05$, $n=7$) greater after treatment with L-NAME.

Discussion

To determine if constant release of nitric oxide could be detected we measured the effect of an active dose of L-NAME on the P_{VP} of low tone and raised tone preparations. Under constant flow conditions the P_{VP} was not changed following inhibition of nitric oxide synthesis, indicating that in our experimental model under constant basal flow conditions there does not appear to be a significant release of nitric oxide. It was found by Barer and colleagues (1993) that L-NAME occasionally induced constriction in normal rats under conditions of constant 50 ml min^{-1} flow which is beyond the range of flow that we studied. In other pulmonary circulation studies various inhibitors of nitric oxide synthesis (Gold *et al.*, 1990; Wiklund *et al.*, 1990; Dinh-Xuan *et al.*, 1991), produced an increase in basal vascular resistance. Inhibitors of G protein function have also produced similar results (Hyman *et al.*, 1989; Fineman *et al.*, 1991). In contrast, some investigators have not found an effect on perfusion pressure following disruption of nitric oxide-mediated vasodilatation by various means (Cherry & Gillis, 1987; Mazmanian *et al.*, 1989; Archer *et al.*, 1990; Nishiwaki *et al.*, 1992). Species differences aside, these studies have not addressed the effect of flow-derived changes in shear stress or the effect of reflexes which can be activated *in vivo* to counteract vascular changes. In our study, under conditions of step augmentation of flow-derived shear stress, treatment with L-NAME produced a significant increase in the resistance of the pulmonary circulation. Similar results were reported by Isaacson and colleagues (1994), where although L-NAME did not significantly change P_{VP} under constant flow conditions, there was an increase in P_{VP} when examined with step augmentations of flow. Although isolated vessel preparations and cultured endothelial cells have been found to release nitric oxide in the absence of flow (Moncada *et al.*, 1991), the contribution to vascular pressure of nitric oxide leaked from endothelial cells in the absence of agonist or flow-derived shear stress does not appear to be significant under the conditions that we studied. Furthermore, although nitric oxide was not detected in the perfusate from control rats with P_{VP} of 17.0 ± 1.4 mmHg, 20–25 nM nitric oxide was found in the perfusate of chronically hypoxic rats with mean pulmonary arterial pressure of 32.5 ± 3.3 mmHg which corroborate our study. Hence, it appears that increased shear stress due to augmented pulmonary vasoconstriction in chronically hypoxic rats causes release of nitric oxide (Barer *et al.*, 1993; Isaacson *et al.*, 1994). This suggests that *in vivo*, pulsatile changes in flow evoke nitric oxide release and that vascular changes evoked by such nitric oxide release can be blocked with L-NAME.

In a preparation in which P_{VP} was raised with U-46619, following blockade of nitric oxide synthesis, the vasodilatation in response to ATP as well as 2-meSATP was abolished and the response changed to vasoconstriction, although, the vasoconstrictor response to α,β-meATP was not changed. In contrast, at basal P_{VP} the vasoconstriction in response to ATP was increased by two fold, unlike the vasoconstriction produced by α,β-meATP which was not changed. The P_{2X}-purinoceptor subtype mediates vasoconstriction of large pulmonary vessels (Liu *et al.*, 1989; Neely *et al.*, 1991), and α,β-meATP is a highly selective agonist for this receptor (Burnstock & Kennedy, 1985), whereas 2-meSATP is selective for P_{2Y}-purinoceptors which mediate endothelium-dependent vasodilatation of pulmonary vessels (Liu *et al.*, 1989; Neely *et al.*, 1989). The natural agonist, ATP, is not selective for P_2-purinoceptor subtypes; therefore, exogenous ATP will stimulate both vasodilator and vasoconstrictor purinoceptors to produce a composite response. Blockade of nitric oxide synthesis with L-NAME removes the vasodilator component of the dual effect to reveal the vasoconstriction.

There is evidence which shows that ATP is released from vascular endothelial cells under conditions of increasing flow (Bodin *et al.*, 1991; 1992; Hasséssian *et al.*, 1993) or following hypoxia (Paddle & Burnstock, 1974; Forrester & Williams, 1977; Clemens & Forrester, 1981; Hopwood *et al.*, 1989). Furthermore, ATP has also been shown to be released from erythrocytes in response to hypoxia (Bergfeld & Forrester, 1992). It is possible that ATP released by flow-derived shear stress, hypoxia or other stimuli, is carried short distances in the blood to act on endothelial cells downstream, or ATP can act on the endothelial cell it is released from, to produce P_{2Y}-purinoceptor-mediated nitric oxide release in addition to possible autoregulation of ATP release. Hence ATP-evoked nitric oxide release from endothelial cells will constantly modulate vasoconstriction evoked by purinergic nerves. Therefore, under conditions where any component of endothelial purinergic mechanism is not fully functional,

vasoconstriction evoked by purinergic nerves will be potentiated.

In forms of pulmonary hypertension where there is loss of endothelial cells or trauma to the endothelium, infusion of ATP may aggravate the situation. In the pathological state, each component in isolation, as well as the interaction between vasoconstrictor and vasodilator mechanisms must be considered for a comprehensive understanding of pulmonary blood vessel reactivity.

The gift of U-46619 from Upjohn is highly appreciated. H.H. is a Postdoctoral Fellow of the Medical Research Council of Canada. This work was funded by a grant to G.B. from the British Heart Foundation.

References

ARCHER, S.L., RIST, K., NELSON, D.P., DEMASTER, E.G., COWAN, N. & WEIR, E.K. (1990). Comparison of the hemodynamic effects of nitric oxide and endothelium dependent vasodilators in intact lungs. *J. Appl. Physiol.*, **68**, 735–747.

BARER, G., EMERY, C., STEWART, A., BEE, D. & HOWARD, P. (1993). Endothelial control of the pulmonary circulation in normal and chronically hypoxic rats. *J. Physiol.*, **463**, 1–16.

BERGFELD, G.R. & FORRESTER, T. (1992). Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnia. *Cardiovasc. Res.*, **26**, 40–47.

BODIN, P., BAILEY, D. & BURNSTOCK, G. (1991). Increased flow-induced ATP release from isolated vascular endothelial cells but not smooth muscle cells. *Br. J. Pharmacol.*, **103**, 1203–1205.

BODIN, P., MILNER, P., WINTER, R. & BURNSTOCK, G. (1992). Chronic hypoxia changes the ratio of endothelin to ATP release from rat aortic endothelial cells exposed to high flow. *Proc. R. Soc. B*, **247**, 131–135.

BURNSTOCK, G. & KENNEDY, C. (1985). Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen. Pharmacol.*, **16**, 433–440.

CHERRY, P.D. & GILLIS, C.N. (1987). Evidence for the role of endothelium derived relaxing factor in acetylcholine-induced vasodilation in the intact lung. *J. Pharmacol. Exp. Ther.*, **241**, 516–520.

CLEMENS, M.G. & FORRESTER, T. (1981). Appearance of adenosine triphosphate in the coronary sinus effluent from isolated working rat heart in response to hypoxia. *J. Physiol.*, **312**, 143–158.

DINH-XUAN, A.T., HIGENBOTTAM, T.W., CLELLAND, C.A., PEPKE-ZABA, J., CREMONA, G., BUTT, A.Y., LARGE, S.R., WELLS, F.C. & WALLWORK, J. (1991). Impairment of endothelium-dependent pulmonary-artery relaxation in chronic obstructive lung disease. *N. Engl. J. Med.*, **324**, 1539–1547.

FINEMAN, J.R., HEYMANN, M.A. & SOIFER, S.J. (1991). N^W-nitro-L-arginine attenuates endothelium-dependent pulmonary vasodilation in lambs. *Am. J. Physiol.*, **260**, H1299–H1306.

FORRESTER, T. & WILLIAMS, C.A. (1977). Release of adenosine triphosphate from isolated adult heart cells in response to hypoxia. *J. Physiol.*, **268**, 371–390.

GOLD, M.E., WOOD, K.S., BYRNS, R.E., FUKUTO, J. & IGNARRO, L.J. (1990). N^G-methyl-L-arginine causes endothelium-dependent contraction and inhibition of cyclic GMP formation in artery and vein. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 4430–4434.

HASSÉSSIAN, H., BODIN, P. & BURNSTOCK, G. (1993). Blockade by glibenclamide of the flow-evoked endothelial release of ATP that contributes to vasodilation in the pulmonary vascular bed of the rat. *Br. J. Pharmacol.*, **109**, 466–472.

HOPWOOD, A.M., LINCOLN, J., KIRKPATRICK, K.A. & BURNSTOCK, G. (1989). Adenosine 5′-triphosphate, adenosine and endothelium-derived relaxing factor in hypoxic vasodilation of the heart. *Eur. J. Pharmacol.*, **165**, 323–326.

HYMAN, A.L., KADOWITZ, P.J. & LIPPTON, H.L. (1989). Methylene blue selectively inhibits pulmonary vasodilator responses in cats. *J. Appl. Physiol.*, **66**, 1513–1517.

HYNES, M.R., DANG, H. & DUCKLES, S.P. (1988). Contractile responses to adrenergic nerve stimulation are enhanced with removal of endothelium in rat caudal artery. *Life Sci.*, **42**, 357–365.

INOUE, T. & KANNAN, M.S. (1988). Nonadrenergic and noncholinergic excitatory neurotransmission in rat intrapulmonary artery. *Am. J. Physiol.*, **254**, H1142–H1148.

ISAACSON, T.C., HAMPL, V., WEIR, E.K., NELSON, D.P. & ARCHER, S.L. (1994). Increased endothelium-derived NO in hypertensive pulmonary circulation of chronically hypoxic rats. *J. Appl. Physiol.*, **76**, 933–940.

LIU, S.F., McCORMACK, D.G., EVANS, T.W. & BARNES, P.J. (1989). Characterization and distribution of P_2-purinoceptor subtypes in rat pulmonary vessels. *J. Pharmacol. Exp. Ther.*, **251**, 1204–1210.

MAZMANIAN, G., BAUDET, B., BRINK, C., CERRINA, J., KIRKIACHARIAN, S. & WEISS, M. (1989). Methylene blue potentiates vascular reactivity in isolated rat lungs. *J. Appl. Physiol.*, **66**, 1040–1045.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.

NISHIWAKI, K., NYHAN, D.P., ROCK, P., DESAI, P.M., PETERSON, W.P., PRIBBLE, C.G. & MURRAY, P.A. (1992). N^W-nitro-L-arginine and pulmonary vascular pressure-flow relationship in conscious dogs. *Am. J. Physiol.*, **262**, H1331–H1337.

NEELY, C.F., KADOWITZ, P.J., LIPPTON, H., NEIMAN, M. & HYMAN, A.L. (1989). Adenosine does not mediate the pulmonary vasodilator response of adenosine 5′-triphosphate in the feline pulmonary vascular bed. *J. Pharmacol. Exp. Ther.*, **250**, 170–176.

NEELY, C.F., HAILE, D.M., CAHIL, B.E. & KADOWITZ, P.J. (1991). Adenosine and ATP produce vasoconstriction in the feline pulmonary vascular bed by different mechanisms. *J. Pharmacol. Exp. Ther.*, **258**, 753–761.

PADDLE, B.M. & BURNSTOCK, G. (1974). Release of ATP from perfused heart during coronary vasodilation. *Blood Vessels*, **11**, 110–119.

RALEVIC, V., LINCOLN, J. & BURNSTOCK, G. (1992). Release of vasoactive substances from endothelial cells. In *Endothelial Regulation of Vascular Tone*. ed. Ryan, U.S. & Rubanyi, G.M. pp. 297–328. New York: Marcel Dekker Inc.

VO, P.A., REID, J.J. & RAND, M.J. (1991). Endothelial nitric oxide attenuates vasoconstrictor responses to nerve stimulation and noradrenaline in the rat tail artery. *Eur. J. Pharmacol.*, **199**, 123–125.

WIKLUND, N.P., PERSSON, M.G., GUSTAFSSON, L.E., MONCADA, S. & HEDQVIST, P. (1990). Modulatory role of endogenous nitric oxide in pulmonary circulation *in vivo*. *Eur. J. Pharmacol.*, **185**, 123–124.

(Received December 16, 1993
Revised October 20, 1994
Accepted October 26, 1994)