Cardiovascular responses attenuate with repeated NO synthesis inhibition in conscious fetal sheep

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Chlorakos, A., B. L. Langille, and S. L. Adamson. Cardiovascular responses attenuate with repeated NO synthesis inhibition in conscious fetal sheep. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1472–H1480, 1998.—The cardiovascular effects of repeated administration of the nitric oxide (NO) synthesis inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) were assessed daily for 3 days in fetal sheep near term (124–126 days gestation) beginning 4 days after surgery (n = 7). In the first hour on day 1, fetal infusion of L-NAME (30 mg bolus, 6 mg/min infusion iv for 3 h) significantly increased fetal arterial pressure from 41 ± 2 to 58 ± 3 mmHg, decreased heart rate from 173 ± 5 to 134 ± 3 beats/min, increased umbilicalplacental resistance from 0.16 ± 0.02 to 0.28 ± 0.07 mmHg·ml⁻¹·min, and inhibited the hypertensive response to acetylcholine (ACh; 2 µg iv bolus). All changes were sustained except for arterial pressure, which decreased significantly to 50 ± 3 mmHg in the third hour. Within 17 h, all cardiovascular variables returned to control. L-NAME readministered on days 2 and 3 had no effect on cardiovascular variables. L-NAME did not potentiate the pressor response to angiotensin II on day 2 and caused a surprising attenuation of the pressor response to endothelin-1 on day 3. We conclude that, whereas NO normally contributes to low arterial pressure, high heart rate, and low umbilicalplacental vascular resistance in fetal sheep near term, the role of NO in these functions is replaced by an alternate mechanism within 17 h after NO synthesis inhibition with L-NAME.

endothelium-derived relaxing factor; Nω-nitro-L-arginine methyl ester; arterial blood pressure; hypertension; placenta

The fetal extra-abdominal umbilicalplacental circulation apparently lacks innervation (32) so that autocrine, paracrine, and humoral substances regulate vasomotor activity of this vascular bed. The umbilicalplacental circulation of sheep is almost maximally dilated in vivo (29), and it receives 30–40% of the fetal biventricular cardiac output at term (33). Umbilicalplacental vessels perfused in vitro release an endothelium-derived relaxing factor [presumably nitric oxide (NO)], and they relax in response to NO donors (11, 21, 35). The preconstricted human placent al cotyledon perfused in vitro vasodilates in response to NO donors (26, 27), and inhibition of basal NO production using L-arginine analogues increases placental vascular resistance in the perfused human placental cotyledon (17, 27). Inhibition of NO synthesis also augments the vasoconstrictor effects of endothelin-1 and angiotensin II in the human placenta (17, 27). In addition, in fetal sheep in utero, NO synthesis inhibition by Nω-nitro-L-arginine (L-NNA) increases umbilicalplacental vascular resistance (10). These findings indicate that endogenous production of NO is important in maintaining low resistance of the placental vasculature.

The current study examines the cardiovascular and umbilicalplacental vascular responses to the L-arginine analogue Nω-nitro-L-arginine methyl ester (L-NAME) in near-term fetal sheep. Our primary hypothesis was that endogenous NO activity suppresses the fetal cardiovascular and umbilicalplacental vascular responses to the vasoconstrictors angiotensin II and endothelin-1. These two constrictors were chosen for study because their effects are augmented by NO synthesis inhibition in the perfused human placenta (17, 27) and because they preferentially constrict different segments of the umbilicalplacental circulation in fetal sheep; angiotensin II constricts the umbilical arteries, whereas endothelin-1 constricts the placental microcirculation (1, 3). We assessed the stability of the cardiovascular response to several hours of NO synthesis inhibition with L-NAME on the first day of the study. On days 2 and 3, we assessed the reproducibility of the cardiovascular response to L-NAME treatment before examining dose responses to angiotensin II and endothelin-1. The most important findings were that the fetal pressor response to L-NAME was not sustained on the first day of administration and that cardiovascular responses were not reproducible on subsequent days, despite a return to baseline cardiovascular function.

METHODS

Animal surgery and experiments were approved by the animal care committee of Mount Sinai Hospital and were conducted in accordance with guidelines approved by the Canadian Council of Animal Care.

Surgical Methods

Seven pregnant Dorset-Suffolk sheep at 120 days gestation (where term is 145 days) were instrumented under general anesthesia using sterile surgical techniques. After ewes were fasted for 24 h, anesthesia was induced with thiopental sodium (1 g iv), and ewes were intubated and artificially ventilated with 1–2% halothane in oxygen to maintain anesthesia. We exposed the fetal hindlimbs through a maternal midline abdominal and uterine incision. Vinyl catheters (1.2 mm OD; V4, Bolab, Lake Havasu City, AZ) were advanced 8 and 10 cm retrogradely into the right femoral artery and vein (two catheters per vessel) such that their tips lay in the distal aorta and vena cava, respectively. Catheter placements were confirmed at necropsy. One femoral artery catheter was used for blood sampling, and the other was used for monitoring fetal arterial blood pressure and heart rate. Fetal venous pressure was monitored from one femoral vein catheter, and the other was used for drug infusions.

The left umbilical artery was exposed via retroperitoneal dissection as described by Berman et al. (6). A snugly fitting
cuff-type electromagnetic flow probe (3–4.5 mm ID; C & C Instruments, Culver City, CA) was used to measure left umbilical artery blood flow. In one of seven fetuses, our smallest flow probe was too large; therefore a 5.0-mm ID probe was used to monitor common umbilical artery blood flow. In this fetus, flow was divided by two before we averaged the flow with left umbilical blood flows measured in the other six fetuses. In all fetuses, a balloon-cuff occluder (6 mm ID; In Vivo Metric, Healdsburg, CA) was implanted around the distal abdominal aorta. The occluder was used to briefly arrest flow to verify accuracy of the electronic zero on the electromagnetic flowmeter during experiments.

Amniotic fluid pressure was measured using a vinyl catheter (2.39 mm OD; V11, Bolab) attached to the fetal skin, and it was used to correct fetal blood pressures for extravascular pressures associated with the intrauterine environment. The fetus was then returned to the uterine cavity, the catheters and flow probe cable were exteriorized, and all incisions were closed.

Vinyl catheters (2.39 mm OD; V11, Bolab) were advanced 20 cm to the in utero vena cava and aortae of the ewe via the femoral vessels (one catheter per vessel). The arterial catheter was used to record maternal blood pressure, and the venous catheter was used to euthanize the ewe.

Postoperative Care

After surgery, ewes were housed in metabolic cages with free access to food and water. Ewes received an analgesic (0.3 mg buprenorphine hydrochloride sc; Temgesic, Reckitt and Colman, Hull, UK) once after surgery and prophylactic antibiotics (8 × 10^6 U procaine penicillin G and 1 g dihydrostreptomycin sulfate; Pen-Di-Strep, Rogar STP, Montreal, Quebec, Canada) injected intramuscularly before and twice daily after surgery. The fetus received antibiotics (1 × 10^6 IU penicillin G sodium, Novopharm, Toronto, Ontario, Canada) into both the femoral vein and the amniotic cavity once daily after surgery. Antibiotics were continued until the end of the study. All catheters were filled with heparinized saline (40 USP U heparin sodium/ml 0.9% NaCl) and were flushed daily to maintain patency. Experiments began on the fourth postoperative day.

Materials

L-NAME (Sigma Chemical, St. Louis, MO) was stored in powder form at −20°C. It was dissolved in 0.9% NaCl (50 mg/ml) on the day of use. Acetylcholine chloride (ACh; 10 mg/ml, Miochol-E, ophthalmic solution) Johnson & Johnson, Peterborough, Ontario, Canada) was diluted in 0.9% NaCl to obtain a solution of 4 µg/ml on the day of use. L-Arginine (Sigma Chemical) was stored in powder form at room temperature, and 4.7 g was dissolved in 10 ml of water on the day of use. Angiotensin II (human synthetic angiotensin II acetate salt; Sigma Chemical) was stored in powder form at −20°C.

On the day of the study, 1-ml vials were reconstituted to 0.5 mg/ml in water and then diluted with 0.9% NaCl to achieve the required dosages. Endothelin-1 (human endothelin-1; Peptide Institute, Osaka, Japan) was stored in powder form at −20°C. On the day of the study, 0.11-ml vials were reconstituted with 0.44 ml 0.1% acetic acid and then diluted with 0.9% NaCl to achieve the required dosages.

Experiments

Experiments were performed on days 124, 125, and 126 of gestation. The average weight of the seven fetuses on the final day of the study was 3.7 ± 0.2 kg. Pressures in the fetal and maternal aorta, fetal inferior vena cava, and amniotic cavity were monitored by attaching the fluid-filled catheters to external pressure transducers (CDX3, Cobb, Lakewood, CO). Pressure transducers were calibrated using a mercury manometer. Blood flow probes were calibrated before and after each use by the timed collection method with steady flow of 0.9% NaCl in vitro. Pressure and flow signals were continuously recorded on a strip-chart recorder (model 78D, Grass Instruments, Quincy, MA), acquired on a computer (IBM compatible) using a ViewDac (Keithley Instruments, Taunton, MA) data acquisition sequence, and recorded on a digital tape recorder (model 4000A, AR Vetter, Rebersburg, PA) as a backup.

Protocol on day 1. On the first day, physiological variables were monitored during a 30-min control period, and then L-NAME was given as a 30-mg bolus followed by a 6 mg/min infusion into the fetal femoral vein for 3 h (n = 7 animals). Fetal arterial blood samples were analyzed immediately for blood gas tensions, hemoglobin concentration, and pH at 37°C using a pH/blood gas analyzer (model 170, Corning Medical, Medfield, MA) and co-oximeter (model 2500, Corning Medical). The fetal arterial blood pressure response to a 2-µg bolus injection of ACh into the fetal femoral vein was tested before the start of the control period and at each hour of L-NAME infusion (n = 4 fetuses). At the end of the 3 h, three fetuses received a 4.7-g bolus injection of L-arginine into the fetal femoral vein, and L-NAME was continued for an additional 30 min. A 30-min recovery period followed the 3.5-h L-NAME infusion. Three fetuses were given a 60-min recovery period immediately after the end of a 3-h L-NAME infusion. In one fetus, the L-NAME infusion was continued for 7 h on day 1; only data for the first 3 h on day 1 were included in the study.

Protocol on days 2 and 3. In four of seven fetuses, we assessed the effect of NO synthesis inhibition on fetal and umbilicalplacental cardiovascular sensitivity to vasoconstrictors. Dose-response curves were generated before and then during L-NAME infusion on day 2 for angiotensin II and on day 3 for endothelin-1. Before L-NAME infusion on these days, a 30-min control period was followed by a dose-response curve generated by infusing (at 0.5 ml/min into the fetal femoral vein) stepwise increasing doses of vasoconstrictor for 15 min each until left umbilical artery blood flow was reduced by 50% or until the maximum dose was reached. Doses used for angiotensin II were 0.38, 0.75, 1.5, 3, and 6 µg/min, and doses used for endothelin-1 were 0.1, 0.2, 0.4, 0.6, and 0.8 µg/min. Two to three hours were allowed for cardiovascular parameters to stabilize before we continued the study. A 30-min control period was then followed by a dose-response curve generated by infusing at 0.5 ml/min into the fetal femoral vein) stepwise increasing doses of vasoconstrictor for 15 min each until left umbilical artery blood flow was reduced by 50% or until the maximum dose was reached. Doses used for angiotensin II were 0.38, 0.75, 1.5, 3, and 6 µg/min, and doses used for endothelin-1 were 0.1, 0.2, 0.4, 0.6, and 0.8 µg/min. Two to three hours were allowed for cardiovascular parameters to stabilize before we continued the study. A 30-min control period was then followed by a dose-response curve generated by infusing at 0.5 ml/min into the fetal femoral vein) stepwise increasing doses of vasoconstrictor for 15 min each until left umbilical artery blood flow was reduced by 50% or until the maximum dose was reached. Doses used for angiotensin II were 0.38, 0.75, 1.5, 3, and 6 µg/min, and doses used for endothelin-1 were 0.1, 0.2, 0.4, 0.6, and 0.8 µg/min. Two to three hours were allowed for cardiovascular parameters to stabilize before we continued the study. A 30-min control period was then followed by a dose-response curve generated by infusing at 0.5 ml/min into the fetal femoral vein) stepwise increasing doses of vasoconstrictor for 15 min each until left umbilical artery blood flow was reduced by 50% or until the maximum dose was reached. Doses used for angiotensin II were 0.38, 0.75, 1.5, 3, and 6 µg/min, and doses used for endothelin-1 were 0.1, 0.2, 0.4, 0.6, and 0.8 µg/min. Two to three hours were allowed for cardiovascular parameters to stabilize before we continued the study.

Data Analysis and Statistics

Fetal blood pressures were expressed relative to intrauterine pressure by subtracting the amniotic fluid pressure. Fetal heart rate was determined from the pulsatile fetal arterial blood pressure signal using a data analysis sequence written in ViewDac (Keithley Instruments). Umbilicalplacental vascular resistance was calculated by dividing the difference between fetal arterial blood pressure and fetal venous blood pressure by left umbilical artery blood flow. Therefore, the reported umbilicalplacental vascular resistance is approxi-
mately two times higher than the resistance of the total umbilicoplacental circulation supplied by the left and right umbilical arteries. The fetal hypotensive response to ACh was determined by measuring on the chart record the difference between the mean fetal arterial blood pressure observed 1 min before ACh injection and the minimum arterial pressure observed after ACh injection.

Statistical analysis was performed on data obtained on day 1 after we averaged the following time periods: 1) the 30-min control period, 2) three consecutive 60-min periods during L-NAME infusion, and 3) the 30-min during which L-arginine was injected and L-NAME infusion was continued (performed on 3 fetuses only). Data obtained during the recovery period (after the end of all infusions) are presented on the figures as 1-min averages, but these data were not used in the statistical analysis. Seven animals were monitored during the control period and during the 3-h L-NAME infusion on day 1 for all variables except umbilical blood flow where six animals were studied due to a faulty flow probe in the seventh. A one-way repeated measures analysis of variance (RM-ANOVA) was performed using SigmaPlot for Windows (Jandel Scientific, San Rafael, CA). If significant, a Student-Newman-Keuls multiple comparison test was used to determine where the significant differences lay.

We also determined the effect of L-NAME infusion on 3 consecutive days for six of seven fetuses (all variables were obtained except blood flow for one fetus because of a faulty flow probe) and on maternal arterial blood pressure in four of seven animals. Statistical analysis was performed on data averaged over the 30-min control period and the first hour of L-NAME infusion, which followed immediately thereafter on the 3 days. A one-way RM-ANOVA was performed using SigmaPlot for Windows. If significant, a Student-Newman-Keuls multiple comparison test was used to identify where the significant differences lay.

For angiotensin II and endothelin-1 dose-response curves that were generated before L-NAME infusion, the averages of the last 5 min of each dose (at which point cardiovascular parameters had stabilized) were expressed as a change from baseline, where baseline was the average of the 30-min control period immediately before the dose-response curve. A similar analysis was performed for dose-response curves generated during L-NAME infusion except that baseline data were averaged over the 60-min L-NAME infusion that preceded the dose-response curves.

The average change from baseline elicited by each dose of either angiotensin II on day 2 or endothelin-1 on day 3 was determined during control and during L-NAME infusion for each fetus. The data for the four animals studied were analyzed using a paired Student's t-test to determine whether the cardiovascular responses obtained before L-NAME infusion were significantly different from those obtained during L-NAME infusion on days 2 and 3.

Results are presented as means ± SE, where n is the number of animals. P < 0.05 was considered statistically significant.

RESULTS

Effects of First L-NAME Infusion (Day 1)

Cardiovascular variables. One-minute and hourly averages of cardiovascular variables obtained on day 1 are presented in Figs. 1 and 2, respectively. The first L-NAME infusion, performed on day 1, caused a rapid and significant increase in the mean fetal arterial blood pressure from 40.8 ± 1.8 during the control period to 57.5 ± 3.2 mmHg during the first hour of L-NAME infusion (Figs. 1 and 2). The increase waned significantly over time so that by the third hour of the infusion arterial pressure was 49.8 ± 2.7 mmHg; however, this pressure was still significantly higher than control (Fig. 2). Fetal venous blood pressure was 3.3 ± 0.4 mmHg during control. In all animals venous pressure initially increased after we started the L-NAME infusion and reached a peak of 2.8 ± 0.4 mmHg above control within 3–11 min (Fig. 1). However, this increase was transient and, as a result, average venous pressure over the first hour of L-NAME infusion was not significantly different from control (Fig. 2). Venous pressure then decreased significantly below the control during the second and third hour of L-NAME infusion to 2.4 ± 0.4 mmHg during the third hour (Fig. 2). Fetal heart rate decreased rapidly and significantly from 173 ± 5 beats/min at control to 134 ± 3 beats/min during the first hour of L-NAME infusion (Figs. 1 and 2). Heart rate then remained constant during L-NAME infusion, in contrast to the waning arterial pressure response (Fig. 2). Left umbilical artery blood flow decreased gradually during L-NAME infusion from 276 ± 49 ml/min during control to 220 ± 35 ml/min in the third hour of infusion (Figs. 1 and 2). Umbilicoplacental vascular resistance increased fairly rapidly in the first 15 min of L-NAME infusion (Fig. 1). It increased significantly from 0.159 ± 0.023 mmHg·ml·min−1 to 0.283 ± 0.072 mmHg·ml·min−1·min during the first hour of L-NAME infusion. Umbilicoplacental vascular resistance remained elevated for the remainder of the infusion (a small decrease in resistance between the first and second hour was not significant; Fig. 2).

Maternal mean arterial blood pressure increased significantly during the first hour of L-NAME infusion (from 88 ± 6 mmHg at control to 92 ± 5 mmHg during the first hour). The increase in maternal arterial pressure was sustained for the duration of the L-NAME infusion (92 ± 5 and 94 ± 6 mmHg at the second and third hours of infusion, respectively).

Arterial blood variables. L-NAME infusion did not significantly affect fetal arterial Pco2 or Po2, whereas it caused small but significant decreases in pH and base excess, and it increased total hemoglobin concentration. These effects were sustained throughout the 3-h infusion (Table 1). Arterial Po2 may have remained unchanged during L-NAME because the 20% increase in total hemoglobin concentration offset the 20% decrease in umbilical blood flow so that fetal oxygen delivery was unchanged. The increase in total hemoglobin concentration could be accounted for by a decrease in fetal blood volume of 16.8 ± 4.0, 18.9 ± 1.7, and 19.5 ± 2.4% from control at the first, second, and third hours of L-NAME infusion, respectively. These changes in blood volume were calculated from changes in hemoglobin concentrations as described previously for fetal sheep (8). Some of the decrease in blood volume was caused by the collection of blood samples, which resulted in a cumulative loss in fetal blood volume of 1.5 ± 0.2, 2.1 ± 0.2, and 2.8 ± 0.3% by the first, second, and third hours of L-NAME infusion, respectively.
Effectiveness and reversibility of NO synthesis inhibition. L-NAME infusion significantly blunted (~50%) the transient decrease in fetal mean arterial blood pressure induced by a 2-µg iv bolus injection of ACh, and this capacity to attenuate the effects of ACh was sustained throughout the 3-h L-NAME infusion (Fig. 2).

A bolus of L-arginine returned fetal heart rate to a value that was not significantly different from control during the last 30 min of a 3.5-h L-NAME infusion (Fig. 1), in the three experiments in which it was tested. In contrast, L-arginine did not significantly reverse the effects of L-NAME on fetal arterial or venous pressures, umbilical blood flow, or umbilicoplacental vascular resistance when averaged over the 30-min period following L-arginine infusion (Fig. 1).

Effect of L-NAME Infusion on 3 Consecutive Days

Cardiovascular and arterial blood variables. All measured fetal cardiovascular and arterial blood variables under control conditions on days 2 and 3 did not significantly differ from those observed during the control period on day 1 except that fetal arterial pH and total hemoglobin concentrations were slightly, but significantly, lower on day 3 than on day 1 (Fig. 3, Table 1). However, in contrast to day 1, the first hour of L-NAME
Infusions on days 2 and 3 had no effect on fetal arterial pressure, heart rate, or placental vascular resistance (Fig. 3) or on arterial pH, base excess, or hemoglobin concentration (Table 1). Loss of responsiveness to L-NAME on days 2 and 3 did not appear to be caused by the vasoconstrictor dose-response curves that were performed before L-NAME infusion on days 2 and 3, because L-NAME infusion also caused virtually no change from control in fetal arterial blood pressure (±57%) and heart rate (±7%) on days 2 and 3 of experimentation in the two fetuses in which no vasoconstrictor dose-response curves were performed. Both fetuses showed marked changes in fetal arterial pressure (+49% and +54%) and heart rate (−17% and −24%) to L-NAME on day 1. These data suggest that loss of responsiveness was caused by prior exposure to L-NAME on day 1.

Cardiovascular Response to Vasoconstrictors

Angiotensin II caused dose-dependent increases in mean fetal arterial blood pressure from baseline values of 40 ± 2 mmHg before and from 39 ± 2 mmHg during L-NAME infusion and a dose-dependent decrease in left umbilical artery blood flow from baseline values of 228 ± 28 ml/min before and from 220 ± 22 ml/min during L-NAME infusion in the four fetuses tested on day 3 (Fig. 5). Contrary to expectations, fetal arterial blood pressure and left umbilical artery blood flow responses to endothelin-1 were significantly smaller when L-NAME was infused (Fig. 5). L-NAME did not significantly affect the fetal heart rate or umbilical-placental vascular resistance responses to endothelin-1 (data not shown).

**DISCUSSION**

Previously, Chang et al. (10) reported that 1-h infusions of L-NNA increased fetal arterial blood pressure, increased umbilical-placental vascular resistance, and caused bradycardia in fetal sheep. We confirmed these cardiovascular responses after 1 h of L-NAME infusion; however, three novel and surprising changes in cardiovascular function occurred at later times. First, when infusion of the drug was maintained for 3 h, the acute pressor response to L-NAME waned substantially. Second, L-NAME treatment on subsequent days had no effect on measured cardiovascular variables. Because baseline cardiovascular variables had returned to normal vascular resistance response to angiotensin II (data not shown).

**Table 1. Arterial blood parameters before (control) and during L-NAME infusion on 3 consecutive days**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 1 (n = 7)</th>
<th>Day 2 (n = 6)</th>
<th>Day 3 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hour 1</td>
<td>Hour 2</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.01a</td>
<td>7.36 ± 0.01f</td>
<td>7.36 ± 0.01f</td>
</tr>
<tr>
<td>PCO₂</td>
<td>51.2 ± 0.6</td>
<td>50.5 ± 0.7</td>
<td>50.1 ± 1.5</td>
</tr>
<tr>
<td>PO₂</td>
<td>20.4 ± 1.2</td>
<td>20.4 ± 1.1</td>
<td>21.1 ± 1.0</td>
</tr>
<tr>
<td>BE</td>
<td>5.9 ± 2.5</td>
<td>2.9 ± 0.7f</td>
<td>2.4 ± 0.8f</td>
</tr>
<tr>
<td>Thb</td>
<td>9.5 ± 0.5a</td>
<td>11.2 ± 0.8f</td>
<td>11.3 ± 0.6f</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of sheep. L-NAME, N-nitro-l-arginine methyl ester; PO₂ and PO₂ in mmHg; BE, base excess (in mmol/L); THb, total hemoglobin concentration (in g/dl). Statistically significant differences on day 1 (n = 7) are represented by differing symbols, and significant differences between control periods on days 1, 2, and 3 (n = 6 each day) are represented by differing letters.
mal on days 2 and 3, we infer that the initial L-NAME infusion caused either a loss of basal NO release or that basal NO release was no longer important in the regulation of these cardiovascular variables. We also infer that an alternative regulatory mechanism reestablishes basal fetal arterial blood pressures in the absence of L-NAME-inhibitable NO release. Finally, L-NAME had no effect on the pressor response to angiotensin II on day 2 but caused a surprising and unexplained suppression of the hypertensive response to endothelin on day 3. Augmentation of pressor responses by L-NAME may be absent because basal release of NO was apparently no longer important in cardiovascular control when these agents were tested.

Vasoconstriction was probably the primary mechanism underlying the pressor response observed during the first hour of infusion on day 1. Inhibition of NO synthesis causes pronounced vasoconstriction in conscious newborn lambs (13), adult rats (15) and dogs (30), and in pregnant (12) and nonpregnant (24, 34) sheep. Furthermore, we observed a 70% increase in the vascular resistance of the umbilical-placental circulation, a bed that receives~40% of the combined ventricular output in fetal sheep (33). Vasoconstrictor-induced increases in blood pressures probably were blunted by a decrease in cardiac output secondary to the 23% reduction in heart rate that we observed.

Vasoconstriction induced by L-NAME may have caused the increase in the venous pressure that occurred in the first 10 min of the first L-NAME infusion. Rapid renormalization of venous pressure was probably due to two factors: a 16% decrease in fetal blood volume plus a venodilatory response associated with baroreceptor activation secondary to the rise in arterial pressure.

The waning of fetal arterial hypertension that occurred during the second and third hour of the first L-NAME infusion must have been due to a decline in cardiac output and/or a decline in total peripheral vascular resistance. Only a substantial decrease in cardiac output during the last 2 h of L-NAME infusion, because fetal heart rate and venous pressure (preload) remained stable and arterial pressure (afterload) declined over this interval. We believe it is more likely that total peripheral vascular resistance decreased during this time, even though no decrease in umbilical-placental vascular resistance was observed. The umbilical-placental bed may have been spared because it is relatively insensitive to some vasomotor agents such as norepinephrine (2) and vasopressin (20) and, at least in the human, it has little innervation (32).

Failure of L-NAME to sustain a fetal arterial hypertension over 3 h is not a general feature of vasoconstric-
tor responses in the fetus because long-term infusion of norepinephrine (7) and angiotensin (4) caused hypertension that is sustained for 24 h to 1 wk in fetal sheep. The rapid waning of the pressor response during NO synthesis inhibition on day 1 in the fetus contrasts with reports of hypertension that is sustained for hours to weeks in adults (14, 23, 25, 34, 36) and chronic hypertension in mice lacking the endothelial NO synthase gene (18).

Although L-arginine reversed the bradycardic response of fetal sheep to L-NAME, the pressor response persisted; however, higher doses of L-arginine than those we administered may have renormalized blood pressure. L-Arginine doses in our study exceeded the cumulative dose of L-NAME by 4-fold, whereas 10-fold excess of L-arginine was used to reverse the pressor response to L-NAME in adult sheep (24), and 100-fold excess of L-arginine reversed the effects of L-NAME on arterial pressure and heart rate in rats (31). Failure of L-arginine to reverse the effects L-NAME in our study was not due to antimuscarinic action of the drug (9), because the muscarinic receptors antagonist, atropine, does not increase blood pressure in fetal sheep (16). Furthermore, L-NNA, which does not antagonize muscarinic receptors (9), causes cardiovascular changes that are almost identical to those we observed with L-NAME (10).

By day 2, cardiovascular variables had returned to normal, and we inferred that L-NAME had been cleared from the fetal circulation. Surprisingly, however, the fetuses no longer exhibited a cardiovascular response to L-NAME. Ward and Angus (36) observed a gradual decrease in the pressor response to daily L-NNA administration in ganglion-blocked adult rabbits. In addition, the pressor response to L-NNA in adult dogs (30) and sheep (28) was abolished when L-NNA treatment was repeated the next day. Even though arterial pressure was near normal on the second day in all three studies, the two studies in which other aspects of baseline cardiovascular function were reported showed marked abnormalities (30, 36). In dogs, total peripheral resistance was increased, cardiac output was decreased, and the dogs were bradycardic. Thus chronic inhibition of NO synthase synthesis was inferred, with the return of normal arterial pressures being attributed to parasympathetically mediated bradycardia and reduced cardiac output. We did not measure cardiac output and total peripheral resistance; however, fetal heart rate and the resistance of a major vascular bed, the umbilicoplacental circulation, were normal on the days following the first infusion.

The restoration of normal blood pressure and umbilicoplacental vascular resistance by day 2, despite ablation of basal effects of NO, indicates that an unidentified vasomotor mechanism has replaced the basal activity of NO. A reduction in sympathetic vasomotor tone may have contributed; however, other mechanisms probably are involved. For example, altered sympathetic activity probably cannot explain normalization of umbilicoplacental vascular resistance, since this bed is relatively insensitive to norepinephrine (2) and it is likely poorly innervated (32). Possibly, umbilicoplacental vascular resistance is renormalized by another endothelial vasodilator system such as prostacyclin (19, 30) or carbon monoxide (22). We speculate that the attenuated pressor response to endothelin-1 following L-NAME treatment may be due to an enhancement of this vasodilatory pathway.

Fetal L-NAME infusion caused only a modest increase in maternal arterial pressure, whereas maternal L-NAME infusion produces a large pressor response (12). Low efficiency of fetal to maternal amino acid transport (5) plus dilution in the maternal circulation probably resulted in low concentrations of L-NAME in maternal blood.

We conclude that NO synthesis normally contributes to regulation of fetal arterial pressure and to the maintenance of a low umbilicoplacental vascular resis-
tance in fetal sheep. However, when inhibition of NO synthase is sustained for hours, then other regulatory mechanisms restore arterial pressure to normal levels, probably through systemic vasodilation. Furthermore, unknown vasomotor controls replace NO in the maintenance of normal cardiovascular function in the days following NO synthesis inhibition. The interactions between NO and the vasoconstrictive responses to angiotensin II or endothelin-1 when investigated in the current study were difficult to interpret, because basal release of NO was apparently no longer important in cardiovascular control on days 2 and 3 when sensitivity to these constrictors was tested.

In perspective, we have shown that the pressor response to NO synthesis inhibition wanes rapidly in fetal sheep and, within 24 h, basal NO synthesis appears to no longer play a role in regulation of fetal arterial pressure. These results contrast with those obtained in adult animals of many species where chronic inhibition of NO synthase induces sustained hypertension and/or a sustained increase in total peripheral resistance. Local and hormonal mechanisms regulating arterial pressure may be particularly important in fetal life because of the late perinatal development of the peripheral sympathetic nervous system. We suggest that the ability of the fetus to return cardiovascular variables to normal and to no longer show a cardiovascular response to subsequent administration of NO synthase inhibitors indicates that a normally redundant vasodilatory mechanism has taken over the role of NO synthase, and we hypothesize that such mechanisms play a more prominent role in cardiovascular regulation in fetal than in adult life.

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REFERENCES


