Inhibition of nitric oxide biosynthesis and carotid arteriovenous anastomotic shunting in the pig

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1 The role of nitric oxide (NO) biosynthesis in the regulation of blood flow through arteriovenous anastomoses was evaluated in the carotid circulation of the anaesthetized pig. For this purpose, the effect of intracarotid (i.c.) administration of the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; 0.1, 0.3 and 1.0 mg kg⁻¹; *n* = 6) or saline (*n* = 6) was studied on the distribution of common carotid blood flow, using the radioactive microsphere method.

2 Apart from the highest dose, L-NAME caused no major changes in the systemic haemodynamic variables. Both cardiac output and systemic vascular conductance were reduced by L-NAME (1 mg kg⁻¹), being reversed partly by L-arginine (100 mg kg⁻¹, i.c.). In both groups, L-arginine slightly reduced mean arterial blood pressure.

3 Total common carotid artery blood flow as well as its distribution over the capillary and arteriovenous anastomotic fraction remained stable after saline injection. In contrast, L-NAME caused a dose-dependent decline in common carotid artery blood flow and conductance and this decline was confined entirely to its arteriovenous anastomotic part.

4 Subsequent intracarotid injection of L-arginine (100 mg kg⁻¹) reversed the reduction in total carotid conductance almost completely and that in the arteriovenous anastomotic region partially. Additionally, L-arginine increased capillary conductance significantly in the L-NAME – as well as the saline-treated animals.

5 These results indicate that the L-arginine-NO pathway contributes little to the regulation of tissue perfusion in the porcine carotid circulation. In contrast, NO seems to play an important role in shunting arterial blood through arteriovenous anastomoses in the anaesthetized pig.

Keywords: Arteriovenous anastomoses; endothelium; N^G-nitro-L-arginine methyl ester; nitric oxide; nitrite; blood flow in pig

Introduction

Arteriovenous anastomoses have been found in animals and man, although their precise physiological function is not yet fully understood. Cutaneous arteriovenous anastomoses may be implicated in the regulation of the body temperature and blood pressure (Hales & Molyneux, 1988). Furthermore, dilatation of cranial arteriovenous anastomoses has been proposed to contribute to the pathogenesis of migraine headache (Heyck, 1969; Saxena, 1978; 1990).

Little is known of the mechanisms involved in opening arteriovenous anastomoses, which are normally under a constrictor tone. However, for several reasons nitric oxide (NO), which is formed from L-arginine by the calcium/calmodulin dependent enzyme NO-synthase (Palmer *et al.*, 1988; Bredt & Snyder, 1990; Förstermann *et al.*, 1991), may be a potential candidate: (i) as indicated by the inhibition of NO biosynthesis using N^G-nitro-L-arginine methyl ester (L-NAME), continuous production of NO apparently contributes to the regulation of arterial blood pressure and tissue perfusion (Gardiner *et al.*, 1990; Rees *et al.*, 1990; Van Gelderen *et al.*, 1991); (ii) compounds that act via the NO system (e.g. nitroglycerin, isosorbide mononitrate) cause headache as well as temporal artery dilatation in man (Iversen, 1992; Iversen *et al.*, 1992); and (iii) we have recently found that systemic administration of L-NAME in anaesthetized pigs decreased the number of microspheres detected in the lungs after left atrial injection (i.e. bronchial plus systemic arteriovenous anastomotic blood flow); however, in these experiments cardiac output was also concomitantly reduced (Van Gelderen *et al.*, 1993).

The present investigation was devoted to study the effect of L-NAME, administered directly into the common carotid

artery to avoid changes in cardiac output, on the distribution of the common carotid artery blood flow into arteriovenous and capillary (tissue) parts in the anaesthetized pig. Furthermore, jugular venous nitrite content was analysed to establish whether the inhibition of NO biosynthesis is reflected by a reduction in plasma nitrite (NO₂) and nitrate (NO₃) levels *in vivo*; NO is rapidly decomposed to finally yield nitrite and nitrate, both detected in biological fluids (Green *et al.*, 1982; Furchgott *et al.*, 1990; Kelm & Schrader, 1990). Preliminary data from the present investigation were presented at the winter meeting of the British Pharmacological Society (Van Gelderen & Saxena, 1993).

Methods

General

Twelve domestic pigs (Yorkshire x Landrace; 15–18 kg) were anaesthetized with azaperone (120 mg, i.m.) and metomidate (150 mg, i.v.), intubated and connected to a respirator (Bear 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Arterial blood gas values were kept within physiological range (pH 7.39–7.49; P_{CO}₂ 37–44; P_O₂ 100–130) by adjusting oxygen supply, respiratory rate and tidal volume. Anaesthesia was maintained by continuous i.v. infusion of pentobarbitone sodium (20 mg kg⁻¹ h⁻¹ during the first hour followed by 12 mg kg⁻¹ h⁻¹) in the right femoral vein. A catheter was introduced into the aortic arch via the left femoral artery, connected to a pressure transducer (Statham P23, Hato Rey, Puerto Rico) for measurement of arterial blood pressure and blood sample withdrawal to determine blood gases (ABL-510, Radiometer, Copenhagen, Denmark). Cardiac output

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and pulmonary arterial blood pressure were measured with a 6F-Swan-Ganz thermodilution catheter (Corodyn, Braun Melsungen AG, Melsungen, Germany) introduced into the pulmonary artery via the left femoral vein and connected to a cardiac output computer (WTI, Rotterdam, The Netherlands) as well as to a pressure transducer. Mean arterial (MAP) and mean pulmonary artery (MPAP) pressures were calculated from respective systolic (SBP) and diastolic (DBP) blood pressures as: $MAP \text{ or } MPAP = DBP + (SBP - DBP)/3$. Heart rate was counted from the blood pressure signals. Systemic and pulmonary vascular conductances were calculated by dividing cardiac output by MAP and MPAP, respectively, with the assumption that the pulmonary capillary wedge pressure remained stable throughout the experiment. The right common carotid artery was dissected free and the total carotid blood flow at the ipsilateral side was measured with a flow probe (internal diameter: 2.5 or 3 mm) connected to a sine-wave electromagnetic flow meter (Trans-flow 600-system, Skalar, Delft, The Netherlands). Two hub-less needles (external diameter 0.5 mm), connected to polyethylene tubing, were inserted into the common carotid artery against the direction of the blood flow for administration of radioactive microspheres or drugs. The right jugular vein was cannulated in order to collect venous blood samples to determine venous blood gases and to analyse nitrite levels. All catheters were filled with a heparin sodium solution (80 iu ml^{-1}) to prevent blood clotting. During the experiment, 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 start of experiments.

Distribution of the common carotid blood flow

The distribution of the common carotid blood flow as measured by repeated injections of radioactive microspheres (mean $\pm$ s.d. diameter: $15 \pm 1 \mu\text{m}$; NEN Company, Dreieich, Germany) with different labels (^{141}Ce , ^{113}Sn , ^{103}Ru , ^{95}Nb or ^{46}Sc) (Johnston & Saxena, 1978). A suspension of approximately 250,000 microspheres was vortexed before each measurement and injected into the common carotid artery against the direction of the blood flow to ensure uniform mixing. At the end of the experiment the animals were killed and the heart, kidneys, lungs and the different cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 10 min in a gamma-scintillation counter (Packard, Minaxi Autogamma 5000) using suitable windows for discriminating between the different isotopes.

Tissue blood flow (capillary blood flow) was calculated by multiplying the ratio between tissue radioactivity and the total radioactivity with the total carotid blood flow at the time of injection. Since radioactivity was absent in the heart and kidneys, all radioactive microspheres reaching the venous side of the circulation had passed through arteriovenous anastomoses to be ultimately sieved in the lungs. Therefore, the amount of radioactivity in the lungs served as an index for the arteriovenous anastomotic fraction of the common carotid blood flow.

Nitrite analysis

The nitrite content of venous blood samples, withdrawn from the ipsilateral jugular vein, was measured by a modification of the colorimetric procedure using the Griess reagent (0.5% sulphanilamide with 0.05% N-(naphthyl)-ethylenediamine in 2% H_3PO_4) (Green *et al.*, 1982). This assay is based on the diazotization of a sulphanilamide by nitrite and a subsequent coupling with N-(naphthyl)-ethylenediamine to yield a azochromophore which can be detected spectrophotometrically. Briefly, following centrifugation $150 \mu\text{l}$ of the supernatant was mixed with an equal amount of the Griess reagent in a 96-wells plate and incubated for 10 min at room temperature. Extinction was measured at 540 nm using a microplate reader

(BIORAD, Model 3550) against a blank containing sulphanilamide. Sample nitrite concentration was calculated from a standard absorption curve constructed with several concentrations of sodium nitrite. Since nitrite is converted rapidly to nitrate in the presence of oxygen, samples were analysed immediately after blood withdrawal.

Additionally, total nitrite and nitrate content in deproteinized samples was measured using Griess reagent following reduction of nitrates with a *Klebsiella pneumoniae* suspension (BBD 900990, RIVM-Pharmacology, Bilthoven, The Netherlands) into nitrites, detected by measuring the absorbance at 540 nm with a spectrophotometer (Perkin-Elmer lambda 15 UV/VIS) against a blank (Phizackerley & Al-Dabbagh, 1983).

Experimental protocol

After the stabilization period the animals were randomly assigned to either the treatment or control group, finally consisting of six animals each. At the start of the experiments, baseline haemodynamic parameters, venous nitrite levels, blood gases as well as the distribution of the common carotid blood flow was measured. Thereafter, the first group was treated with three consecutive doses of L-NAME (0.1, 0.3 and 1.0 mg kg^{-1} , i.c.), whereas an equal volume of saline was injected three times into the control animals group. In both groups, L-arginine (100 mg kg^{-1} , i.c.) was injected after the last dose of L-NAME or saline. Ten minutes after each dose all parameters were reassessed and blood samples collected.

Data presentation and analysis

All data, calculated with a personal computer (Olivetti PCS 286) using a set of specially developed computer programmes (Saxena *et al.*, 1980), are presented in the text as mean $\pm$ s.e.mean. The effect of treatment was analysed in each group by a repeated measurement of analysis of variance. When the samples represented different populations, the values after the different treatments were compared to baseline values by use of a Duncan's new multiple range test. Subsequently, the changes to baseline values among the two groups were tested using a Student's *t* test. Statistical significance was accepted at a level of $P \leq 0.05$.

Chemicals

The chemicals used in this study were: azaperone, metomidate (both from Janssen Pharmaceutica, Beerse, Belgium), L-arginine, N-(naphthyl)-ethylenediamine, N^G -nitro-L-arginine methyl ester (L-NAME), sulphanilamide (All from Sigma Chemical Co., St. Louis, U.S.A.), pentobarbitone sodium (Narcovet, Apharmo, Arnhem, The Netherlands) and heparin sodium (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands). Both L-arginine and L-NAME were dissolved in saline. All doses refer to their respective salts.

Results

Systemic haemodynamics

The systemic haemodynamic variables in both groups at baseline, followed by either saline or L-NAME (0.1, 0.3 and 1.0 mg kg^{-1} , i.c.), and L-arginine (100 mg kg^{-1} , i.c.) are depicted in Table 1. There were no significant differences between the two groups in the systemic haemodynamic values at baseline, and no major changes were observed in the saline-treated animals. L-NAME also did not significantly affect heart rate or mean arterial blood pressure but after the highest dose, pulmonary artery pressure increased ($18 \pm 5\%$) and cardiac output decreased ($-20 \pm 3\%$) significantly. Consequently, the calculated systemic ($21 \pm 6\%$) and pulmonary

Table 1 Systemic haemodynamic variables measured at baseline and after intracarotid injections of saline (control; $n = 6$) or N^G -nitro-L-arginine methyl ester (L-NAME) ($n = 6$), followed by L-arginine (100 mg kg^{-1})

	Baseline	Saline or L-NAME (mg kg^{-1})			L-Arginine
		0.1	0.3	1.0	
HR (beats min^{-1})					
Saline	93 $\pm$ 7	91 $\pm$ 6	91 $\pm$ 6	91 $\pm$ 6	91 $\pm$ 6
L-NAME	104 $\pm$ 5	102 $\pm$ 5	100 $\pm$ 6	94 $\pm$ 3	92 $\pm$ 4
MAP (mmHg)					
Saline	96 $\pm$ 5	95 $\pm$ 7	94 $\pm$ 6	90 $\pm$ 5	82 $\pm$ 4†
L-NAME	100 $\pm$ 2	100 $\pm$ 5	104 $\pm$ 6	109 $\pm$ 8	91 $\pm$ 6†
MPAP (mmHg)					
Saline	21.5 $\pm$ 1.0	21.7 $\pm$ 0.9	23.2 $\pm$ 1.3	22.2 $\pm$ 1.6	21.5 $\pm$ 1.3
L-NAME	20.7 $\pm$ 0.5	21.3 $\pm$ 0.4	23.0 $\pm$ 0.7	24.5 $\pm$ 1.4*	21.2 $\pm$ 0.7†
CO (l min^{-1})					
Saline	2.01 $\pm$ 0.2	1.94 $\pm$ 0.2	1.90 $\pm$ 0.2	1.83 $\pm$ 0.1	1.86 $\pm$ 0.1
L-NAME	2.10 $\pm$ 0.2	1.97 $\pm$ 0.2	1.83 $\pm$ 0.1	1.68 $\pm$ 0.1*	1.80 $\pm$ 0.1
SVC ($\text{ml min}^{-1} \text{ mmHg}^{-1}$)					
Saline	21.3 $\pm$ 2.7	20.9 $\pm$ 2.2	20.4 $\pm$ 1.7	20.7 $\pm$ 1.7	22.7 $\pm$ 1.1
L-NAME	21.1 $\pm$ 1.9	19.6 $\pm$ 1.4	17.7 $\pm$ 1.6	16.2 $\pm$ 1.0*	19.9 $\pm$ 0.5†
PVC ($\text{ml min}^{-1} \text{ mmHg}^{-1}$)					
Saline	96.3 $\pm$ 14	89.6 $\pm$ 7.0	81.8 $\pm$ 3.5	84.0 $\pm$ 7.2	87.0 $\pm$ 4.1
L-NAME	102.5 $\pm$ 10	92.6 $\pm$ 9.8	80.7 $\pm$ 7.0	70.6 $\pm$ 8.9*	84.9 $\pm$ 5.3

Data are mean $\pm$ s.e.mean. * $P < 0.05$ versus baseline; † $P < 0.05$ L-arginine versus L-NAME (1.0 mg kg^{-1}) or saline. HR, heart rate; MAP, mean arterial blood pressure; MPAP, mean pulmonary artery pressure; CO, cardiac output; SVC, systemic vascular conductance; PVC, pulmonary vascular conductance.

(37 $\pm$ 7%) vascular conductances were significantly decreased at the highest dose of L-NAME (1 mg kg^{-1}). L-Arginine (100 mg kg^{-1} , i.c.) reversed the L-NAME-induced increase in mean pulmonary artery pressure as well as the reduction in systemic vascular conductance, and it also moderately lowered mean arterial blood pressure in both groups.

Carotid haemodynamics

As shown in Figure 1, no significant effects were observed in either total carotid blood flow or its distribution in saline-treated animals. In contrast, L-NAME caused a dose-dependent decline in total common carotid artery blood flow (maximum change: $-37 \pm 3\%$). This effect was confined entirely to the arteriovenous anastomotic part with little change in the capillary part. The effects of L-NAME were partially reversed by L-arginine.

The effects of saline and L-NAME on carotid vascular bed conductance are shown in Figure 2. While no changes were observed with saline, intracarotid injections of the three consecutive doses of L-NAME (0.1 to 1.0 mg kg^{-1}) elicited dose-dependent decreases in total carotid conductance; the maximum change following the highest dose was $-41 \pm 4\%$. Coincident with this reduction, a decrease in arteriovenous anastomotic conductance was observed ($-64 \pm 2\%$), whereas capillary conductance was unaffected. Subsequent intracarotid injection of L-arginine (100 mg kg^{-1}) reversed the reduction in total carotid conductance almost completely ($93 \pm 16\%$) and the arteriovenous anastomotic conductance partially ($50 \pm 6\%$). Additionally, L-arginine increased capillary conductance significantly in the L-NAME-treated animals ($+50 \pm 18\%$), whereas in the saline-treated animals the conductance was increased when compared to baseline ($+55 \pm 18\%$; Figure 2).

Regional carotid blood flow

Although L-NAME failed to alter capillary blood flow, tissue blood flow to the eyes was reduced by L-NAME (0.3 and 1.0 mg kg^{-1}); the maximum changes observed from baseline ($21 \pm 4 \text{ ml min}^{-1} 100 \text{ g}^{-1}$) were $52 \pm 7\%$ and $43 \pm 17\%$, respectively. No significant changes were observed in all other dissected extracerebral tissues. L-Arginine restored blood flow to the eyes ($16 \pm 2 \text{ ml min}^{-1} 100 \text{ g}^{-1}$). As depicted in Figure 3, total brain flow increased following L-NAME ($261 \pm 106\%$)

Figure 1 Total carotid, arteriovenous (AVA) and capillary blood flow at baseline (BL), after the three intracarotid injections with either saline (left panels; $n = 6$) or N^G -nitro-L-arginine methyl ester (L-NAME) (right panels; 0.1 , 0.3 and 1.0 mg kg^{-1} ; $n = 6$) and following L-arginine (Arg, 100 mg kg^{-1} ; solid columns). In each set of panels, the hatched columns represent three increasing doses of saline or L-NAME. Values are given as mean $\pm$ s.e.mean. † $P < 0.05$ versus BL; * $P < 0.05$ versus saline.

and this was unaffected by L-arginine. A further subdivision of the brain revealed a similar increase in all parts, also unaffected by L-arginine.

Similarly, L-NAME failed to alter total capillary conductance, but reduced vascular conductance in the eyes significantly by $54 \pm 6\%$ (baseline: $1.6 \pm 0.3 \text{ ml min}^{-1} \text{ mmHg} \times 100$) following L-NAME (0.3 and 1.0 mg kg^{-1}), whereas no significant changes were observed in all other dissected extracerebral tissues. L-Arginine reversed the vascular conductance in the eyes ($1.3 \pm 0.2 \text{ ml min}^{-1} \text{ mmHg}^{-1} \times 100$). Furthermore, as shown in Figure 4, total brain conductance was enhanced by L-NAME (maximum: $212 \pm 77\%$), which was unaffected by L-arginine. A further subdivision of the brain revealed a similar increase in all parts, also being unaffected by L-arginine (Figure 4).

Arteriovenous oxygen saturation difference and nitrite content

In both groups arterial oxygen tension and carbon dioxide tension remained stable throughout the experiments. The difference in arteriovenous oxygen saturation increased significantly following the highest dose of L-NAME with $92 \pm 33\%$, whereas no changes were observed in the saline-treated control group (Table 2). Similarly, a reduction in venous

oxygen pressure was observed with this dose ($19 \pm 4\%$), whereas venous carbon dioxide pressure remained stable. At baseline, total venous nitrite and nitrate content was $125 \pm 20 \text{ nmol ml}^{-1}$ ($n = 12$, 6 each in saline and L-NAME groups), which remained stable throughout the experiment in both saline and L-NAME-treated animals (Table 2). At baseline, plasma nitrite content varied largely between saline and L-NAME-treated animals; the pooled nitrite level was $3.3 \pm 0.6 \text{ nmol ml}^{-1}$ ($n = 8$; 4 each in saline and L-NAME groups). The nitrite level remained essentially unchanged in both groups, though with L-NAME (1.0 mg kg^{-1} ; $n = 4$) some decrease ($34 \pm 19\%$) was observed compared to baseline values. L-Arginine restored venous nitrite levels fully in the L-NAME-treated animals, but was without effect in the control group (Table 2).

Discussion

Systemic haemodynamics and distribution of the common carotid blood flow

Using the radioactive microsphere technique, we have previously shown that inhibition of NO biosynthesis in the anaesthetized pig by systemic administration of L-NAME reduces blood flow and vascular conductance in a number of tissues as well as the microsphere content of the lungs (i.e. a sum of bronchial artery and total systemic arteriovenous anastom-

Figure 2 Total carotid, arteriovenous (AVA) and capillary vascular conductance at baseline (BL), after the three intracarotid injections with either saline (left panels; $n = 6$) or N^G-nitro-L-arginine methyl ester (L-NAME) (right panels: 0.1, 0.3 and 1.0 mg kg^{-1} ; $n = 6$) and following L-arginine (Arg, 100 mg kg^{-1} ; solid columns). In each set of panels, the hatched columns represent three increasing doses of saline or L-NAME. Values are given as mean $\pm$ s.e.mean. $\dagger P < 0.05$ versus BL; $* P < 0.05$ versus saline.

Figure 3 Blood flow distribution to cerebral and extracerebral tissues and to dura mater at baseline (BL), after the three intracarotid injections with either saline (a, c; $n = 6$) or N^G-nitro-L-arginine methyl ester (L-NAME) b,d; 0.1, 0.3 and 1.0 mg kg^{-1} ; $n = 6$) and following L-arginine (100 mg kg^{-1} ; solid columns). In each set of panels, the hatched columns represent three increasing doses of saline or L-NAME. Values are given as mean $\pm$ s.e.mean. $\dagger P < 0.05$ versus BL.

otic flows) (Van Gelderen *et al.*, 1993). In these experiments the reduction in arteriovenous anastomotic blood flow was not unequivocal and, moreover, it was accompanied by a decline in cardiac output. Therefore, in the present study we

Figure 4 Vascular conductance in cerebral and extracerebral tissues and dura mater at baseline (BL), after the three intracarotid injections with either saline (a,c; $n = 6$) or N^G -nitro-L-arginine methyl ester (L-NAME) (b,d; 0.1, 0.3 and 1.0 mg kg^{-1} ; $n = 6$) and following L-arginine (100 mg kg^{-1} ; solid columns). In each set of panels, the hatched columns represent three increasing doses of saline or L-NAME. Values are given as mean $\pm$ s.e.mean. $\dagger P < 0.05$ versus BL.

evaluated the effects of local intra-arterial injections of L-NAME (0.1, 0.3 and 1 mg kg^{-1}) on the porcine carotid vascular bed, where arteriovenous anastomoses are abundant (Saxena & Verdouw, 1982). Indeed, the effects of i.c. application of L-NAME were largely confined to the carotid vascular bed, since little changes in systemic haemodynamic variables were observed; only the highest dose significantly affected mean pulmonary artery pressure, cardiac output and systemic and pulmonary vascular conductances. It is unlikely that these changes were due to the carotid haemodynamic effects of L-NAME and, therefore, it seems that pharmacologically effective concentrations of L-NAME were present in the systemic circulation following its highest i.c. dose. These changes as well as the ability of L-arginine to reverse them are in accordance with our previous observations following systemic administration of L-NAME (Van Gelderen *et al.*, 1993).

Independent of any systemic haemodynamic effect, total common carotid blood flow and conductance were decreased by L-NAME. Moreover, it is remarkable that L-NAME caused no change in the capillary part of the carotid circulation and that the reductions in carotid blood flow and conductance were entirely confined to the arteriovenous anastomotic part. The carotid arteriovenous anastomoses have been localized, as demonstrated by simultaneous injections of radioactive microspheres of different sizes, particularly in the skin, ears and dura mater, but also in the eyes and tongue (Saxena & Verdouw, 1985; Den Boer *et al.*, 1992). Although arteriovenous anastomoses are anatomically distinct from the rest of the microvasculature, light and electron microscopy studies have shown the presence of an endothelial layer in both human and rabbit peripheral arteriovenous anastomoses (Gorgas *et al.*, 1977; Fujiwara & Iijima, 1990). The finding that L-arginine, the endogenous substrate for NO-synthase, partly reversed the L-NAME-induced reductions in the carotid arteriovenous anastomotic blood flow and conductance suggests that NO, released from the endothelium, may provide a vasodilator tone in these specialized vessels. Interestingly, it has also been reported that sodium nitroprusside, which releases NO spontaneously (Feelisch & Noack, 1987), tends to increase systemic arteriovenous anastomotic shunt flow in anaesthetized rabbits (Hof & Hof, 1989).

Regional carotid blood flow

Total capillary and extracerebral blood flow were unaffected by L-NAME and L-arginine caused no significant increases in blood flows to or conductances in the various tissues, though

Table 2 Jugular venous blood PO_2 ($V PO_2$), PCO_2 ($V PCO_2$), difference in arterial and jugular venous oxygen saturation (A-V O_2 sat) and jugular venous blood nitrite levels, both as nitrite alone (NO_2) and as nitrate and nitrite (NO_3) measured at baseline and after intracarotid injection of either saline or N^G -nitro-L-arginine methyl ester (L-NAME) and following L-arginine (100 mg kg^{-1})

	Saline or L-NAME (mg kg^{-1})				
	Baseline	0.1	0.3	1.0	L-Arginine
VPO_2 (mmHg)					
Saline	58 $\pm$ 3	56 $\pm$ 4	54 $\pm$ 5	59 $\pm$ 4	53 $\pm$ 5
L-NAME	60 $\pm$ 5	60 $\pm$ 8	57 $\pm$ 6	47 $\pm$ 3*	50 $\pm$ 4
$VPCO_2$ (mmHg)					
Saline	43 $\pm$ 2	46 $\pm$ 6	45 $\pm$ 2	42 $\pm$ 2	44 $\pm$ 2
L-NAME	43 $\pm$ 1	39 $\pm$ 2	43 $\pm$ 1	45 $\pm$ 2	43 $\pm$ 2
A-V O_2 sat (%)					
Saline	15.2 $\pm$ 3	17.6 $\pm$ 4	19.1 $\pm$ 4	14.9 $\pm$ 3	19.3 $\pm$ 4
L-NAME	15.5 $\pm$ 4	16.4 $\pm$ 6	17.4 $\pm$ 5	24.8 $\pm$ 4*	18.5 $\pm$ 4
NO_2 (nmol ml^{-1})					
Saline	134 $\pm$ 34	135 $\pm$ 28	127 $\pm$ 29	120 $\pm$ 28	116 $\pm$ 23
L-NAME	117 $\pm$ 23	93 $\pm$ 29	133 $\pm$ 32	116 $\pm$ 26	128 $\pm$ 27
NO_3 (nmol ml^{-1})					
Saline	2.4 $\pm$ 1	2.7 $\pm$ 1	2.7 $\pm$ 1	2.6 $\pm$ 1	2.3 $\pm$ 1
L-NAME	4.2 $\pm$ 1	3.8 $\pm$ 1	4.4 $\pm$ 1	3.1 $\pm$ 1†	3.5 $\pm$ 1

Data are means $\pm$ s.e.mean.

* $P < 0.05$ versus corresponding saline value; † $P < 0.05$ versus baseline value ($n = 4$).

total capillary conductance was increased by the latter in both saline and L-NAME treated animals. It therefore seems that, unlike the arteriovenous anastomoses, a NO-dependent vascular tone is absent in the capillary part of the cranial circulation of the anaesthetized pig. However, it may be noted that under pentobarbitone anaesthesia, capillary blood flow is approximately one-third of the total carotid blood flow (Saxena & Verdouw, 1982; 1985; Den Boer *et al.*, 1992; present results) and thus the basal release of NO may already be too low to detect a significant decrease.

In contrast to a number of studies which reported a moderate reduction in cerebral blood flow following NO-synthase inhibition, suggesting a basal NO-release in this part of the cranial circulation (Kováč *et al.*, 1992; Prado *et al.*, 1992; Seligsohn & Bill, 1993), in our present experiments L-NAME failed to reduce the cerebral component of carotid blood flow. The absence of an endothelial NO-dependent dilator tone is supported by the recent finding that cultured rat brain microvessel endothelial cells do not show basal release of NO (Durieu-Trautmann *et al.*, 1993). Moreover in our experiments, L-NAME increased blood flow to the brain as well as to its constituent parts. This increase cannot be explained by hypercapnia, since arterial carbon dioxide tension remained unchanged. One possible explanation may be a redistribution of blood from the constricted arteriovenous anastomoses towards the cerebral blood vessels. If this is the case, it is of interest that the redistribution is solely confined to the brain tissue without affecting extracerebral blood flow. Since the increase in cerebral blood flow was not susceptible to L-arginine, a NO-independent mechanism seems likely. Finally, the inability of L-NAME to reduce cerebral blood flow is probably not due to the low doses used, since 1 mg kg⁻¹ of L-NAME has been shown to block NO-production, as reflected by the increase in mean arterial blood pressure and blood flow reductions in a number of peripheral tissues (e.g. Gardiner *et al.*, 1990; Van Gelderen *et al.*, 1991). In doses up to 30 mg kg⁻¹, both L-NAME and N^G-nitro-L-arginine (L-NOARG) are without effect on brain blood flow (Van Gelderen *et al.*, 1991; Faraci & Heistad, 1992), though in higher doses these agents can significantly reduce cerebral blood flow (Kováč *et al.*, 1992; Prado *et al.*, 1992; Seligsohn & Bill, 1993). It is tempting to speculate that at high doses NO-synthase inhibitors may act at a different site within the brain. Apart from the endothelium, NO-synthase is abundantly present in neural tissues including perivascular nerves surrounding cerebral vessels (Bredt *et al.*, 1990; Nozaki *et al.*, 1993). The activity of these nerves, denoted as nitroergic, is inhibited by L-NOARG (Toda *et al.*, 1993) and topical application of N^G-monomethyl-L-arginine (L-NMMA) reduces the diameter of cerebral arteries (Faraci, 1991). Hence, it is conceivable that these NO-synthase inhibitors do not readily pass the blood brain barrier and can only inhibit the activity of nitroergic nerves when used in high doses.

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Changes in arteriovenous oxygen saturation difference and nitrite content

L-NAME caused an increase in arteriovenous oxygen saturation difference which is in keeping with an effect on arteriovenous anastomoses. A reduced difference between arterial and venous oxygen has been observed by Heyck (1969) in migraine patients and the difference is increased by ergot alkaloids together with a reduction in arteriovenous anastomoses blood flow (Johnston & Saxena, 1978; Den Boer *et al.*, 1992). Since no changes were observed following L-NAME in the capillary fraction of the carotid blood flow, it is unlikely that the increase in percentage difference in arteriovenous oxygen saturation is due to an enhanced oxygen consumption in the capillaries.

Concomitant with the decrement in arteriovenous anastomotic blood flow by L-NAME, a reduction of the plasma nitrite content in jugular venous blood was observed, which was restored by endogenous substrate L-arginine. Though this finding is in line with the proposed competition of L-NAME and L-arginine for the enzyme NO-synthase, basal plasma nitrite content was within the low micromolar range and a marked difference between the saline and L-NAME group was observed. Since NO may bind to haemoproteins or be converted into nitrite and nitrate it is not clear if this is a true reflection of the basal endothelial NO release. In contrast, total nitrite and nitrate content was approximately 40 times higher than nitrite levels and no changes were observed following L-NAME. In human plasma, an approximately 3 fold lower nitrate level was measured by Wennamalm and co-workers (1992), together with low nitrite concentrations. These authors suggested that NO is converted predominantly within red blood cells to nitrate. If this is the case, it is still unclear to what extent endothelial NO release contributes to the plasma nitrate content. Therefore, NO-synthase inhibition may not be *per se* reflected by a reduction in nitrate levels. However, the inability of L-NAME to reduce nitrate levels in jugular venous blood samples may be explained in part by the reduced renal blood flow observed following L-NAME (Van Gelderen *et al.*, 1993). An augmented nitrate elimination due to a reduced renal perfusion may oppose the expected reduction in plasma nitrate levels.

In conclusion, the results of the present investigation indicate that, under resting conditions, the L-arginine-NO pathway has little contribution to the regulation of tissue perfusion within the carotid circulation of the anaesthetized pig. In contrast, NO may participate in controlling shunting of arterial blood through arteriovenous anastomoses.

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