Effect of NO, phenylephrine, and hypoxemia on ductus venosus diameter in fetal sheep

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Kiserud, Torvid, Takashi Ozaki, Hidenori Nishina, Charles Rodeck, and Mark A. Hanson. Effect of NO, phenylephrine, and hypoxemia on ductus venosus diameter in fetal sheep. Am J Physiol Heart Circ Physiol 279: H1166–H1171, 2000.—To study the regulation of the ductus venosus (DV) inlet in vivo, we measured the effect of vasoactive substances and hypoxemia on its diameter in nine fetal sheep in utero at 0.9 gestation under ketamine-diazepam anesthesia. Catheters were inserted into an umbilical vein and a fetal common carotid artery, and a flowmeter was placed around the umbilical veins. Ultrasound measurements of the diameter of the fetal DV during normoxic baseline conditions [fetal arterial Po2 (PaO2) 24 mmHg] were compared with measurements during infusion of sodium nitroprusside (SNP; 1.3, 2.6, and 6.5 μg·kg−1·min−1) or the α1-adrenergic agonist phenylephrine (6.5 μg·kg−1·min−1) into the umbilical vein or during hypoxemia (fetal PaO2 reduced to 10 mmHg). SNP increased the DV inlet diameter by 23%, but phenylephrine had no effect. Hypoxemia caused a 61% increase of the inlet diameter and a distension of the entire vessel. We conclude that the DV inlet is tonically constricted, because nitric oxide dilates it but an α1-adrenergic agonist does not potentiate constriction. Hypoxemia causes a marked distension of the entire DV.

IN THE HUMAN FETUS during the second half of gestation, 20–30% of the umbilical venous return flows through the ductus venosus (DV) bypassing the fetal liver (17). This fraction is higher in fetal monkeys and sheep (40–50%) (3, 9), and the shunting increases during hypoxemia (3, 10) and hypervolemia (21). Barron (2) suggested a sphincter at the entrance of the DV as a key regulator of this flow. The idea was supported when adrenergic nerve fibers were identified in the area with the use of histochemical techniques in specimens from human fetuses at 20–24 wk gestation (11). These specimens showed contractile responses to norepinephrine, acetylcholine, and 5-hydroxytryptamine. Later studies in the fetal lamb confirmed the adrenergic innervation of the DV and demonstrated α-adrenergic constriction and β-adrenergic relaxation but showed an inconsistent response to acetylcholine (4). The DV relaxes under the influence of prostaglandin E1 (1, 24), but the response is weaker than in the ductus arteriosus (23). A recent study applying ultrasound techniques showed increased shunting through the DV of fetal sheep during hypoxemia (30). However, apart from experiments in fetal and neonatal rats in which the whole body was frozen after exposure to indomethacin (22), there have been no direct measurements of the diameter of the DV inlet in utero to support the operation of the regulatory mechanisms suggested from in vitro studies. We hypothesize that the DV is actively regulated and that this regulation can be observed in vivo, using ultrasound techniques, as a change in diameter in response to agonists.

Therefore, on the basis of previous reports of increased shunting through the DV during hypoxemia and in vitro results suggesting an active control of DV diameter, we aimed to determine whether the DV diameter does in fact change during hypoxemia, whether α1-adrenergic constriction can be reproduced in the fetal sheep in utero, and whether nitric oxide (NO), a mediator for smooth muscle relaxation and vasodilatation in most organs, has an effect on the diameter of the DV.

METHODS

Animal preparation. Ten ewes of Mule crossbreed, time dated and carrying singleton fetuses, were subjected to acute experiments at 130 (range 118–137) days gestation (term = 145 days). One fetus developed severe acidosis (pH 6.91) during the early stages of the experiment and never reached acceptable baseline values, and the experiment was discontinued. The remaining nine animals were included in the statistics given here.

The ewes were fasted for 12–16 h before the experiment. General anesthesia was induced by 250 mg of ketamine and 20 mg of diazepam (iv) and maintained by 150 mg of ketamine every 10 min and 10 mg of diazepam every 40–60 min through a catheter inserted in a pedal vein. A continuous intravenous infusion of 0.9% saline was administered to the ewe throughout the surgical procedure. Maternal arterial blood gases and pH were determined in samples drawn from

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a catheter in the femoral artery inserted through a peripheral branch. The ewe was put in a supine position, and the pregnant horn of the uterus was exposed through a longitudinal laparotomy. The fetal head and neck were delivered through a small incision, and a polyethylene catheter was introduced into the common carotid artery and connected to a pressure transducer (SensoNor 840, SensoNor, Horten, Norway). Through a separate uterotomy, a second polyethylene catheter was inserted into one of the umbilical veins through a peripheral cotyledonary branch and connected to an infusion pump for the administration of vasoactive substances. A Transonic flowmeter (Transonic Systems, Ithaca, NY) was mounted around the intra-abdominal common umbilical vein through a small incision in the fetal abdominal wall (n = 4 fetuses) or directly around the two umbilical veins in the cord (n = 6 fetuses). The fetus was then returned to the uterine cavity, and the uterotomy was closed. The pressure recorder and flowmeter were connected to a Vingmed CFM 800 ultrasound scanner (GE Vingmed Sound, Horten, Norway) through an amplifier (Neurolog, Digitimer). After the experimental procedure, which lasted 2.5–4 h, the ewe was killed with an overdose of pentobarbital, and the weight of the fetus was noted. All procedures were conducted in accordance with United Kingdom Home Office regulations and the Guidance for the Operation of the Animals (Science Procedures) Act (1986).

**Experimental protocol and measurements.** Fetal baseline measurements of arterial blood gases [arterial PO₂ (PaO₂), arterial PCO₂ (PaCO₂)] and pH, mean arterial blood pressure (MAP), heart rate (HR), and umbilical venous blood flow were recorded under stable anesthesia in normoxemia. The DV was visualized by ultrasound imaging using a multifrequency mechanical annular array transducer with a center frequency of 5 or 7.5 MHz; the axial resolution is 0.7 mm, and the minimum diameter measurements compared with either the DV inlet or on the degree of shunting (Table 1).

The NO donor sodium nitroprusside (SNP) was infused into the umbilical vein at doses of 5, 10, and 25 μg/min. After the procedure, when the fetal weight was known, the normalized dosages were calculated to be 1.3 (range 0.9–1.8), 2.6 (range 1.8–3.6), and 6.5 (range 4.6–9.1) μg·kg⁻¹·min⁻¹, respectively. After a 10-min stabilizing period at each level, the measurements were repeated. The NO donor sodium nitroprusside (SNP) was infused into the umbilical vein at doses of 5, 10, and 25 μg/min. After the procedure, when the fetal weight was known, the normalized dosages were calculated to be 1.3 (range 0.9–1.8), 2.6 (range 1.8–3.6), and 6.5 (range 4.6–9.1) μg·kg⁻¹·min⁻¹, respectively. After a 10-min stabilizing period at each level, the measurements were repeated. The NO donor sodium nitroprusside (SNP) was infused into the umbilical vein at doses of 5, 10, and 25 μg/min. After the procedure, when the fetal weight was known, the normalized dosages were calculated to be 1.3 (range 0.9–1.8), 2.6 (range 1.8–3.6), and 6.5 (range 4.6–9.1) μg·kg⁻¹·min⁻¹, respectively. After a 10-min stabilizing period at each level, the measurements were repeated.

Hypoxemia was imposed by mixing air, N₂, and CO₂ in the proportions of 7:12:1 l/min in the inspirate. The mixture was adjusted to achieve the desired level of hypoxemia in the fetal carotid artery before the measurements started. Hypoxemia was started 30 min after the phenylephrine infusion was discontinued. The measurements were taken when fetal PaO₂ had reached ≤15 mmHg. Two fetuses died during hypoxemia.

**Calculations and statistics.** DV flow was calculated as \((0.5D^2) \cdot \frac{V_{max}}{2}\), where \(D\) is the diameter of the DV and \(V_{max}\) is the time-averaged maximum velocity. Together with the umbilical venous flow recorded with the Transonic flowmeter, these results were used to calculate the fraction of umbilical flow (%) that was shunted through the DV. The results are presented as means ± SE. Analysis of variance for repeat measurements and a post hoc test were used to compare data during baseline conditions with data during SNP infusion. Two-tailed t-test was used to assess differences between paired observations, and Wilcoxon signed-rank test was used for data with nonnormal distribution. \(P \leq 0.05\) was considered significant.

**RESULTS**

The nine fetuses included in the statistics had a mean weight of 4.0 kg (range 2.8–5.4 kg) and a mean Hb of 9.1 g/dl (range 7.7–10.1 g/dl). The baseline values of PaO₂ (24 ± 1.4 mmHg), PaCO₂ (44 ± 2.3 mmHg), and pH (7.27 ± 0.02) were maintained during SNP (mean PaO₂; 25 mmHg) and phenylephrine infusion (mean PaO₂: 24 mmHg). The NO donor sodium nitroprusside (SNP) had no impact on HR but reduced MAP in a dose-dependent manner (Fig. 1). There was no change in umbilical blood flow (Fig. 1).

SNP caused a distension of the DV inlet (Table 1). Compared with baseline conditions, the effect on the diameter and the relative change calculated in percent distension did not extend beyond the dose of 2.6 μg·kg⁻¹·min⁻¹ (Fig. 2). There was a correlation between the normalized dosage (μg·kg⁻¹·min⁻¹) of SNP and the diameter changes of the DV inlet (calculated as % change from baseline value) [correlation coefficient of 0.856, 95% CI (0.613;0.951), \(P < 0.0001\)].

No significant change in the diameter could be demonstrated at the midportion of the DV (Table 1). There was no effect on the time-averaged maximum blood velocity at the DV inlet or on the degree of shunting (Table 1).

**Effect of phenylephrine.** Compared with baseline values, phenylephrine did not change HR but increased MAP and umbilical venous flow (Fig. 1). A constrictor effect of phenylephrine could not be found from the DV diameter measurements compared with either the baseline values or the measurements taken during SNP infusion 0.5 h earlier (Fig. 2). The higher time-averaged blood velocity at the DV inlet during the phenylephrine infusion (80 cm/s) was not significantly different from the velocity during baseline conditions (69 cm/s) (Table 1).

**Effect of hypoxemia.** Fetal hypoxemia had a profound effect on the circulation and led to a marked reduction in HR, MAP, and umbilical venous flow (Fig. 1). The DV inlet was substantially distended (Figs. 2 and 3).
Table 1), and a higher fraction of umbilical blood flow was shunted through the vessel, but with no change in the blood velocity (Table 1).

Figure 3 illustrates the effect of hypoxemia on the DV. In addition to distension of the inlet, there is a corresponding widening of the rest of the vessel that is reflected in the measurements of the midportion (Table 1).

Table 1. Results of measurements in fetal sheep

<table>
<thead>
<tr>
<th></th>
<th>Sodium Nitroprusside, mg·min⁻¹·kg⁻¹</th>
<th>Phenylephrine (6.5 mg·min⁻¹·kg⁻¹)</th>
<th>Hypoxemia</th>
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<tr>
<td></td>
<td>Baseline 1.3 2.6 6.5</td>
<td></td>
<td>Baseline 1.3 2.6 6.5</td>
</tr>
<tr>
<td>Ductus venosus inlet diameter, mm</td>
<td>1.4 ± 0.3 1.6 ± 0.3 1.7 ± 0.3* 1.7 ± 0.3* 1.8 ± 0.4* 2.1 ± 0.3*</td>
<td></td>
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<tr>
<td>Ductus venosus, blood velocity, cm/s</td>
<td>69 ± 5 65 ± 6 60 ± 7 63 ± 8 80 ± 8 61 ± 12</td>
<td></td>
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<tr>
<td>Ductus venosus shunt, % of umbilical flow</td>
<td>17 ± 9 25 ± 14 25 ± 13 32 ± 18 19 ± 9 29 ± 5*</td>
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<tr>
<td>Ductus venosus midpoint diameter, mm</td>
<td>2.4 ± 0.5 2.2 ± 0.5 2.3 ± 0.5 2.4 ± 0.4 3.7 ± 0.7*</td>
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Values are means ± SE; n = 5–9 sheep. Differences were tested using analysis of variance for repeat measurements and post hoc test for the baseline and sodium nitroprusside data, otherwise paired t-test or Wilcoxon signed-rank test. *P ≤ 0.05 vs. baseline.

DISCUSSION

The present study has demonstrated by ultrasound measurements of the DV in utero that the inlet of the vessel is under active control, having a tonic constriction that is relaxed by NO but not augmented by the α₁-adrenergic agonist phenylephrine, and that hypoxemia causes a powerful widening of the DV inlet and the entire vessel (Fig. 3).

Whether the inlet of the DV (or a sphincter) actually controls the shunting and regulates the distribution of umbilical blood to the liver has not been clear until recently. Hemodynamic studies of autonomic blockade with atropine or phentolamine in fetal sheep have not demonstrated any distinct effect on distribution (8). A more recent study showed that infusion of norepinephrine and epinephrine caused some redistribution to the DV, whereas vasopressin and angiotensin II had no effect (26). However, an increase in hemoglobin concentration during these norepinephrine and epinephrine infusions is a confounding variable because it will produce an effect via an increase in viscosity (18). Prostaglandins are also candidates for regulating distribution, but prostaglandin synthesis inhibition with indomethacin did not cause any redistribution between the liver and the DV (25).
This leaves the possibility that fluid dynamic forces are the main determinants for this flow distribution (7). In line with this, it has been shown that low umbilical venous pressure and high viscosity (i.e., hematocrit) lead to a marked increase in the proportion shunted through the DV, and, conversely, increased venous pressure and reduced hematocrit both favor a redistribution to the liver (19).

The results in Fig. 1 suggest that MAP is the main determinant for umbilical flow during the experiments. This pattern reflects the relative inertia of the placental bed rather than the resistance of the liver and the DV, which is generally low (20, 25, 26). The corresponding pressure gradient between the intra-abdominal umbilical vein and the inferior vena cava is \( \approx 4 \) mmHg. In the Bernoulli equation, the blood velocity at the DV inlet reflects this pressure gradient with an error of \( \approx 30\% \) (13, 27, 28). The blood velocity in the DV in our experiments suggests that changes in the umbilical pressure were not significant (Table 1).

It is still possible that the increase in shunting during hypoxemia could be caused by slight changes in the fluid dynamic forces (as the reduced MAP and umbilical venous flow suggest) or an increase in resistance in the liver. However, without an increase in transmural pressure (and intravascular pressure) fluid dynamic forces cannot explain the distension of the DV, whereas active regulation can.

Although in vitro studies suggest that prostaglandins maintain patency, \( \alpha \)-adrenergic agonists cause constriction, and \( \beta \)-agonists cause distension of the DV (5), this has not been verified in vivo. The main reason for this is that direct study of the DV in utero is a technical challenge, because the DV is a narrow vessel embedded dorsally in the visceral surface of the liver, hardly accessible for conventional surgery.

One answer to such a challenge would be a noninvasive approach using ultrasound, which has proved valuable in the human fetus (14). We previously used the method to determine the diameter of the DV inlet during the second half of human pregnancy and found it to be 0.5–2 mm (17). In the present study of near-term fetal sheep, we found a comparable mean diameter of 1.4–2.1 mm (Fig. 2). The variation of such measurements cannot be ignored. Previous evaluations have shown that by using an average of at least five measurements the upper 95\% confidence limit for the random error is restricted to \( < 0.15 \text{ mm} \) (16, 18). Taking such details into account, the present study shows that ultrasound technology is suitable for this type of research.

Both the normalized umbilical flow (119 ml kg\(^{-1} \cdot \text{min}^{-1}\)) and the fraction of umbilical blood shunted through the DV (16–61%) are lower in our study than in some previous studies (\( \approx 200 \text{ ml kg}^{-1} \cdot \text{min}^{-1} \) and 48–70\%, respectively; Refs. 3 and 7–10). These studies used isotope-labeled microspheres and electromagnetic flowmeters to estimate flow and flow distribution. Methodological considerations may explain the difference in results. Electromagnetic flowmeters can suffer from baseline drift after implantation and cannot be zeroed physiologically in situ; we used Transonic flowmeters to avoid this. The use of microspheres gives only point measurement of flow distribution, and we preferred to use velocity measurements to calculate distribution. This is illustrated by a recent ultrasound study in human fetuses. The normalized umbilical flow was 70 ml kg\(^{-1} \cdot \text{min}^{-1}\) near term, and the corresponding average shunting through the DV was 20–25\%, both considerably lower than in previous sheep experiments and lower than in the present study, demonstrating methodological differences and the physiological differences between species.

The necessary use of anesthesia in the present study may constitute a confounding factor. Ketamine is known to increase maternal MAP (7\%), cardiac output (16\%), cerebral flow, and circulating epinephrine (6) but is still much used for acute experiments. The reason for this is that ketamine preserves respiratory reflexes and exerts a relatively mild effect on hypoxic and acidemic responses (12, 29). Although the introduction of catheters and flow probes required a minimum of surgery, we must acknowledge that endocrine...
responses to surgical trauma constitute another confounding factor compared with chronic preparations. On the other hand, we speculate that chronic preparations may suffer from the long-lasting effect of surgery, which may be reflected in some of the variation of results discussed in the previous paragraph. We believe that our results, obtained during stable anesthesia, represent physiological responses. However, the true normal tuning of such responses can only be described with noninvasive and atraumatic approaches.

NO is a ubiquitous mediator of vasodilatation (20), and it is not surprising that it also dilates the DV inlet during direct infusion into the umbilical vein. The effect seems to be blunted with SNP doses > 2.6 μg·kg⁻¹·min⁻¹ (Fig. 2), which indicates that the DV in vivo is under the influence of other mechanisms capable of modifying the response.

Our results underscore the importance of testing the results of in vitro studies in a more physiological setting to determine the significance of putative mechanisms. This seems to be particularly true for the α₁-adrenergic agonist phenylephrine, which did not cause the constriction of the DV expected from in vitro studies although other circulatory changes showed that the dose was physiological (Figs. 1–2). It may be that other regulatory mechanisms override an effect on the diameter in vivo (e.g., prostaglandin and β-adrenergic). Moreover, phenylephrine infusion did not constrict the vessel compared with its distended state during SNP infusion 0.5 h earlier (Fig. 2), which supports the idea that humorally mediated α₁-adrenergic constriction is not a prominent physiological mechanism in the DV. The present study concentrated on the use of agonist drugs. Studying the effect of antagonists is now the logical experiment to rule out any possible tonic α-adrenergic constriction.

The profound and immediate distension of the DV inlet during hypoxemia demonstrates that the tone of its vascular smooth muscle is actively regulated. Now that we have established this experimental design for assessing the diameter of the DV inlet in vivo, further experiments are possible to determine the role of prostaglandins, β-adrenergic mechanisms, and other vasodilators such as adenosine in mediating the effect of hypoxia.

In fetal and neonatal rats, Momma et al. (22) demonstrated that in addition to the inlet, the entire length of the DV seemed to be actively constricted. Our diameter measurements of the midportion do indeed show a distension during hypoxemia (Fig. 3, Table 1) and hence support the concept that the entire DV is involved in the regulation of the shunt.

In conclusion, we have shown that the DV inlet is under active regulation, demonstrated by its distension during infusion of an NO donor or hypoxemia. In addition, we have shown that, in a more physiological situation, an α₁-adrenergic agonist did not have the constrictor effect expected from in vitro studies; further studies are merited to assess any tonic adrenergic function. Ultrasound measurements of the DV diameter provide a noninvasive method of the study of the mechanisms regulating it.

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