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# Vasodilator Action of the S-nitrosothiol, SNAP, in Rat Isolated Perfused Lung

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## Summary

The pulmonary vasodilator action of an S-nitrosothiol, S-nitroso acetylpenicillamine (SNAP), was investigated in the rat pulmonary vasculature. The influence of its nitric oxide donator property was studied by comparison with the effect of acetylpenicillamine (AP), SNAP minus the nitroso group, and the blockade of nitric oxide release by the L-arginine analogue, L-NAME. In the isolated rat lung perfused with autologous blood at a constant flow rate (IPL), changes in pulmonary artery pressure (Ppa) reflect changes in pulmonary vascular resistance. Dose-response relationships to both SNAP and AP (0.1, 1, 10 and 100 $\mu$g) were established both during normoxic ventilation (air + 5 % $CO_2$; low Ppa) and when Ppa was raised by alveolar hypoxic vasoconstriction (2 % $O_2$ + 5 % $CO_2$). SNAP caused small dose-dependent fall in normoxic Ppa (mean±S.D. 17.4±3.0 mm Hg). In 11 rat IPL % fall of Ppa was 1, 3 and 4 % for 1, 10 and 100 $\mu$g, respectively ($p<0.01$). This fall was more obvious when Ppa was raised by hypoxia (mean Ppa rise (HPV) 11.5±3.8 mm Hg); there was a 22, 55 and 79 % fall in HPV for 1, 10 and 100 $\mu$g in 11 rat IPL. The dilatation after 10 $\mu$g SNAP was not consistently affected by 100 $\mu$g L-NAME (% fall in HPV pre L-NAME 45±22 % vs 42±23 % post L-NAME). AP had no significant effect on Ppa, causing only small falls in Ppa, equivalent to solvent (saline). There was occasionally a small rise in Ppa with 10 and 100 $\mu$g AP. Thus, the dilator action of SNAP is most likely due to its NO donator property, and is not consistently affected by blockade of endogenous NO release.

**Key words**

SNAP – Rat – Pulmonary vasodilatation

## Introduction

The pulmonary circulation, unlike the systemic, is a low-pressure system, mean pulmonary artery pressure (Ppa) being 15 mm Hg. This may be elevated in certain disease states, such as hypoxic pulmonary hypertension in chronic lung disease and primary pulmonary hypertension in both adults and neonates. This rise in Ppa is associated with both vasoconstriction and vascular restriction due to remodelling. Although large intrapulmonary vessels are muscularised, more distal arterioles are usually thin-walled with a single elastic lamina and little or no muscle. These may become muscularised and less compliant in certain lung diseases, the muscle encroaching into the lumen.

Severe pulmonary hypertension increases the work of the heart leading to right ventricular hypertrophy and possible heart failure. Treatment with oxygen for the relief of hypoxic pulmonary hypertension has partial success but more potent dilators such as prostacyclin (prostaglandin $PGI_2$) are not specific for the pulmonary circulation and may produce profound systemic hypotension. Many vasodilators stimulate the release of endothelial derived relaxant factor (EDRF), considered to be NO (Moncada *et al.* 1991), and are referred to as endothelial-dependent. Endothelial NO is released from L-arginine and diffuses, probably combined with a carrier such as a thiol (Ignarro *et al.* 1981), into the underlying smooth muscle where it causes relaxation by increasing cyclic 3'5'-guanosine monophosphate (cGMP) through activation of guanylate cyclase. The NO formed in the lung is detected in the exhaled air of man and other species (Gustafsson *et al.* 1991). Its combination with cellular thiols, such as cysteine and glutathione, forms a more stable compound which may resemble EDRF more closely than NO itself (Myers *et*

*al.* 1990). S-nitrosothiols have been detected in bronchoalveolar fluid (Barnes and Belvisi 1993).

In the case of endothelial damage, both in systemic and pulmonary vascular hypertensive disease, dilators which are independent of an intact functioning endothelium would be advantageous. Thus endothelial-independent dilators such as exogenous NO and NO donator drugs may provide essential treatment. Inhaled NO is a selective pulmonary vasodilator which has been successfully used in the treatment of severe pulmonary hypertension (Rossaint *et al.* 1993, Pepke-Zaba *et al.* 1991). However, due to its short half-life and its potential toxicity, it must be given continuously under carefully controlled conditions. The development of a biologically safe, stable nitric oxide donator drug, which could be delivered *via* the airway, may provide a novel treatment. The S-nitrosothiols could be one such group of compounds.

This study investigates the vasodilator action of a stable S-nitrosothiol, S-nitroso acetylpenicillamine (SNAP), on the pulmonary circulation of the rat. A comparison is made with the action of the non-nitrosylated form, acetylpenicillamine (AP), to determine the effect of the nitric oxide group. Like other NO donators, e.g. sodium nitroprusside, the dilator action of SNAP does not work through nitric oxide synthase (NOS) although the presence of endogenous NO may be influential. We have therefore investigated the action of SNAP in the presence of L-NAME, an inhibitor of NOS.

## Materials and Methods

*Isolated blood-perfused lung (IPL)*

Male Wistar strain rats, body weight 200 to 250 g, were anaesthetized with 60 mg/kg sodium pentobarbitone (Sagatal, Rhone Merieux, France). The lungs were isolated and perfused as described by Emery *et al.* (1981). Briefly, the pulmonary artery and left atrium were cannulated and the lungs were perfused *in situ* with autologous blood at a constant flow rate of 20 ml/min. The lungs were ventilated through the cannulated trachea by a Harvard Ideal ventilator at constant tidal volume of 3–4 ml and rate 50 breaths/min. Pulmonary artery pressure (Ppa) was measured from a side arm in the circuit close to the artery, connected to a pressure transducer. Normoxic ventilation was air + 5 % $CO_2$. Blood pH was adjusted within a physiological range, 7.35–7.45 by the addition of 8 % sodium bicarbonate and maintained at 37 °C.

*Dose-response to S-nitroso acetylpenicillamine (SNAP) and acetylpenicillamine (AP)*

Dilutions of S-nitroso acetylpenicillamine (SNAP) and acetylpenicillamine (AP) were made up daily in normal saline and kept away from light and heat. Doses tested (0.1, 1, 10 and 100 $\mu$g) were given as a bolus 0.1 ml into the perfusion circuit close to the pulmonary artery. 0.1 ml saline acted as a placebo dose.

Individual IPLs were tested with either SNAP or AP, both during normoxia and when the Ppa was raised by hypoxic vasoconstriction (HPV), produced by ventilation with 2 % $O_2$ + 5 % $CO_2$. During acute hypoxia, the drugs were injected when Ppa had achieved a stable plateau; reventilating with air + 5 % $CO_2$ caused Ppa to return to prehypoxic levels.

*Effect of nitro-L-arginine methyl ester (L-NAME) on SNAP response*

Having established a response to 10 $\mu$g SNAP during HPV, 100 $\mu$g L-NAME was given in a 0.1 ml bolus dose into the blood reservoir. After a 15–20 min period the response to SNAP was retested.

*Statistics*

Results are expressed as means ± S.D. The changes in Ppa to the drugs are expressed as either % fall in normoxic Ppa or % fall in the rise of Ppa with hypoxia (= fall in HPV). Statistical differences were established using either paired or unpaired Student's t-test. $P < 0.05$ was considered significantly different.

## Results

*Effects of SNAP and AP during normoxia*

SNAP (11 rats, mean normoxic Ppa 17.4±3.0 mm Hg) caused a small fall in Ppa, increasing with the dose. AP (6 rats, mean normoxic Ppa 19.6±2.2 mm Hg) caused small vasodilatation, independent of the dose, with an occasional rise in Ppa with 10 or 100 $\mu$g. The fall was usually sustained. Fig. 1 shows the mean changes in normoxic Ppa, expressed as a % fall, for SNAP, AP and 0.1 ml saline.

*Effects of SNAP and AP during HPV*

Acute hypoxia caused a rise in Ppa (HPV), which was reproducible after the initial 2–3 challenges. Mean HPV was 11.5±3.8 mm Hg in 11 rats in which SNAP caused a dose-dependent fall in HPV (Figs 1 and 2). In another 5 rats (mean HPV 11.1±1.9 mm Hg) AP caused only small falls in Ppa, independent of the dose, showing occasional rises. Fig. 1 shows the mean changes, expressed as a % fall in HPV, with SNAP, AP and saline control. The dilatation was usually persistent for the period of hypoxia (up to 10 min) but occasionally a slight reversal was seen.

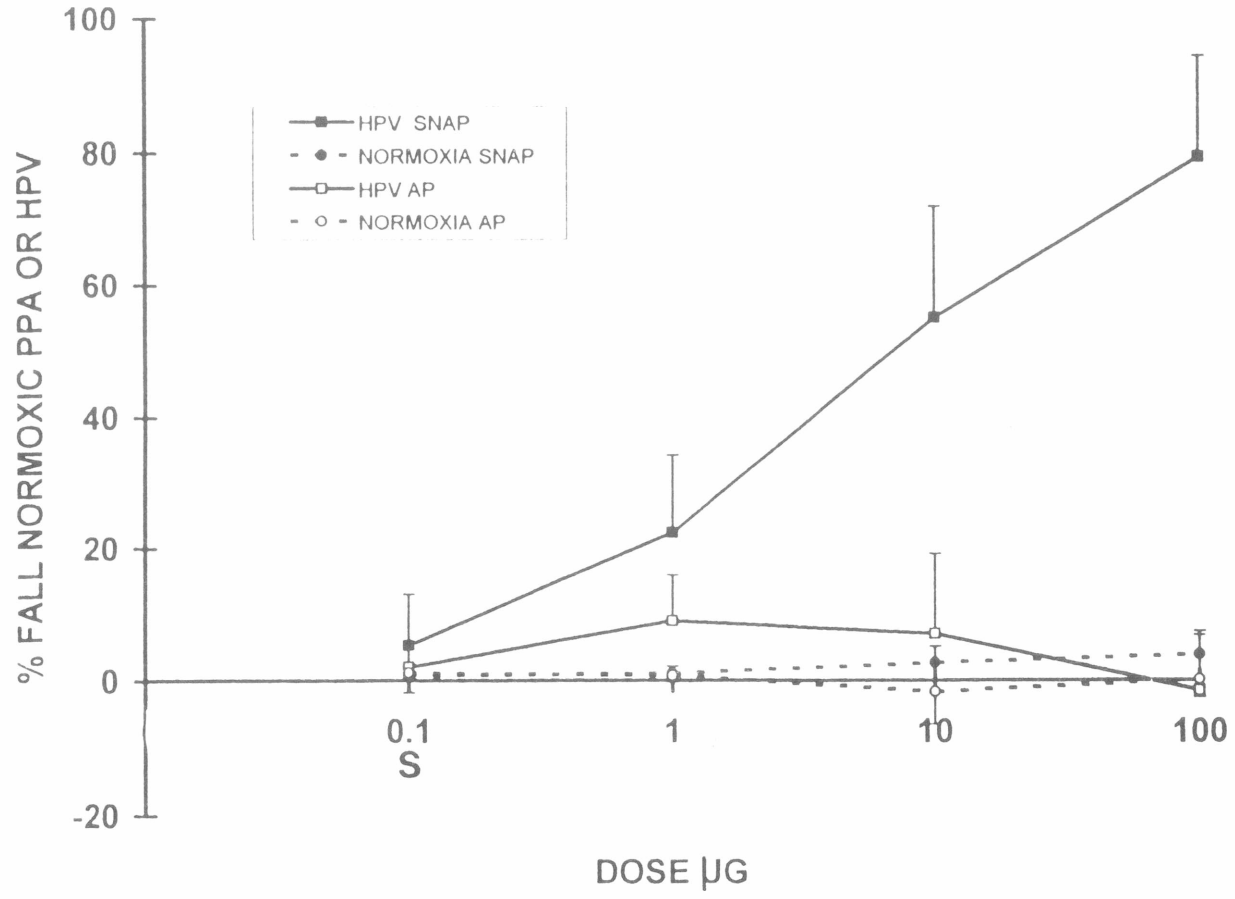


**Fig. 1**
Effect of S-nitroso acetylpenicillamine (SNAP) and acetylpenicillamine (AP) (1, 10 and 100 $\mu$g) on pulmonary artery pressure (PPA) during normoxia and hypoxic vasoconstriction (HPV). Results are expressed as either a % fall in normoxic PPA or % fall in the rise in PPA with hypoxia (2 % $O_2$) (HPV). Negative values indicate a mean rise in PPA. Values are given as mean ± SD. Responses to SNAP doses during hypoxia were significantly different from each other and saline control (S) ($p<0.01$) and from equivalent doses of AP ($p<0.01$).

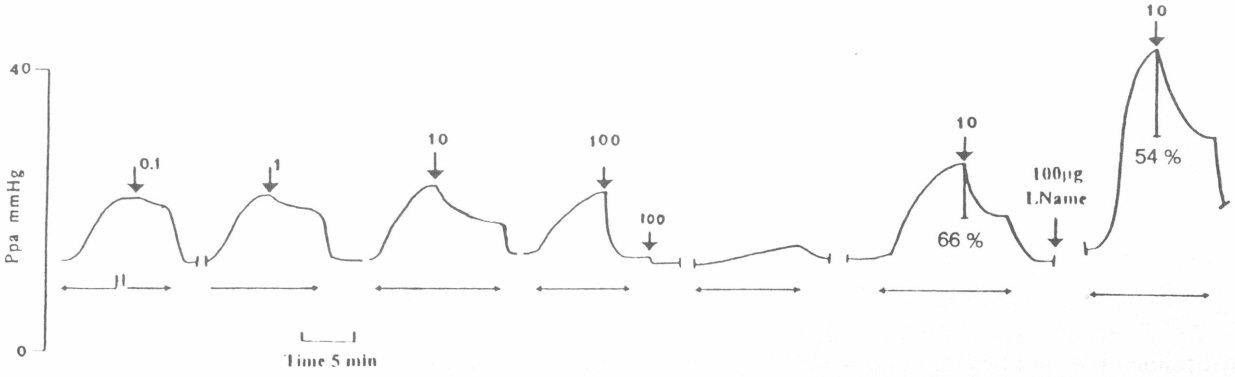


**Fig. 2**
Trace of effect of S-nitroso acetylpenicillamine (SNAP) in one rat IPL. Bars indicate periods of hypoxia. Injection of SNAP and L-NAME ($\mu$g) are indicated by the arrows.

*Effect of L-NAME*

100 $\mu$g L-NAME had either no effect or led to a small increase in normoxic Ppa (1.0±0.9 mm Hg). HPV was enhanced (pre L-NAME 10.3±7.7 mm Hg vs 21.0±15.3 mm Hg post L-NAME, $p<0.01$, $n=9$). The % fall in HPV after 10 $\mu$g SNAP was not significantly altered, being either unchanged, slightly greater or smaller (45±22 % pre vs 42±23 % post, NS, $n=9$). After L-NAME the dilatation was less persistent, tending to show slight reversal over the period studied (5–10 min).

*Effect of SNAP on HPV*

Large doses of SNAP (>100 $\mu$g), tended to reduce subsequent HPV (Fig. 2) but small doses had no effect. HPV was not significantly lowered by 1 $\mu$g SNAP (before: 10.1±2.1 mm Hg; after: 7.8±3.5 mm Hg, NS, $n=6$). The effect became significant when 100 $\mu$g SNAP was given (11.6±4.9 vs 7.4±3.2 mm Hg, $p<0.01$, $n=10$).

## Discussion

In the rat isolated lung, S-nitroso acetylpenicillamine (SNAP) caused profound dose-dependent vasodilatation, particularly when the pulmonary vascular tone was raised by hypoxia. In contrast, AP, a similar molecule to SNAP but with an SH group substituted for the SNO group, had no significant effect. This suggests that the nitric oxide donator property of SNAP was the major cause of the vasodilatation. Blockade of endogenous release of NO by L-NAME had no consistent effect on the dilator response to SNAP.

Nitric oxide, or a labile nitroso derivative such as S-nitrosocysteine, is generally considered to be EDRF (Moncada *et al.* 1991). It is released from cellular L-arginine by nitric oxide synthase (NOS) and diffuses into the adjacent vascular smooth muscle where it activates soluble guanylate cyclase. This raises the levels of guanosine 3'5'-cyclic monophosphate (cGMP), leading to vasorelaxation.

NOS is present in a variety of cells including vascular endothelium, smooth muscle, airway epithelium, inflammatory cells and certain neurones (Barnes and Belvisi 1993). There are at least two forms of the enzyme a) constitutive (cNOS) which is $Ca^{2+}$-dependent, basally expressed in vascular endothelial cells. cNOS is rapidly activated by agonists such as acetylcholine and bradykinin, producing NO within seconds. b) inducible (iNOS), which is $Ca^{2+}$-independent and expressed in inflammatory cells after exposure to endotoxin and some cytokines. iNOS involves gene transcription and thus NO production occurs several hours after exposure. NO released by either enzyme activates soluble guanylate cyclase to raise cGMP.

Dilators which work through this mechanism are termed endothelial-dependent, such as acetylcholine, bradykinin and substance P. Although this may not be an exclusive pathway, their dilator action is reduced or abolished if the production of EDRF is disrupted (Dinh-Xuan *et al.* 1991). Others, e.g. VIP, ANP and $\beta_2$ agonists, work through an endothelial-independent mechanism, raising cGMP through particulate guanylate cyclase, or cAMP through adenylate cyclase, to cause vasodilatation. Exogenous NO bypasses the endothelium.

Both NOS enzymes are inhibited by L-arginine analogues, such as nitro L-arginine methyl ester (L-NAME), nitro monomethyl L-arginine (L-NMMA) and nitro L-arginine (L-NA); the inhibition may often be overcome by addition of excess arginine. iNOS is selectively inhibited by corticosteroids. Target selective NOS inhibitors have also been developed (Barnes and Belvisi 1993).

The possible role of NO in the control of vascular resistance has been studied using both inhibitors of NO production and also inhibitors of soluble guanylate cyclase, such as methylene blue. In the systemic vasculature there appears to be a basal release of NO, modulating vascular tone (Moncada *et al.* 1991). Indeed, the vasodilatation associated with increased flow appears to be due to release of NO in systemic vessels (Rubanyi *et al.* 1986, Griffith *et al.* 1987). It has been suggested that the low pulmonary vascular tone may be due to continuous release of NO and that vasoconstriction to hypoxia is due to a reduction of NO production. Studies in different species and preparations have produced varying results. Overall, it appears that there is little release of NO under normal conditions in both rat and dog (Barer *et al.* 1993, Barnard *et al.* 1993, McMurtry *et al.* 1992, Nishiwaki *et al.* 1992, Perrella *et al.* 1992) although in other species, e.g. cats, rabbit, pig, this may not be true (Hyman *et al.* 1989, Wiklund *et al.* 1990, Cremona *et al.* 1994). There is some evidence that EDRF function may be reduced in severe hypoxia, but there is also evidence that EDRF release is increased in response to acute hypoxia and other vasoconstrictor agents, such as endothelin, in the rat and dog (Barer *et al.* 1993, Brashers *et al.* 1988, Leeman *et al.* 1994, McMurtry *et al.* 1992, Perrella *et al.* 1992) and appears to be continuously released in the normoxic, pulmonary hypertensive rat lung after exposure to chronic hypoxia (Barer *et al.* 1993, Oka *et al.* 1993). Thus, although EDRF release may not play as important a role in the maintenance of normal pulmonary vascular tone as in the systemic vasculature, NO does appear to be released in response to a rise in Ppa, acting as a braking mechanism. Interestingly, Barer *et al.* (1993) found evidence that this release was stimulated by

vasoconstrictor-induced but not passive rises in pressure, including increased flow, in rat isolated lungs.

The combination of NO with cellular thiols produces a more stable and more potent compound, which may resemble EDRF more closely than NO (Myers *et al.* 1990). In the cat left lower lobe preparation McMahon *et al.* (1993) showed that SNAP and S-nitrosocysteine caused a dose-dependent fall in pulmonary vascular resistance (PVR), when raised by the prostaglandin-endoperoxide analogue U-46619, in a similar manner to NO. In the current work, a similar dose-response relationship was found in the isolated rat lung, preconstricted by hypoxia. In this preparation, the fall in Ppa reflects change in PVR, as blood flow and left atrial pressure are maintained constant. Thus a fall in pressure reflects dilatation. The onset of the dilatation to SNAP was immediate and usually persisted for the period of hypoxic challenge (5–10 min) although occasionally partial reversal was seen. From normoxia, the fall in Ppa was also sustained. Large doses of SNAP tended to cause a temporary reduction of the subsequent HPV, which may be due to the persistence of released NO.

This dilatation, expressed as percentage fall in HPV (the rise in Ppa with 2 % $O_2$), was not consistently affected by L-NAME, it was occasionally reduced but sometimes enhanced. As AP did not cause any significant vasodilatation, it is reasonable to assume that the NO component of SNAP was responsible for the observed dilatation. Thus, it could be considered that blockade of endogenous release of NO during vasoconstriction might allow an enhancement of the action of released NO from SNAP. As expected, L-NAME enhanced HPV and the absolute fall in Ppa with SNAP was also increased. However, when expressed as a % fall in HPV, there was no consistent increase (Fig. 2). McMahon *et al.* (1993) found that Zaprinast, which inhibits the hydrolysis of cGMP, caused vasodilatation which was dependent on the endogenous release of NO. The size of the fall in Ppa to NO and NO donators, as well as to other endothelial-independent and -dependent dilators, was unaltered by Zaprinast. Although they investigated the effect of L-NAME on the dilatation to Zaprinast, its effect on NO dilatation was not studied. Interestingly, Hampl *et al.* (1993) found that the dilator response to the NO donator, sodium nitroprusside, was enhanced in the rat lung after chronic treatment with L-NAME. In the current work, L-NAME revealed a tendency to reversal of the dilatation to SNAP. Whether this reflects the instability of the PVR after NO blockade or an effect of L-NAME on the dilator action to SNAP, cannot be ascertained from this work. McMahon *et al.* (1993) found that Zaprinast increased the duration of the dilatation to SNAP suggesting that SNAP increased the formation of cGMP. They concluded that their results support the involvement of cGMP in the dilator mechanism of SNAP.

Inhaled NO is both a vaso- and bronchodilator and has provided a novel form of treatment for pulmonary hypertensive crises in the critical care situation. It has many advantages over other dilator therapies. a) It is endothelial-independent, there is evidence that endothelial function is reduced in chronic lung disease (Dinh-Xuan *et al.* 1991), b) given *via* the airway NO is potentially pulmonary selective, as any NO entering the blood is rapidly removed by the red blood cells due to its high affinity with haemoglobin, c) vasodilatation is dose-dependent with a rapid onset. However, NO has a short half-life and must be given continuously under carefully controlled conditions, to minimize its toxic potential.

The development of an endothelial-independent nitric oxide donator drug which could be administered *via* the airway could be advantageous by providing a potent stable form of NO delivery with pulmonary selectivity. Any concomitant bronchodilatation may also be advantageous. The S-nitrosothiols could be such a group of compounds. Inhalation of nitrosothiols is feasible; an inhaled thiol, glutathione, was found to be biologically safe and efficacious in reducing superoxide anion release from alveolar macrophages in patients with inflammatory lung disease (Borok *et al.* 1991).

In conclusion, SNAP causes a profound dose-dependent fall in pulmonary arterial pressure more obvious when the vasculature is preconstricted by hypoxia. This is independent of endogenous NO production. At present, inhalation of NO provides a more ideal treatment for severe pulmonary hypertension than other dilators. Whether inhaled nitric oxide donator drugs may provide more sustained, controlled release of NO requires further study.

## References

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

BARNARD J.W., WILSON P.S., MOORE T.M., THOMPSON W.J., TAYLOR A.E.: Effect of nitric oxide and cyclooxygenase products on vascular resistance in dog and rat lungs. *J. Appl. Physiol.* **74:** 2940–2948, 1993.

BARNES P.J., BELVISI M.G.: Nitric oxide and lung disease. *Thorax* **48:** 1034–1043, 1993.

BOROK Z., BUHL R. CRIMES G.J., BOKSER A.D., HUBBARD R.C., HOLROYD K.J., ROUM J.H., CZERSKI D.B., CANTIN A.M., CRYSTAL R.G.: Effect of glutathione aerosol on oxidant-antioxidant imbalance in idiopathic pulmonary fibrosis. *Lancet* **338:** 215–216, 1991.

BRASHERS V.L., PEACH M.J., ROSE C.E.: Augmentation of hypoxic pulmonary vasoconstriction in the isolated perfused rat lung by in vitro antagonists of endothelium-dependent relaxation. *J. Clin. Invest.* **82:** 1495–1502, 1988.

CREMONA G., WOOD A.M., HALL L.W., BOWER E.A., HIGENBOTTAM T.: Effect of inhibitors of nitric oxide release and action on vascular tone in isolated lungs of pig, sheep, dog and man. *J. Physiol. Lond.* **481:** 185–195, 1994.

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.: Impairment of endothelial-dependent pulmonary artery relaxation in chronic obstructive lung disease. *N. Engl. J. Med.* **324:** 1539–1547, 1991.

EMERY C.J., BEE D., BARER G.R.: Mechanical properties and reactivity in isolated lungs of chronically hypoxic rats. *Clin. Sci.* **61:** 569–580, 1981.

GRIFFITH T.M., EDWARDS D.H., DAVIES R.L., HARRISON T.J., EVANS K.T.: EDRF coordinates the behaviour of vascular resistance vessels. *Nature* **329:** 442–445, 1987.

GUSTAFSSON L.E., LEONE A.M., PERSSON M.G., WIKLUND N.P., MONCADA S.: Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem. Biophys. Res. Commun.* **181:** 852–857, 1991.

HAMPL V., ARCHER S.L., NELSON D.P., WEIR E.K.: Chronic EDRF inhibition and hypoxia: effects on pulmonary circulation and systemic blood pressure. *J. Appl. Physiol.* **75:** 1748–1757, 1993.

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

IGNARRO L.J., LIPPTON H.L., EDWARDS J.C., BARRICOS W.H., HYMAN A.L., KADOWITZ P.J., GRUETTER C.A.: Mechanisms of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide; evidence for the involvement of S-nitrosothiols as active intermediates. *J. Pharmacol. Exp. Ther.* **218:** 739–749, 1981.

LEEMAN M., DE BEYL V.Z., DELCROIX M., NAEIJE R.: Effects of endogenous nitric oxide on pulmonary vascular tone in intact dogs. *Am. J. Physiol.* **266:** H2343–H2347, 1994.

MCMAHON T.J., IGNARRO L.J., KADOWITZ P.J.: Influence of Zaprinast on vascular tone and vasodilator responses in the cat pulmonary vascular bed. *J. Appl. Physiol.* **74:** 1704–1711, 1993.

MCMURTRY I., MASAHIKO O. HASUNUMA K., YAMAGUCHI T., MORRIS K., RODMAN D.: Role of EDRF in control of normoxic and hypoxic pulmonary vascular tone. In: *High Altitude Medicine,* G. UEDA *et al.* (eds), Shinshu University Press, Matsumoto, Japan, 1992, pp. 131–138.

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

MYERS P.R., MINOR R.L.Jr., GUERRA R.Jr., BATES J.N., HARRISON D.G.: Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. *Nature* **345:** 161–163, 1990.

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

OKA M., HASUNUMA K., WEBB S.A., STELZNER T.J., RODMAN D.M., MCMURTRY I.F.: EDRF suppresses an unidentified vasoconstrictor mechanism in hypertensive rat lungs. *Am. J. Physiol.* **264:** L587–L597, 1993.

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

PERRELLA M.A., EDELL E.S., KROWKA M.J., CORTESE D.A., BURNETT J.C.Jr.: Endothelium-derived relaxing factor in pulmonary and renal circulations during hypoxia. *Am. J. Physiol.* **258:** R45–R50, 1992.

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

RUBANYI G.M., ROMERO J.C., VANHOUTTE P.M.: Flow-induced release of endothelium-derived relaxing factor. *Am. J. Physiol.* **250:** H1145–H1149, 1986.

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

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