Nitric oxide blunts the endothelin-mediated pulmonary vasoconstriction in exercising swine

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We have previously shown that vasodilators and vasoconstrictors that are produced by the vascular endothelium, including nitric oxide (NO), prostanoids and endothelin (ET), contribute to the regulation of systemic and pulmonary vascular tone in swine, in particular during treadmill exercise. Since NO and prostanoids can modulate the release of ET, and vice versa, we investigated the integrated endothelial control of pulmonary vascular resistance in exercising swine. Specifically, we tested the hypothesis that increased NO and prostanoid production during exercise limits the vasoconstrictor influence of ET, so that loss of these vasodilators results in exaggerated ET-mediated vasoconstriction during exercise. Fifteen instrumented swine were exercised on a treadmill at 0–5 km h^{-1} before and during ET_A/ET_B receptor blockade (tezosentan, 3 mg kg^{-1} I.V.) in the presence and absence of inhibition of NO synthase (N^{ω}-nitro-L-arginine, 20 mg kg^{-1} I.V.) and/or cyclo-oxygenase (indometacin, 10 mg kg^{-1} I.V.). In the systemic circulation, ET receptor blockade decreased vascular resistance at rest, which waned with increasing exercise intensity. Prior inhibition of either NO or prostanoid production augmented the vasodilator effect of ET receptor blockade, and these effects were additive. In contrast, in the pulmonary bed, ET receptor blockade had no effect under resting conditions, but decreased pulmonary vascular resistance during exercise. Prior inhibition of NO synthase enhanced the pulmonary vasodilator effect of ET receptor blockade, particularly during exercise, whereas inhibition of prostanoids had no effect, even after prior NO synthase inhibition. In conclusion, endogenous endothelin limits pulmonary vasodilatation in response to treadmill exercise. This vasoconstrictor influence is blunted by NO but not by prostanoids.

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The pulmonary vascular bed is a low-resistance system that is capable of accommodating large amounts of blood flow at low levels of pulmonary artery pressure (Reeves & Taylor, 1996). Consequently, under basal resting conditions the normal arterio-venous pressure difference across the pulmonary vascular bed is approximately 10 mmHg, which contrasts sharply with the 90–100 mmHg arterio-venous pressure gradient across the systemic bed. Despite the low pulmonary vascular resistance under resting conditions, a small further decrease in pulmonary resistance occurs during exercise, albeit significantly less (10–30%) than the 60–80% decrease in systemic vascular resistance (Reeves & Taylor, 1996). The exercise-induced decrease in pulmonary vascular resistance involves both passive pulmonary vasodilatation, including vascular recruitment and passive distension due to the exercise-induced increase in pulmonary artery pressure, as well as an active reduction in pulmonary vasomotor tone (Reeves & Taylor, 1996). The mechanism of vasomotor tone regulation in pulmonary resistance vessels during exercise is still incompletely understood, but differs from that in the systemic vascular bed. For example, while endogenous adenosine and K^+_{ATP} channel activity have been shown to exert a vasodilator influence in the systemic bed during treadmill exercise, they are not mandatory for the regulation of tone in pulmonary resistance vessels (Duncker *et al.* 1998, 2001).

The vascular endothelium releases a variety of vasoactive substances, including nitric oxide (NO), prostanoids and endothelin (ET), that contribute to vasomotor control. However, the endothelial lining is not a homogeneous compartment as it is characterized by significant structural and functional heterogeneity. For example, the endothelium in the pulmonary bed differs markedly in ultrastructure and function from the systemic endothelium (Aird, 2003; Budhiraja *et al.* 2004). In support

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of this concept, recent studies in swine indicate that while both NO and prostanoids exert a vasodilator influence on the systemic vascular bed during exercise, only NO, but not prostanoids, contributes to the exercise-induced pulmonary vasodilatation (Duncker *et al.* 2000; Merkus *et al.* 2004). Furthermore, we recently observed in exercising swine that in the systemic circulation, the vasoconstrictor influence of ET wanes with increasing exercise intensities, whereas in the pulmonary circulation an ET vasoconstrictor influence emerges during exercise (Merkus *et al.* 2003). Since ET can increase the production of NO and prostanoids, which in turn can blunt the release of ET (Rubanyi & Polokoff, 1994; Haynes & Webb, 1998; Schiffrin & Touyz, 1998) or modify the responsiveness of its receptors (Wiley & Davenport, 2001), the present study was undertaken to investigate the integrated vasomotor control of pulmonary vascular resistance by NO, prostanoids and ET, in chronically instrumented swine under resting conditions and during graded treadmill exercise.

Methods

Animals

Studies were performed in accordance with the Council of Europe Convention (ETS123)/Directive (86/609/EEC) for the protection of vertebrate animals used for experimental and other scientific purposes, and with approval of the Animal Care Committee of the Erasmus Medical Center. Fifteen 2–3-month-old Yorkshire X Landrace swine (22 ± 1 kg at the time of surgery) of either sex entered the study.

Surgery

Swine were sedated with ketamine (30 mg kg^{-1} I.M.), anaesthetized with thiopental (10 mg kg^{-1} I.V.), intubated and ventilated with a mixture of O_2 and N_2O (1 : 2) to which 0.2–1% (v/v) isoflurane was added (Stubenitsky *et al.* 1998; Duncker *et al.* 2001). Anaesthesia was maintained with midazolam (2 mg kg^{-1} + 1 mg kg^{-1} h^{-1} I.V.) and fentanyl (10 μg kg^{-1} h^{-1} I.V.). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid-filled polyvinylchloride catheter was inserted into the aortic arch for aortic blood pressure measurement (Combitrans pressure transducers, Braun) and blood sampling. An electromagnetic flow probe (14–15 mm, Skalar) was positioned around the ascending aorta for measurement of cardiac output. Polyvinylchloride catheters were inserted into the left atrium to measure pressure, and into the pulmonary artery to measure pressure, administer drugs and collect mixed venous blood samples. Catheters were tunnelled to the back, and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine I.M.) for 2 days and antibiotic prophylaxis (25 mg kg^{-1} amoxicillin and 5 mg kg^{-1} gentamicin I.V.) for 5 days.

Experimental protocols

Studies were performed 1–3 weeks after surgery with animals exercising on a motor driven treadmill. The excellent reproducibility of consecutive exercise trials has been reported previously (Duncker *et al.* 1998, 2000, 2001; Stubenitsky *et al.* 1998). In the present study, four exercise protocols were performed on different days and in random order.

Endothelin. With swine ($n = 11$) lying quietly on the treadmill, resting haemodynamic measurements, consisting of heart rate, cardiac output, mean aortic pressure (MAP), mean pulmonary artery pressure (MPAP), and mean left atrial pressure (MLAP) were obtained and blood samples collected. Haemodynamic measurements were repeated, and rectal temperature was measured with animals standing on the treadmill. Subsequently, a five-stage (1–5 km h^{-1}) treadmill exercise protocol was started; each exercise stage lasted 2–3 min. Haemodynamic variables were continuously recorded and blood samples collected during the last 45 s of each stage. After completing the exercise protocol animals were allowed to rest on the treadmill for 90 min, after which the mixed ET_A and ET_B receptor (ET_A/ET_B) antagonist tezosentan (a gift from Dr Clozel, Actelion Pharmaceuticals Ltd) was intravenously administered over 10 min in a dose of 3 mg kg^{-1}, followed by a continuous infusion of 6 mg kg^{-1}·h^{-1} I.V. (Merkus *et al.* 2003), and the exercise protocol was repeated.

Prostanoids and endothelin. Ninety minutes after nine swine had undergone a control exercise trial (as described above), animals received the cyclo-oxygenase inhibitor indometacin ((Sigma) 10 mg kg^{-1} I.V. over 10 min (Merkus *et al.* 2004)), and 5 min later underwent a second exercise trial. Ninety minutes later, animals received indometacin in a dose of 5 mg kg^{-1} I.V., which resulted in haemodynamic conditions that were identical to those following administration of 10 mg kg^{-1} prior to the second exercise trial. Subsequently, animals received tezosentan (3 mg kg^{-1} I.V. + 6 mg kg^{-1} h^{-1} I.V.), and underwent a third exercise trial.

NO and endothelin. Ninety minutes after seven swine had undergone a control exercise trial, animals received the NO-synthase inhibitor N^{ω}-nitro-L-arginine (NLA (Sigma), 20 mg kg^{-1} I.V.; (Duncker *et al.* 2000)), and underwent a second exercise trial. Ninety minutes later, animals received tezosentan (3 mg kg^{-1}

Table 1. Haemodynamics of swine at rest and during exercise

	Rest		Exercise level (km h^{-1})				
Treatment	Lying	Standing	1	2	3	4	5
HR (bpm)							
Control	126 ± 5	147 ± 5*	169 ± 6*	186 ± 5*	202 ± 6*	232 ± 8*	265 ± 5*
Tezo	150 ± 5‡	163 ± 5*‡	184 ± 6*	198 ± 8*	214 ± 7*‡	243 ± 9*‡	267 ± 6*
Indo	86 ± 7†	97 ± 5*†	121 ± 6*†	134 ± 6*†	150 ± 6*†	174 ± 6*†	204 ± 7*†
Indo + Tezo	97 ± 7†‡	112 ± 5*†‡	128 ± 6*†	141 ± 6*†	157 ± 7*†	178 ± 8*†	212 ± 9*†
NLA	97 ± 5†	115 ± 3*†	129 ± 6*†	139 ± 6*†	156 ± 6*†	190 ± 9*†	225 ± 9*†
NLA + Tezo	112 ± 6†	136 ± 7*†‡	146 ± 8*†‡	154 ± 7*†‡	176 ± 8*†‡	201 ± 8*†‡	234 ± 10*†
NLA + Indo	82 ± 7†	93 ± 8*†	110 ± 8*†	122 ± 12*†	134 ± 10*†	164 ± 13*†	193 ± 12*†
NLA + Indo + Tezo	100 ± 10†	116 ± 8*†‡	127 ± 6*†‡	138 ± 7*†	153 ± 13*†‡	171 ± 11*†	194 ± 10*†
CO (l min^{-1})							
Control	3.6 ± 0.2	4.5 ± 0.2*	5.3 ± 0.3*	5.9 ± 0.3*	6.4 ± 0.3*	7.1 ± 0.3*	7.8 ± 0.4*
Tezo	4.0 ± 0.2‡	4.7 ± 0.3*	5.5 ± 0.3*	6.1 ± 0.4*	6.8 ± 0.4*‡	7.6 ± 0.4*‡	8.2 ± 0.4*
Indo	2.6 ± 0.3†	3.2 ± 0.3*†	4.1 ± 0.3*†	4.5 ± 0.3*†	5.1 ± 0.3*†	6.2 ± 0.3*†	7.2 ± 0.3*
Indo + Tezo	3.0 ± 0.2†	3.8 ± 0.3*†‡	4.4 ± 0.3*†	5.2 ± 0.4*‡	5.6 ± 0.4*‡	6.4 ± 0.4*	7.5 ± 0.5*
NLA	3.1 ± 0.4†	3.8 ± 0.2*†	4.5 ± 0.3*†	4.9 ± 0.3*†	5.4 ± 0.3*†	6.4 ± 0.4*†	7.1 ± 0.4*†
NLA + Tezo	3.8 ± 0.4‡	4.7 ± 0.4*‡	5.2 ± 0.4*†‡	5.6 ± 0.4*†‡	6.4 ± 0.3*†‡	7.3 ± 0.4*†‡	8.2 ± 0.5*‡
NLA + Indo	2.0 ± 0.3†	2.3 ± 0.3†	3.1 ± 0.3*†	3.7 ± 0.4*†	4.1 ± 0.3*†	5.1 ± 0.5*†	6.0 ± 0.3*
NLA + Indo + Tezo	3.1 ± 0.3	3.8 ± 0.4‡	4.2 ± 0.3*‡	4.9 ± 0.3*	5.2 ± 0.3*‡	5.8 ± 0.3*	6.6 ± 0.4*‡
MAP (mmHg)							
Control	97 ± 3	90 ± 3*	89 ± 2*	90 ± 3*	89 ± 2*	92 ± 2*	93 ± 2
Tezo	91 ± 2	82 ± 3*‡	81 ± 2*‡	79 ± 2*‡	82 ± 2*‡	83 ± 2*‡	85 ± 2*‡
Indo	124 ± 7†	120 ± 7†	107 ± 6*†	106 ± 6*†	107 ± 5*†	104 ± 5*	101 ± 4*
Indo + Tezo	111 ± 3†	93 ± 3*†‡	89 ± 2*†‡	89 ± 3*†‡	89 ± 3*‡	88 ± 3*‡	89 ± 3*‡
NLA	124 ± 3†	118 ± 3†	115 ± 3†	115 ± 2*†	118 ± 2†	119 ± 2†	119 ± 3†
NLA + Tezo	112 ± 5†	103 ± 3†‡	99 ± 4*†‡	99 ± 3*†‡	100 ± 3*†‡	98 ± 3*†‡	98 ± 2*†‡
NLA + Indo	166 ± 8†	163 ± 8†	151 ± 6*†	145 ± 5†	140 ± 3*†	134 ± 4*†	128 ± 5*†
NLA + Indo + Tezo	128 ± 5†‡	118 ± 7*†‡	113 ± 6*†‡	114 ± 6*†‡	115 ± 7*†‡	113 ± 6*†‡	113 ± 8*†‡
MPAP (mmHg)							
Control	15 ± 1	15 ± 2	19 ± 1*	21 ± 1*	23 ± 1*	28 ± 2*	33 ± 1*
Tezo	16 ± 1	13 ± 2	17 ± 2	18 ± 2	21 ± 2*‡	25 ± 2*‡	28 ± 2*‡
Indo	19 ± 1	18 ± 2	18 ± 2	20 ± 2	22 ± 2*	26 ± 2*	29 ± 2*
Indo + Tezo	17 ± 2	14 ± 2*‡	15 ± 2	17 ± 2	20 ± 2	23 ± 2*	26 ± 2*
NLA	25 ± 2†	22 ± 2	25 ± 2†	27 ± 2†	31 ± 2*†	38 ± 2*†	44 ± 1*†
NLA + Tezo	18 ± 2	19 ± 2	18 ± 2†‡	21 ± 1‡	25 ± 2*†‡	29 ± 3*‡	33 ± 3*‡
NLA + Indo	24 ± 2†	26 ± 3†	27 ± 3	29 ± 3	31 ± 3*	35 ± 2*†	37 ± 3*
NLA + Indo + Tezo	19 ± 2	17 ± 3	20 ± 2	23 ± 3	25 ± 3	28 ± 4*‡	31 ± 3*‡
MLAP (mmHg)							
Control	4 ± 1	2 ± 2	3 ± 1	4 ± 1	6 ± 1	7 ± 1*	11 ± 1*
Tezo	4 ± 1	0 ± 1*	3 ± 1	3 ± 1	6 ± 1	8 ± 1*	10 ± 1*
Indo	10 ± 2†	7 ± 2	3 ± 2*	6 ± 1*	6 ± 1*	7 ± 2	8 ± 2
Indo + Tezo	8 ± 1	2 ± 1*‡	3 ± 2*	4 ± 1*	6 ± 1*	7 ± 1	8 ± 2
NLA	11 ± 1†	5 ± 2*	7 ± 1*	8 ± 1*	9 ± 1*	10 ± 1*	12 ± 1*
NLA + Tezo	3 ± 3	5 ± 1*	4 ± 2*	7 ± 1*	9 ± 1*	11 ± 2*	13 ± 2*
NLA + Indo	16 ± 3†	15 ± 4†	13 ± 4	14 ± 3†	14 ± 3†	12 ± 3	10 ± 3
NLA + Indo + Tezo	7 ± 3‡	4 ± 4	5 ± 2	8 ± 2	9 ± 2	11 ± 3	12 ± 2

HR: heart rate, CO: cardiac output, MAP: mean arterial pressure, MPAP: mean pulmonary arterial pressure, MLAP: mean left atrial pressure. Tezo: tezosentan, Indo: indometacin, NLA: N$^{\omega}$-nitro-L-arginine. Data are mean ± S.E.M.; *$P < 0.05$ *versus* rest (lying); †$P < 0.05$ *versus* corresponding control, ‡$P < 0.05$ effect of tezosentan.

I.V. + 6 mg kg^{-1} h^{-1} I.V.) and underwent a third exercise trial.

NO, prostanoids and endothelin. Ninety minutes after five swine underwent an exercise trial in the presence of NLA (20 mg kg^{-1} I.V.), animals received indometacin (10 mg kg^{-1} I.V.) and underwent a second exercise trial. Ninety minutes later, animals received indometacin (5 mg kg^{-1} I.V.) and tezosentan (3 mg kg^{-1} I.V. and 6 mg kg^{-1} h^{-1} I.V.), and underwent a third exercise trial.

Blood gas measurements

Blood samples were kept in iced syringes until the conclusion of each exercise trial. Measurements of P_{O_2}(mmHg), P_{CO_2}(mmHg) and pH were then immediately performed with a blood gas analyser (Acid-Base Laboratory Model 505, Radiometer, Copenhagen, Denmark). Oxygen saturation (%) and haemoglobin (g (100 ml)$^{-1}$) were measured with a haemoximeter (OSM3, Radiometer). Blood O_2 content (μmol ml^{-1}) was computed as (Hb $\times$ 0.621 $\times$ O_2 saturation) + (0.00131 $\times$ P_{O_2}). Body O_2 consumption (BV_{O_2}) was calculated as the product of cardiac output and the difference in O_2 content between arterial and mixed venous blood (Stubenitsky *et al.* 1998; Duncker *et al.* 2001).

Data analysis

Digital recording and off-line analysis of haemodynamics have been previously described (Duncker *et al.* 1998; Stubenitsky *et al.* 1998). Systemic vascular resistance was computed as mean aortic blood pressure divided by cardiac output. Pulmonary vascular resistance was computed as mean pulmonary artery pressure minus mean left atrial pressure divided by cardiac output (Merkus *et al.* 2004).

Statistical analysis

Analysis of variance (ANOVA) for repeated measures or analysis of covariance (ANCOVA) were used as appropriate. *Post hoc* testing for exercise and drug effect was performed using Scheffe's test. Statistical significance was accepted when $P < 0.05$. Data are presented as mean ± S.E.M.

Results

The role of prostanoids and NO in the regulation of vascular tone

Systemic circulation. Table 1 shows that exercise up to 5 km h^{-1} resulted in more than a doubling of cardiac output which was principally due to an increase in heart

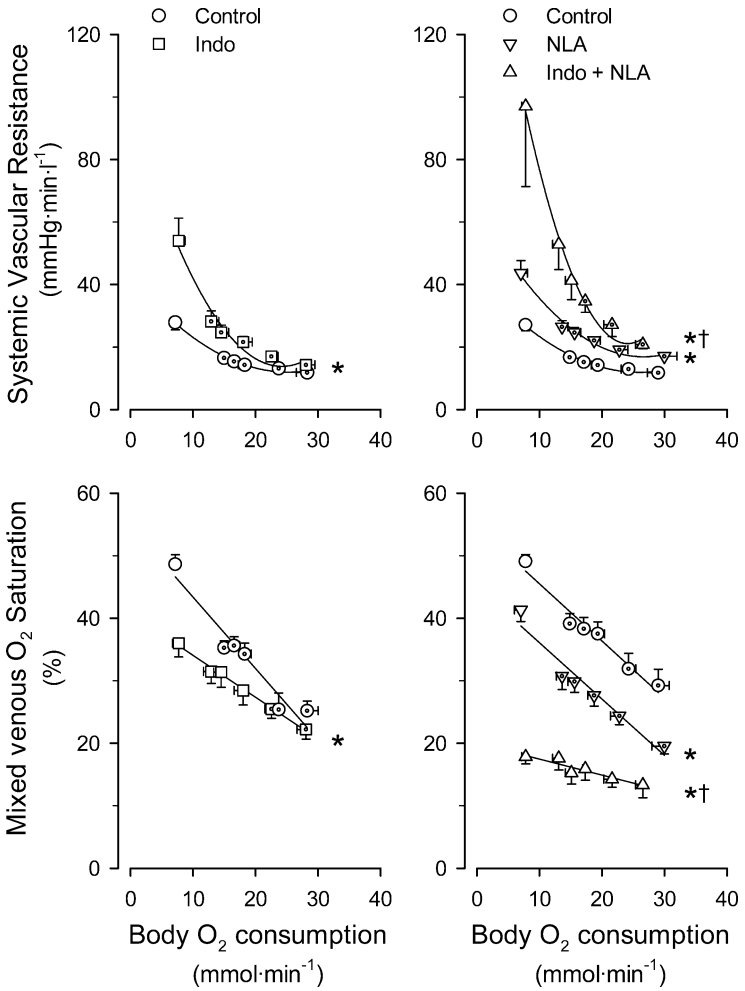

Figure 1. Role of NO and prostanoids in the regulation of systemic vascular tone
Effect of cyclo-oxygenase inhibition (indometacin (Indo), left panels) and NO synthase inhibition (NLA, right panels) and combined inhibition of cyclo-oxygenase and NO synthase (Indo + NLA, right panels) on the relation between body O_2 consumption and systemic vascular resistance (upper panels) and the mixed venous O_2 saturation (lower panels). Data are mean ± S.E.M. Dot inside symbol denotes $P < 0.05$ *versus* corresponding value at rest, *$P < 0.05$ *versus* control; †$P < 0.05$ *versus* NLA.

rate (up to 85% of maximum heart rate), as stroke volume increased by only 15% (not shown). The increase in cardiac output, was balanced by a similar decrease in systemic vascular resistance (Fig. 1), so that mean aortic blood pressure was minimally affected (Table 1).

Administration of the NO synthase inhibitor NLA or the cyclo-oxygenase inhibitor indometacin resulted in a marked increase in aortic blood pressure, which was due to systemic vasoconstriction (Table 1, Fig. 1). The accompanying decrease in cardiac output resulted from a (probably baroreflex-mediated) decrease in heart rate. The systemic vasoconstriction necessitated an increase in O_2 extraction resulting in a decreased mixed venous O_2 saturation. During exercise, the pressor and vasoconstrictor responses to cyclo-oxygenase inhibition were progressively blunted, whereas the responses to NO synthase inhibition were maintained (Table 1, Fig. 1).

Pretreatment with NLA enhanced the indometacin-induced vasoconstriction in the systemic circulation, as indicated by the exaggerated increase in systemic vascular resistance and exaggerated decrease in mixed venous O_2 saturation. Importantly, despite the marked potentiation of the systemic vasoconstrictor response to cyclo-oxygenase inhibition by NO synthase inhibition under resting conditions, the exercise-induced vasodilatation was unmitigated in the presence of indometacin and NLA (Fig. 1).

Pulmonary circulation. Pulmonary artery pressure doubled during exercise (Table 1). However, the transpulmonary pressure gradient (MPAP – MLAP) increased slightly less than cardiac output, reflecting a 20% decrease in pulmonary vascular resistance (Fig. 2). In contrast to the systemic bed, inhibition of cyclo-oxygenase had no effect on pulmonary vascular resistance either at rest or during exercise. NO synthase inhibition produced vasoconstriction in the pulmonary circulation, resulting in an increase in pulmonary artery pressure. Subsequent inhibition of cyclo-oxygenase had no additional effect on pulmonary vascular resistance (Fig. 2).

The role of endothelin in the regulation of vascular tone

Systemic circulation. Administration of the mixed ET_A/ET_B antagonist tezosentan resulted in a small decrease in aortic blood pressure under resting conditions (Table 1), which was caused by systemic vasodilatation as demonstrated by a decrease of systemic vascular resistance and an increase in mixed venous O_2 saturation (Fig. 3). The vasodilator response to tezosentan waned progressively with incremental exercise intensity. Pretreatment with indometacin enhanced the systemic vasodilatation by tezosentan, particularly at rest. In contrast, pretreatment with the NO synthase inhibitor NLA enhanced the vasodilator response to tezosentan both at rest and during exercise. Finally, combined pretreatment with indometacin and NLA caused a further increase in the vasodilator responses to tezosentan (Fig. 3). These observations indicate that both prostanoids and NO blunt the endothelin vasoconstrictor influence in the systemic circulation at rest and during exercise.

Pulmonary circulation. Tezosentan had no effect on the pulmonary circulation under resting conditions (Table 1, Fig. 4). In contrast during exercise, tezosentan reduced pulmonary artery pressure, with no effect on left atrial pressure and cardiac output (Table 1), reflecting a decrease in pulmonary vascular resistance (Fig. 4). Pretreatment with indometacin did not change the vasodilator response to tezosentan. In contrast, in the presence of NLA, the pulmonary vasodilator response to tezosentan was markedly enhanced. Additional pretreatment with indometacin did not further enhance pulmonary vasodilatation by tezosentan, as compared to NLA alone. These findings indicate that NO, but not prostanoids, blunts the vasoconstrictor response to ET in the pulmonary circulation during exercise.

Discussion

The main findings in the present study in awake swine, free from the effects of anaesthesia and acute surgical trauma are that: (i) prostanoids blunt the vasoconstrictor influence

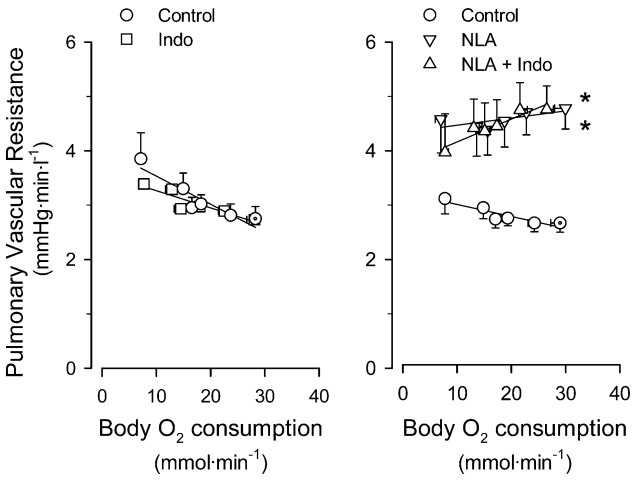

Figure 2. Role of NO and prostanoids in the regulation of pulmonary vascular tone
Effect of cyclo-oxygenase inhibition (Indo, left panels) and NO synthase inhibition (NLA, right panels) and combined inhibition of cyclo-oxygenase and NO synthase (Indo + NLA, right panels) on the relation between body O_2 consumption and pulmonary vascular resistance. Indometacin had no effect on pulmonary vascular resistance either under control conditions or in the presence of NLA. Data are mean ± S.E.M. Dot inside symbol denotes $P < 0.05$ *versus* rest ; $*P < 0.05$ *versus* control.

of ET in the systemic but not the pulmonary circulation; (ii) NO blunts the vasoconstrictor influence of ET in both the systemic and pulmonary circulation, particularly during exercise; (iii) prostanoids and NO blunt the vasoconstrictor influence of ET on the systemic bed in an additive manner; but (iv) loss of NO does not unmask a role of prostanoids in blunting the vasoconstrictor influence of ET in the pulmonary circulation.

Pulmonary vascular resistance during exercise

Exercise produced a small decrease in pulmonary vascular resistance, which was probably due to a decrease in vasomotor tone in the pulmonary resistance vessels. Pulmonary vasomotor tone is the resultant of an interplay between vasodilator and vasoconstrictor influences, as is also illustrated by previous findings from our laboratory that α-adrenoceptor blockade (Stubenitsky *et al.* 1998) and ET receptor blockade (Merkus *et al.* 2003) induce pulmonary vasodilatation, while β-adrenoceptor blockade (Stubenitsky *et al.* 1998) as well as NO synthase inhibition (Duncker *et al.* 2000; Merkus *et al.* 2004) result in pulmonary vasoconstriction in exercising pigs. Importantly, the increase in pulmonary vascular resistance produced by blockade of vasodilator pathways was accompanied by an increase in pulmonary arterial pressure. Since an increase in pulmonary arterial pressure would act to cause a passive decrease in resistance, these studies indicate that the increase in pulmonary vascular resistance must have been the result of an increase in vasomotor tone, and further support the concept that the exercise-induced decrease in pulmonary vascular resistance is principally due to a decrease in pulmonary resistance vessel tone (Dawson, 1984).

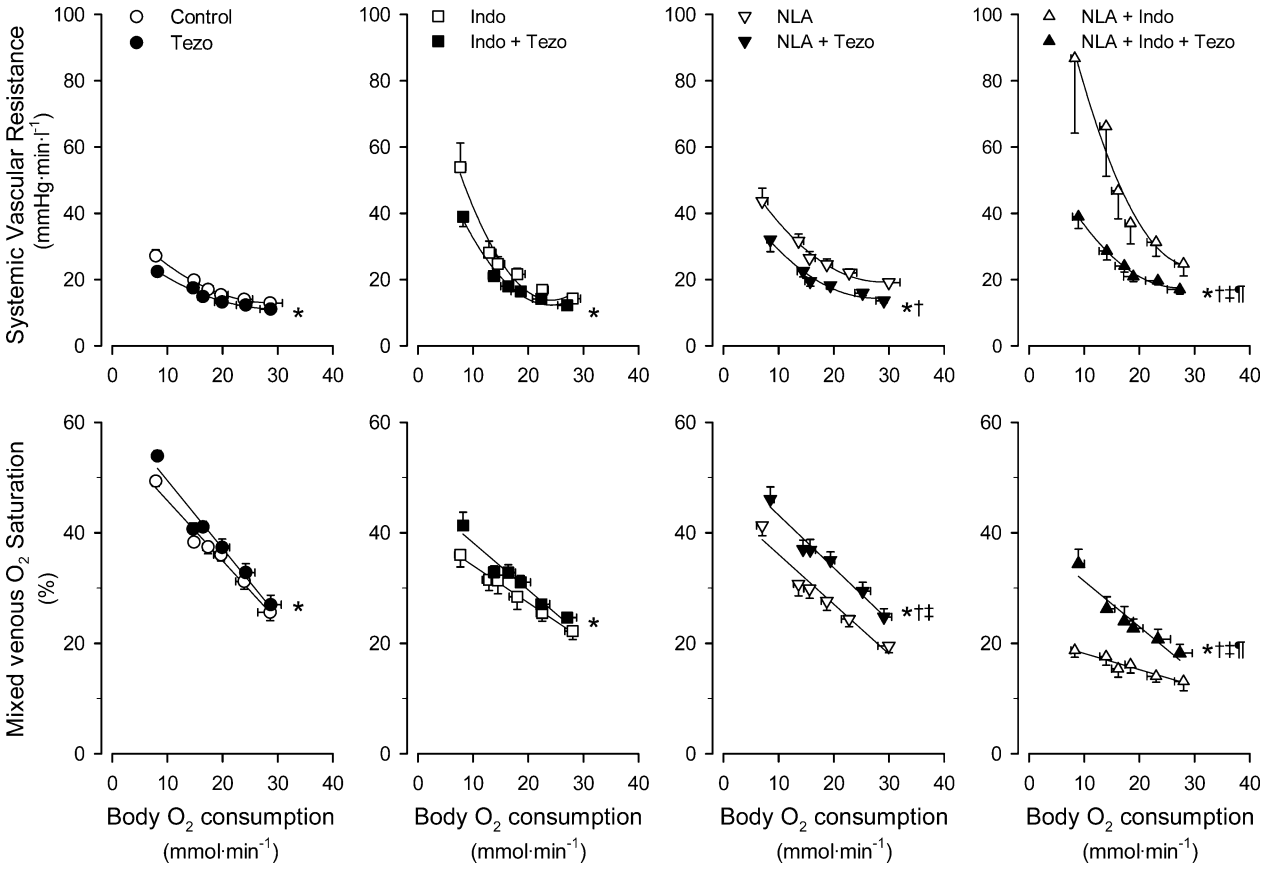

Figure 3. Interaction between NO, prostanoids and endothelin in the regulation of systemic vascular tone

From left to right: effect of endothelin receptor blockade (tezosentan (Tezo)), cyclo-oxygenase inhibition and endothelin receptor blockade (Indo + Tezo), NO synthase inhibition and endothelin receptor blockade (NLA + Tezo) and combined inhibition of cyclo-oxygenase and NO synthase and endothelin receptor blockade (NLA + Indo + Tezo) on the relation between body O_2 consumption and systemic vascular resistance (upper panels) and the mixed venous O_2 saturation (lower panels). Data are mean ± S.E.M. *$P < 0.05$ effect of Tezo *versus* corresponding control; †$P < 0.05$ effect of Tezo in presence of Indo, NLA or NLA + Indo *versus* Tezo alone; ‡$P < 0.05$ effect of Tezo in presence of NLA or NLA + Indo *versus* effect of Tezo in the presence of Indo; ¶$P < 0.05$ effect of Tezo in presence of NLA + Indo *versus* effect of Tezo in the presence of NLA.

Role of endothelium in regulation of pulmonary vascular resistance

Nitric oxide. The role of endogenous NO in maintaining the low basal pulmonary vascular resistance is species dependent. Thus, while the majority of studies suggest a role for NO in the regulation of basal pulmonary vascular resistance in swine, sheep, horses and humans, most studies do not support such a role of NO in dogs (see Hampl & Herget (2000) for references). The role of NO in the pulmonary vasodilatation during exercise is similarly species dependent. Thus, despite causing a significant pulmonary vasoconstriction under resting conditions, NLA had no effect on the exercise-induced pulmonary vasodilatation in sheep (Koizumi *et al.* 1994) or horses (Manohar & Goetz, 1998). In contrast, we have consistently observed that NO synthase inhibition blunts the exercise-induced pulmonary vasodilatation in swine (Duncker *et al.* 2000; Merkus *et al.* 2004). The results of the present study confirm our previous observations and indicate that significant interspecies differences exist with respect to vasomotor control mechanisms within the pulmonary resistance vessels (Hampl & Herget, 2000).

Prostanoids. Inhibition of endogenous prostanoid production does not alter basal pulmonary vascular resistance in sheep (Newman *et al.* 1986) and swine (Merkus *et al.* 2004), but causes vasoconstriction in dogs (Lindenfeld *et al.* 1983; Endredi *et al.* 1992). In most species, endogenous prostanoids also do not appear to contribute to the exercise-induced pulmonary vasodilatation (Lindenfeld *et al.* 1983; Newman *et al.* 1986; Merkus *et al.* 2004). It could be argued that the dose of indometacin used in the present study was not sufficient to inhibit cyclo-oxygenase in the pulmonary circulation. This is unlikely, however, in view of our observation that indometacin in a dose of 10 mg kg^{-1} does not produce a greater vasoconstrictor response in the systemic bed than 1 mg kg^{-1} (Merkus *et al.* 2004), suggesting a maximal effect. On the other hand, it cannot be excluded that cyclo-oxygenase inhibition may have gone without an apparent effect on pulmonary vascular resistance due to the opposing effects of simultaneously blocking vasodilator and vasoconstrictor prostanoids. Interestingly, the vasoconstrictor response to indometacin in the systemic circulation was enhanced following NO synthase inhibition, which is probably due to the inhibition of prostacyclin synthase activity by NO and peroxynitrite (Zou *et al.* 1997). In contrast, cyclo-oxygenase inhibition in the presence of NLA still had no effect on pulmonary vascular resistance (Albertini *et al.* 1996; Merkus *et al.* 2004), suggesting that prostacyclin activity was not increased following NO synthase inhibition. Future studies, using thromboxane A_2 receptor antagonists or thromboxane A_2 synthase inhibitors in conjunction with cyclo-oxygenase inhibitors, are required to evaluate in detail the integrated control of pulmonary vascular

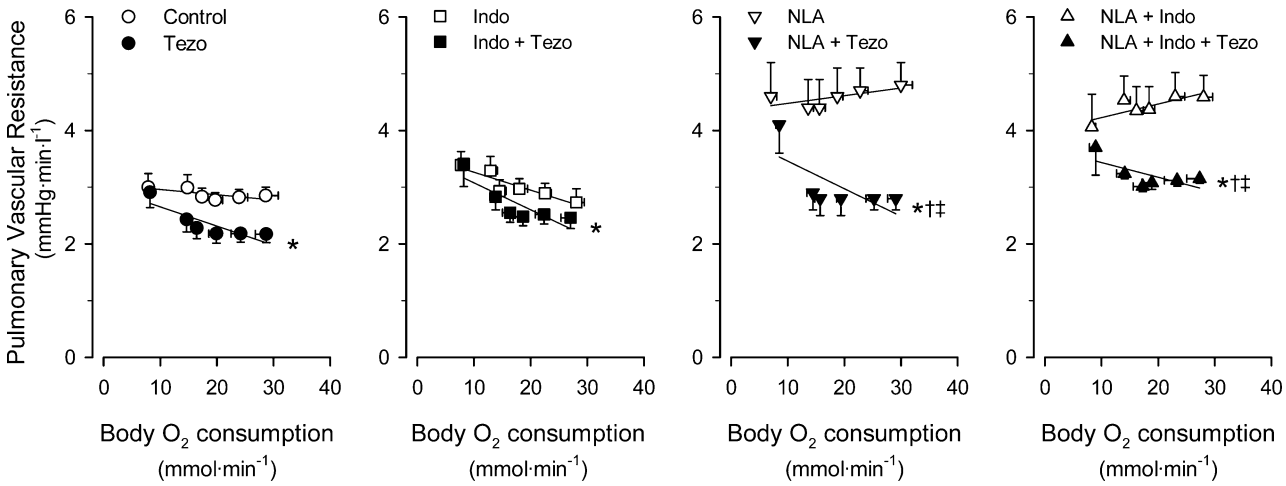

Figure 4. Interaction between NO, prostanoids and endothelin in the regulation of pulmonary vascular tone

From left to right: effect of endothelin receptor blockade (tezosentan (Tezo)), cyclo-oxygenase inhibition and endothelin receptor blockade (Indo + Tezo), NO synthase inhibition and endothelin receptor blockade (NLA + Tezo) and combined inhibition of cyclo-oxygenase and NO synthase and endothelin receptor blockade (NLA + Indo + Tezo) on the relation between body O_2 consumption and pulmonary vascular resistance. Indometacin had no effect on the response of pulmonary vascular resistance to tezosentan either under control conditions or in the presence of NLA. Data are mean ± S.E.M. *$P < 0.05$ effect of Tezo *versus* corresponding control; †$P < 0.05$ effect of Tezo in presence of Indo, NLA or NLA + Indo *versus* Tezo alone; ‡$P < 0.05$ effect of Tezo in presence of NLA or NLA + Indo *versus* effect of Tezo in the presence of Indo.

resistance by vasodilator and vasocontrictor prostanoids during exercise.

Endothelin. ET-induced constriction is mediated by ET_A receptors in the large pulmonary arteries, whereas it is mediated by ET_B receptors in the smaller pulmonary resistance vessels (MacLean *et al.* 1994). In accordance with these findings, the density of ET_A receptors in the lung decreases with decreasing vessel size, whereas the density of ET_B receptors, in both the endothelium and smooth muscle increases (Soma *et al.* 1999). In our study, blockade of both ET_A and ET_B receptors did not affect pulmonary vascular resistance at rest, indicating that endogenous ET does not contribute to resting tone in the pulmonary resistance vessels. During exercise however, an ET-mediated vasoconstriction became apparent, which contrasts with the blunted ET-mediated constriction in the systemic bed (Merkus *et al.* 2003).

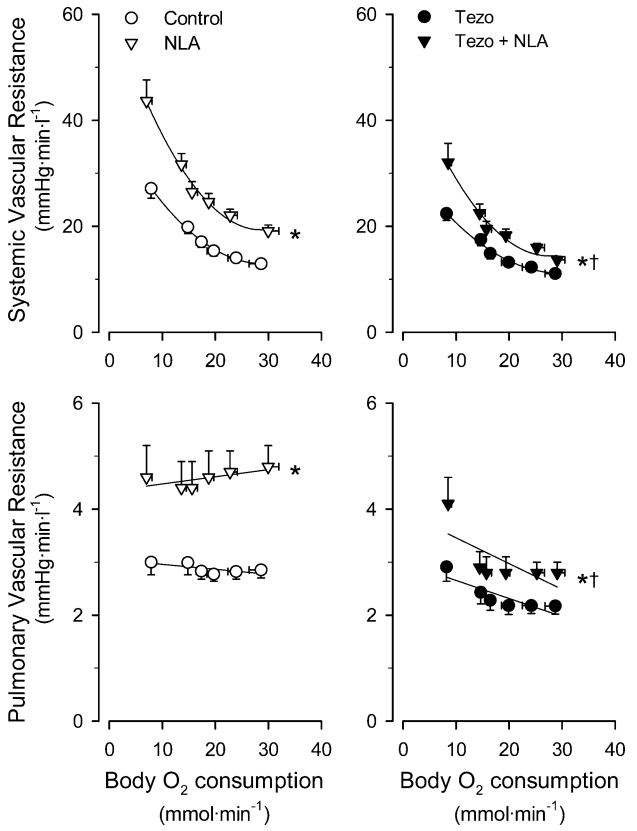

Figure 5. Effect of NO synthase inhibition in the absence and presence endothelin receptor blockade
Effect of NO synthase inhibition (NLA) in the absence (left) and presence (right) of endothelin receptor blockade (Tezo) on the relation between body O_2 consumption and systemic vascular resistance (upper panels) and on the relation between body O_2 consumption and pulmonary vascular resistance (lower panels). Data are mean ± S.E.M. *$P < 0.05$ effect of NLA *versus* corresponding control; †$P < 0.05$ effect of NLA in presence of Tezo *versus* NLA alone.

Interactions between NO, prostanoids and endothelin in the regulation of pulmonary vascular resistance

The present study shows that there is an interaction between NO and ET in the pulmonary and the systemic circulation. This interaction was demonstrated by the larger effect on systemic and pulmonary vascular resistance of combined ET_A and ET_B receptor blockade in the presence of NO synthase inhibition, as compared to the effect of combined ET_A and ET_B receptor blockade under control conditions. Possible mechanisms behind this interaction include modification by NO of ET production or ET receptor binding affinity (Lavallee *et al.* 2001; Alonso & Radomski, 2003). Indeed Kelly *et al.* (2004) showed *in vitro*, that in pulmonary arterial endothelial cells, NO decreases endothelin-1 secretion through the activation of soluble guanylyl cyclase. Moreover, Wiley & Davenport (2001) showed that NO can modulate the binding of ET to the ET receptor. It has been suggested that the principal vasodilator effect of NO occurs through the inhibition of ET-induced constriction (Lavallee *et al.* 2001). If this were the case, the level of vasodilatation reached by ET receptor blockade alone would be identical to the level of vasodilatation obtained by the combined effect of ET receptor blockade and NO synthase inhibition. At rest, pulmonary vascular resistance was significantly higher after combined administration of NLA and tezosentan compared to tezosentan alone, which indicates that NO acts predominantly in a direct manner (Fig. 5). However during exercise, pulmonary vascular resistance was only slightly higher ($P < 0.05$ by ANCOVA) after combined administration of NLA and tezosentan, as compared to administration of tezosentan alone. This suggests that, although a significant part of the vasodilator effect of NO on the pulmonary vasculature during exercise occurs via inhibition of ET, NO also has a direct vasodilator effect on the pulmonary circulation. In the systemic vasculature, the effect of NLA under control conditions is approximately twice as large as the effect of NLA after tezosentan, indicating that the direct vasodilator effect of NO is approximately equal to the vasodilator effect of the NO-mediated inhibition of ET (Fig. 5).

In the systemic circulation, prostanoids limit ET-induced vasoconstriction, particularly after NO synthase inhibition. The mechanism behind the interaction between prostanoids and ET is not fully understood, but may involve inhibition of transcription, translation, secretion and/or action of ET by prostacyclin (Prins *et al.* 1994). In contrast with the findings in the systemic circulation, our data do not support an interaction between prostanoids and ET in the pulmonary circulation under physiological conditions. Exogenous prostacyclin can induce pulmonary vasodilatation (Owall *et al.* 1991; Albertini *et al.* 1996; Max *et al.* 1999), indicating that prostacyclin receptors are present in the pulmonary

circulation of swine. However, we previously found that inhibition of endogenous prostanoid production did not affect the low basal pulmonary vascular resistance either under normal conditions or in the presence of NO synthase inhibition (Merkus *et al.* 2004). Although we cannot exclude that cyclo-oxygenase inhibition may have gone without an apparent effect due to the opposing effects of simultaneously blocking vasodilator and vasoconstrictor prostanoids, it could also be argued that endogenous prostanoid production in the pulmonary circulation is too small under physiological conditions. This concept is supported by *in vitro* data by Wort *et al.* (2002), who found no cyclo-oxygenase protein in human pulmonary arterial smooth muscle cells under normal conditions. However, under stimulation with cytokines, which mimics pathological conditions, cyclo-oxygenase-2 protein expression could be detected, and inhibited the production of ET. Also the prostacyclin mimetic cicaprost inhibited ET production (Wort *et al.* 2002). Taken together, these data suggest that prostanoids can exert an inhibitory influence on endothelin production in the pulmonary circulation, similar to what we found in the systemic vasculature. However, under physiological conditions, prostanoid levels may be insufficient to modulate pulmonary vasomotor tone and blunt the ET-induced pulmonary vasoconstriction during exercise.

Clinical relevance

Pulmonary arterial hypertension can arise from a multitude of aetiologies. However, in the later stages of the disease, endothelial dysfunction becomes a central feature (Hampl & Herget, 2000; Budhiraja *et al.* 2004). Endothelial dysfunction is associated with a loss of endothelial vasodilator substances, including NO and prostanoids. The present study shows that under conditions of endothelial dysfunction, in particular a reduced NO bioavailability, exercise-induced pulmonary vasodilatation is blunted and pulmonary hypertension during exercise is exacerbated, which is due to 'unopposed' ET-mediated pulmonary vasoconstriction. These observations support clinical studies that have shown the efficacy of ET receptor antagonism and/or exogenous NO in the treatment of chronic pulmonary arterial hypertension (Hill & Pearl, 1999; Channick *et al.* 2001; Rubin *et al.* 2002; Wang *et al.* 2003).

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