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Activation of sympathoadrenomedullary system increases pulmonary nitric oxide production in the rabbit

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Abstract

Nitric oxide (NO) is continuously produced in the lung and can be measured in exhaled gas of different species. To investigate a possible neuro-humoral regulation of pulmonary NO production in vivo we injected veratrine, an activator of Na^+ channels known to activate the sympathoadrenal system, in anaesthetized, mechanically ventilated and laparotomized rabbits. Exhaled NO concentration increased by $38 \pm 3\%$ when plasma adrenaline rose from 12.3 ± 3.1 to 49.5 ± 10.7 pmol ml^{-1} in response to veratrine (500 $\mu g\ kg^{-1}$, i.v.). Pretreatment with atenolol, a β_1-adrenoceptor antagonist (1 mg kg^{-1}), or bilateral ligation of adrenal blood vessels inhibited the increase in exhaled NO in response to veratrine. Atenolol also decreased basal NO, suggesting an endogenous regulation of pulmonary NO by adrenaline. Neither phentolamine (1 mg kg^{-1}), atropine (1 mg kg^{-1}) nor vagotomy inhibited the veratrine-induced pulmonary NO production. These results suggest a role of the sympathoadrenal system in the regulation of pulmonary NO production.

Keywords: Adrenal; Atenolol; β-Adrenoceptor; Catecholamine; Lung; Nitric oxide (NO)

1. Introduction

Nitric oxide (NO) is continuously produced by cells in the respiratory system where NO has ubiquitous functions. Nitric oxide is a vasodilator in the tracheobronchial and pulmonary circulation (Alving et al., 1992; Higenbottam, 1995; Kuo et al., 1992; Carvalho et al., 1998; Stamler et al., 1994; Cooper et al., 1996; Celermajer et al., 1994) and NO modulates pulmonary vascular resistance during conditions of increased tone, e.g. during acute hypoxia (Persson et al., 1990; Archer et al., 1989). It appears that NO is of cardinal importance in the transition of pulmonary circulation at birth (Abman et al., 1990; Fineman et al., 1994). In the airways, mucosal NO production suppresses plasma exudation (Erjefalt et al., 1994; Bernareggi et al., 1997, 1999) and mucus secretion (Ramnarine et al., 1996), and promotes mucociliary clearance (Jain et al., 1993). Furthermore, NO counteracts the obstructive effects of bronchoconstrictive mediators and allergens (Persson et al., 1995a; Nijkamp et al., 1993; Ricciardolo et al., 1996) and NO has been identified as a major bronchodilator pathway in human airways (Belvisi et al., 1992). Nitric oxide can also directly influence ion channel activity in different pulmonary cells (Kamosinska et al., 1997; Guo et al., 1998; Schobersberger et al., 1997; Jain et al., 1998). Most likely NO plays a role in airway host defense since the nasal cavities holds cytotoxic concentrations of NO (Lundberg et al., 1995) known to kill viruses, bacteria, fungi and parasites (Lowenstein et al., 1994; Tsai et al., 1997).

Respiratory-produced NO can directly be detected in the exhaled air (Gustafsson et al., 1991) although the sites of origin of exhaled NO cannot be differentiated. Thus, the extent to which the above mentioned functions of NO are reflected in the concentrations of exhaled NO merits further study. Nevertheless, measurement of exhaled NO have revealed a variety of factors important for the physio-

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logical regulation of pulmonary NO production, e.g. airway concentrations of the respiratory gases oxygen and carbon dioxide (Bannenberg et al., 1997; Adding et al., 1999b; Gustafsson et al., 1991; Strömberg et al., 1997) and availability of L-Arginine, the substrate for nitric oxide synthase (NOS) (Gustafsson et al., 1991; Kharitonov et al., 1995). We have also found that stretching the lungs of rabbits and guinea pigs increases exhaled NO (Persson et al., 1995b; Strömberg et al., 1997; Bannenberg and Gustafsson, 1997; Artlich et al., 1999) an effect that pharmacologically could be inhibited with the stretch-activated Ca^{2+} channel blocker gadolinium chloride but not with the L-type Ca^{2+} channel blocker verapamil (Bannenberg and Gustafsson, 1997; Adding et al., 1998). Veratrine, an activator of voltage-gated Na^{+} channels has previously been found to stimulate slowly adapting pulmonary stretch receptors (Matsumoto and Shimizu, 1995). Therefore, we aimed at investigating whether veratrine might also enhance pulmonary NO production via activation of vagal pulmonary stretch receptors. The findings reported below indicate that veratrine indeed can increase NO production, but rather lend support to a role for catecholamines in both endogenous and veratrine-stimulated NO production.

2. Materials and methods

2.1. Surgical procedures

The local animal ethics committee approved the experiments. Male New Zealand white rabbits (2–3 kg) were anaesthetized with pentobarbital sodium (6 mg ml^{-1}, 45 mg kg^{-1} body weight) via an ear vein, were placed in the supine position on the operating table and tracheotomized. The animals were ventilated via a two way valve (model 2200 A; Hans Rudolph, Kansas City, MO, USA) by means of a Harvard model 683 rodent ventilator (Harvard Apparatus, South Natick, MA, USA). The ventilator was supplied with NO-free air using a charcoal filter (150×12 cm). Ventilation rate was 40 min^{-1} and the tidal volume was adjusted to keep end-tidal CO_2 at 4.5–5.5% as determined by a ventilatory monitor (Oscar-Oxy, Datex, Helsinki, Finland). A pressure transducer connected to a side arm of the tracheal cannula recorded insufflation pressure. Pressure recordings were performed by means of Statham pressure transducers (Hato Rey, Puerto Rico). The animals were paralyzed by injection of pancuronium bromide (0.5 mg kg^{-1}). Polyethylene catheters were inserted into the right carotid artery and the left jugular vein for recordings of arterial blood pressure, heart rate, sampling of arterial blood for catecholamine and blood gas determination and administration of drugs. A continuous infusion of glucose (2.75 g 100 ml^{-1}), dextran 70 (Macrodex® 2.8 g 100 ml^{-1}), $NaHCO_3$ (0.7 g 100 ml^{-1}) and pentobarbital sodium (180 mg 100 ml^{-1}) was administered i.v. at a rate of 10 ml kg^{-1} h^{-1} by means of an infusion pump (Terumo STC-521; Terumo, Tokyo, Japan). Drugs were administered by a microinfusion pump (CMA 100, Carnegie Medicine, Stockholm, Sweden) into the catheter inserted in the left jugular vein. Blood samples were analyzed on a Radiometer ABL 300 acid–base laboratory blood gas analyzer (Radiometer, Copenhagen, Denmark). Body temperature, measured rectally, was maintained at 37–38°C by means of a heating pad connected to a thermostat (Heater control LB 700, PRODAB, Uppsala, Sweden). In 19 rabbits, a midline laparotomy was performed and the intestines were exteriorized and wrapped into plastic foil to prevent desiccation. The adrenals were then dissected from the surrounding tissue so that only a stalk of connective tissue, containing the adrenal vessels, remained attached to the adrenal. In five rabbits, the adrenal vessels were crushed and tightly ligated, the remaining animals were used as sham controls. Hereafter, the intestines were placed back into the abdominal cavity and the abdomen was closed.

2.2. NO measurements in exhaled air

NO concentration was continuously measured in respiratory gas with a single-breath analysis system (Aerocrine, Danderyd, Sweden) as previously described (Adding et al., 1999b). Briefly respiratory gas sampling (70 ml min^{-1}) was from the tracheal cannula and detection limit < 1.5 ppb. The NO analyzer was calibrated with dilutions of NO in filtered air using certified NO standard gas in nitrogen (AGA Specialgas, Lidingö, Sweden).

2.3. Plasma catecholamine determination

Arterial plasma adrenaline and noradrenaline concentrations were determined with high-performance liquid chromatography (HPLC) as described by Hallman et al. (1978). Briefly, 1.5 ml of arterial whole blood (mixed with 30 μl EGTA (95 mg ml^{-1}) and glutathione (60 mg ml^{-1})) was drawn for each sample. The samples were centrifuged at $1000 \times g$ for 15 min at 4°C and the plasma supernatant was removed and frozen at −80°C until analysis. Catecholamines were extracted from each plasma specimen in aluminum oxide slurry, were eluted from the aluminum oxide with 0.1 M perchloric acid and subsequently quantified using HPLC. Catecholamines were resolved on a 0.4×15 cm 5-μm C18 reverse-phase column using an isocratic mobile phase at 600 ml min^{-1} consisting of 25 mM citric acid, 50 mM sodium acetic acid, 60 mM sodium hydroxide and acetic acid 0.21%. Amperometric detection was accomplished with a carbon electrode (Coulochem II; ESA, Chelmsford, MA, USA) set at a potential of +400 mV and 50 nA. Detection limits were 0.1 pmol for both adrenaline and noradrenaline.

Quantification was done by comparison with an internal standard of 1 pmol dihydroxybensylaminohydrobromide added to each sample before extraction.

2.4. Experimental protocol

Pilot experiments revealed that veratrine enhanced pulmonary NO production only at high doses (>300 μg kg^{-1}) which indicated the involvement of the sympathoadrenal system (Pilati et al., 1992). In a recent pharmacological study, we found that exogenous adrenaline infusions stimulated pulmonary NO production and that preferentially β_1-adrenoceptors were involved in this process (Adding et al., 1999a). Bearing these observations in mind, the following protocol was constructed.

In the first set of sham-operated rabbits (controls, $n = 6$), we studied the effect of veratrine on pulmonary NO production and arterial plasma catecholamine concentrations. For comparison, in the same animals, we administered an infusion of adrenaline (0.3 μg kg^{-1} min^{-1}, a dose that increased pulmonary NO production but caused little hemodynamic activity in our previous study (Adding et al., 1999a). Thus, a two min infusion of adrenaline was first administered and was 20 min later followed by a one min infusion of veratrine (500 μg kg^{-1} total dose). Arterial blood for catecholamine and blood gas analysis was sampled immediately prior to the start of and during the respective infusion of adrenaline or veratrine, at a time when exhaled NO reached its highest concentration.

The involvement of β_1-adrenoceptors in the veratrine-induced pulmonary NO production was investigated in sham-operated rabbits pre-treated with the β_1-adrenoceptor selective antagonist atenolol (1 mg kg^{-1}, $n = 6$). Adrenaline and veratrine were administered as described above.

The role of the adrenal glands in mediating the veratrine-induced pulmonary nitric oxide production was studied in rabbits where the adrenal blood vessels were crushed and ligated ($n = 5$). Adrenaline and veratrine were administered as described above. In two rabbits, we examined the effect of alpha (α)-adrenoceptor inhibition with phentolamine (1 mg kg^{-1}) or bilateral vagotomy (including section of the depressor nerves) and subsequent atropine (1 mg kg^{-1}) pretreatment on the response to veratrine infusion.

The NO concentration in inhaled and exhaled air was, together with arterial blood pressure, heart rate, end-tidal CO_2 and insufflation pressure continuously recorded on a Grass model 7 Polygraph (Grass Instruments, Quincy, MA, USA).

2.5. Statistics

Data are expressed as means ± S.E.M. Repeated measures of heart rate and arterial blood pressure were analyzed by one-way analysis of variance (ANOVA) (Tukey's test) or, if Kolmogorov–Smirnov test suggested a non-normality distribution of data, by Friedman ANOVA on Ranks (Dunnett's Method). Student's paired *t*-test or Wilcoxon signed rank test, if normality distribution test failed, evaluated pairwise comparisons. First-order linear regression were used for correlation analysis of NO and plasma

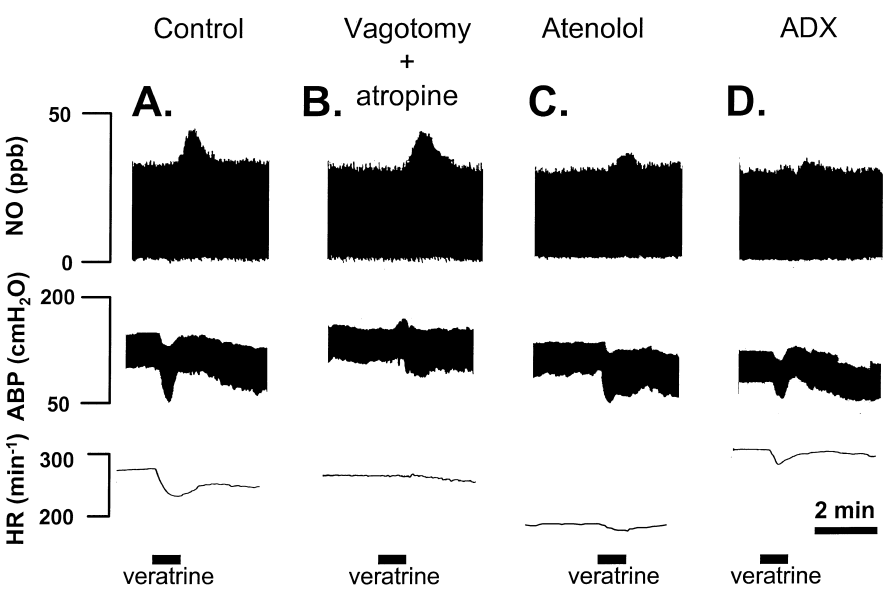

Fig. 1. Representative recordings of the effect of veratrine infusion (500 μg kg^{-1}, horizontal lines) on exhaled nitric oxide measured breath by breath, arterial blood pressure and heart rate in four pentobarbital anaesthetized rabbits. (A) No pre-treatment (control), (B) after bilateral vagotomy and a bolus dose of atropine (1 mg kg^{-1}; vagotomy + atropine), (C) after a bolus dose of atenolol (1 mg kg^{-1}; atenolol), and (D) after adrenal vessels were crushed and ligated (ADX). Note the rapid increase in exhaled NO in response to veratrine infusion and how this effect was abolished in the rabbit after the adrenal vessels were ligated.

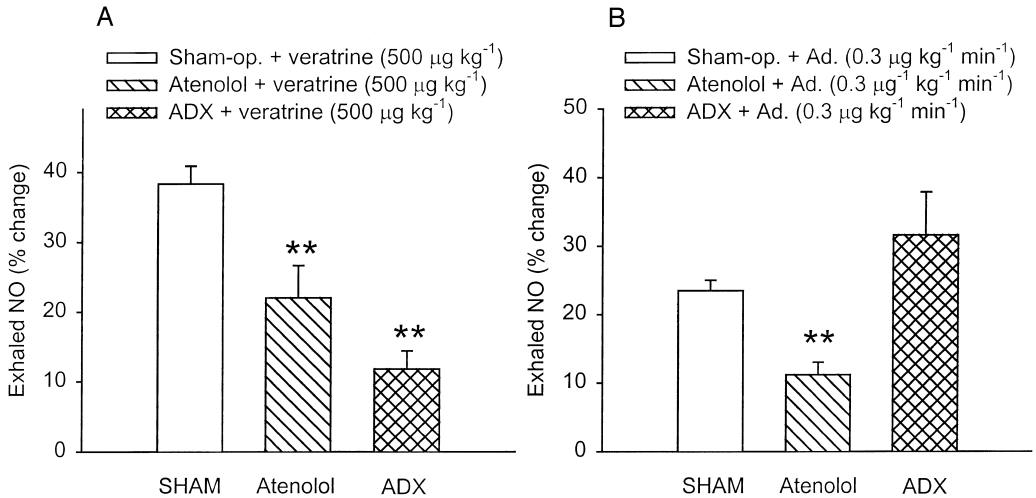

Fig. 2. The effect of veratrine (500 μg kg^{-1}, panel A) and adrenaline infusion (Ad. 3 μg kg^{-1} min^{-1}, panel B) on exhaled nitric oxide in sham-operated, control rabbits (open bars), sham-operated rabbits pretreated with atenolol (1 mg kg^{-1}, hatched bars) and rabbits with ligated adrenal blood vessels, crosshatched bars). ** Denotes significantly different ($P < 0.01$) from sham-operated, control rabbits. Means ± S.E.M. ($n = 5–6$).

adrenaline concentrations. A computer program (Sigma-Stat, Jandel, San Rafael, USA) processed all statistical analysis of data.

2.6. Drugs

Adrenaline was from NM Pharma (Stockholm, Sweden); Atenolol (Tenormine®) from Zeneca (Göteborg, Sweden); Phentolamine (Regitina®) from Ciba-Geigy (Basel, Switzerland); heparin from Kabi Vitrum (Stockholm, Sweden); pancuronium bromide (Pavulon®) from Organon (Oss, Holland); pentobarbital sodium from Apoteksbolaget (Stockholm, Sweden); atropine and veratrine hydrochloride from Sigma, St Louis, MO, USA; Dextran 70 (Macrodex®) from Pharmacia Infusion (Uppsala, Sweden).

3. Results

3.1. Effects of veratrine or adrenaline infusion in control rabbits

Breath by breath NO concentrations in exhaled air averaged 34 ± 1 ppb under control conditions ($n = 19$). Rapid increases in exhaled NO were detected in response to infusion of veratrine (500 μg kg^{-1}, Figs. 1 and 2A) or adrenaline (0.3 μg kg^{-1} min^{-1}, Fig. 2B) in sham-operated, control rabbits. Parallel to the maximal increase in exhaled NO arterial plasma adrenaline concentration increased significantly in response to veratrine (Fig. 3A) or adrenaline infusion (Fig. 3B). Plasma noradrenaline increased slightly in response to veratrine infusion (Fig. 3A)

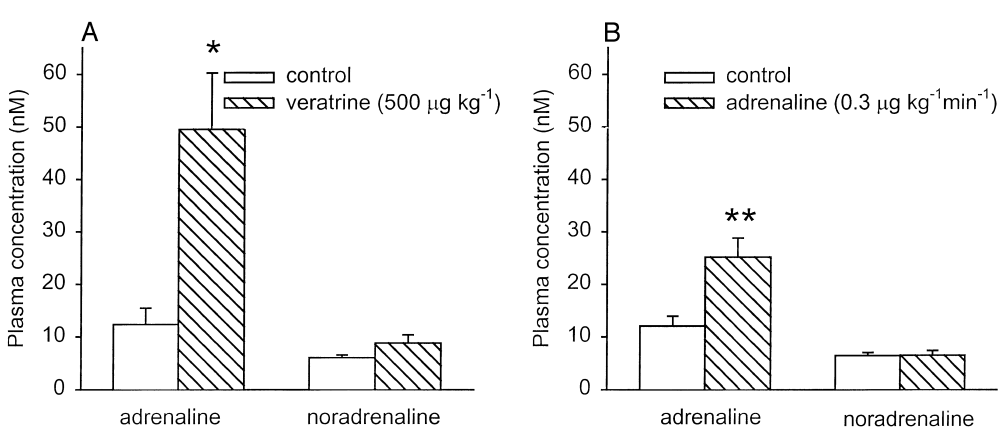

Fig. 3. The effect of veratrine (500 μg kg^{-1}, panel A) or adrenaline infusion (3 μg kg^{-1} min^{-1}, panel B) on arterial plasma catecholamine concentration in sham-operated rabbits. Determinations were made immediately before (open bars) and after respective infusion at a time (usually 1–2 min) exhaled nitric oxide had attained its highest value (hatched bars). * and ** denote significantly different ($P < 0.01$ and $P < 0.001$) from baseline. Means ± S.E.M. ($n = 6$).

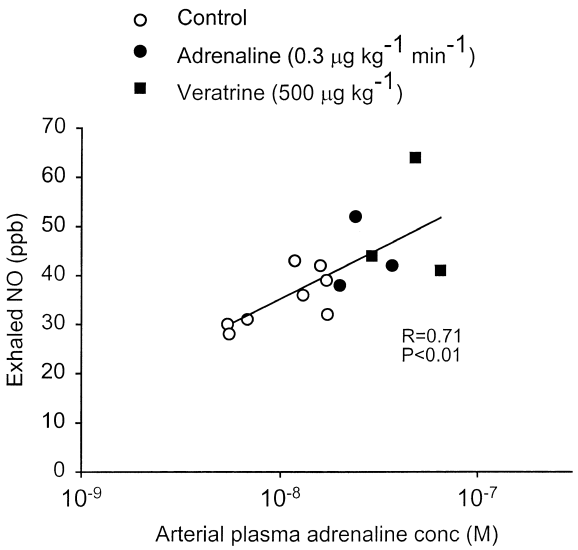

Fig. 4. Pentobarbital anaesthetized sham-operated rabbits. Exhaled nitric oxide as a function of the logarithm of arterial plasma adrenaline concentration. Data derived from baseline values and during respective infusion with veratrine (500 μg kg^{-1}) and adrenaline (0.3 μg kg^{-1} min^{-1}). First-order linear regression calculated using the least squares method, data from 14 determinations in three animals.

but did not change in response to adrenaline infusion (Fig. 3B). There was a positive correlation ($R = 0.71$; $P < 0.01$) between plasma levels of adrenaline and exhaled NO concentrations in these animals (Fig. 4). Veratrine induced a rapid and significant depression of heart rate and arterial blood pressure (Figs. 1 and 5) but caused no change in insufflation pressure (data not shown).

3.2. Effects of veratrine or adrenaline infusion in rabbits pretreated with atenolol

Pretreatment with atenolol (1 mg kg^{-1}) significantly reduced baseline levels of NO, from 37 ± 2 to 31 ± 1 ($P < 0.01$), and antagonized increments in exhaled NO concentrations in response to both veratrine (Fig. 2A) or adrenaline (Fig. 2B). Increments in arterial plasma catecholamine concentrations, in atenolol treated rabbits, were equivalent to control rabbits during infusion of adrenaline or veratrine. Atenolol-treated rabbits compared to control rabbits had a significantly lower baseline heart rate than control rabbits, 223 ± 8 and 291 ± 9, respectively ($P < 0.001$). Baseline arterial blood pressure was also lower in the atenolol-treated rabbits compared to control animals 96 ± 5 and 110 ± 5, respectively ($P = 0.08$). Veratrine infusion in atenolol-treated rabbits induced a significant fall in heart rate but the marked drop in arterial blood pressure seen in control animals was markedly reduced (Fig. 5).

3.3. Effects of veratrine or adrenaline infusion in rabbits with ligated adrenal vessels

In rabbits with crushed and ligated adrenal vessels, the increment in exhaled NO in response to veratrine was markedly inhibited (Fig. 2A), however the increase in exhaled NO in response to adrenaline was not different compared to control animals (Fig. 2B). In these animals baseline arterial blood pressure was significantly lower than control rabbits 84 ± 5 and 110 ± 5, respectively ($P = 0.005$), though baseline heart rate was not different. Veratrine infusion caused slight but non-significant drops in heart rate and arterial blood pressure in rabbits with ligated adrenal vessels (Fig. 5).

The effect of veratrine on exhaled NO concentration could neither be altered by pretreatment with phentolamine (1 mg kg^{-1}, data not shown) nor with bilateral vagotomy and atropine (1 mg kg^{-1}), even though the drop in arterial blood pressure and heart rate was inhibited (Fig. 1).

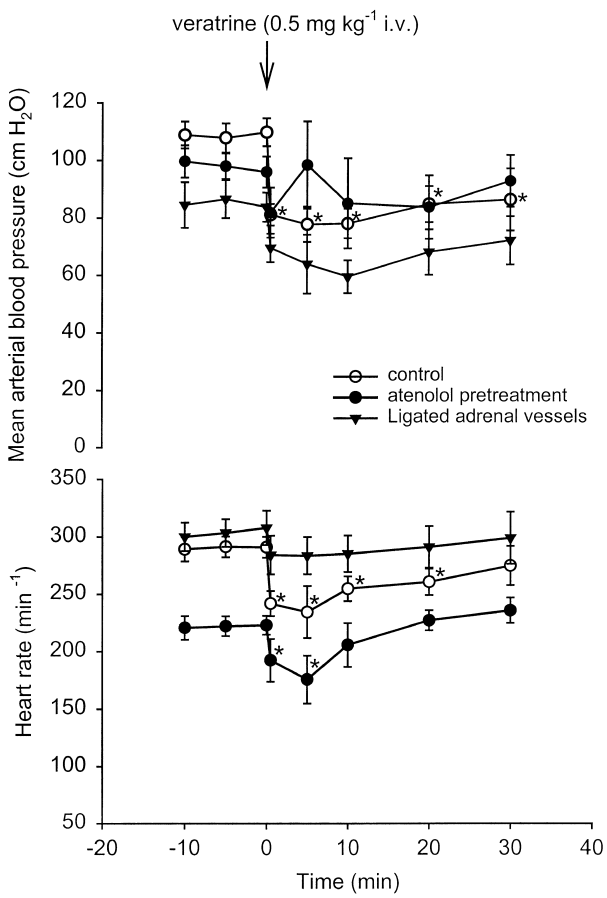

Fig. 5. Time-course of the effect of veratrine (500 μg kg^{-1}) on mean arterial blood pressure (upper panel) and heart rate (lower panel) in pentobarbital, mechanically ventilated and laparotomized rabbits. Control group (open circles), atenolol-pretreated rabbits (closed circles), rabbits with crushed and ligated adrenal vessels (closed triangles). * Denotes significantly different ($P < 0.05$) from time 0 min. Means ± S.E.M. (n = 5–6).

Table 1
Effects of adrenaline (0.3 μg kg^{-1} min^{-1}) and veratrine (500 μg kg^{-1} min^{-1}) on pH and blood gases in three groups of pentobarbital anaesthetized and mechanically ventilated rabbits

	pH	pCO_2 (kPa)	pO_2 (kPa)
Sham			
Control	7.33 ± 0.00	10.8 ± 0.7	4.8 ± 0.1
Adrenaline	7.34 ± 0.01	10.8 ± 0.6	4.7 ± 0.1
Veratrine	7.33 ± 0.01	11.3 ± 0.7	4.7 ± 0.1
Atenolol			
Control	7.35 ± 0.02	11.3 ± 0.7	5.0 ± 0.3
Adrenaline	7.33 ± 0.03	11.7 ± 0.6	5.2 ± 0.4
Veratrine	7.35 ± 0.02	11.2 ± 0.7	4.8 ± 0.1
ADX			
Control	7.32 ± 0.04	10.0 ± 1.2	4.8 ± 0.4
Adrenaline	7.31 ± 0.03	9.6 ± 0.4	4.6 ± 0.3
Veratrine	7.33 ± 0.03	9.8 ± 0.7	4.9 ± 0.4

Sham-operated (sham), sham-operated + atenolol-pretreated (atenolol) and operated with ligation of adrenal vessels (ADX). Values were obtained immediately before (control) and within 1–2 min after the start of respective infusion (during the peak of exhaled NO). Means ± S.E.M. (n = 3–5).

Blood gases in all three groups of rabbits were comparable to sham-operated rabbits during control conditions, pH 7.33 ± 0.01, pO_2 10.8 ± 0.7 and pCO_2 4.8 ± 0.1, at all times, and did not significantly change in response to neither adrenaline nor veratrine infusions (Table 1).

4. Discussion

The present study demonstrates that pulmonary NO production is enhanced by activation of the sympathoadrenomedullary system via stimulation of β-adrenoceptors and that this pathway may be pharmacologically stimulated by an intravenous infusion of veratrine. Furthermore, it is likely that adrenal secretion of catecholamines, particularly adrenaline, plays a key role in the pulmonary NO production in response to veratrine since ligation of adrenal blood vessels inhibited the increase in exhaled NO.

Endogenous pulmonary NO is continuously produced and is involved in the physiological regulation of airway and pulmonary vascular function. To fully appreciate the role of NO in pulmonary function, it is necessary to consider the physiological regulation of pulmonary NO production. Stretch-dependent pulmonary NO formation may involve a vagally mediated component (Persson et al., 1995b) and pulmonary vagal afferent neurons express the mRNA encoding NO synthase (Lawrence et al., 1996). Since it has been shown that veratrine stimulates slowly adapting pulmonary stretch receptors and their vagal afferents (Matsumoto and Shimizu, 1995; Matsumoto et al., 1998) we thought that similar mechanisms were involved in the veratrine-induced stimulation of pulmonary NO production. However, we found that vagotomy and atropine pretreatment had no effects on the veratrine-induced pulmonary NO production, which makes the involvement of muscarinic receptors or a vagally mediated reflex unlikely. Nevertheless, vagotomy and atropine pretreatment fully antagonized the veratrine-induced bradycardia and hypotension (Bezold–Jarisch reflex, coronary chemoreflex; Dawes and Widdicombe, 1951), which makes hemodynamic changes as possible cause for the veratrine-induced increases in exhaled NO unlikely. Furthermore, as mentioned above, we found that veratrine stimulated pulmonary NO production only at high doses yet hemodynamic changes occurred at lower doses (30–100 μg kg^{-1}, data not shown).

Veratrine has been used to directly stimulate the secretion of catecholamines from perfused adrenal glands (Ito and Ohga, 1978; Kirpekar and Prat, 1979) and bovine adrenal medullary cells in culture (Knight and Whitaker, 1978). Indeed, we found that plasma adrenaline increased almost fourfold in response to veratrine infusion and that the increase in exhaled NO was attenuated in the rabbits with ligated adrenal vessels. In these rabbits the increase in exhaled NO in response to exogenously administered adrenaline was unaltered indicating an intact functional receptor response. The fact that ligation of the adrenal vessels did not totally abolish the increase in exhaled NO in response to veratrine suggests either residual secretion of catecholamines from extra-adrenal tissue or from pulmonary sympathetic nerve terminals.

In a recent study, we showed that β- but not α-adrenoceptors were activated by exogenous adrenaline to stimulate pulmonary NO production and that administration of a β_1-adrenoceptor agonist (prenalterol) was more than 100 times more potent than a β_2-adrenoceptor agonist (terbutaline) in that respect (Adding et al., 1999a). In the present study a β_1-adrenoceptor antagonist (atenolol) but not an unselective α-adrenoceptor antagonist (phentolamine) significantly reduced the baseline levels of exhaled NO clearly demonstrating involvement of β_1-adrenoceptors in the endogenous regulation of pulmonary NO production, at least in rabbits in vivo after major surgery. Atenolol was not able to fully antagonize the increase in NO production in response to either veratrine or exogenously administered adrenaline. Thus, we cannot exclude that another subtype of β-adrenoceptor is also involved.

The origin of exhaled NO is not known and multiple cell types in the respiratory tract express NOS and produce NO (Kobzik et al., 1993). Furthermore, endogenously formed *S*-nitrosothiols, in particular *S*-nitrosoglutathion, are found in the airways (Gaston et al., 1993) and may decompose to NO that may influence exhaled NO levels. We cannot, from the present study, advance our understanding of the cellular source of exhaled NO. However, non-enzymatic NO formation seems unlikely since previous studies have shown that NO synthase inhibitors totally abolish exhaled NO in this rabbit model (Persson et al., 1994; Gustafsson et al., 1991; Adding et al., 1998).

Exhaled NO levels is not only determined by NO production but also NO degredation, e.g. oxidation by hemoglobin (Rimar and Gillis, 1993) and oxygen radicals (Freeman, 1994), the latter also known to deactivate catecholamines (Wolin and Belloni, 1985). Consequently, excess production of oxygen radicals in pathologic conditions may be detrimental to exhaled NO, partly due to rapid degradation of NO and possible due to suppression of catecholamine stimulated NO formation.

The physiological role of this endogenous catecholamine-stimulated pulmonary NO production is not obvious. Catecholamines are crucial for the physiological adaptation of the lungs at birth (Lagercrantz and Slotkin, 1986) where NO is a powerful vasodilator. Furthermore, measurements of airway NO concentrations in newborn humans reveal extremely high airway NO levels (Schedin et al., 1995). Recently it has been shown that autoinhalation of airway NO exerts vasodilator effects in the pulmonary circulation (Settergren et al., 1998). Therefore, it is tempting to speculate that catecholamine-induced pulmonary NO production plays a role at birth, however further studies are warranted.

In conclusion, we have shown that β-adrenoceptors are involved in the endogenous production of pulmonary NO and that veratrine can stimulate pulmonary NO production via the release of adrenal catecholamines in rabbits. Thus, activation of the sympathoadrenomedullary system might have effects on pulmonary function via NO.

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References

Abman, S.H., Chatfield, B.A., Hall, S.L., McMurtry, I.F., 1990. Role of endothelium-derived relaxing factor during transition of pulmonary circulation at birth. Am. J. Physiol. 259, H1921–H1927.

Adding, L.C., Bannenberg, G.L., Gustafsson, L.E., 1998. Gadolinium chloride inhibition of pulmonary nitric oxide production and effects on pulmonary circulation in the rabbit. Pharmacol. Toxicol. 83, 8–15.

Adding, L.C., Agvald, P., Artlich, A., Persson, M.G., Gustafsson, L.E., 1999a. Beta-adrenoceptor agonist stimulation of pulmonary nitric oxide production in the rabbit. Br. J. Pharmacol. 126, 833–839.

Adding, L.C., Agvald, P., Persson, M.G., Gustafsson, L.E., 1999b. Regulation of pulmonary nitric oxide by carbon dioxide is intrinsic to the lung. Acta Physiol. Scand. 167, 167–174.

Alving, K., Fornhem, C., Weitzberg, E., Lundberg, J.M., 1992. Nitric oxide mediates cigarette smoke-induced vasodilatory responses in the lung. Acta Physiol. Scand. 146, 407–408.

Archer, S.L., Tolins, J.P., Raij, L., Weir, E.K., 1989. Hypoxic pulmonary vasoconstriction is enhanced by inhibition of the synthesis of an endothelium derived relaxing factor. Biochem. Biophys. Res. Commun. 164, 1198–1205.

Artlich, A., Adding, C., Agvald, P., Persson, M.G., Lonnqvist, P.A., Gustafsson, L.E., 1999. Exhaled nitric oxide increases during high frequency oscillatory ventilation in rabbits. Exp. Physiol. 84, 959–969.

Bannenberg, G.L., Gustafsson, L.E., 1997. Stretch-induced stimulation of lower airway nitric oxide formation in the guinea-pig: inhibition by gadolinium chloride. Pharmacol. Toxicol. 81, 13–18.

Bannenberg, G.L., Giammarresi, C., Gustafsson, L.E., 1997. Inhaled carbon dioxide inhibits lower airway nitric oxide formation in the guinea pig. Acta Physiol. Scand. 160, 401–405.

Belvisi, M.G., Stretton, C.D., Yacoub, M., Barnes, P.J., 1992. Nitric oxide is the endogenous neurotransmitter of bronchodilator nerves in humans. Eur. J. Pharmacol. 210, 221–222.

Bernareggi, M., Mitchell, J.A., Barnes, P.J., Belvisi, M.G., 1997. Dual action of nitric oxide on airway plasma leakage. Am. J. Respir. Crit. Care Med. 155, 869–874.

Bernareggi, M., Radice, S., Rossoni, G., Oriani, G., Chiesara, E., Berti, F., 1999. Hyperbaric oxygen increases plasma exudation in rat trachea: involvement of nitric oxide. Br. J. Pharmacol. 126, 794–800.

Carvalho, P., Johnson, S.R., Charan, N.B., 1998. Non-cAMP-mediated bronchial arterial vasodilation in response to inhaled beta-agonists. J. Appl. Physiol. 84, 215–221.

Celermajer, D.S., Dollery, C., Burch, M., Deanfield, J.E., 1994. Role of endothelium in the maintenance of low pulmonary vascular tone in normal children. Circulation 89, 2041–2044.

Cooper, C.J., Landzberg, M.J., Anderson, T.J., Charbonneau, F., Creager, M.A., Ganz, P., Selwyn, A.P., 1996. Role of nitric oxide in the local regulation of pulmonary vascular resistance in humans. Circulation 93, 266–271.

Dawes, M.J., Widdicombe, J.G., 1951. The depressor action of the veratrum alkaloids. Br. J. Pharmacol. Chemother. 6, 675–681.

Erjefalt, J.S., Erjefalt, I., Sundler, F., Persson, C.G., 1994. Mucosal nitric oxide may tonically suppress airways plasma exudation. Am. J. Respir. Crit. Care Med. 150, 227–232.

Fineman, J.R., Wong, J., Morin III, F.C., Wild, L.M., Soifer, S.J., 1994. Chronic nitric oxide inhibition in utero produces persistent pulmonary hypertension in newborn lambs. J. Clin. Invest. 93, 2675–2683.

Freeman, B., 1994. Free radical chemistry of nitric oxide. Looking at the dark side. Chest 105, 79S–84S.

Gaston, B., Reilly, J., Drazen, J.M., Fackler, J., Ramdev, P., Arnelle, D., Mullins, M.E., Sugarbaker, D.J., Chee, C., Singel, D.J. et al., 1993. Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. Proc. Natl. Acad. Sci. U. S. A. 90, 10957–10961.

Guo, Y., DuVall, M.D., Crow, J.P., Matalon, S., 1998. Nitric oxide inhibits Na^+ absorption across cultured alveolar type II monolayers. Am. J. Physiol. 274, L369–L377.

Gustafsson, L.E., Leone, A.M., Persson, M.G., Wiklund, N.P., Moncada, S., 1991. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. Biochem. Biophys. Res. Commun. 181, 852–857.

Hallman, H., Farnebo, L.O., Hamberger, B., Johnsson, G., 1978. A sensitive method for the determination of plasma catecholamines using liquid chromatography with electrochemical detection. Life Sci. 23, 1049–1052.

Higenbottam, T., 1995. Lung disease and pulmonary endothelial nitric oxide. Exp. Physiol. 80, 855–864.

Ito, S., Ohga, A, 1978. Pharmacological evidence for the involvement of Na^+ channels in the release of catecholamines from perfused adrenal glands. Br. J. Pharmacol. 62, 359–361.

Jain, B., Rubinstein, I., Robbins, R.A., Leise, K.L., Sisson, J.H., 1993. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. Biochem. Biophys. Res. Commun. 191, 83–88.

Jain, L., Chen, X.J., Brown, L.A., Eaton, D.C., 1998. Nitric oxide inhibits lung sodium transport through a cGMP-mediated inhibition of epithelial cation channels. Am. J. Physiol. 274, L475–L484.

Kamosinska, B., Radomski, M.W., Duszyk, M., Radomski, A., Man, S.F., 1997. Nitric oxide activates chloride currents in human lung epithelial cells. Am. J. Physiol. 272, L1098–L1104.

Kharitonov, S.A., Lubec, G., Lubec, B., Hjelm, M., Barnes, P.J., 1995. L-Arginine increases exhaled nitric oxide in normal human subjects. Clin. Sci. (Colch) 88, 135–139.

Kirpekar, S.M., Prat, J.C., 1979. Release of catecholamines from perfused cat adrenal gland by veratridine. Proc. Natl. Acad. Sci. U. S. A. 76, 2081–2083.

Knight, D.E., Whitaker, M.J., 1978. Veratridine-induced secretion in medullary cells isolated from the bovine adrenal gland. J. Physiol. (London) 281, 18P–19P [proceedings].

Kobzik, L., Bredt, D.S., Lowenstein, C.J., Drazen, J., Gaston, B., Sugarbaker, D., Stamler, J.S., 1993. Nitric oxide synthase in human and rat lung: immunocytochemical and histochemical localization. Am. J. Respir. Cell Mol. Biol. 9, 371–377.

Kuo, H.P., Liu, S., Barnes, P.J., 1992. The effect of endogenous nitric oxide on neurogenic plasma exudation in guinea-pig airways. Eur. J. Pharmacol. 221, 385–388.

Lagercrantz, H., Slotkin, T.A., 1986. The "stress" of being born. Sci. Am. 254, 100–107.

Lawrence, A.J., Krstew, E., Jarrott, B., 1996. Actions of nitric oxide and expression of the mRNA encoding nitric oxide synthase in rat vagal afferent neurons. Eur. J. Pharmacol. 315, 127–133.

Lowenstein, C.J., Dinerman, J.L., Snyder, S.H., 1994. Nitric oxide: a physiologic messenger. Ann. Intern. Med. 120, 227–237.

Lundberg, J.O., Farkas-Szallasi, T., Weitzberg, E., Rinder, J., Lidholm, J., Anggaard, A., Hokfelt, T., Lundberg, J.M., Alving, K., 1995. High nitric oxide production in human paranasal sinuses. Nat. Med. 1, 370–373.

Matsumoto, S., Shimizu, T., 1995. Flecainide blocks the stimulatory effect of veratridine on slowly adapting pulmonary stretch receptors in anaesthetized rabbits without changing lung mechanics. Acta Physiol. Scand. 155, 297–302.

Matsumoto, S., Takahashi, T., Tanimoto, T., Saiki, C., Takeda, M., Ojima, K., 1998. Excitatory mechanism of veratridine on slowly adapting pulmonary stretch receptors in anesthetized rabbits. Life Sci. 63, 1431–1437.

Nijkamp, F.P., van der Linde, H.J., Folkerts, G., 1993. Nitric oxide synthesis inhibitors induce airway hyperresponsiveness in the guinea pig in vivo and in vitro. Role of the epithelium. Am. Rev. Respir. Dis. 148, 727–734.

Persson, M.G., Gustafsson, L.E., Wiklund, N.P., Moncada, S., Hedqvist, P., 1990. Endogenous nitric oxide as a probable modulator of pulmonary circulation and hypoxic pressor response in vivo. Acta Physiol. Scand. 140, 449–457.

Persson, M.G., Midtvedt, T., Leone, A.M., Gustafsson, L.E., 1994. $Ca^{(2+)}$-dependent and $Ca^{(2+)}$-independent exhaled nitric oxide, presence in germ-free animals, and inhibition by arginine analogues. Eur. J. Pharmacol. 264, 13–20.

Persson, M.G., Friberg, S.G., Gustafsson, L.E., Hedqvist, P., 1995a. The promotion of patent airways and inhibition of antigen-induced bronchial obstruction by endogenous nitric oxide. Br. J. Pharmacol. 116, 2957–2962.

Persson, M.G., Lonnqvist, P.A., Gustafsson, L.E., 1995b. Positive end-expiratory pressure ventilation elicits increases in endogenously formed nitric oxide as detected in air exhaled by rabbits. Anesthesiology 82, 969–974.

Pilati, C.F., Bosso, F.J., Maron, M.B., 1992. Factors involved in left ventricular dysfunction after massive sympathetic activation. Am. J. Physiol. 263, H784–H791.

Ramnarine, S.I., Khawaja, A.M., Barnes, P.J., Rogers, D.F., 1996. Nitric oxide inhibition of basal and neurogenic mucus secretion in ferret trachea in vitro. Br. J. Pharmacol. 118, 998–1002.

Ricciardolo, F.L., Geppetti, P., Mistretta, A., Nadel, J.A., Sapienza, M.A., Bellofiore, S., Di Maria, G.U., 1996. Randomised double-blind placebo-controlled study of the effect of inhibition of nitric oxide synthesis in bradykinin-induced asthma. Lancet 348, 374–377.

Rimar, S., Gillis, C.N., 1993. Selective pulmonary vasodilation by inhaled nitric oxide is due to hemoglobin inactivation. Circulation 88, 2884–2887.

Schedin, U., Frostell, C., Gustafsson, L.E., 1995. Nitric oxide occurs in high concentrations in monkey upper airways. Acta Physiol. Scand. 155, 473–474.

Schobersberger, W., Friedrich, F., Hoffmann, G., Volkl, H., Dietl, P., 1997. Nitric oxide donors inhibit spontaneous depolarizations by L-type Ca^{2+} currents in alveolar epithelial cells. Am. J. Physiol. 272, L1092–L1097.

Settergren, G., Angdin, M., Astudillo, R., Gelinder, S., Liska, J., Lundberg, J.O., Weitzberg, E., 1998. Decreased pulmonary vascular resistance during nasal breathing: modulation by endogenous nitric oxide from the paranasal sinuses. Acta Physiol. Scand. 163, 235–239.

Stamler, J.S., Loh, E., Roddy, M.A., Currie, K.E., Creager, M.A., 1994. Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. Circulation 89, 2035–2040.

Strömberg, S., Lonnqvist, P.A., Persson, M.G., Gustafsson, L.E., 1997. Lung distension and carbon dioxide affect pulmonary nitric oxide formation in the anaesthetized rabbit. Acta Physiol. Scand. 159, 59–67.

Tsai, W.C., Strieter, R.M., Zisman, D.A., Wilkowski, J.M., Bucknell, K.A., Chen, G.H., Standiford, T.J., 1997. Nitric oxide is required for effective innate immunity against *Klebsiella pneumoniae*. Infect. Immun. 65, 1870–1875.

Wolin, M.S., Belloni, F.L., 1985. Superoxide anion selectively attenuates catecholamine-induced contractile tension in isolated rabbit aorta. Am. J. Physiol. 249, H1127–H1133.