Prolonged uterine artery nitric oxide synthase inhibition modestly alters basal uteroplacental vasodilation in the last third of ovine pregnancy

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Rosenfeld CR, Roy T. Prolonged uterine artery nitric oxide synthase inhibition modestly alters basal uteroplacental vasodilation in the last third of ovine pregnancy. Am J Physiol Heart Circ Physiol 307: H1196–H1203, 2014. First published August 15, 2014; doi:10.1152/ajpheart.00996.2013.—Mechanisms regulating uteroplacental blood flow (UPBF) in pregnancy remain unclear, but they likely involve several integrated signaling systems. Endothelium-derived nitric oxide (NO) is considered an important contributor, but the extent of its involvement is unclear. Bolus intra-arterial infusions of nitro-L-arginine methyl ester (L-NAME) modestly decrease ovine basal UPBF; however, the doses and duration of infusion may have been insufficient. We, therefore, examined prolonged uterine artery nitric oxide synthase inhibition modestly alters ovine basal UPBF; however, the doses and duration of infusion may have been insufficient. We, therefore, examined prolonged uterine artery nitric oxide synthase inhibition with L-NAME throughout the last third of ovine pregnancy by performing either continuous 30-min UA infusion dose responses (n = 4) or 72-h UA infusions (0.01 mg/ml) at 104–108, 118–125, and 131–137 days of gestation (n = 7) while monitoring mean arterial pressure (MAP), heart rate (HR), and UPBF. Uteroplacental vascular resistance (UPVR) was calculated, and uterine cGMP synthesis was measured. Thirty-minute UA L-NAME infusions did not dose dependently decrease UPBF, increase UPVR, or decreased cGMP synthesis (P > 0.1); however, MAP rose and HR fell modestly. Prolonged continuous 72-h UA L-NAME infusions decreased UPBF ~32%, increased UPVR ~68% (P ≤ 0.001), and decreased uterine cGMP synthesis 70% at 54–72 h (P ≤ 0.004); the noninfused uterine horn was unaffected. These findings were associated with ~10% increases in MAP and decreases in HR that were greater at 104–108 than 118–125 and 131–137 days of gestation (P = 0.006). Although uterine and UA NO and cGMP synthesis increase severalfold during ovine pregnancy, they contribute modestly to the maintenance and rise in UPBF in the last third of gestation. Thus, local UA NO may primarily modulate vasconstrictor responses. Notably, the systemic vasculature appears more sensitive than the uterine vasculature to NO synthase inhibition.

nitric oxide synthase inhibition; mean arterial pressure; cGMP synthesis; sheep; uteroplacental blood flow

THE SUCCESS OF PREGNANCY is dependent on the processes associated with implantation, placentation with vasculogenesis, and the subsequent rise and maintenance of maternal and fetal placental blood flows. In most species, basal maternal uteroplacental blood flow (UPBF) increases severalfold in the last third of gestation, paralleling logarithmic increases in fetal weight (40, 43). Although this is primarily due to progressive vasodilation and maintenance of low vascular resistance in the maternal uteroplacental circulation, there is also a modest contribution by vasculogenesis in sheep (12, 39, 47). In contrast, the rise in fetal placental blood flow is primarily due to increasing angiogenesis and branching morphogenesis in the last third of gestation, thereby substantially increasing the placental surface area and capacity for oxygen and nutrient transport (12, 47). Alterations in the rise in maternal UPBF are often associated with disorders resulting in abnormalities of fetal growth and well-being, e.g., maternal hypertension (11, 16, 44). The mechanisms responsible for these alterations are unclear.

The pattern of rise in maternal UPBF and fall in uteroplacental vascular resistance (UPVR) in the last third of gestation are well described in most species (12, 39, 43). The mechanisms remain unclear, but they are likely to be complex (2, 46, 49). Prostaglandins, in particular prostacyclin, were considered important modulators of basal systemic and uterine vasodilation in pregnancy, but this has been questioned (3, 22, 24, 30). Nitric oxide (NO), a potent smooth muscle relaxing factor, vasodilates via cGMP-dependent mechanisms, is produced by NO synthase (NOS) expressed in uterine artery (UA) endothelium of several species and by endothelial NOS (eNOS). Moreover, its expression in pregnancy increases in all species studied, including sheep (5, 15, 18, 24–26, 46, 51–54). Thus, NO may be essential to the rise and maintenance of basal maternal UPBF in the last third of pregnancy, as well as maintenance of systemic vascular resistance and blood pressure. The former, however, is not supported by studies of prolonged systemic NOS inhibition in pregnant rats or eNOS null (eNOS<sup>−/−</sup>) mice (1, 17, 19, 20, 28). In these studies, NOS inhibition was associated with relative increases in blood pressure exceeding the fall in UPBF, which was only modestly affected, and suggesting differences in vascular sensitivity. These studies may have been limited by activation of adaptive mechanisms during prolonged systemic NOS inhibition (7) and increases in perfusion pressure that would attenuate the magnitude of the fall in UPBF by offsetting increases in UPVR. In near-term pregnant ewes, isolated UA NO inhibition with a broad range of doses of nitro-L-arginine methyl ester (L-NAME) infused over 1–2 min decreased basal UPBF only 30% (27, 35). L-NAME was infused directly into the uterine arterial circulation to avert systemic effects; nonetheless, mean arterial pressure (MAP) increased, again suggesting differences in regional vascular sensitivity to NOS inhibition. It is possible that the L-NAME doses or the durations of the infusions were insufficient to optimize tissue uptake, vascular NOS inhibition, and the fall in UPBF. Furthermore, NOS activity increases during...
the last third of pregnancy, and these studies were only done at full term.

Accumulating evidence suggests broad similarities in vascular function in pregnant women and sheep (6, 14, 36, 37, 42). Moreover, vasoactive agonists or antagonists can be infused intra-arterially into one uterine horn of pregnant ewes, limiting systemic effects and allowing the contralateral uterine horn to serve as a control (27, 35, 40). Moreover, steady-state arterial concentrations of infused agents can be estimated if UPBF is continuously monitored (35, 40), and uterine cGMP synthesis and the role of perfusion pressure can be assessed. We, therefore, used this model to study the effect of prolonged UA NOS inhibition on basal UPBF throughout the last third of ovine pregnancy when UA eNOS activity and expression parallel increases in UPBF. We studied dose responses to 30-min UA infusions of l-NAME, hypothesizing that this would optimize tissue uptake and local NOS inhibition. We also examined responses to 72 h of continuous UA l-NAME infusions to further optimize endothelial exposure, uptake, and local inhibitory effects on UA NOS. Uterine cGMP synthesis was measured in the treated uterine horn to demonstrate decreases in local NO synthesis and, thus, inhibition of the NOS-NO-cGMP pathway during the fall in UPBF and rise in UPVR.

METHODS

Animal model. The animal model is described in detail elsewhere (35, 40). Briefly, time-dated pregnant ewes (n = 11) of mixed Western breed were brought to the University of Texas Southwestern Medical Center and allowed 5–7 days to acclimate. Two groups of animals were studied: animals in group 1 (n = 4; 3 twins, 1 singleton) underwent surgery at ~98 days of gestation and were used for dose-response studies, and animals in group 2 (n = 7; 3 twins, 4 singletons) underwent surgery at ~100 days (full term ~150 days) and were used in studies of prolonged NOS inhibition. All animals were fasted overnight. In the morning, they received intramuscular atropine sulfate, and a percutaneous jugular venous catheter was implanted for infusion of prazosin pentobartital and ketamine hydrochloride. After intubation, animals were prepared for sterile abdominal surgery during inhalation anesthesia with isoflurane. Aortic arterial and uterine venous catheters were placed retrograde just distal to the bifurcation of the main UA. After 5 days of recovery from surgery, animals were studied: animals in group 1 underwent a UPBF dose-response study using unilateral UA infusions of l-NAME into the gravid uterine horn calculated to result in steady-state arterial concentrations of 0.025, 0.05, 0.1, 0.25, 0.5, and 1.0 mg/ml for 30 min. Since the uterine and systemic clearance of l-NAME is unclear, but the effects may be prolonged (31, 48), only one randomly selected dose was studied each day; studies were ~80 h apart, at which time all hemodynamic parameters had returned to baseline. Steady-state arterial levels (mg/ml) were estimated using the l-NAME infusion rate (mg/min) ÷ baseline UPBF (ml/min) obtained from the continuous measurement of unilateral UPBF (35, 40). Continuous recordings of bilateral UPBF (ml/min), heart rate (HR; beats/min), and MAP (mmHg) were initiated 30–60 min before l-NAME infusions and maintained for 60 min after completion of each infusion. Blood samples were collected simultaneously from an aortic arterial and uterine venous catheter to assess cGMP levels and uterine synthesis rate before, during, and 60 min after termination of the UA l-NAME infusion. Animals in group 2 underwent a 72-h continuous UA infusion of l-NAME in the gravid uterine horn to further optimize uterine vascular uptake and NOS inhibition that may not have been optimal in previous studies or during 30-min infusions (27, 35). Infusion rates were established to achieve a steady-state UA concentration of 0.01 mg/ml using the method described above and adjusted daily. In preliminary studies, this was the maximum rate of infusion over 72 h that did not result in adverse effects on maternal appetite and general well-being. Since UA NO synthesis increases with advancing gestation (25, 26, 51, 52), studies were performed at 104–108 (n = 6), 118–125 (n = 7), and 131–137 (n = 7) days of gestation to detect gestational age-dependent differences in the uterine responses to l-NAME in the last third of pregnancy that might relate to the increase in NO synthesis. Studies were >10 days apart to take into account the potential for prolonged NOS inhibition (30, 47). Hemodynamic parameters were monitored 2 h after the initiation of the 72-h infusions and each morning and late afternoon for 1 h. Uterine venous and arterial blood samples were simultaneously collected twice, 30 min apart, before initiation of intra-arterial l-NAME infusions, at 2, 4, 6, 24, 30, 48, 54, and 72 h during infusions and at 72 and 96 h after completion of the infusions to examine the extent of inhibition of uterine synthesis of cGMP.

cGMP assay. Blood samples were simultaneously collected from an aortic arterial and uterine venous catheter into chilled, heparinized plastic syringes (35, 40) and centrifuged at 10,000 g, plasma was removed, equal volumes of 10% trichloroacetic acid were added, and samples were frozen at -20°C until assayed. Plasma cGMP was measured by radioimmunoassay (NEX-133, PerkinElmer, Boston, MA). The validity of the assay is reported elsewhere (35, 40). Inter- and intra-assay variabilities were 11.4% and 1.86%, respectively. Uterine cGMP synthesis (pmol/min) was calculated as venous-arterial concentration differences of cGMP (pmol/ml) × UPBF (ml/min).

Statistical analysis. One-way analysis of variance (ANOVA) was used to examine dose responses and changes in hemodynamic parameters across gestational age. The postinfusion measurements at 72 and 96 h were excluded to accurately examine the significance of changes “during” l-NAME infusions independent of the recovery phase. Multiple-comparison procedures (Tukey’s test and the Holm-Sidak method) were used to identify significant differences across each infusion of l-NAME. Two-way ANOVA was used to examine differences in baseline data across doses and gestational ages and between hemodynamic parameters. Student’s t-test was used where appropriate. Values are means ± SE.

RESULTS

Dose responses. Unilateral 30-min UA infusions of l-NAME were performed using six doses as described in METHODS. There were no differences in baseline or preinfusion hemodynamic parameters for any dose of l-NAME studied (P > 0.1 by ANOVA; Table 1). Although UPBF appeared greater and UPVR less in the infused than noninfused uterine horn, they

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did not differ significantly \((P > 0.1\) by 2-way ANOVA). When responses to each dose of L-NAME were examined, there was no dose effect for any hemodynamic parameter \((P > 0.1\) by 2-way ANOVA), including UPBF in the infused uterine horn (Table 2), which fell an average of 9% across doses, while UPVR rose an average of 22%. The fall in UPBF was likely attenuated by a modest 7% rise in MAP or perfusion pressure, demonstrating that L-NAME had entered the systemic circulation and elicited an increase in MAP during the continuous infusions. This is substantiated by a 14% fall in HR likely due to a baroreflex response.

**Prolonged L-NAME infusions.** The uterine vascular uptake of infused L-NAME may be time- and dose-dependent. Moreover, the response may differ across the last third of gestation due to increases in UA NOS expression and activity during pregnancy. Therefore, we continuously infused L-NAME for 72 h via a UA catheter in the gravid horn to achieve and maintain a level of 0.01 mg/ml in seven additional pregnant ewes for 2 wk during the last third of gestation. There were no differences in baseline hemodynamic parameters prior to infusion of L-NAME for the three study times \((P > 0.1\) by 2-way ANOVA and regression analysis; Table 3). Notably, the infused or gravid uterine horn had a higher UPBF and lower UPVR \((P < 0.02\) by 2-way ANOVA).

A systemic response, i.e., a rise in MAP and fall in HR, occurred within 2–4 h of initiation of the prolonged UA infusions of L-NAME in each time period and was maintained until the infusion was stopped (Fig. 1A). This was greater at 104–108 than 118–125 and 131–137 days of gestation, which was associated with simultaneous decreases in HR averaging 13% that did not differ across gestation (Fig. 1B; \(P = 0.2\) by ANOVA). As noted above, the HR response is likely due to a baroreflex mechanism throughout pregnancy.

**Baseline UPBF in the infused uterine horn decreased during each study period (Fig. 2, left; \(P \leq 0.001\) by 1-way repeated-measures ANOVA), falling \(7.8 \pm 7.8, 37 \pm 6.6,\) and \(31 \pm 4.8\%\) by 72 h of infusion. UPVR also rose in each study period (Fig. 2, right; \(P \leq 0.003\) by repeated-measures ANOVA), increasing \(58 \pm 15, 81 \pm 22,\) and \(66 \pm 12\%,\) respectively. There were no differences between study periods for the fall in UPBF or the rise in UPVR \((P > 0.5\) by 2-way ANOVA). The relative fall, i.e., percent change, in UPBF was less than the rise in UPVR, demonstrating an attenuating effect of the rise in perfusion pressure or MAP during each study period. Importantly, neither UPBF nor UPVR in the contralateral or noninfused uterine horn was significantly changed in any study period \((P > 0.2\) by repeated-measures ANOVA), nor did they differ across study periods.

**Uteroplacental cGMP synthesis.** The model permits evaluation of uterine or uteroplacental NOS activity by examining local cGMP synthesis \((35, 40).\) Although limited uterine venous samples were available from the L-NAME dose-response studies due to the loss of functional venous catheters, the number of samples was sufficient to perform a regression analysis comparing the change in uterine cGMP synthesis at 30 min in the infused or gravid uterine horn and the L-NAME dose. There was no significant dose effect \((P = 0.9, R = 0.01, n = 9;\) data not shown); however, uterine cGMP synthesis decreased 17% on average across doses.

### Table 1. Baseline systemic and uterine hemodynamic parameters in infused and noninfused uterine horn of pregnant ewes

<table>
<thead>
<tr>
<th>Dose of L-NAME, mg/ml</th>
<th>0.025</th>
<th>0.05</th>
<th>0.10</th>
<th>0.25</th>
<th>0.50</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>83.5 ± 8.6</td>
<td>82.8 ± 7.2</td>
<td>83.3 ± 5.9</td>
<td>82.4 ± 6.2</td>
<td>86.5 ± 8.6</td>
<td>90.2 ± 12</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>87.6 ± 6.7</td>
<td>83.2 ± 4.5</td>
<td>96.0 ± 9.3</td>
<td>90.4 ± 6.0</td>
<td>92.3 ± 6.0</td>
<td>83.5 ± 11</td>
</tr>
<tr>
<td>UPBF, ml/min</td>
<td>Infused</td>
<td>508 ± 93</td>
<td>557 ± 109</td>
<td>536 ± 105</td>
<td>568 ± 133</td>
<td>421 ± 51</td>
</tr>
<tr>
<td></td>
<td>Noninfused</td>
<td>460 ± 118</td>
<td>510 ± 122</td>
<td>395 ± 107</td>
<td>403 ± 63</td>
<td>486 ± 139</td>
</tr>
<tr>
<td>UPVR, mmHg.min⁻¹</td>
<td>Infused</td>
<td>0.18 ± 0.03</td>
<td>0.16 ± 0.03</td>
<td>0.17 ± 0.04</td>
<td>0.18 ± 0.06</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Noninfused</td>
<td>0.21 ± 0.04</td>
<td>0.19 ± 0.05</td>
<td>0.27 ± 0.08</td>
<td>0.23 ± 0.05</td>
<td>0.21 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 4\) ewes. N-nitro-L-arginine methyl ester (L-NAME) was infused for 30 min via a uterine arterial catheter; each dose was infused on separate days ≥80 h apart. MAP, mean arterial pressure; HR, heart rate; UPBF, uteroplacental blood flow; UPVR, uteroplacental vascular resistance. See methods for definition of doses.

### Table 2. Percent change from baseline in systemic and uterine hemodynamic parameters in infused and noninfused uterine horn at 30 min during unilateral uterine artery infusions of L-NAME in pregnant ewes

<table>
<thead>
<tr>
<th>Dose of L-NAME, mg/ml</th>
<th>0.025</th>
<th>0.05</th>
<th>0.10</th>
<th>0.25</th>
<th>0.50</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>4.66 ± 2.3</td>
<td>10.2 ± 5.4</td>
<td>15.1 ± 1.7</td>
<td>3.37 ± 3.4</td>
<td>2.53 ± 3.5</td>
<td>8.18 ± 14</td>
</tr>
<tr>
<td>HR</td>
<td>-14.5 ± 6.4</td>
<td>-11.6 ± 5.8</td>
<td>-24 ± 6.5</td>
<td>-10.9 ± 0.4</td>
<td>-20.6 ± 9.5</td>
<td>-3.45 ± 6.1</td>
</tr>
<tr>
<td>UPBF</td>
<td>Infused</td>
<td>-5.55 ± 10</td>
<td>-11.3 ± 6.2</td>
<td>-13.4 ± 7.8</td>
<td>-5.64 ± 13</td>
<td>-12.2 ± 9.9</td>
</tr>
<tr>
<td></td>
<td>Noninfused</td>
<td>4.61 ± 11</td>
<td>-15.2 ± 6.2</td>
<td>-1.28 ± 15</td>
<td>-11.6 ± 9.8</td>
<td>-27.6 ± 11</td>
</tr>
<tr>
<td>UPVR</td>
<td>Infused</td>
<td>14.1 ± 11</td>
<td>26.1 ± 11</td>
<td>36.5 ± 13</td>
<td>20.1 ± 26</td>
<td>19.2 ± 11</td>
</tr>
<tr>
<td></td>
<td>Noninfused</td>
<td>2.52 ± 8.0</td>
<td>30.9 ± 5.7</td>
<td>26.1 ± 21</td>
<td>24.0 ± 22</td>
<td>47.7 ± 20</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 4\) ewes. Percent change from baseline was calculated as difference in response between baseline values and values at 30 min of infusion divided by baseline. Each dose was infused on separate days ≥80 h apart. See methods for definition of doses.
Uterine venous and arterial catheters remained patent in three to four studies in each time period during prolonged 72-h UA infusions of L-NAME in the last third of gestation. An example of the simultaneous changes in uterine venous and arterial cGMP concentrations at 104–108 days of gestation is shown in Fig. 3. Uterine venous cGMP concentrations always exceeded arterial levels before and during local L-NAME infusions (Table 3); however, the venous-arterial differences were no longer significant after 6 h of infusion. The parallel fall in both suggests that uterine cGMP is a major contributor to systemic levels. Importantly, there were no significant differences in preinfusion levels of cGMP in the venous and arterial blood across study periods (P > 0.1 by repeated-measures ANOVA), demonstrating that prolonged NOS inhibition was unlikely at 118–125 and 131–137 days of gestation. When these data were analyzed for each time period in the last third of gestation as uterine cGMP synthesis (Fig. 4), values decreased significantly in each time period (P = 0.004 by 2-way ANOVA), and the magnitude and pattern were similar throughout the last third of gestation (P = 0.8 by 2-way ANOVA), with uterine cGMP synthesis decreasing ~70% at 54–72 h of infusion.

### DISCUSSION

The rise in UPBF in the last third of pregnancy is essential for normal logarithmic increase in fetal growth and well-being. The mechanisms are likely complex and involve several integrated signaling pathways (2, 46, 49). UA eNOS expression and/or activity also increase in several species during pregnancy (5, 18, 25, 26, 51–54). Thus the NOS-NO-cGMP pathway has been considered a major contributor. However, UPBF decreases only modestly after prolonged systemic NOS inhibition in pregnant rats and eNOS−/− mice (17, 19, 20) and in

#### Table 3. **Baseline hemodynamic parameters and uterine venous and arterial cGMP concentrations in pregnant ewes prior to 72-h uterine artery infusion of L-NAME**

<table>
<thead>
<tr>
<th>Days of Gestation</th>
<th>104–108 (n = 6)</th>
<th>118–125 (n = 7)</th>
<th>131–137 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>82 ± 2.1</td>
<td>82 ± 2.2</td>
<td>81 ± 3.3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>87 ± 6.8</td>
<td>92 ± 5.7</td>
<td>93 ± 4.9</td>
</tr>
<tr>
<td>UPBF, ml/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infused</td>
<td>541 ± 82</td>
<td>570 ± 40</td>
<td>563 ± 64*</td>
</tr>
<tr>
<td>Noninfused</td>
<td>378 ± 59</td>
<td>341 ± 64</td>
<td>316 ± 91</td>
</tr>
<tr>
<td>UPVR, mmHg·min·ml⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infused</td>
<td>0.17 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>0.16 ± 0.02*</td>
</tr>
<tr>
<td>Noninfused</td>
<td>0.25 ± 0.06</td>
<td>0.34 ± 0.13</td>
<td>0.34 ± 0.15</td>
</tr>
<tr>
<td>cGMP, pmol/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous</td>
<td>14.7 ± 1.0</td>
<td>15.1 ± 1.5</td>
<td>12.2 ± 1.8</td>
</tr>
<tr>
<td>Arterial</td>
<td>11.2 ± 2.0</td>
<td>10.8 ± 1.3</td>
<td>8.51 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.02 by 2-way ANOVA (all pairwise multiple comparisons, Holm-Sidak method).
near-term pregnant sheep following local UA NOS inhibition with doses of L-NAME infused over 1–2 min (27, 35). It is possible that NOS inhibition was not optimal in the latter studies due to short exposures. In the present study we used the same pregnant sheep model to examine “isolated” uterine vascular responses to NOS inhibition throughout the last third of pregnancy; however, NOS inhibition was prolonged. A wide range of doses resulted in no dose effect of L-NAME on UPBF, UPVR, or uterine cGMP synthesis during 30-min UA infusions, but MAP increased modestly. When the UA infusion of L-NAME was increased to 72 h using a single dose, UPBF fell 30% and UPVR rose 70%; importantly, simultaneous uterine cGMP synthesis fell 70%. This was similar throughout the last third of pregnancy. MAP increased, despite anticipated decreases in L-NAME concentrations in the peripheral circulation, suggesting regional differences in vascular sensitivity to NOS inhibition (48). Although NO contributes modestly to basal UPBF and blood pressure in ovine pregnancy, it is not the predominant mechanism that regulates basal uteroplacental vasodilation or the rise in UPBF in the last third of pregnancy.

NO is a potent vasodilator, and its synthesis in the uteroplacental circulation has been examined in depth. UA eNOS expression and activity increase substantially during pregnancy in all species studied and are associated with increased NO and cGMP synthesis (2, 35, 46). Type 1 (neuronal) NOS (nNOS) is also expressed in ovine UA smooth muscle and increases after estradiol exposure in nonpregnant ewes (45). Furthermore, UA nNOS expression increases in pregnant sheep and mice, suggesting that it may also contribute to UA NO synthesis and regulation of basal UPBF (21, 33). The rise in UA NO synthesis is paralleled by >30-fold increases in uterine cGMP.
We (35) previously observed that 2-min UA infusions of NOS-NO-cGMP pathway in regulating basal UPBF (27, 35). Studies into the uterine arterial circulation in conscious pregnant ewes (P nights of gestation. Synthesis decreased significantly in each group (P < 0.001 by 1-way ANOVA) and did not differ between study periods (P > 0.1).

![Graph](image)

Fig. 4. Effect of 72-h unilateral uterine intra-arterial infusions of L-NAME on uterine cGMP synthesis in pregnant ewes in the last third of gestation. Studies were performed at 104–108 (n = 3; A), 118–125 (n = 4; B), and 131–137 (n = 4; C) days of gestation. Synthesis decreased significantly in each group (P < 0.001 by 1-way ANOVA) and did not differ between study periods (P > 0.1).

NOS synthesis in pregnant ewes (35). Thus, existing evidence supports an association between uterine NO and cGMP synthesis and the rise in and maintenance of UPBF in pregnancy, as well as abnormalities in NO synthesis and decreased UPBF in women with preeclampsia (15). However, in the presence of systemic NOS inhibition and the absence of eNOS in eNOS−/− pregnant mice, the rise in basal UPBF is only modestly attenuated (1, 17, 19, 20). This could reflect activation of compensatory mechanisms, as reported for prostaglandins during chronic NO inhibition in the mid-term-pregnant rat kidney (7). However, Naden et al. (30) reported that local prostaglandin inhibition did not alter basal UPBF or UPVR in pregnant ewes. Thus the extent to which NO synthesis contributes to the regulation of basal UPBF remains unclear.

We infused L-NAME, a nonspecific NOS inhibitor, directly into the uterine arterial circulation in conscious pregnant ewes remote from surgery, permitting the study of local uterine NOS inhibition with minimal systemic effects and the role of the UA NOS-NO-cGMP pathway in regulating basal UPBF (27, 35). We (35) previously observed that 2-min UA infusions of L-NAME, estimated to achieve concentrations of 0.5–25 mg/ml, decreased UPBF ~26% and uterine cGMP synthesis 66%, but there was no dose effect. Using the same model, Miller et al. (27) observed that 2 and 20 mg/kg L-NAME infused into a UA over 1 min decreased UPBF ~35% with both doses. Since L-NAME was rapidly infused as a bolus, tissue uptake and NOS inhibition may not have been optimal in either study due to insufficient exposure time and/or concentration, since basal UPBF exceeded 300–400 ml/min. In the present study, we increased the infusion time to 30 min in the dose-response studies and used a wider range of doses based on steady-state levels. There was no dose effect, and UPBF fell only 9% while UPVR rose 22%. Thus, 30-min tissue exposures to L-NAME also appear to be insufficient pharmacologically.

In contrast, prolonged (72-h) L-NAME infusions consistently decreased UPBF ~30% throughout the last third of ovine gestation while increasing UPVR ~70%. The fall in UPBF was attenuated by increases in MAP or perfusion pressure, demonstrating the importance of examining simultaneous changes in each parameter. Uterine cGMP synthesis also fell, confirming substantial inhibition of the local NOS-NO-cGMP pathway. There was no evidence of prolonged NOS inhibition before each study period, since baseline levels of cGMP in uterine arterial and venous blood did not differ and fetal growth was not compromised. The limited effect of NOS inhibition on basal UPBF might still reflect an insufficient dose; however, higher doses similarly infused caused poor feeding and disorientation, reflecting entry into the systemic circulation. Alternatively, the limited effect on UPBF might be due to activation of compensatory mechanisms, e.g., prostanoids, plus the rise in perfusion pressure (7). No one has simultaneously inhibited UA NO and prostanoid synthesis.

UA eNOS expression/activity and NO synthesis increase during the last third of ovine pregnancy (26, 53, 54). Thus the dependency of UPBF on NO synthesis should increase and the effect of NOS inhibition on UPBF should have been greater at full term; yet this was not seen. In fact, the effect of NOS inhibition on cGMP synthesis was similar throughout the last third of pregnancy. Since UPBF fell only 30% while cGMP synthesis decreased 70%, the major source of uterine NO synthesis may be nonvascular, i.e., placental, and involved in nonvascular functions (4, 10, 29, 55). While it is clear that NO contributes to basal UPBF regulation, it is not the primary pathway, confirming earlier reports (27, 35). We and others (13, 34, 38) have shown that large-conductance Ca2+-activated K+ (BKCa) channels are major contributors to the maintenance of basal ovine UPBF; e.g., unilateral BKCa channel inhibition decreases basal UPBF ≥80% without affecting the noninfused uterine horn or cGMP synthesis. BKCa channel activation occurs via the NOS-NO-cGMP-PKG pathway and/or direct activation by estrogens via the β1-regulatory subunit, which is upregulated in the UA during the last third of ovine pregnancy (8, 9, 41, 50). It is noteworthy that simultaneous inhibition of UA NOS and BKCa channel completely abolished uterine responses to estradiol (41). Although the contribution of these pathways to BKCa channel activation is unclear, BKCa channel activation appears to be very important.

Pregnant rats receiving systemic NOS inhibitors demonstrate greater systemic than uterine vascular responses (1, 17); i.e., twofold greater increases in peripheral vascular resistance than UPVR. This also occurs in NOS null pregnant mice, which have overt hypertension but only modest decreases in basal UPBF (19, 20). Thus there is evidence of regional differences in sensitivity to NOS inhibition (48), and the peripheral vasculature appears more sensitive to NOS inhibi-
tion. NO, therefore, appears to be more important in regulating blood pressure than basal UPBF. In the present study, L-NAME entered the systemic circulation, as evidenced by a rise in MAP and a reflex fall in HR. The pressor effects during 72-h L-NAME infusions were greater at 104–108 than 118–125 and 131–137 days of gestation. This is consistent with decreases in systemic arterial levels in later pregnancy due to the progressive rise in cardiac output in the last third of pregnancy (43); i.e., concentrations of L-NAME should have been less in the peripheral arterial circulation than in the uterine venous effluent at all gestational ages, but more so at full term. Thus the ovine systemic vasculature appears more sensitive to NOS inhibition, suggesting that NO is more important in maintaining peripheral than utero-placental vascular tone in pregnancy. This is supported further by the observation that the noninfused uterine horn was unaffected by the levels in the arterial perfusate. If uterine NO is not a major contributor to the maintenance of basal UPBF, it may serve to modulate vasoconstrictor responses to endogenous agonists such as α-adrenergic agents (20, 28, 32, 53, 54).

In the present study we examined the effects of prolonged UA NOS inhibition with L-NAME. We did not elicit a dose effect with 30-min UA infusions of L-NAME. However, 72-h infusions decreased UPBF 30% while increasing UPVR 70% throughout the last third of ovine pregnancy, and this was paralleled by substantial inhibition of the uterine NOS-NO-cGMP pathway. Importantly, the magnitude of the fall in UPBF was partially attenuated by simultaneous increases in perfusion pressure. Thus it is essential to examine relative changes in blood flow, resistance, and MAP to take into account the effects of perfusion pressure. Nonetheless, the present data support the hypothesis that NO contributes only modestly to the regulation of basal UPBF in ovine pregnancy, and other pathways, e.g., the BKCa channel, are more important. We believe the elevation of UA NO synthesis contributes to protecting the uterine circulation by attenuating vasoconstrictor responses to increased sympathetic activity in normotensive pregnancy (5, 20, 53, 54), but this was not addressed in the present study.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

NITRIC OXIDE AND UTEROPLACENTAL BLOOD FLOW


