Insignificant response of the fetal placental circulation to arterial hypotension in sheep

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Insignificant response of the fetal placental circulation to arterial hypotension in sheep. J Appl Physiol 111: 1042–1047, 2011. First published June 30, 2011; doi:10.1152/japplphysiol.00345.2011.—Infusion of the angiotensin-converting enzyme inhibitor enalaprilat into fetal sheep caused a profound arterial hypotension within days. Five fetal lambs were infused with enalaprilat for 8 days starting at day 128 of gestation. Total accumulated dose was 0.30 ± 0.11 mg/kg. Arterial pressure decreased from 43.6 to 25.6 mmHg; venous pressure did not change. Biventricular output was not statistically significantly changed; placental blood flow decreased almost in proportion to the decrease in pressure but the increase in somatic pressure was not statistically significant. There were no significant changes in pressure 30 min after the initial 50-μg loading dose of enalaprilat. However, the arterial pressure responses to test doses of ANG I were largely abolished. After 1 day, however, there was a significant decrease in somatic vascular resistance, which became stronger with time, but almost no decrease in the placental resistance. We conclude that the fetal somatic circulation exhibits a slow but strong decrease in resistance both in the presence of elevated angiotensin concentrations (7) and in the presence of reduced angiotensin concentrations (9). These responses, however, took days to develop. In the latter study we also found an increase in the resistance of the fetal somatic circulation.

The purpose of the present study was to determine if the reverse is also true so that decreases in fetal arterial pressure lead to vasodilation in the fetal placental circulation. Since fetal oxygenation is a function of flow rather than pressure, such a response would significantly ameliorate the deleterious effects of fetal hypotension. While studying the effects of a reduced systolic load on the development of the fetal heart in a previous study (14), we found that intravenous infusion of enalaprilat into the fetal sheep caused a sustained fall in fetal arterial pressure. We, therefore, used this approach in the present study to investigate the long-term effect of fetal arterial hypotension on placental flow and resistance.

This study was facilitated by the fact that the fetal circulation in the sheep differs from that in the human fetus in that the single brachiocephalic artery is, after the coronary arteries, the only vessel to branch from the aortic arch before the aorta is joined by the ductus arteriosus. It is thus possible to measure the fetal biventricular cardiac output by placing flow sensors on the brachiocephalic artery and on the postdental aorta. The sum of these flows is the biventricular output, except only for the pulmonary flow, which is ~7% of the output (15) and the coronary flow, ~3% (5). By placing a third flow sensor on the common umbilical artery (a continuation of the aorta beyond the iliac bifurcation in fetal sheep), we could separate the thoracic flows into the somatic (except coronary) and placental components and calculate the resistances of both.

METHODS

Surgical procedures. All surgical and experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC). Five time-bred ewes of mixed Western breeds, carrying singletons, were obtained from a commercial source. At 123 days of gestational age (range 121–125 days) the ewes were premedicated with 7.5 mg atropine (im) and given 10 mg diazepam and 400 mg ketamine (iv) to induce anesthesia. They were then intubated and mechanically ventilated with a mixture of 2,000 ml/min oxygen and 700 ml/min nitrous oxide with isoflurane at 1–2%, as required for a surgical level of anesthesia in both the ewe and the fetus.

Using strictly sterile surgical technique, we placed indwelling catheters in a fetal carotid artery and jugular vein. The carotid artery catheters were advanced only a few centimeters into the vessel so as not to interfere with the subsequently placed flow sensor on the brachiocephalic artery. We accessed the thoracic vessels through an incision in the fourth left intercostal space and placed flow sensors (Transonic, Ithaca, NY) on the brachiocephalic artery and the postdental aorta without incising the pericardium. We then approached the left flank of the fetus through a separate uterine incision and placed a third flow sensor on the common umbilical artery. Catheters sewn to the fetal skin served to later record amniotic fluid pressure. All incisions were closed in layers, the catheters were filled with heparinized saline; 1 million units of penicillin-G and 2 mg of ciprofloxacin were instilled into the amniotic fluid. The free ends of all cables and catheters were tunneled under the skin of the ewe and exteriorized at the flank where they were kept in a pouch attached to her flank. Typical duration of surgery was three and a half hours. Postoperatively, the ewes received 0.6 mg buprenorphine twice a day for 2 days.

Animal laboratory procedures. Three to 6 days after surgery, the ewes were placed in stanchions in the laboratory. The animals were watered and fed ad libitum. The experiments were begun on day 128 or 129 of gestation. We obtained control measurements for 3 days, which we labeled days −2, −1, and 0 in the figures. The reason for a long control period is that, in our experience, chronically instrumented fetuses are not quite unstressed immediately after their catheters have been opened and flushed. It can be seen, for instance, in

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Figs. 3 and 4 in RESULTS that there were increases in biventricular output and $P_{\text{O}_2}$ during the period from day −2 to day 0. At the end of the control period, the fetuses were given intravenous test doses of ANG I (Bachem, Torrance, CA) to determine the pressure responses before blockade. We then started fetal infusions of enalaprilat (Novaplus Vasotec) with a loading dose of 50 μg (iv); this dose in a 3-kg fetus corresponded to the maximally effective dose in human patients with hypertension (18), and a continuous infusion was initiated by means of a roller pump with an enalaprilat concentration of 2.5 or 5 μg/ml of lactated Ringer’s solution. We tested the adequacy of the loading dose in two fetuses with test doses of 7.5 μg of ANG I. Since there was large interfetus variability in the sensitivity of arterial pressure to enalaprilat, we adjusted the infusion rate to obtain an arterial blood pressure of between 25 and 30 mmHg during the final 4 days of the infusion. As a result, the total amounts of enalaprilat given to each fetus in the course of the experiments varied from 0.27 to 2.42 mg (0.30 ± 0.11 mg/kg autopsy body wt). Eight days after the start of the enalaprilat infusions, the experiments were terminated. Test doses of ANG I again established the adequacy of the converting enzyme block, after which the animals were euthanized, as approved by the IACUC. All fetuses were autopsied to verify the placement of the flow sensors and the catheters.

Measurements. Fetal arterial, venous, and amniotic fluid pressures as well as all flows were continuously recorded for the duration of the experiments. Pressures were recorded by means of Abbott Transpac transducers (Abbott Park, IL). To maintain catheter patency, we infused lactated Ringer’s solution with 2,000 units of heparin per liter through the transducers and catheters at a slow rate that represented a total fetal fluid load of <70 ml/day. The slow infusion rates made no differences in the recorded pressures. Transducer zeros were checked each day, after which we measured pressures and flows for 1 h for statistical analysis. Pressures were accurate to ±0.5 mmHg. Intravascular pressures will be reported with amniotic fluid pressure being considered zero. Flows were recorded by means of synchronized Transonic flowmeters, and all data were processed through AD Instruments (Colorado Springs, CO) electronics and an Apple computer (Cupertino, CA) and stored on disk. Daily samples of fetal arterial blood were analyzed for pH, $P_{\text{CO}_2}$, $P_{\text{O}_2}$, hemoglobin concentration, hematocrit, oxygen content, and lactate concentration with an ABL 700 blood gas analyzer (Radiometer America, Westlake, OH), and total plasma protein concentration was measured by refractometry. In four of the five animals, plasma samples anticoagulated with EDTA were analyzed for plasma renin activity (Diasorin, Stillwater, MN), as previously described (9).

Statistical methods. Statistical analyses of the data were performed with Prism-4 software (GraphPad Software, San Diego CA). Repeated-measures ANOVA with Dunnett’s multiple-comparison test was used to determine if, and when, the daily measurement differed from the day 0 preinfusion value. Results are presented as means ± 1 SE.

RESULTS

Adequacy of converting enzyme block. Figure 1 shows that at a maximum dose of 20 ± 3 μg ANG I, there was an average increase in arterial pressure of 21 mmHg ($P < 0.001$) before the administration of the angiotensin-converting enzyme (ACE) inhibitor. At the end of the enalaprilat infusion period of 8 days, the far larger dose of 64 ± 14 μg of ANG I produced only a very small increase of 3 mmHg in arterial pressure that was not statistically significant. In the two fetuses in which the adequacy of the loading dose was tested by comparing the responses to 7.5 μg of ANG I before and after the loading dose, arterial pressure increased 14 mmHg before the loading dose and 4 mmHg after in the first fetus and 19 mmHg before and 3 mmHg after in the second. We considered this a sufficient block for the purpose at hand.

Circulatory effects of enalaprilat. Figure 2 shows the changes in fetal arterial blood pressure, venous blood pressure, biventricular stroke volume, and heart rate. Mean arterial pressure was reduced by the second day of infusion, and this decrease was sustained for the duration of the enalaprilat infusion. Mean fetal arterial pressure on day 0 was 43.6 ± 1.8 mmHg as opposed to 25.6 ± 3.2 mmHg on the eighth and last day of infusion. Fetal central venous pressure was not statistically significantly affected at any time, being 2.5 ± 0.3 and 2.5 ± 0.5 mmHg on days 0 and 8, respectively. Biventricular stroke volume did not change significantly during the infusion period. Heart rate showed a moderate decrease from 170 ± 3 to 150 ± 1 beats/min on days 0 and 8, respectively, and the decline over time was statistically significant at $P < 0.03$, consistent with the normal gestational decrease in fetal heart rate over time.

Figure 3 illustrates the changes in biventricular output, somatic flow, placental flow, total resistance, somatic resistance, and placental resistance. In contrast to the steep decline in arterial blood pressure, biventricular output was almost unaffected, although the normal slight increase with fetal gestational age appeared to be absent after day 3. Figure 3 does show, however, that the near constancy of biventricular output derived from the fact that while placental blood flow was statistically significantly decreased on day 3 and thereafter, this decrease was balanced by a statistically nonsignificant increase in somatic flow.

A near constant biventricular output in the face of a steep decline in arterial blood pressure signified a decrease in total peripheral resistance. The decrease in total peripheral resistance was already statistically significant after 1 day and remained significant through the remainder of the experiment.

Somatic resistance measured 10 min before the loading dose of enalaprilat was still unchanged 30 min later; it actually increased by 0.0005 ± 0.0040 mmHg-min/ml (not significant). However, the large decrease in somatic resistance shown in Fig. 3 was already significant 24 h later and intensified during the course of the experiment. Placental resistance measured 10 min before and 30 min after the loading dose decreased insignificantly by 0.0006 ± 0.0020 mmHg-min/ml and was
significantly reduced later only on the third day of the infusion (Fig. 3). It follows that the large decrease in the somatic vascular resistance accounted for almost the entire decrease in total resistance.

In addition to the total somatic resistance, mentioned above, we also analyzed the resistance of that part of the somatic vascular bed that was supplied by the brachiocephalic artery because its flow depended on only one flow sensor, as opposed to the three needed for the calculation of the total somatic resistance. Brachiocephalic resistance was, and remained, significantly decreased after 2 days of infusion ($P < 0.05$).

Figure 4 shows the changes in fetal arterial blood in pH, hematocrit, PCO$_2$, hemoglobin concentration, PO$_2$, oxygen content, plasma protein concentration, and lactate concentration before and during 8 days of enalaprilat infusion. Figure 5 shows the plasma renin activities recorded in four of the five fetuses.

Figure 3 shows the changes in fetal arterial pressure, heart rate, and biventricular stroke volume 3 days before and during 8 days of enalaprilat infusion. The changes were compared with day 0.

**DISCUSSION**

The major findings of this study are that enalaprilat infusion is associated with a deep and sustained decrease in fetal arterial pressure, little change in fetal biventricular output, a substantial decrease in somatic resistance, and little change in placental resistance. These changes, in concert with the elevations in hematocrit and hemoglobin concentration, increased somatic hemoglobin flow and maintained somatic oxygen delivery. These beneficial responses accounted for the surprisingly mild deleterious effects of arterial hypotension on fetal tissue oxygenation.
Variability in enalaprilat sensitivity. In the adult, enalaprilat is normally eliminated almost entirely by renal excretion (11). In the fetus this is excretion into the amniotic and allantoic fluids. Depending on the balance between the rates of excretion into these fluids, and fetal reabsorption from those fluids, the concentrations of enalaprilat may be very different in the extrafetal fluids and fetal intravascular water. Without analysis of these fluids and fetal plasma this possibility cannot be further explored.

Effects of fetal arterial hypotension on fetal somatic and cardiac growth. The average fetal body weight was $4.4 \pm 0.5$ kg. The mean body weights of the group of control fetuses of similar gestational age in a previous study (14), who received only lactated Ringer’s solution, was $3.9 \pm 0.4$ kg. The mean body weight of the present fetuses was essentially the same. In this previous study also, the body weights of the control group and the hypotensive group were the same. The maintenance of body growth in the hypotensive fetuses is consistent with the
observed biventricular outputs and hemoglobin flows. However, as previously shown (14) the result of arterial hypoten-
sion was a reduction in the ratio of heart weight to body
weight. Normally that ratio is about 7.4 g/kg in singletons (12)
or 7.0 g/kg in a mixed group of singletons and twins (14). In
the present study, the fetal hearts were smaller as indicated by
the fetal heart weight-to-body weight ratio of 5.2 ± 0.6 g/kg.
This heart weight-to-body weight ratio is similar to our previ-
ous finding in hypotensive fetuses where the ratio was 5.6 ± 0.5 g/kg after 8 days of enalapril-induced fetal arterial hypo-
tension (14).

Effects of enalapril-induced hypotension on fetal cardiac
function and resistances. The slow decrease in arterial pressure
may in part reflect a slow rate of fluid loss to the maternal
circulation in the placenta by the mechanism first proposed by
Adamson and coworkers (1). These investigators reasoned that
an increase in the precapillary resistance of the fetal placenta
mediated by angiotensin would decrease fetal placental capil-
lar pressure and thereby promote fluid transfer from mother
to fetus. Inhibiting the production of angiotensin, therefore,
would do the opposite. This would be consistent with the
increases in hemoglobin concentration, hematocrit, and plasma
protein in Fig. 4. The ability of the heart to maintain output in
the face of a decreased vascular filling may well have been due
to the large decrease in afterload. With the biventricular output
almost unchanged, most, perhaps all, of the reduction in
arterial pressure reflects a decrease in resistance. However, the
results were more complex than anticipated because the
changes in somatic and placental resistances were very differ-
ent.

The near absence of a change in placental resistance came as
a surprise. A physiologically plausible hypothesis is that an-
giotensin not only vasoconstricts the fetal placental precapil-
lar resistance but also dilates the fetal placental postcapillary
resistance. This would make fetal renal control of fetal fluid
volume feasible without causing the significant changes in fetal
placental resistance that would follow if only the precapillary
resistance was modulated. Resolution of this aspect of fluid
control awaits further study. It must be remembered that
vascular resistance depends not only on the vascular geometry
but also on the viscosity of blood. The increases in the
concentrations of hemoglobin and plasma protein may have
masked a small decrease in placental geometric resistance and
thus a weak form of fetal placental dilation. For the same
reason, the response of the somatic circulation may have been
stronger than is apparent from our data. The most direct
explanation for the presence of an increase in resistance in the
placenta when arterial pressure is increased (9) but a near
absence of a decrease when pressure is reduced may well be
that the fetal placental circulation is already almost completely
dilated. A previous study in which fetal placental flow was
reduced for long periods of time by mechanical means also
failed to show vasodilation (2).

Potential shortcomings. Although the ultimate effects ob-
served during this study likely arose from the block of ACE
demonstrated on day 8 of the experiment, unknown effects of
ACE inhibition cannot be excluded. However, the fact that the
effects of losartan are similar to those of captopril led other
investigators to conclude that the results of converting enzyme
inhibition are independent of any direct effects on bradykinin
or prostaglandin levels (16).

The fetal nephrotoxicity of ACE inhibitors is well docu-
mented (4, 10). It should be noted, however, that the total dose
of enalapril accumulated over the 8 days of infusion was only
0.30 ± 0.11 mg/kg fetus autopsy weight compared with, for
instance, a total of 18.5 mg/kg administered previously over a
period of only 3 days (8).

Conclusions. We conclude that the resulting combination of
a fetal arterial hypotension and a strong vasodilation of the
somatic circulation is beneficial in the sense of protecting
somatic hemoglobin flow and oxygen delivery. However, any
response of the fetal placental circulation is weak, if it exists at
all.

We further conclude that the fetal somatic circulation is
uniquely suited to the study of the control of flow. Fetal arterial
blood pressure can be modulated up or down over the neces-
sary long period of time in a manner that would be nearly

![Graph](image_url)
impossible to duplicate in the adult circulation. This is due to the fact that fetal arterial pressure is extremely sensitive to intravascular volume (6) and that intravascular volume can be easily modulated by a variety of experimental interventions that invoke the mechanism proposed by Adamson et al. (1), such as those exemplified in other studies (6, 9, 14).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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