Enhanced Umbilical Blood Flow During Acute Hypoxemia After Chronic Umbilical Cord Compression
A Role for Nitric Oxide
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Background—The continuing incidence of intrapartum morbidity may be partly due to antenatal compromise, which influences the fetal defense to labor and delivery. We have shown that antenatal exposure of the ovine fetus to partial compression of the umbilical cord suppresses femoral vasoconstriction during subsequent acute hypoxemia through elevated nitric oxide (NO) activity. This study investigated whether elevated NO activity in cord-compressed fetuses enhanced the vasodilator response to hypoxemia in circulations in which blood flow is known to increase during acute hypoxemia, such as the umbilical vascular bed.

Methods and Results—Fifteen fetal sheep were chronically instrumented between 117 and 120 days of gestation with vascular catheters and an umbilical flow probe. In 8 of these fetuses, umbilical blood flow was reduced by 30% for 3 days between 125 and 128 days. The remaining 7 fetuses acted as sham-operated controls. Between 2 and 7 days after umbilical cord/sham compression, fetuses were exposed to 2 episodes of acute hypoxemia, on separate days, during infusion with either saline or treatment with a combination of N\textsuperscript{G}-nitro-L-arginine methyl ester and sodium nitroprusside. The data show that umbilical cord compression significantly enhances the umbilical hyperemia through NO-dependent mechanisms during a subsequent episode of acute hypoxemia.

Conclusions—Increased fetal NO activity after chronic cord compression has opposing effects in circulations that either constrict or dilate during subsequent acute hypoxemia. The data imply that antenatal compromise switches the fetal strategy to withstand episodes of subsequent acute hypoxemia of the type that may occur during labor and delivery from a reliance on vasoconstrictor mechanisms to those promoting NO-dependent vasodilation. 

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Key Words: pregnancy ■ hypoxia ■ nitric oxide

Clinical evidence suggests that perinatal morbidity arising from birth hypoxia or asphyxia has remained high despite marked improvements in obstetric practice and the management of labor in recent years.\textsuperscript{1} It has therefore been suggested that perinatal mortality in these circumstances may reflect the cumulative effects of antenatal compromise and the conditions encountered during labor rather than the effects of labor per se.\textsuperscript{2} We have previously shown that the ovine fetal adaptive responses to an antenatal challenge, in the form of a 3-day period of reversible, controlled compression of the umbilical cord, are retained up to 1 week beyond the period of actual compromise.\textsuperscript{3} Furthermore, these persistent, adaptive responses result in a marked attenuation of femoral vasoconstriction during a subsequent episode of acute hypoxemia\textsuperscript{3} of the type that may be encountered during labor and delivery.\textsuperscript{4}

In the healthy fetus, survival during acute hypoxemia is promoted through a redistribution of the combined ventricular output away from ancillary and toward essential vascular beds such as the cerebral, myocardial, adrenal, and umbilical circulations.\textsuperscript{5} The redistribution of blood flow is partly mediated by peripheral vasoconstriction\textsuperscript{5,6} and partly by maintained or increased perfusion of the essential vascular beds through either passive, pressure-dependent changes in blood flow\textsuperscript{7} or active vasodilation.\textsuperscript{8-11} In the compromised fetus that has been exposed to a period of cord compression, there is an increase in nitric oxide (NO) activity that overcomes vasoconstrictor influences on the fetal femoral circulation during acute hypoxemia.\textsuperscript{3} The resulting diminished femoral vasoconstriction may therefore weaken the fetal defense and render the fetus more susceptible to the acute hypoxemia associated with relatively uncomplicated labor and delivery. On the other hand, increased NO activity in the cord-compressed fetus may further enhance vasodilator responses in essential vascular beds, thereby compensating for the weakening effect that a diminished femoral vasoconstriction may have on the redistribution of the fetal combined ventricular output during acute hypoxemia.
In this study, we tested the hypothesis that in compromised fetuses exposed to an antenatal challenge there is a compensatory increase in NO activity that has opposing effects on circulations that undergo vasoconstriction or vasodilation during acute hypoxemia. Previously, we have reported that in the healthy ovine fetus, umbilical blood flow increases during an episode of acute hypoxemia. In the present study, we investigated in the cord-compressed fetus: (1) whether the increase in umbilical blood flow during subsequent acute hypoxemia is enhanced and (2) whether this enhancement is mediated by increased NO activity.

Methods

Surgery

Fifteen Welsh Mountain ewes of known gestational age were used in the study. All procedures were performed under the UK Animals (Scientific Procedures) Act, 1986. All animals were fasted for 24 hours before surgery. Surgery was performed under strict aseptic conditions between 117 and 120 days of gestation (dGA; term being 140 to 145 dGA). Anesthesia was induced with sodium thiopentone (20 mg/kg IV Intravial sodium; Rhone Mérieux) and maintained with 1% to 2% halothane in 50:50 O2/N2O. Fetal vascular (ascending and descending aorta, inferior vena cava) and amniotic catheters were inserted, and a transit-time flow transducer (Transonics) was placed around a umbilical artery, close to the common umbilical artery, inside the fetal abdominal cavity. An inflatable occluder cuff (In Vivo Metrics) was positioned around the proximal end of the umbilical cord, as described previously in detail.3,12

Experimental Protocol

No experiment was performed until at least 5 days after surgery. At 124±1 days, baseline mean unilateral umbilical blood flow was recorded in all fetuses for 1 day. At 125±1 days, the animals were divided randomly into 2 experimental groups; in 8 of the fetuses, the occluder cuff was inflated to reduce umbilical blood flow by 30% for 3 days (umbilical cord compressed, UCC), as previously described.3,12 The duration of the challenge was chosen arbitrarily. The occluder cuff was then deflated, allowing the return of umbilical blood flow to baseline. In the remaining 7 fetuses, the occluder cuff was not inflated throughout the duration of the protocol (sham compressed). Between 2 and 7 days after the end of umbilical cord compression or sham compression, 6 UCC and 5 control fetuses were exposed to an episode of acute hypoxemia (induced for 1 hour by alteration of inhaled maternal gases)6,7 either during fetal intravenous infusion with saline (0.9% NaCl at 0.25 mL/min) or during fetal combined intravenous treatment with Nω-nitro-L-arginine methyl ester (L-NAME) and sodium nitroprusside (the NO clamp: L-NAME, Sigma; 100 mg/kg bolus dissolved in 1 mL saline, injected intravenously, and a 5.1±2.0 μg/kg per minute, mean±1 SD, infusion of nitroprusside dissolved in saline; Sigma). The NO clamp is a well-established technique that combines fetal treatment with the NO synthase inhibitor L-NAME with the NO donor sodium nitroprusside to block de novo synthesis of NO while compensating for the tonic production of the gas and thereby maintaining basal cardiovascular function.3,7,13 In all fetuses, acute hypoxemia was induced during saline infusion first and then, 1 to 2 days later, during treatment with the NO clamp. The order of treatment was not randomized to minimize potential confounders of the long-term effects of fetal exposure to L-NAME alone.6,10 Fetal descending aortic perfusion pressure, amniotic pressure, and umbilical blood flow were recorded continuously during each episode of acute hypoxemia. At the end of all protocols, the ewes and fetuses were humanely killed with a lethal dose of sodium pentobarbital (200 mg/kg, Pentoject; Animal Ltd), and the positions of implanted catheters, occluder, and flow probe were confirmed.

Blood Sampling

Fetal arterial blood samples (0.5 mL) were drawn into sterile syringes daily and, at appropriate intervals, during the acute hypoxemia protocols for measurement of arterial blood gases and pH (ABL5 blood gas analyzer, Radiometer; blood corrected to 39.5°C).

Measurements and Calculations

Fetal mean descending aortic and perfusion (arterial minus venous) pressures were corrected for amniotic pressure. Changes in umbilical vascular resistance were calculated by dividing descending aortic blood pressure (corrected for amniotic pressure) by umbilical blood flow. All analogue signals for calibrated fetal descending aortic blood pressure, amniotic pressure, and umbilical blood flow were recorded continuously at 1-second intervals throughout the study with the use of a data acquisition system.3,7

Statistical Analysis

Values for all variables are expressed as mean±SEM unless otherwise stated and were compared by means of 2-way ANOVA with repeated measures (Sigma-Stat; SPSS Inc), comparing the effect of time, group (control versus UCC), and interactions between group and time. Summary measures analysis was conducted on cardiovascular data obtained during the acute hypoxemia protocols to focus the number of comparisons.14 Where a significant effect of time or group was indicated, the post hoc Student’s t test for unequal data was used. For all comparisons, statistical significance was accepted at a level of P<0.05.

Results

Umbilical Cord Compression

Baseline descending aortic perfusion pressure (45.7±3.7 versus 37.4±4.5 mm Hg), unilateral umbilical blood flow (195±16 versus 198±27 mL/min), and umbilical vascular conductance (4.39±0.63 versus 5.13±0.16 (mL/min per mm Hg) were similar in control and UCC fetuses, respectively, and, in control fetuses, remained unchanged from the baseline value for the duration of the experimental protocol. In UCC fetuses, unilateral umbilical blood flow was reduced on average by 28% (range, 25% to 31%; 95% CI) from a baseline of 209±25 mL/min to 148±18 mL/min for 3 days. Umbilical cord compression led to a significant increase in fetal descending aortic perfusion pressure (Figure 1A) and significant decreases in umbilical blood flow (Figure 1B) and vascular conductance (Figure 1C). On deflation of the cord occluder, while umbilical vascular conductance returned to baseline, fetal descending aortic perfusion pressure remained significantly elevated, producing a significant overcompensation in umbilical blood flow, which remained at a level significantly greater than baseline flow before compression for between 30 and 36 hours.

Carotid arterial blood gases and pH in control fetuses were within the normal range (pHa, 7.35±0.01; Paco2, 51.0±1.9 mm Hg; PacO2, 24.2±1.8 mm Hg) and remained unchanged throughout the experimental period. In contrast, umbilical cord compression reduced carotid arterial pH from 7.35±0.01 to 7.32±0.07 and PacO2 from 24.0±1.0 to 20.4±1.3 mm Hg and increased PacO2 from 51.6±1.4 to 56.4±1.2 mm Hg (P<0.05). After 1 day of recovery from cord compression, values for all these variables had returned to basal conditions.
### Umbilical Hemodynamic Responses to Acute Hypoxemia During Saline Infusion

Baseline fetal descending aortic perfusion pressure (43.6±4.0 versus 45.5±2.4 mm Hg), unilateral umbilical blood flow (182±14 versus 174±17 beats/min), and umbilical vascular conductance (3.43±0.25 versus 3.24±0.29 (mL/min per mm Hg) were similar in sham control and UCC fetuses, respectively. In control fetuses, acute hypoxemia led to significant hypertension (Figure 2A) that tended to drive an increase in umbilical blood flow (Figure 2B; *P<0.08), as no change in umbilical vascular conductance was calculated (Figure 2C). In contrast, in UCC fetuses, hypertension during acute hypoxemia and recovery was markedly attenuated relative to control fetuses (Figure 2D), and there was a pronounced increase in umbilical vascular conductance (Figure 2F) that allowed for a significant increase in umbilical blood flow during acute hypoxemia (Figure 2E).

### Umbilical Hemodynamic Responses to Acute Hypoxemia During NO Clamp

Treatment with the NO clamp did not affect baseline fetal descending aortic perfusion pressure (48.5±4.8 versus 45.2±6.7 mm Hg), unilateral umbilical blood flow (196±17 versus 167±19 mL/min), or umbilical vascular conductance (4.43±0.64 versus 3.55±0.54 (mL/min per mm Hg) in sham control or UCC fetuses, respectively. In control fetuses, treatment with the NO clamp did not affect the umbilical hemodynamic responses to acute hypoxemia (Figure 3, A through C). In contrast, in UCC fetuses, hypertension during acute hypoxemia and recovery was markedly attenuated relative to control fetuses (Figure 3D), diminished the increase in umbilical blood flow (Figure 3E), and abolished the

### Table: Fetal Arterial Blood Gas Status During Acute Hypoxemia With Saline Infusion or Nitric Oxide Clamp Treatment

<table>
<thead>
<tr>
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<th>Hypoxemia With Saline Infusion</th>
<th>Hypoxemia With Nitric Oxide Clamp</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>H15</td>
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<tr>
<td>pH</td>
<td></td>
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<tr>
<td>Control</td>
<td>7.34±0.01</td>
<td>7.31±0.01*</td>
</tr>
<tr>
<td>UCC</td>
<td>7.33±0.01</td>
<td>7.32±0.01</td>
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<tr>
<td>PaCO₂, mm Hg</td>
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<tr>
<td>Control</td>
<td>51.3±1.9</td>
<td>58.5±1.9</td>
</tr>
<tr>
<td>UCC</td>
<td>55.0±1.5</td>
<td>54.4±1.4</td>
</tr>
<tr>
<td>PaO₂, mm Hg</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>24.4±0.8</td>
<td>13.4±0.2*</td>
</tr>
<tr>
<td>UCC</td>
<td>19.9±0.8</td>
<td>12.1±0.3*</td>
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Data are for blood gases and pH during normoxia (baseline), at 15 (H15) and 45 minutes (H45) of hypoxemia, and after 45 minutes (R45) of recovery from hypoxemia. Values are mean±SEM for n=6 UCC and n=5 control fetuses during acute hypoxemia. *P<0.05, baseline vs hypoxemia or recovery.
Discussion

Fetuses whose umbilical-placental perfusion has been reduced temporarily but then restored show a markedly diminished capacity to constrict their femoral circulation in response to the subsequent acute challenge of reduced oxygenation of the magnitude that may be encountered during labor and delivery. This attenuation in vasoconstrictor capacity is due to enhanced NO activity, which masks the increase in femoral vascular resistance during acute hypoxemia, as the vasoconstriction can be restored by treating the compromised fetus with the NO clamp. However, such fetuses do not metabolically deteriorate to a greater extent than sham control fetuses during or after acute hypoxemia (ie, the decreases in arterial pH, acid-base excess, and the increase in lactate are similar during the period of subsequent acute hypoxemia). This suggests a degree of tolerance to the acute hypoxic challenge in cord-compressed fetuses even after antecedent compromise. In the present study, we tested the hypothesis that in compromised fetuses, the compensatory increase in NO activity has opposing effects on circulations that undergo constriction and dilation during acute hypoxemia, thereby enhancing the vasodilator responses of essential vascular beds, such as the umbilico-placental circulation, and compensating for the weakening effects of the diminished femoral vasoconstriction. The results of this study support the hypothesis and suggest for the first time that antecedent compromise switches the fetal strategy to withhold subsequent acute hypoxemia from a reliance on peripheral vasoconstriction to an increased dependence on umbilical vasodilation to maintain or enhance perfusion in essential vascular beds.

The current data confirm that in fetuses undergoing compression of the umbilical cord, release of the vascular occluder leads to a distinct overcompensation in umbilical blood flow and suggest that this temporary umbilical hyperemia is mediated entirely by passive, pressure-dependent mechanisms, as no change in umbilical vascular conductance was calculated. However, the potential for active vasodilation in the umbilico-placental circulation clearly develops over the next few days. During subsequent acute hypoxemia (between 2 and 7 days later), despite an attenuated hypertensive response, a pronounced increase in umbilical blood flow occurs as the result of a distinct increase in umbilical vascular conductance (Figure 3F) during acute hypoxemia.
tance. Additional findings demonstrate that this enhanced umbilical vasodilation in cord-compressed fetuses is NO-dependent, as it could be totally abolished by fetal treatment with the NO clamp during acute hypoxemia. The mechanism for increased NO-dependent activity in the umbilical vascular bed of the compromised fetus during subsequent acute hypoxemia may be due to a number of factors, including greater generation of NO, increased expression of NO synthase in fetoplacental tissue, increased sensitivity of the placental vascular tissue to NO, and/or greater synthesis of vasoactive factors that reduce umbilical-placental tone through NO-dependent mechanisms. Candidate factors include adenosine, estrogen, and/or adrenomedullin. In addition, we have previously shown that fetuses preexposed to umbilical cord compression have elevated basal plasma concentrations of cortisol. Anwar et al. reported enhanced acetylcholine-dependent vasodilation in fetal sheep treated with glucocorticoids. Therefore, an alternative possibility is that fetuses preexposed to umbilical cord compression may have enhanced acetylcholine-dependent vasodilation in the umbilical placenta vascular bed as a result of elevated plasma cortisol concentrations.

It is possible that this altered response, that is, attenuated femoral vasoconstriction but enhanced umbilical vasodilation to a subsequent acute stress in the previously compromised fetus, represents an adaptive mechanism that first, protects peripheral circulations from excessive reductions in oxygen delivery and pH, and, second, facilitates further perfusion of essential circulations. Although the results of the current study support the latter hypothesis, there are some data in the current model to support the former. The output of lactate from skeletal muscle in the fetal hind limb, which ordinarily increases during acute hypoxemia in the healthy fetus, is attenuated in the cord-compressed fetus despite similar hind limb oxygen and glucose delivery.

In addition, the greater ability to maintain flow to the periphery in cord-compressed fetuses and to further increase flow to other circulations such as the placenta suggests that cord-compressed fetuses must have a greater reliance on either increased cardiac output and/or venoconstriction to increase central blood volume and accommodate the acute episode of oxygen deprivation. Although we have no direct evidence to support this contention, venous pressures are ≈5 mm Hg higher and the rate-pressure product is greater in cord-compressed fetuses [controls, 7.92±0.75 versus UCC, 8.99±0.79 (beats/min per mm Hg)×10], which may indicate the potential for greater cardiac preload and increased myocardial work, respectively. Estrogen and adenosine have been shown to increase coronary flow through induction of NO, which may aid greater cardiac work.

In conclusion, fetal exposure to a reversible period of adverse intrauterine conditions upregulates vasodilator, in particular NO-dependent, mechanisms in the fetus. This enhanced NO activity has opposing effects on circulations that constrict or dilate during acute hypoxemia. We propose that preadynamic fetal compromise shifts the balance of the defense mechanisms that enable the fetus to adapt successfully to an episode of acute oxygen deprivation from diminished vasoconstrictor-mediated to enhanced vasodilator-mediated redistribution of the combined ventricular output. An example of such enhanced NO-dependent vasodilator responses to acute hypoxemia in the compromised fetus is the response that occurs in the umbilical vascular bed.

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References
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