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N^G-nitro L-arginine methyl ester: systemic and pulmonary haemodynamics, tissue blood flow and arteriovenous shunting in the pig

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Summary. The effects of N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of the endothelial nitric oxide (NO) biosynthesis, on systemic and pulmonary haemodynamics, and tissue as well as arteriovenous anastomotic blood flows were investigated in the anaesthetized pig, using simultaneous injections of radioactive microspheres of two different sizes (diameter: 15 and 50 μm). L-NAME (1, 3 and 10 $mg \cdot kg^{-1}$) reduced systemic and pulmonary artery conductance and cardiac output, but heart rate and mean arterial blood pressure remained unchanged. L-arginine reversed the systemic and pulmonary haemodynamic changes induced by L-NAME. As detected with 15 μm microspheres, L-NAME (1 and 3 $mg \cdot kg^{-1}$) decreased tissue blood flow to and vascular conductance in the eyes, lungs, atria, kidneys, adrenals and liver. Furthermore, the difference between blood flows simultaneously measured with 15 and 50 μm microspheres, which can be equated to blood flow through arteriovenous anastomoses with a diameter between about 28 and 90 μm, was reduced by L-NAME (3 $mg \cdot kg^{-1}$) in the skin of head and gluteal regions and, as indicated by the microsphere content of the lungs, in the total systemic circulation. These results suggest that in the anaesthetized pig (i) NO is involved in the regulation of both systemic and pulmonary vascular conductance, (ii) the decrease in systemic vascular conductance is in part due to constriction of systemic arteriovenous anastomoses, and (iii) the decrease in pulmonary vascular conductance, leading to reduction of cardiac output, seems to negate the expected rise in arterial blood pressure observed, for example, in rats and rabbits following inhibition of NO-synthesis.

Key words: Arteriovenous anastomoses – Endothelium – L-arginine – Nitric oxide – N^G-nitro-L-argininie methyl ester – Pig – Regional haemodynamics

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Introduction

Vascular smooth muscle tone is regulated by the release of endothelium-derived relaxing factor (EDRF), which has now been identified as nitric oxide (NO) (Ignarro et al. 1987; Palmer et al. 1987) or a related nitrosothiol (Myers et al. 1990). Its synthesis from the precursor L-arginine is mediated by the enzyme NO-synthase present in endothelial cells (Palmer et al. 1988; Förstermann et al. 1991). Analogues of L-arginine, including N^G-nitro-L-arginine (L-NNA) and its methyl ester (L-NAME), have been shown to inhibit NO biosynthesis (Mülsch and Busse 1990; Rees et al. 1990). These NO-synthase inhibitors induce a sustained rise in arterial blood pressure in rats (Gardiner et al. 1990; Rees et al. 1990; Van Gelderen et al. 1991) and rabbits (Humphries et al. 1991; Du et al. 1992), indicating a basal NO release mediating vasodilatation. In these species, blood pressure elevation is usually accompanied by a reflex bradycardia. Less pronounced effects on blood pressure as well as heart rate were found with NO-synthase inhibitors in lambs (Fineman et al. 1991), goats (Garcia et al. 1992), dogs (Richard et al. 1991; Klabunde et al. 1991) and cats (Van Gelderen et al. 1991; McMahon et al. 1992); in cats, microsphere content of the lungs, used as an *index* for systemic arteriovenous shunting, was decreased (Van Gelderen et al. 1991). The reason for the poor pressor response following inhibition of NO synthesis in these animal species is not known, but it is possible that the pressor response due to rise in systemic vascular resistance is offset by an additional decrease in cardiac output as a consequence of a more marked increase in pulmonary vascular resistance. Indeed, NO-synthesis inhibitors have been shown to block pulmonary vasodilator response to vagal stimulation in cats (McMahon et al. 1992), attenuate endothelium-dependent pulmonary vasodilatation, leading to pulmonary hypertension, in lambs (Fineman et al. 1991), and to produce pulmonary hypertension and reduced cardiac output in dogs (Klabunde et al. 1991). The purpose of this investigation was to evaluate the effects of L-NAME on systemic and

pulmonary haemodynamics, tissue blood flows and total and regional arteriovenous shunting, using radioactive microsphere of two different diameters (15 and 50 μm) in anaesthetized pigs. Preliminary findings have been reported elsewhere (Van Gelderen et al. 1992).

Methods

General. Twelve male and female pigs (weight 17.8±0.3 kg) were anaesthetized with metomidate (120 mg, i.v.) following sedation with azaperone (150 mg, i.m.). After intubation, the animals were connected to a respirator (Bear 2E, BeMeds AG, Baar, Switzerland) for ventilation with a mixture of oxygen and room air. Arterial blood gases were measured (ABL-2, Radiometer, Copenhagen, Denmark) and kept within a physiological range (pH 7.45–7.55, PO_2 90–120 mmHg, PCO_2 35–45 mmHg) by adjusting the ventilation rate and the oxygen-content of the mixture. Anaesthesia was maintained by infusing pentobarbitone sodium in the right femoral vein; 20 $mg \cdot kg^{-1} \cdot h^{-1}$ during the first hour followed by 12 $mg \cdot kg^{-1} \cdot h^{-1}$. A catheter was introduced in the left femoral artery for blood pressure measurement. Cardiac output was measured with the help of a 6F-Swan-Ganz thermodilution catheter (Corodyn, Braun Melsungen AG, Melsungen, Germany), which was advanced into the pulmonary artery via the left femoral vein and connected to a cardiac output computer (WTI, Rotterdam, The Netherlands). Another catheter was placed in the left ventricle via the left common carotid artery to inject radioactive microspheres. To calibrate the radioactive microsphere method, arterial reference samples were collected from the aorta via a catheter in the right femoral artery. Blood pressures were continuously measured in the aorta, the pulmonary artery and the left ventricle, using pressure transducers (Statham P23, Hato Rey, Puerto Rico). Mean arterial blood pressure was calculated as the sum of the diastolic pressure and one third of the difference between systolic and diastolic pressure. Heart rate, left ventricular end diastolic pressure and the peak rate of rise in left ventricular pressure (LV dP/dt) were derived from the ventricular pulse pressure signal. Pulmonary capillary wedge pressure was measured by inflating the Swan-Ganz-balloon and pulmonary artery resistance was calculated by dividing the difference between mean pulmonary artery and capillary wedge pressures by cardiac output. In some experiments the last measurement (after L-arginine) of pulmonary capillary wedge pressure could not be measured and, therefore, pulmonary vascular resistance was not calculated. Drugs were injected into the aorta to prevent high concentrations of drugs reaching the heart. All catheters were filled with a heparin sodium solution (80 $IU \cdot ml^{-1}$) to prevent clotting. Body temperature was kept above 37 °C and physiological saline was infused to compensate for fluid loss. The animals were allowed to stabilize for at least 45 min before the experiment started.

Radioactive microsphere technique. The regional distribution of the cardiac output was determined with the radioactive microsphere technique using the reference blood sample method (Heymann et al. 1977), modified for use of simultaneous injection of microspheres of different diameter (see Saxena and Verdouw 1985; Den Boer et al. 1992a, b). The microspheres used in this investigation were of 15 and 50 μm diameter and labelled with either ^{141}Ce (15 μm), ^{113}Sn (50 μm), ^{103}Ru (50 μm), ^{95}Nb (15 μm) or ^{46}Sc (15 μm) (NEN Company, Dreiech, Germany). The order of 15 and 50 μm spheres injected before and after the drugs was randomized. The approximate number of microspheres given per isotope was about 1000000 for 15 μm and 50000 for 50 μm. The microspheres were vortexed for about 30 s and then injected into the left ventricle against the direction of the flood blow to ensure uniform mixing. An arterial reference sample was withdrawn (rat: 10.5 $ml \cdot min^{-1}$) using a pump (Braun-Melsungen, Melsungen, Germany), starting about 15 s before and continuing until 1 min after the injection of the microspheres. At the end of the experiment the animals were killed by an overdose of sodium pentobarbitone and the different tissues were dissected out, weighed and put in vials. The radioactivity in these vials and in the blood samples was counted for 5–10 min in a gamma-scintillation counter (Packard, Minaxi Autogamma 5000) using suitable windows for discriminating the different isotopes. For each tissue, blood flow (Q_{tis}) was calculated using the formula: $Q_{tis} = (I_{tis}/I_{ref}) \times 10.5$, in which I_{tis} and I_{ref} represent, respectively, radioactivity in the tissue and in the reference blood sample. Tissue vascular conductance was calculated by dividing the tissue blood flow value by mean arterial blood pressure. Tissue blood flow and conductance values have been expressed per 100 g of tissue. All data were processed by a personal computer (Olivetti PCS 286) using a set of specially developed computer programs, based on those described earlier (Saxena et al. 1980).

As extensively discussed elsewhere (Johnston and Saxena 1978; Saxena and Verdouw 1985; Den Boer et al. 1992a, b), not all microspheres are trapped in tissues; they escape entrapment via arteriovenous anastomoses to appear into the venous blood and are ultimately sieved in the lungs. Lung radioactivity, therefore, represents for the greatest part arteriovenous anastomotic blood flow, although a small part (1–1.5% of cardiac output) is derived from the bronchial arteries (Baille et al. 1982; Wu et al. 1988). Based on the demonstration in the hamster cheek pouch that microspheres of 15 and 24 μm diameter are already trapped in blood vessels with a diameter of 27.7 and 42.7 μm, respectively (Dickhoner et al. 1978), it may be assumed that 15 μm spheres detected in a certain tissue reflect blood flow through vessels with a diameter smaller than about 28 μm (capillaries and small arteriovenous anastomoses), whereas 50 μm spheres reflect blood flow through vessels under 90 μm diameter (capillaries and medium-sized arteriovenous anastomoses). Therefore, the difference between blood flow values simultaneously obtained with 15 and 50 μm microspheres may serve as an *index* of the blood flow in arteriovenous anastomoses with a diameter between 28 and 90 μm.

Experimental protocol. At baseline values of systemic and pulmonary haemodynamic variables were collated and a mixture of 15 and 50 μm microspheres was injected in the left ventricle to determine baseline regional tissue blood flows. The animals were then divided at random into two groups ($n = 6$ each), which were treated every 25 min with three consecutive doses of L-NAME (1, 3 and 10 $mg \cdot kg^{-1}$) or saline (3 ml each). Systemic and pulmonary haemodynamic values were collected after each dose of L-NAME or saline. After the first dose 15 μm microspheres were injected, whereas after the second dose 15 and 50 μm spheres were injected simultaneously. Finally, in both groups five animals were treated with L-arginine (1000 $mg \cdot kg^{-1}$) and, 15 min later, the haemodynamic variables, except pulmonary capillary wedge pressure, were collated again.

Data presentation and analysis. All data in the text are mentioned as mean±SEM. The effects of treatments were analyzed in each group using a repeated measurements analysis of variance with the factors size and treatment. Once the samples represented different populations, the values after the different treatments were compared to baseline values by use of Duncan's new multiple range test. In each group the difference in blood flow values obtained with 15 and 50 μm microspheres before and after treatment were analyzed with a Student's *t*-test for paired data. The changes among the two groups were tested by a Student's *t*-test. Statistical significance was accepted at $P < 0.05$ (two-tailed).

Drugs. The drugs used in this study were: azaperone, metomidate (both from Janssen Pharmaceutica, Beerse, Belgium), L-arginine, N^G-nitro-L-arginine methyl ester (both from Sigma, St. Louis, Mo., USA), pentobarbitone sodium (Narcovet, Apharmo, Arnhem, The Netherlands) and heparin sodium (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands). All doses refer to the respective salts.

Results

Systemic and pulmonary haemodynamics

Systemic and pulmonary haemodynamic variables in the two groups at baseline and following treatment with L-NAME or saline are depicted in Fig. 1. Apart from mean arterial blood pressure, which was somewhat higher in

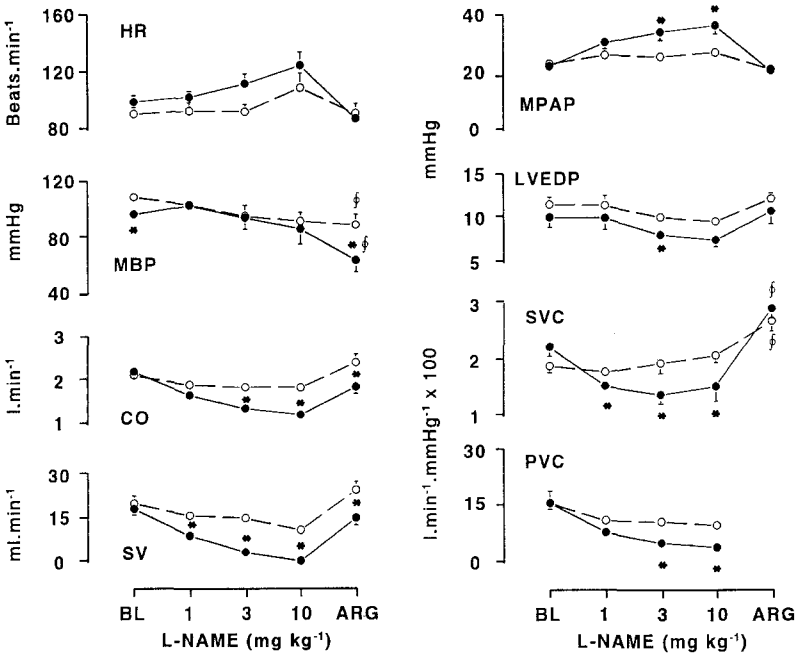

Fig. 1. Systemic haemodynamics in anaesthetized pigs before (BL) and after saline (*open circles*) or L-NAME (*closed circles*), followed in both groups ($n = 6$ each) by L-arginine (1000 mg·kg^{-1}; ARG). The haemodynamic variables are abbreviated: heart rate (HR), mean arterial blood pressure (MBP), cardiac output (CO), stroke volume (SV), mean pulmonary artery pressure (MPAP), left ventricular end diastolic pressure (LVEDP), systemic vascular conductance (SVC) and pulmonary artery conductance (PVC). All values are shown as mean±SEM, but in some cases error bars fall within the limits of the symbol. *$P<0.05$ vs. corresponding saline value; §$P<0.05$ vs. baseline value, in case of ARG only

the saline group, no differences were observed initially between the groups. L-NAME significantly reduced cardiac output (by 40±2 and 45±4% after 3 and 10 mg·kg^{-1}, respectively) and stroke volume (by 28±3, 46±5 and 53±5% after the three doses, respectively), but did not significantly affect heart rate, mean arterial blood pressure, LV dP/dt or pulmonary capillary wedge pressure (both not shown in Fig. 1). Left venticular end diastolic pressure decreased significantly after 3 mg·kg^{-1} L-NAME (−17±8%), whereas mean pulmonary artery pressure increased significantly after the two highest doses (51±11 and 62±11%, respectively). L-NAME significantly reduced systemic (by 30±3, 36±6 and 28±11% after the three doses, respectively) and pulmonary (by 65±5 and 65±2% after 3 and 10 mg·kg^{-1}, respectively) vascular conductances (Fig. 1).

Except for arterial blood pressure, administration of L-arginine (1000 mg·kg^{-1}) partially or fully reversed the systemic and pulmonary haemodynamic changes induced by L-NAME.

Tissue blood flow and vascular conductance

Tissue blood flows measured with 15 µm radioactive microspheres at baseline and after L-NAME are shown in Fig. 2. L-NAME, 1 and 3 mg·kg^{-1}, respectively, decreased blood flow to the eyes (42±5 and 62±4%), atria (37±8 and 44±9%), lungs (42±8 and 68±5%), kidneys (31±2 and 51±4%), and adrenals (35±7 and 60±7%). When compared to baseline values, blood flow was reduced in the bones (32±5 and 39±6%) and liver (32±12, 1 mg·kg^{-1} only). L-NAME induced no significant change in blood flow to the brain as well as to the skin, tongue, spleen, stomach, mesentery, intestines or skeletal muscles (not shown in Fig. 2).

Since mean arterial blood pressure changed only moderately during the course of the experiment, similar changes were observed in tissue vascular conductances. The vascular conductance was decreased in the adrenals (37±7 and 59±5%), eyes (45±5 and 60±4%), kidneys (35±4 and 48±4%), bones (36±5 and 35±8%) and lungs (46±7 and 67±5%) by L-NAME (1 and 3 mg·kg^{-1}, respectively). Additionally, the lower dose of L-NAME also reduced vascular conductance in the liver (35±12%) and heart (16±5%). In the heart, the effect of L-NAME was not observed in the ventricles and was confined to the atria, where vascular conductance was decreased by 42±6 and 44±6% by 1 and 3 mg·kg^{-1} of L-NAME, respectively.

Total systemic and regional arteriovenous anastomotic blood flow

The presence of a substantial number of 15 and 50 µm microspheres, with the latter in preponderance, in the lungs demonstrated that abundant systemic arteriovenous shunting occurs in anaesthetized pigs (Table 1; Fig. 3). L-NAME (3 mg·kg^{-1}) clearly reduced the number of both 15 and 50 µm microspheres as well as the difference between these two spheres (62±6%), indicating a decrease in total systemic shunting through arteriovenous anastomoses between 28 and 90 µm diameter (Fig. 3). At the tissue level, similar blood flow values were found with the two sizes of microspheres in all tissues dissected, except in the skin and tongue (Table 1). The difference in the blood flows measured with 15 and 50 µm microspheres in the skin and tongue reflects the presence of arteriovenous anastomoses in these tissues. Administration of L-NAME (3 mg·kg^{-1}) significantly reduced this difference in the skin (by 75±5%), but not in the tongue (Fig. 3).

It may be noted that skin obtained from different regions showed heterogeneity (Fig. 4). Thus, the head and gluteal skin exhibited a large difference between 15 and

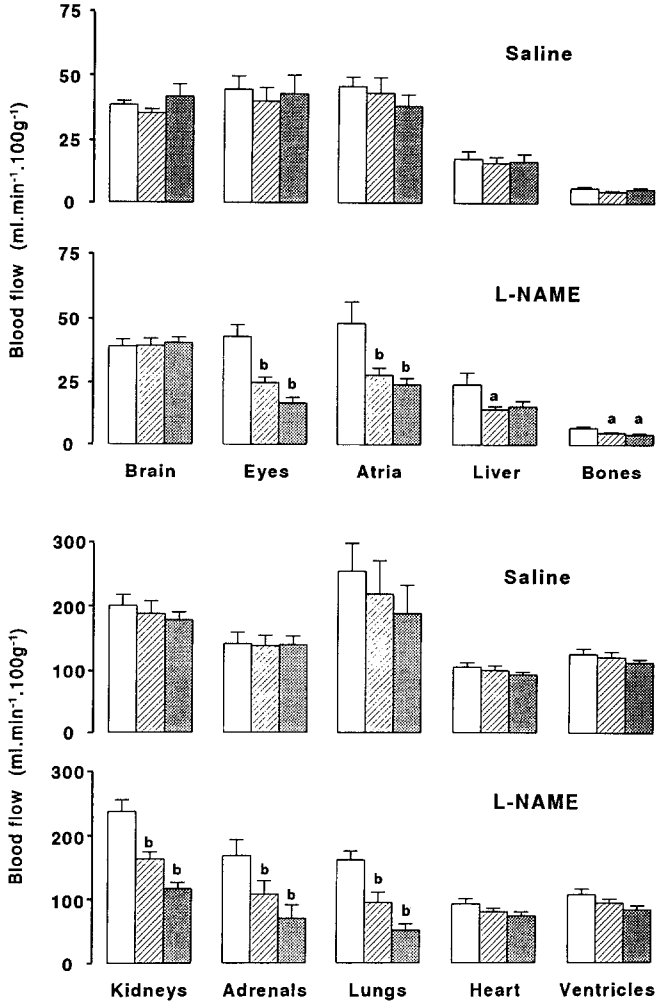

Fig. 2. Tissue blood flows measured with 15 μm radioactive microspheres in pigs treated with either saline (*upper panels; n* = 6) or L-NAME (*lower panels; n* = 6). In each set of panels, baseline blood flow is shown by the *open bars,* whereas increasing doses of L-NAME (1 and 3 $mg \cdot kg^{-1}$) or saline are represented by the *hatched and cross hatched bars,* respectively. Values are shown as mean±SEM. *a,* $P<0.05$ vs baseline only; *b,* $P<0.05$ vs saline experiments

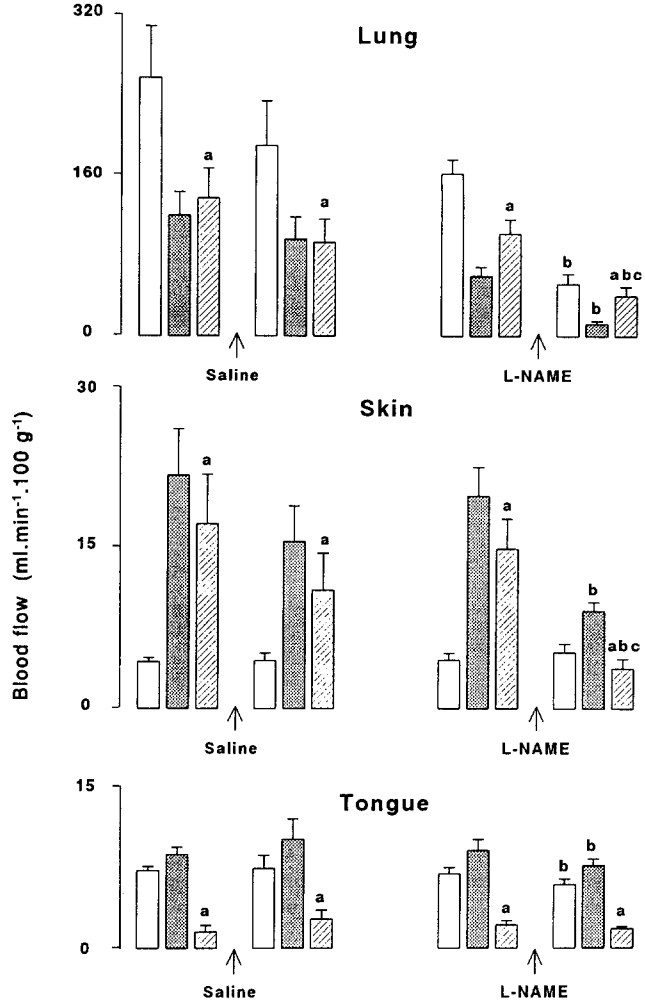

Fig. 3. The effect of saline (n = 6) or L-NAME (3 $mg \cdot kg^{-1}$; n = 6) on total systemic (lung) and regional arteriovenous anastomoses blood flow, represented by the difference in blood flow measured with 15 μm (*open bars*) and 50 μm (*cross-hatched bars*) radioactive microspheres. In each set of panels the absolute difference is depicted by the *hatched bar.* Values are given as mean±SEM. *a,* Denotes significant difference between 15 μm and 50 μm; *b,* denotes significant difference from baseline ($P<0.05$); *c,* denotes significant change in difference betwen 15 and 50 μm ($P<0.05$)

50 μm microsphere blood flow values, while those from the abdominal and back region did not. The blood flow differences in were significantly decreased by L-NAME (3 $mg \cdot kg^{-1}$) in the gluteal and head skin by 70±5% and 70±4%, respectively (Fig. 4).

Discussion

The present investigation was devoted to study the effects of NO-synthase inhibition in anaesthetized pigs using L-NAME. Recently, Buxton and coworkers reported that several alkyl esters of L-arginine analogues, including L-NAME, have atropine-like properties in both radioligand binding and functional in vitro studies. However, muscarinic cholinergic blockade is not likely to play a meaningful role in our studies. Firstly, in vivo L-NAME is rapidly dealkylated into L-NNA, which seems to be responsible for the pharmacological actions in intact animals (Schwarzacher and Raberger 1992), but is devoid of antimuscarinic property (Buxton et al. 1993). Secondly, in our previous studies in both rats and cats, L-NAME (up to 30 $mg \cdot kg^{-1}$) failed to attenuate the magnitude of hypotensive responses to acetylcholine (Van Gelderen et al. 1991). Lastly, the effects of L-NAME in the present study were partly reversed by L-arginine, which does not affect the antimuscarinic action of L-NAME (Buxton et al. 1993).

Systemic and pulmonary haemodynamics

Several studies have demonstrated a pronounced increase in blood pressure following administration of L-NNA or L-NAME to rats (Gardiner et al. 1990; Rees et al. 1990; Van Gelderen et al. 1991) and rabbits (Wiklund et al. 1990); this effect is thought to be due to vasoconstriction

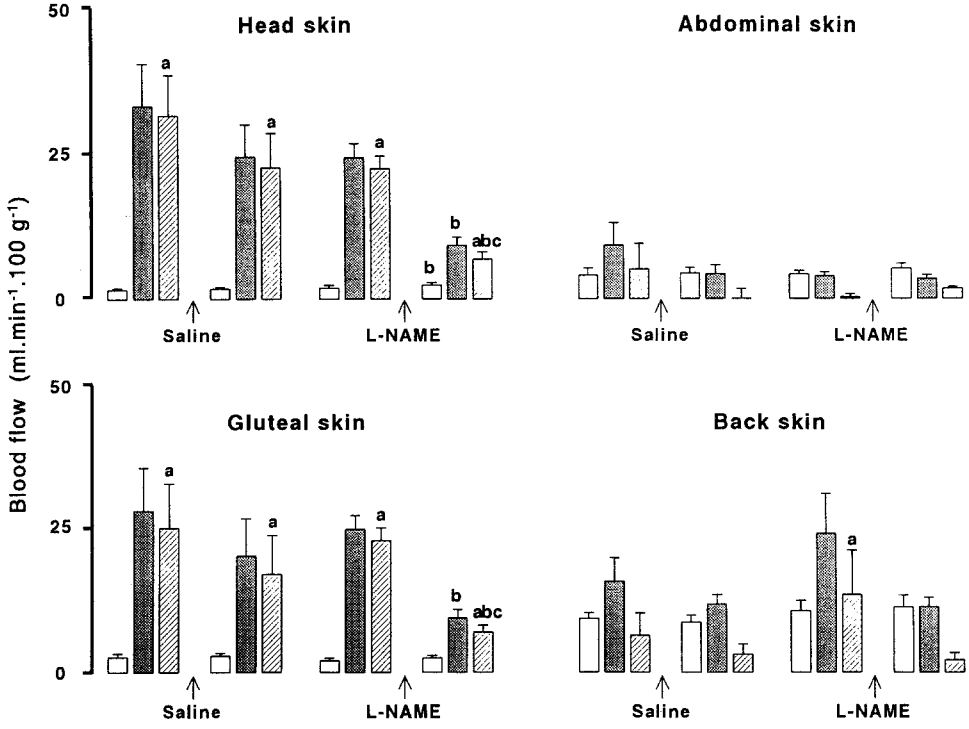

Fig. 4. The effect of saline ($n = 6$) or L-NAME (3 mg·kg^{-1}; $n = 6$) on arteriovenous anastomoses blood flow in several skin regions, represented by the difference in blood flow measured with 15 μm (*open bars*) and 50 μm (*cross-hatched bars*) radioactive microspheres. In each set of panels the absolute difference is depicted by the *hatched bar*. Values are given as mean±SEM. *a*, Denotes significant difference between 15 and 50 μm; *n*, denotes significant difference from baseline ($P<0.05$); *c*, denotes significant change in difference between 15 and 50 μm ($P<0.05$)

resulting from attenuation of basal NO-release. In contrast, the pressor responses are often quite moderate or even absent in dogs, goats, lambs and cats (Fineman et al. 1991; Klabunde et al. 1991; Van Gelderen et al. 1991; Garcia et al. 1992). Similar to our previous observations in cats (Van Gelderen et al. 1991), the present experiments show that L-NAME did not increase blood pressure in anaesthetized pigs, though systemic vascular conductance was significantly decreased. Coincident with the reduction in systemic vascular conductance, pulmonary vascular conductance, left ventricular end diastolic pressure, stroke volume and, ultimately, cardiac output declined dose-dependently. It is therefore likely that the expected rise in arterial blood pressure due to systemic vasoconstriction was negated by the fall in cardiac output in the pig. A comparable fall in cardiac output has also been observed in the anaesthetized dog after the NO-synthase inhibitors, L-NNA and N^G-monomethyl-L-arginine (Klabunde et al. 1991; Perrella et al. 1992).

Table 1. Tissue blood flows (ml·min^{-1}·100 g^{-1}) at baseline measured with 15 μm and 50 μm radioactive microspheres, as well as their difference ($n = 12$)

Tissue	Microsphere size		Difference 50−15 μm
	15 μm	50 μm	
Lungs*	210±29	90±15**	−120 ±17
Brain	39± 1.5	41± 2.1	2.8± 1.0
Eyes	44± 3.4	48± 3.1	4.6± 2.0
Tongue	7± 0.4	9± 0.6**	1.8± 0.4
Bones	6± 0.4	7± 0.6	0.6± 0.5
Heart	99± 5.6	104± 4.5	5.1± 1.7
Liver	21± 2.8	22± 3.2	1.6± 0.6
Spleen	111±15	119±17	8.1± 3.3
Stomach	18± 1.6	19± 1.8	1.0± 0.7
Mesentery	3± 0.4	6± 1.4	2.5± 1.3
Intestines	39± 1.3	40± 1.5	1.0± 0.8
Kidneys	220±13	232±14	12 ± 4.5
Adrenals	155±15	153±20	−2.2± 7.2
Skin	5± 0.3	21± 2.4**	16 ± 2.6
Muscles	5± 0.4	5± 0.4	0.4± 0.2

* Indicates the sum of total systemic arteriovenous anastomotic and bronchial blood flows

** denotes significant difference from the corresponding 15 μm value ($P<0.05$)

The reduction of stroke volume and cardiac output is best explained by the marked decrease in pulmonary vascular conductance, leading to high pulmonary artery pressure, though the reduction in systemic vascular conductance may have partly contributed. Indeed, NO-release can be involved in the maintenance of a low vascular tone within the pulmonary circulation (Wiklund et al. 1990; Fineman et al. 1991; Perrella et al. 1991) and exogenous NO may reverse pulmonary hypertension in man (Pepke-Zaba et al. 1991). The rise in mean pulmonary artery pressure following L-NAME and the ability of L-arginine to reverse the changes induced by L-NAME in our experiments are in keeping with this view.

In conscious rats a direct negative inotropic action of L-NAME has been proposed, based upon the reduction in stroke volume, LV dP/dt_{max} and peak aorta blood flow (Gardiner et al. 1990). Though N^G-methyl-L-arginine (L-NMA) decreased contactility and cAMP and cGMP levels in isoprenaline-stimulated rat perfused hearts, the compound failed to reduce LV dP/dt_{max}, despite a decrease in myocardial cGMP content in unstimulated hearts (Klabunde et al. 1992). In anaesthetized dogs, LV dP/dt_{max} was only slightly decreased by L-

NMA (Klabunde et al. 1991), and intracoronary application of L-NAME did not affect myocardial tissue perfusion measured with radioactive microspheres, though L-NAME did induce a local coronary constriction (Richard et al. 1991). Similarly, in the present experiments in pigs, no evidence for a major negative inotropic effect of L-NAME was uncovered as the compound did not lower LV dP/dt or ventricular vascular conductance significantly. Therefore, a reduction in myocardial oxygen supply due to coronary artery constriction by L-NAME is also unlikely.

Tissue blood flow and vascular conductance

In the anaesthetized pig, L-NAME reduced tissue blood flow and vascular conductance in a limited number of tissues, being most apparent in the adrenals, eyes, kidneys and lungs. These findings differ from our previous results in cats in which a higher dose ($30\ mg \cdot kg^{-1}$) of L-NAME reduced blood flows to abdominal tissues without affecting renal blood flow (Van Gelderen et al. 1991). Furthermore, as measured by the radioactive microsphere technique, reduced blood flow was observed in most of the tissues in rats following the NO-synthase inhibitor N^G-monomethyl-L-arginine (Greenblatt et al. 1993). These differences may be related to differences in basal NO-release between species and tissues or to differences in sensitivity of the vascular smooth muscle cells. Thus, isolated porcine renal and coronary arteries show a similar EDRF release but display a difference in sensitivity to the released EDRF (Christie and Lewis 1991). In any case, the reduced renal conductance following administration of L-NAME, an inhibitor of NO-synthase, is in accordance with studies in rats (Gardiner et al. 1990; Greenblatt et al. 1993), rabbits (Humphries et al. 1991) and dogs (Perrella et al. 1992), and are indicative of a regulatory role for NO in the renal circulation.

Total systemic and regional arteriovenous shunting

The use of radioactive microspheres to measure total systemic (Johnston and Saxena 1978; Saxena et al. 1980; Saxena and Verdouw 1982) and regional (Saxena and Verdouw 1985; Den Boer et al. 1992a, b) arteriovenous anastomotic blood flows has been discussed previously. Briefly, microspheres (10 μm or larger in diameter) escaping entrapment in peripheral tissues and sieved by the lung capillaries can be used as an *index* of total systemic arteriovenous shunting, whereas the difference between tissue blood flows simultaneously measured with microspheres of different diameters indicates blood flow through arteriovenous anastomoses at the tissue level. In our case, the difference between blood flows measured with 15 and 50 μm spheres represents blood flow through arteriovenous anastomoses between approximately 28 and 90 μm diameter, as suggested by the relative sizes of arterial microvessels and the therein trapped microspheres (Dickhoner et al. 1978).

In the present experiments, 21% of the 15 μm microspheres injected into the heart were detected in the lungs. Since bronchial blood flow has been shown to be only 1 to 1.5% of the cardiac output (Baille et al. 1982; Wu et al. 1988), more than 90% of the microspheres sieved by the lungs had passed through arteriovenous anastomoses. As indicated by the difference between tissue blood flows measured simultaneously with 15 and 50 μm spheres, lungs seem to completely sieve microspheres escaping entrapment mainly in the skin, but also in the tongue and, possibly, in some other tissues which we did not dissect. Interestingly, skin exhibited regional heterogeneity, with the head and gluteal skin having a much higher arteriovenous shunting than the skin from the abdominal or back region. These results are in agreement with our previous observations (Saxena and Verdouw 1985; Den Boer et al. 1992b). L-NAME substantially decreased the total systemic arteriovenous shunting as judged by the decrease in the 'lung blood flow' measured with 15 μm and 50 μm microspheres as well as the difference between 'lung blood flow' measured with the microspheres of the two sizes. At the tissue level, L-NAME significantly decreased the difference between 15 and 50 μm sphere blood flows and, therefore, shunting in medium-sized arteriovenous anastomoses in the head and gluteal skin; the apparent lack of effect in abdominal and back skin and tongue may be due to a less pronounced shunting in these tissues.

The effectiveness of L-NAME tempts us to suggest that the release of NO from endothelium regulates the tone of arteriovenous anastomoses demonstrated in porcine skin. Cutaneous arteriovenous anastomoses are supposed to have a role in temperature and, perhaps, also in blood pressure regulation (Hales and Molyneux 1988); NO-release may provide a rapid mechanism to adapt to temperature changes. However, at this moment we cannot fully exclude that blood flow through arteriovenous anastomoses following L-NAME was reduced as a result of the fall in cardiac output, causing a diminished blood supply to these vessels.

In conclusion, our results demonstrate that basal NO release regulates both systemic and pulmonary conductance in the anaesthetized pigs. The expected increase in blood pressure due to NO-synthase inhibition is offset by the coincident reduction in cardiac output. A part of the reduced vascular conductance may be attributed to a constriction of arteriovenous anastomoses.

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