Vasodilator tone in the llama fetus: the role of nitric oxide during normoxemia and hypoxemia

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Sanhueza, Emilia M., Raquel A. Riquelme, Emilio A. Herrera, Dino A. Giussani, Carlos E. Blanco, Mark A. Hanson, and Aníbal J. Llanos. Vasodilator tone in the llama fetus: the role of nitric oxide during normoxemia and hypoxemia.

The fetal llama responds to hypoxemia, with a marked peripheral vasoconstriction but, unlike the sheep, with little or no increase in cerebral blood flow. We tested the hypothesis that the role of nitric oxide (NO) may be increased during hypoxemia in this species, to counterbalance a strong vasoconstrictor effect. Ten fetal llamas were operated under general anesthesia. Mean arterial pressure (MAP), heart rate, cardiac output, total vascular resistance, blood flows, and vascular resistances in cerebral, carotid and femoral vascular beds were determined. Two groups were studied, one with nitric oxide synthase (NOS) blocker N^G-nitro-L-arginine methyl ester (L-NAME), and the other with 0.9% NaCl (control group), during normoxemia, hypoxemia, and recovery. During normoxemia, L-NAME produced an increase in fetal MAP and a rapid bradycardia. Cerebral, carotid, and femoral vascular resistance increased and blood flow decreased to carotid and femoral beds, while cerebral blood flow did not change significantly. However, during hypoxemia cerebral and carotid vascular resistance fell by 44% from its value in normoxemia after L-NAME, although femoral vascular resistance progressively increased and remained high during recovery. We conclude that in the llama fetus: *1)* NO has an important role in maintaining a vasodilator tone during both normoxemia and hypoxemia in cerebral and femoral vascular beds and *2)* during hypoxemia, NOS blockade unmasked the action of other vasodilator agents that contribute, with nitric oxide, to preserving blood flow and oxygen delivery to the tissues.

N^G-nitro-L-arginine methyl ester; arterial pressure; blood flow; vascular resistance; hypoxia

THE LLAMA, a species that dwells at the high altitudes of the South American altiplano, uses a different means of withstanding hypoxia than species evolved in lowland environments such as sheep. These differences can be seen even in fetal life (see Ref. 30 for a review). Characteristically, the fetal llama has a greater total peripheral vascular resistance than the sheep, both in normoxemia and in hypoxemia (28, 34). Furthermore, unlike fetuses of lowland species (34), the fetal llama responds to acute hypoxemia with a marked peripheral vasoconstriction and with little or no increase in cerebral blood flow (13, 14, 27, 29). This response to hypoxemia suggests differences in the gain of chemoreflexes or in the balance between endocrine and autocrine vasoconstrictor and vasodilator mechanisms between fetuses of highland and lowland species. However, the fetal sheep submitted to long-term hypoxia by acclimatization to high-altitude hypoxemia also has a cerebrovascular response with a near-normal cerebral blood flow (31) and has been proposed as a possible model for prenatal programming of adult disease (31). In this sense, the characterization of fetal llama response to hypoxia as the species evolved at high altitude also could contribute to that knowledge.

Carotid sinus denervation does not modify the intense peripheral vasoconstriction during hypoxemia in the fetal llama (13), indicating a major role for endocrine/autocrine mechanisms. The increase in fetal plasma AVP in hypoxemia is 7 to 8 times greater in the llama than sheep, suggesting a major role of this hormone in regulating the peripheral vascular resistance (13). However, the administration of a V_1 AVP receptor blocker does not modify the marked peripheral vasoconstriction or cardiovascular function in the fetal llama (14, 20). In contrast, when an α-adrenergic antagonist is administered in hypoxemia, there is a profound decrease in systemic arterial pressure, carotid blood flow and femoral blood flow, leading to cardiovascular collapse and fetal death (14). This result can be partially explained by higher plasma catecholamine concentrations in the fetal llama, both in normoxemia and in hypoxemia (36), than in sheep fetuses of the same gestational age (11). However, the vascular endothelium, which acts as an oxygen sensor, can also release vasoactive agents such as NO, endothelin-1, prostaglandins, and CO, which modify vascular resistance and therefore organ blood flows (10, 40). In the late-gestation ovine fetus, nitric oxide synthase (NOS) inhibition increases the basal systemic vascular tone and blocks the rise in carotid blood flow and the decrease in carotid vascular resistance during hypoxemia (17), although it does not prevent the increase in cerebral blood flow during hypoxemia (19). Differences in cardiovascular responses to acute hypoxemia between the fetal llama and sheep may therefore be due to differences in NO-mediated vasodilatation. However, the role

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of NO in the control of vascular tone in the llama fetus is unknown. We hypothesized that nitric oxide counterbalances the marked vasoconstrictor tone present during both normoxemia and hypoxemia in the llama fetus.

Therefore, this study investigates the effect of blockade of NO synthesis on cardiovascular function during basal and hypoxemic conditions in the chronically instrumented llama fetus. The blockade of nitric oxide synthase (NOS) was achieved with N^G-nitro-L-arginine methyl ester (L-NAME), which inhibits the three distinct isoforms of NOS, endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) (1, 23). These three isoforms have been identified in fetuses, and they appear to be developmentally regulated (18, 42).

MATERIALS AND METHODS

Animals. Ten time-mated pregnant llamas were obtained from the University of Chile farm at Rinconada de Maipú, at 580 m above sea level. After arrival in Santiago (585 m above sea level), the llamas were housed in an open yard with access to food and water ad libitum, and they were familiarized with the study metabolic cage and the laboratory conditions for 1–2 wk before surgery. The fetal llamas had a mean weight of 3.68 ± 0.18 kg (means ± SE), ranging from 3.09 to 4.60 kg (at postmortem), at 221–246 days, that is, 64–71% of gestation. Term gestational age and term weight in fetal llama are 342 ± 4 days and 10.3 ± 0.3 kg, respectively (21).

Surgical preparation. Maternal and fetal surgeries were carried out on consecutive days using well-established techniques previously described in detail (4, 27). Briefly, following food and water deprivation for 24 h, the pregnant llamas were premedicated with atropine (1 mg im, Atropina Sulfato, Laboratorio Chile, Santiago, Chile). Polyvinyl catheters (1.3 mm ID) were placed in the descending aorta and inferior vena cava via a hindlimb artery and vein under light general anesthesia (ketamine, 10 mg kg^{-1} im, Ketostop, Drug Pharma-Invetec, Santiago, Chile) with additional local infiltration of lidocaine (2% lidocaine hydrochloride, Dimecaina, Laboratorio Beta, Santiago, Chile). The catheters were then tunneled subcutaneously to exit at a keyhole incision in the maternal flank.

The following day the fetuses were surgically prepared under maternal premedication with atropine (2 mg iv, Atropina Sulfato, Laboratorio Chile, Santiago, Chile) and general anesthesia (5–7 mg kg^{-1} sodium thiopentone, Tiopental Sódico, Laboratorio Biosano SA, Santiago, Chile for induction, and 0.5–2% halothane in 50:50 O_2 with N_2O for maintenance). After a midline laparotomy, a fetal hindlimb was withdrawn through a small hysterotomy. Polyvinyl catheters (0.8 mm ID) were inserted into the fetal aorta (below the kidneys), via a hindlimb artery and into the inferior vena cava via a hindlimb vein, and an ultrasonic flow probe (2R Transonics, Systems, Ithaca, NY) was implanted around a femoral artery (13). The fetal head and a forelimb were exposed through a second hysterotomy, a catheter was inserted into the ascending aorta via a brachial artery and a second ultrasonic flow probe was implanted around a carotid artery. A catheter was placed in the amniotic cavity for pressure reference, and the uterine and abdominal incisions were closed. All catheters were filled with heparinized saline (1,000 IU heparin/ml 0.9% NaCl), plugged with a copper pin, exteriorized with the flow transducer leads through the maternal flank, and kept in a pouch sewn onto the maternal skin.

During surgery, maternal arterial pressure and heart rate were continuously recorded on a polygraph. All maternal llamas were hydrated with a warm intravenous 0.9% NaCl solution (15–20 $ml \cdot kg^{-1} \cdot h^{-1}$) to compensate for fluid loss during the procedures. At the end of the surgery and daily after the surgery, ampicillin (500 mg; Ampicilina, Laboratorio Best Pharma, Santiago, Chile) and gentamicin (80 mg; Gentamicina Sulfato, Laboratorio Biosano SA, Santiago, Chile) were administered into the amniotic cavity, and intramuscularly to the mother. After surgery the animals were returned to the yard, and at least 4 days of postoperative recovery were allowed before the experiments were commenced. Vascular catheters were maintained patent by daily flushing with heparinized saline (200 IU/ml).

All experimental protocols were reviewed and approved by the Faculty of Medicine Ethics Committee of the University of Chile. All animal care, maintenance, procedures and experimentation were performed in accordance with the UK Animals (Scientific Procedures) Act, 1986, and the American Physiological Society's "Guiding Principles for Research Involving Animals and Human Beings" (2).

Experimental procedure. All experiments were based on a 3-h protocol divided into three periods of 60 min: 1 h of normoxemia, 1 h of hypoxemia and 1 h of recovery. Infusions of NaCl 0.9% or L-NAME were started 45 min into the control normoxemia period. Measurements were made at six time points: *1)* Normoxemia (0–45 min); *2)* Normoxemia during the beginning of the infusion (46–60 min); *3)* Early hypoxemia (61–75 min); *4)* Late hypoxemia (76–120 min); *5)* Early recovery (121–135 min), and *6)* Late recovery (136–180 min). The continuous date was averaged every minute for each time period. A transparent, loosely tied polyethylene bag was placed over the llama's head into which a controlled mixture of air, N_2, and CO_2 was passed at ~50 l/min. Following 1 h of breathing air (normoxemia), hypoxemia (9% O_2 and 2–3% CO_2 in N_2) was induced in the maternal llama, which reduced maternal P_{O_2} from ~92 to ~34 mmHg and hemoglobin saturation from ~96 to ~71%. This, in turn, reduced fetal descending aortic P_{O_2} and hemoglobin saturation from ~22 to ~12 mmHg and from ~51 to ~22%, respectively, without altering fetal arterial P_{CO_2}. After the hour of hypoxemia, the pregnant llama was returned to breathing air, and recordings continued for a further 60 min (recovery). The five fetal llamas constituting the L-NAME group were treated with a N^G-nitro-L-arginine methyl ester solution (20 mg/kg iv bolus followed by intravenous infusion of 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$ dissolved in 0.9% NaCl; L-NAME, Sigma Chemical Co). The 5 fetal llamas in the control group received the same volume of saline infusion of 0.9% NaCl without NOS inhibitor. The corresponding solution (L-NAME or 0.9% NaCl) was infused into the fetal circulation via the vena cava. It started 15 min before hypoxemia and runs continuously until the end of the hypoxemic challenge.

Arterial blood samples (0.5 ml) were taken in heparinized syringes from the mother and fetus at 15 and 45 min of normoxemia, and after 15 min of infusion during normoxemia; at intervals of 15 min during the hypoxemic hour; and at 15 and 45 min of recovery. Arterial pH, P_{O_2}, P_{CO_2} (BMS 3Mk2 Blood Microsystem and PHM 73 pH/Blood gas Monitor, Radiometer, Copenhagen, Denmark; measurements corrected to 39° C), percentage of saturation of hemoglobin and hemoglobin concentration (OSM2 Hemoximeter, Radiometer, Copenhagen, Denmark) were measured. Fetal arterial, venous, and amniotic pressures (Statham Transducers, Hato Rey, Puerto Rico), heart rate, and mean carotid and femoral blood flows were recorded continuously throughout the experiment on a polygraph (Gilson ICM-5, Emeryville, CA). In addition, the fetal combined ventricular output and cerebral blood flows were determined after 45 and 60 min of normoxemia and after 45 min of both hypoxemia and recovery (during late hypoxemia and late recovery, respectively), in the 0.9% NaCl and L-NAME-infused fetuses. To do this, 15-μm diameter radionuclide-labeled microspheres (^{57}Co, ^{113}Sn, ^{46}Sc, and ^{103}Ru labels; New England Nuclear, Boston, MA) were injected into the inferior vena cava, while reference samples from the ascending and descending aorta were obtained (22). The rate at which the reference samples were drawn was 3.2 ml/min for 1.5 min. This method allows blood flow determination to all organs except the lung (22).

On completion of the experiments, the llama and the fetus were anesthetized with intravenous sodium thiopentone (1 g; Tiopental Sódico, Laboratorio Biosano SA, Santiago, Chile) and humanely euthanized with saturated potassium chloride injected intravenously.

Measurements and calculations. At postmortem, fetuses were weighed, and the transducer position around the arteries was checked. The uterus and individual fetal organs were dissected and weighed. All dissected material was carbonized, ground to coarse powder, placed in vials, and counted with a multichannel gamma pulse analyzer (Minaxi 5000, Packard, Canberra, Australia). To ascertain adequate mixing of the microspheres, the right and left cerebral hemispheres and the right and left kidneys were counted separately. Microsphere mixing was considered appropriate when the percent difference in calculated blood flow to the cerebral hemispheres and kidneys was less than 10%. To minimize error in the calculation of organ blood flow, sufficient microspheres were injected to ensure an organ distribution of more than 400 microspheres (22).

Blood flow to all fetal organs was calculated by comparing the organ radioactivity with the activity and flow rate of the appropriate reference sample (ascending aorta for upper body organs and descending aorta for lower body organs). Fetal combined ventricular output was calculated in the following equation as the sum of the absolute blood flow to all organs except the lungs (22): $\dot{Q}$ organ (ml/min) = C organ (cpm) × $\dot{Q}$ reference (ml/min)/C reference (cpm), where $\dot{Q}$ is blood flow in milliliters per minute, and C is the radioactivity in counts per minute.

Total vascular resistance was calculated by dividing perfusion pressure (arterial minus venous pressure) by combined cardiac output. Cerebral vascular resistance was calculated by dividing perfusion pressure (arterial minus venous pressure) by cerebral blood flow at the time of microsphere injection. Carotid and femoral vascular resistance was calculated by dividing perfusion pressure by the corresponding arterial blood flow.

Statistical analysis. All values are expressed as means ± SE for each experimental period. Changes in the variables during the experiment in both groups were tested for statistical significance using two-way ANOVA for repeated measures, followed by the Student-Newman-Keuls test. A difference was considered significant when the P value was less than 0.05 (15).

RESULTS

Maternal blood gases. In the pregnant llama, the Pa_{O_2} decreased from 92 ± 2 mmHg to 34 ± 3 mmHg during hypoxemia ($P < 0.05$), whilst maternal Pa_{CO_2} remained constant. Maternal hemoglobin saturation decreased from 96 ± 2% to 71 ± 6% ($P < 0.05$). No significant changes in maternal pH were observed. These values correspond to the 0.9% NaCl-infused group and they were not different from the L-NAME group (data not shown).

Fetal arterial blood gas status. Fetal treatment with L-NAME had no effect on basal blood gas status. During acute hypoxemia, there was a similar reduction in fetal Pa_{O_2} and hemoglobin saturation, which was accompanied by a fall in pH in the recovery period in both groups of fetuses (Table 1). No significant changes in fetal Pa_{CO_2} were observed. While the hemoglobin concentration remained unchanged from baseline in control fetuses, it increased in L-NAME-treated fetuses, reaching values 15% greater than normoxemic values and remaining elevated during recovery (Table 1).

Mean systemic arterial pressure and heart rate. In the L-NAME-treated fetuses, a marked rise in mean systemic arterial pressure during normoxemia was observed, and this was maintained until the end of the experimental protocol (Figs. 1 and 2). In addition, there was a decrease in fetal heart rate in normoxemia, which persisted during the early hypoxemic period (Figs. 1 and 2). In the saline-treated fetuses, there was a transient bradycardia at the beginning of hypoxemia, after which heart rate returned to basal values. No significant changes in mean systemic arterial pressure were observed in saline-treated fetuses (Figs. 1 and 2).

Carotid blood flow and carotid vascular resistance. In the L-NAME group, there was a profound reduction in carotid blood flow from the beginning of L-NAME infusion, a change that tended to recover during hypoxemia, but the value remained low during recovery and always lower than that of the control group (Figs. 1 and 3). Carotid vascular resistance increased during L-NAME infusion in normoxemia and remained elevated during the whole experiment (Figs. 1 and 3). Carotid blood flow and vascular resistance did not change significantly from baseline during acute hypoxemia in the control fetuses (Figs. 1 and 3).

Femoral blood flow and femoral vascular resistance. Femoral blood flow decreased from the beginning of L-NAME infusion and remained low during hypoxemia and recovery (Figs. 1 and 4). The femoral vascular resistance increased

Table 1. *Arterial blood gases during acute hypoxemia in saline and L-NAME-treated fetuses*

	Normoxemia			Hypoxemia		Recovery	
	N15	N45	N+INF	H15	H45	R15	R45
pH_a							
Saline	7.40±0.03	7.34±0.04	7.37±0.03	7.32±0.07	7.28±0.05	7.22±0.04*	7.23±0.03*
L-NAME	7.38±0.02	7.37±0.02	7.37±0.03	7.36±0.04	7.25±0.05	7.19±0.07*†	7.14±0.08*†
Pa_{O_2}, mmHg							
Saline	23.0±2.0	22.4±1.2	23.0±1.3	11.4±0.8*†	12.0±0.9*†	21.0±1.9	20.2±1.6
L-NAME	21.0±2.0	21.4±0.9	20.6±1.2	12.8±1.1*†	11.0±0.7*†	19.0±1.6	19.1±1.5
Pa_{CO_2}, mmHg							
Saline	44.3±1.2	42.3±0.7	43.6±0.7	41.5±3.9	40.8±2.0	40.0±0.9	42.0±1.2
L-NAME	42.0±0.6	42.2±1.1	42.6±1.3	41.5±0.6	42.6±1.4	42.8±0.5	43.4±1.7
Hb Sat, %							
Saline	52.8±4.5	51.6±3.7	53.2±3.7	22.1±3.8*†	22.1±4.3*†	43.3±4.2	40.5±4.6*†
L-NAME	50.5±7.6	50.1±4.1	47.0±6.0	23.7±4.6*†	21.3±4.1*†	41.5±5.0	32.9±2.8*†
Hb, g/dl							
Saline	11.9±0.4	11.6±0.4	11.8±0.4	12.7±0.4	12.7±0.4	11.8±0.3	11.7±0.4
L-NAME	11.4±0.5	11.8±0.6	11.9±0.6	13.2±0.4*†	13.6±0.5*†	13.3±0.5*†‡	13.7±0.4*†‡

Values are presented as means ± SE, taken at 15 (N15) and 45 (N45) minutes of normoxemia, at 60 (N+INF) minutes of normoxemia, at 15 (H15) and 45 (H45) minutes of hypoxemia, and 15 (R15) and 45 (R45) minutes of recovery, in saline (NaCl 0.9%) or N^G-nitro-L-arginine methyl ester (L-NAME) infusion-treated fetuses. Significant differences are $P < 0.05$, *vs. normoxemia (average of N15+N45), †vs. N+INF, and ‡L-NAME vs. saline.

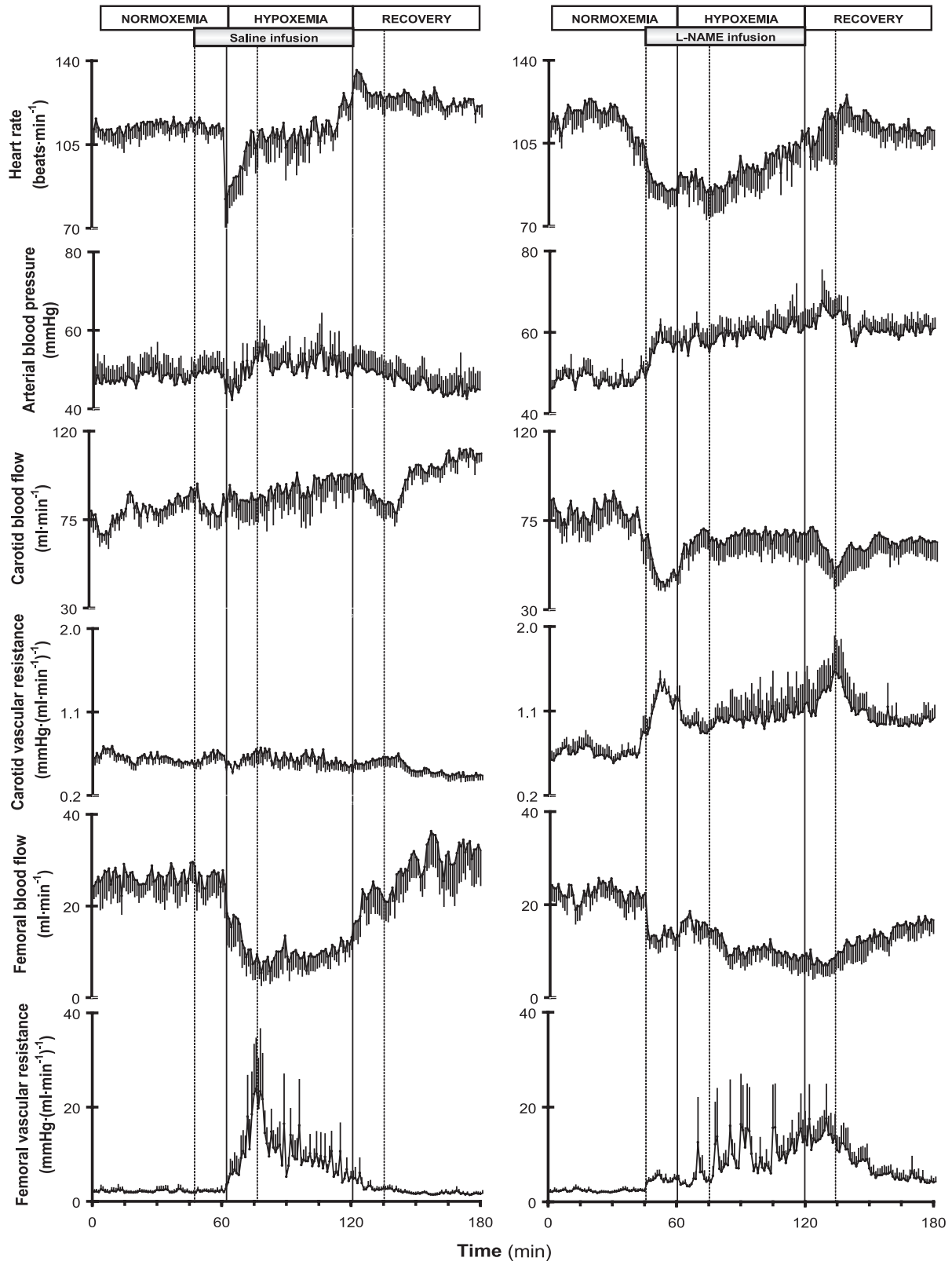

Fig. 1. Cardiovascular responses to acute hypoxemia with 0.9% NaCl or N^G-nitro-L-arginine methyl ester (L-NAME) infusion in llama fetuses. Values are means ± SE for cardiovascular variables calculated every minute of the experimental protocol. Fetuses were infused with either 0.9% NaCl (Hypoxia saline) or with L-NAME (Hypoxia L-NAME). Treatment started 15 min before onset of acute hypoxemia and ran continuously until the end of the hypoxemic episode.

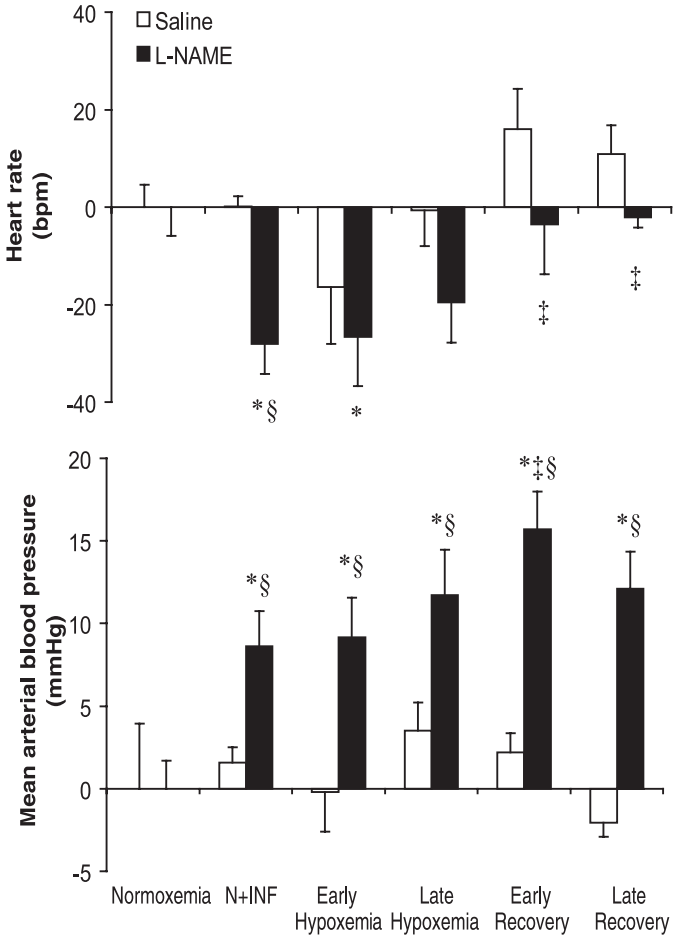

Fig. 2. Heart rate and mean arterial blood pressure during acute hypoxemia with saline or L-NAME infusion in the llama fetuses.Values are presented as means ± SE of the absolute changes in heart rate and mean arterial blood pressure of fetuses infused with saline (open bars) or treated with L-NAME (solid bars). Significant differences are $P < 0.05$ * vs. normoxemia period, ‡ vs. N+INF, and §L-NAME vs. saline.

during late hypoxemia and remained high during early recovery in the L-NAME group (Figs. 1 and 4). In contrast to the L-NAME group, femoral blood flow did not change in control animals during normoxemia but decreased briskly at the onset of hypoxemia, remaining low until the end of the hypoxemic period. However, during recovery, femoral blood flow in control fetuses returned to baseline values, and a mirror image of the femoral blood flow was observed for femoral vascular resistance values in this group (Figs. 1 and 4).

Cardiac output and total vascular resistance. Fetal combined ventricular output decreased during the recovery period in the L-NAME-treated fetuses, whereas this variable remained unchanged in the controls (Fig. 5). In the L-NAME-treated fetuses total vascular resistance increased markedly from the beginning of the infusion until the recovery period. In contrast, total vascular resistance did not change in the saline-infused group (Fig. 5).

Cerebral blood flow and vascular resistance. Cerebral blood flow did not change either in the L-NAME group or in the controls (Fig. 5). During normoxemia, L-NAME infusion increased cerebral vascular resistance. This resistance fell during hypoxemia to reach basal values, but remained higher than that of saline-infused fetuses (Fig. 5). During recovery, the cerebral vascular resistance increased again, and although this value was lower than that of L-NAME in normoxemia, it remained elevated compared with that of saline-infused fetuses (Fig. 5). In the saline-infused fetuses, cerebral vascular resistance decreased during hypoxemia and remained low in recovery (Fig. 5).

DISCUSSION

This study tested the hypothesis that NO plays an important vasodilator role, counteracting the marked vasoconstrictor tone present during both normoxemia and hypoxemia in the llama fetus. The results show that NO plays a major role in the control of mean systemic arterial pressure, cerebral and peripheral vascular resistance during normoxemia, hypoxemia, and recovery, supporting the hypothesis. In addition, blockade of NO uncovers a NO-independent vasodilatation in the cerebral vascular bed during hypoxemia in the llama fetus.

In the present study, NOS inhibition by L-NAME in normoxemia produced an increase in mean systemic arterial pressure through a marked increase in total vascular resistance. This effect was maintained during hypoxemia and recovery. Harris et al. (19) reported elevated systemic arterial pressure in

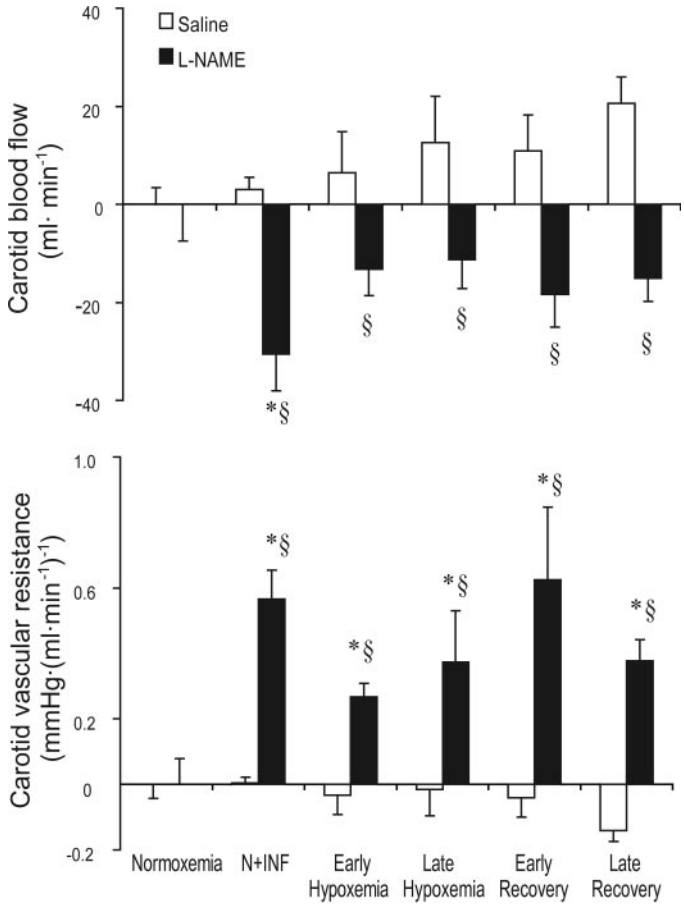

Fig. 3. Hemodynamic changes in the carotid vascular bed during acute hypoxemia with saline or L-NAME infusion in the llama fetuses.Values are means ± SE of the absolute changes in carotid blood flow and carotid vascular resistance in fetuses infused with saline (open bars) or treated with L-NAME (solid bars). Significant differences are $P < 0.05$ * vs. normoxemia period and §L-NAME vs. saline.

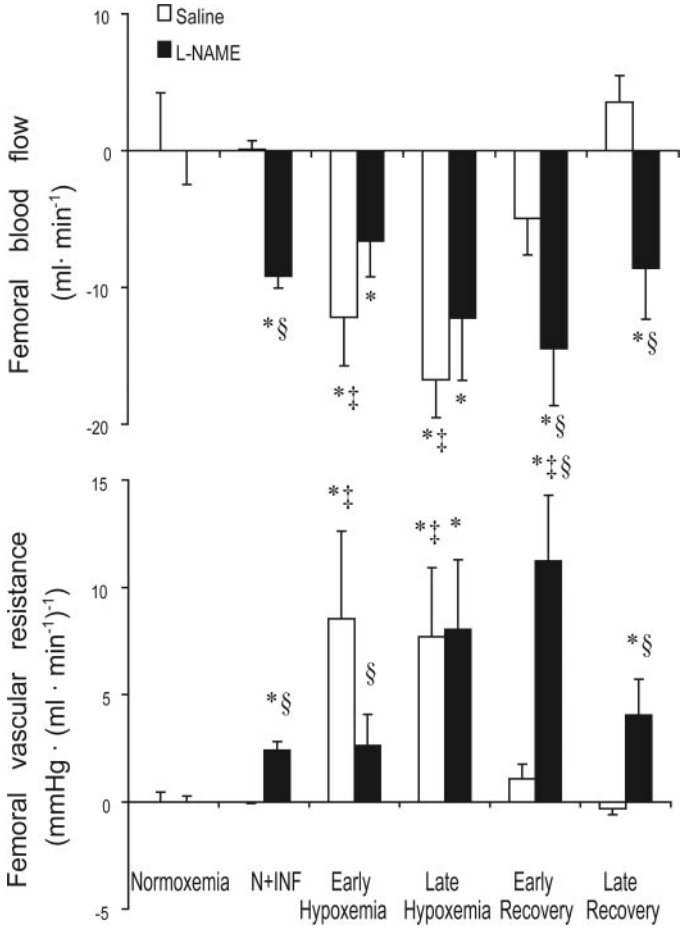

Fig. 4. Hemodynamic changes in the femoral vascular bed during acute hypoxemia with saline or L-NAME infusion in the llama fetuses. Values are means ± SE of the absolute changes in femoral blood flow and femoral vascular resistance in fetuses infused with saline (open bars) or treated with L-NAME (solid bars). Significant differences are $P < 0.05$ * vs. normoxemia period, ‡ vs. N+INF, and §L-NAME vs. saline.

normoxemia and hypoxemia with administration of L-NAME in fetal sheep at 0.6 and 0.9 of gestation. However, the increase in total vascular resistance observed in the fetal sheep is much less than that observed in the fetal llama, suggesting that the vasodilator role of NO is less in the former. In addition, the marked increase in plasma norepinephrine concentration produced by L-NAME in the llama fetus (36), is likely to contribute to the rise in mean systemic arterial pressure and total vascular resistance that we observed during the whole experimental protocol. Further studies with α-adrenergic blockade are needed to confirm this.

In L-NAME-treated fetuses, we measured a decrease in the combined ventricular output during the experimental protocol, although this only reached significance during recovery. The decrease in fetal combined ventricular output may be due to several mechanisms, including an increase in afterload and or a decrease in heart rate, coronary blood flow, or in preload. There is evidence for all these possibilities. First, in this study, we found an increase in systemic vascular resistance and a fall in heart rate. In unpublished data, we have found an increase in heart vascular resistance after L-NAME administration, which could alter myocardial function. Moreover, Reller et al. (35) reported that NOS blockade reduced coronary blood flow in fetal sheep, which could affect the myocardial contractility. Kubes and Granger reported that NO inhibition increased microvascular permeability (25), which could reduce blood

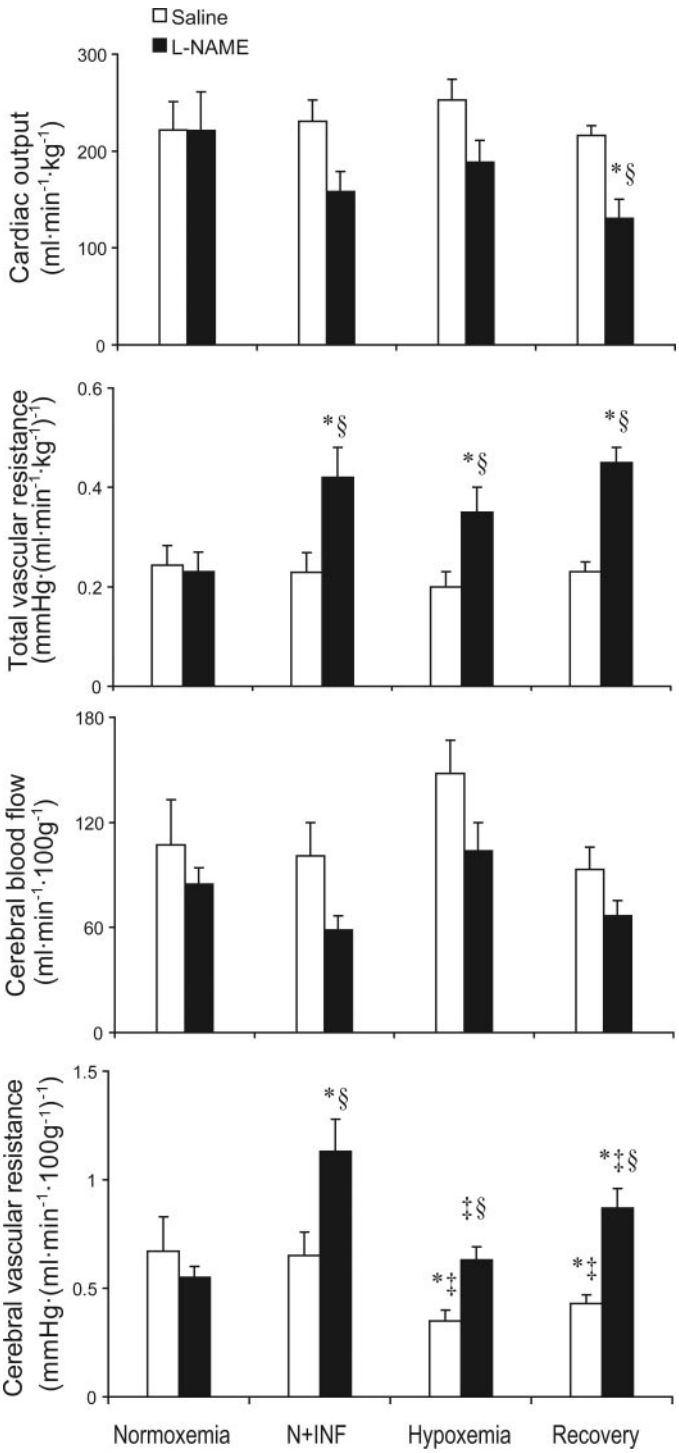

Fig. 5. Cardiac output, total vascular resistance, and cerebral hemodynamic responses to acute hypoxemia in the llama fetus during saline or L-NAME infusion. Values are presented as means ± SE for cardiovascular variables calculated at 45 (Normoxemia) and 60 (N+INF) min of normoxemia, 45 min of hypoxemia (Hypoxemia) and 45 min of recovery (Recovery) in saline (open bars) or L-NAME (solid bars)-treated fetuses. Significant differences are: $P < 0.05$ * vs. normoxemia, ‡ vs. N+INF, and § L-NAME vs. saline.

volume and thereby decrease cardiac preload. Finally, a reduction in combined ventricular output due to bradycardia, an increase in mean arterial pressure, decreased coronary blood flow and hemoconcentration induced by L-NAME has also been described in fetal sheep at 0.75 of gestation (6).

In our experiments, we found an increase in hemoglobin concentration in the L-NAME-treated fetuses, which could result from hemoconcentration due to an increase in vascular permeability. This is supported by the finding of Smolich et al. (38) that NOS inhibition in fetal sheep produced a fall in the circulating blood volume, increased hemoglobin concentration, and a fall in combined ventricular output (38).

In the llama fetuses, carotid vascular resistance increased markedly with L-NAME during the whole experimental protocol, suggesting that NO plays a major role in the regulation of vascular tone in this bed as for the sheep fetus (17). A decrease in carotid blood flow may reflect a reduction in blood flow, to both noncerebral and cerebral regions of the head. Moreover, Covert et al. (7) showed that the common carotid artery flow, measured by a blood flow transducer, provides and accurate prediction of total brain blood flow, measured with microspheres, over a wide range of values in fetal sheep. Nevertheless, there is no data available regarding the percent of carotid blood flow that goes into the brain in the llama fetus. However, in the present study, we showed an increase in both carotid and cerebral vascular resistance during normoxemia. Interestingly, during hypoxemia the increase in the cerebral vascular resistance was diminished, suggesting that blockade of NO reduces vasoconstrictor or enhances vasodilator influences. Possible candidates for vasodilators are carbon monoxide (26), prostaglandins (33), arginine vasopressin (34). They may also be potassium channel activation, which may hyperpolarize the vascular smooth muscle cells (9), adenosine (3, 5), adenosine diphosphate. (41), and adrenomedullin (12). Possible vasoconstrictors include neuropeptide Y (37), endothelin (8), or changes in the adrenergic action that are gestational age-dependent (16). Any or all of these could be responsible, together with NO, in maintaining cerebral oxygen and nutrient delivery to the fetal brain during hypoxemia, an effect that could be different in postnatal life due to cerebral endothelial maturation (41).

A decrease in cerebral blood flow with an increase in cerebral vascular resistance has been reported during NOS blockade in normoxemia in the fetal sheep (19, 24, 32, 39). Moreover, the decrease in cerebral vascular resistance during hypoxemia was not abolished by L-NAME administration (19). Green et al. (17) and Harris et al. (19) concluded that NO exerts a basal vasodilator influence on the brain, but it is not an important mechanism for hypoxic vasodilatation in the fetal brain. The changes in the cerebral vascular bed that we observed in the fetal llama after NOS blockade are in accordance with this concept.

When L-NAME was infused, femoral vascular resistance increased four-fold between normoxemia and the early recovery period. In contrast, femoral blood flow and vascular resistance were not significantly different in L-NAME-treated vs. control sheep fetuses during hypoxemia (17). The initial increase of vascular resistance during hypoxia in fetal llama is not mediated by a carotid sinus reflex (13), indicating that endocrine and autocrine functions are more relevant in the early stages of hypoxemia. It is interesting that in early hypoxemia the increase in femoral vascular resistance was smaller in L-NAME-treated fetuses than in controls; this suggests a role for vasoactive agents other than NO as in the cerebral bed, discussed previously).

In summary, the results of this study indicate that NO is a major determinant of vascular tone during fetal life in the llama. Even though we do not know the levels of NO production in the llama fetus, it appears to counterbalance the high vascular resistance in this species, perhaps to maintain an adequate perfusion and oxygen delivery. Moreover, NOS inhibition unmasked other vasoactive agents that appear to play a role in the control of the cerebral and femoral hemodynamic responses to acute hypoxemia in this species.

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