Nonimmune hydrops fetalis and activation of the renin-angiotensin system after asphyxia in preterm fetal sheep

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Nonimmune hydrops fetalis and activation of the renin-angiotensin system after asphyxia in preterm fetal sheep. Am J Physiol Regulatory Integrative Comp Physiol 280: R1045–R1051, 2001.—This study examined the hypothesis that the development of hydrops fetalis after asphyxia in the 0.6 gestation sheep fetus would be associated with activation of the fetal renin-angiotensin system (RAS). Fetuses were randomly assigned to either sham occlusion (n = 7) or to 30 min of asphyxia induced by complete umbilical cord occlusion for 30 min (n = 8). Asphyxia led to severe bradycardia and hypotension that resolved after release of occlusion. After occlusion, plasma renin concentration was significantly increased in the asphyxia group compared with controls (P < 0.005) after 3 min (16.3 ± 5.3 vs. 4.1 ± 1.3 ng·ml⁻¹·h⁻¹), and 72 h (30.6 ± 6.3 vs. 3.7 ± 1.2 ng·ml⁻¹·h⁻¹). Renal renin concentrations and mRNA levels were significantly greater in the asphyxia group after 72 h of recovery. All fetuses in the asphyxia group showed generalized tissue edema, ascites, and pleural effusions after 72 h of recovery. In conclusion, asphyxia in the preterm fetus caused sustained activation of the RAS, which was associated with hydrops fetalis.

FETAL HYDROPS in its most severe form is the result of a general increase in interstitial fluid volume, characterized by ascites and pleural effusions. The umbilical cord and placenta may also be edematous. This condition remains a significant cause of fetal and neonatal mortality, and although there are many known causes of nonimmune hydrops, a significant proportion (∼20%) remains unexplained (3, 19, 20). Hydrops can occur at any time during gestation, but the incidence and timing of hydrops have been difficult to determine. With greater use of ultrasound in pregnancy, an increasing incidence of early to midgestation fetal hydrops is now being recognized, which may resolve spontaneously by term (20).

Although there are many factors known to contribute to the development of hydrops, the pathogenesis of this condition ultimately involves dysfunctional regulation of the fluid balance between vascular and interstitial compartments, i.e., changes in capillary and tissue hydrostatic and onotic pressures, capillary permeability, or lymphatic return. The fetus may be relatively susceptible to accumulation of excess interstitial fluid because of its relatively greater capillary filtration coefficient and greater interstitial compliance (3, 6).

At present, little consideration has been given to the concept that fetal asphyxia per se may cause hydrops, particularly during midgestation, when the fetus may be more vulnerable because of the fragility of its vascular ultrastructure. Increasing evidence shows that asphyxia can occur in utero and in midgestation (24). We have recently demonstrated (4) that the immature fetus can tolerate prolonged periods of severe asphyxia. During such episodes, the preterm fetus became profoundly hypotensive, with both central and peripheral hypoperfusion. It is likely that this would lead to significant tissue and vascular injury resulting in increased microvascular permeability. There was evidence of continued vascular dysfunction after reperfusion from asphyxia, as shown by secondary impairment of central and peripheral blood flow unrelated to perfusion pressure. We now report a secondary observation from this study, that the asphyxiated fetuses developed hydrops fetalis.

A role for the kidney and the renin-angiotensin system (RAS) in the pathogenesis of hydrops fetalis has been suggested by recent studies. Infusions of large doses of ANG I in the fetus cause polyhydramnios (2). In nephrectomized fetuses, such infusions lead to hydrops fetalis (13), whereas fetuses in which the ureters are bilaterally ligated also show hydrops (15). Surprisingly, the possible involvement of the RAS in hydrops has not been considered clinically. The RAS is active during fetal life. The release of renin from the ovine kidney occurs even at this very young age (0.6 gestation) in response to hypotension caused by hemorrhage...
Hypoxia is known to be only a weak-to-modest stimulus to renin and ANG II production in the fetus (8, 17, 25, 31); however, there have been no studies of the effects of asphyxia on the fetal RAS. From this literature, we hypothesized that the development of hydrops fetalis after asphyxia in the 0.6 gestation sheep fetus would be associated with activation of the fetal RAS.

**METHODS**

**Animal preparation.** All surgery and experiments were approved by the Animal Ethics Committee of the University of Auckland, New Zealand. Romney-Suffolk fetal sheep were instrumented at 86–89 days of gestation (term = 147 days) as previously described (4). Surgery was performed under general anesthesia (2% halothane in O2) by use of sterile techniques. Ewes were given 5 ml of Streptopen (Pitman-Moore, Wellington, New Zealand) intramuscularly for prophylaxis. Fetal catheters were placed in the left femoral artery and vein, right axillary artery, and the amniotic cavity. Ultrasound blood flow probes (2S, Transonic Systems, Ithaca, NY) were placed around the right femoral artery and the left carotid artery for the measurement of femoral and carotid blood flow. An inflatable silicone occluder was placed around the umbilical cord of fetuses (In Vivo Metric, Healdsburg, CA). Electrodes (Cooner Wire, Chatsworth, CA) were implanted to measure the fetal electroencephalogram (EEG) and electrocardiogram (EGC) (4). All leads were exteriorized through the maternal flank. Gentamicin (80 mg, Roussel, Auckland, New Zealand) was administered into the amniotic cavity before closure. A maternal tarsal vein was catherized.

After surgery, sheep were housed together in separate metabolic cages with access to water and food ad libitum. They were kept in a temperature-controlled room (16 ± 1°C, humidity 50 ± 10%), in a 12:12-h light-dark cycle. Experiments were carried out 3–5 days after surgery (91.7 ± 0.3 days). Ewes were given Crystapen (600 mg, Biocheme, Vienna, Austria) and 80 mg Gentamicin intravenously daily for 4 days. Patency of the fetal catheters was maintained by continuous infusion of heparinized saline (10 U/ml at 0.15 ml/h).

**Experimental procedures.** Experiments were conducted at 90–93 days gestation. Fetal arterial and venous blood pressure were corrected for amniotic fluid pressure (Novatrans II, MX860, Medex, Dublin, OH). Fetal heart rate (FHR) was derived from the ECG. These variables and EGG activity and carotid and femoral blood flow were recorded continuously from 12 h before the experiment until 72 h afterward. Data were collected and stored to disk with the use of custom software (Labview for Windows, National Instruments, Austin, TX). We have previously reported the cardiovascular and EEG responses to asphyxia in some of these fetuses (4).

Fetuses were randomly assigned to either the sham occlusion group (n = 7) or the asphyxia group (n = 8). In the sham group, tissues were taken from all seven fetuses but blood samples from only six of the seven fetuses. In the occlusion group, blood samples were taken from all eight fetuses, but tissues from only seven of the eight fetuses. Fetal asphyxia was induced in the occlusion group by rapid inflation of the umbilical occluder for 30 min with a defined volume of sterile saline known to completely inflate the occluder. The success of occlusion was confirmed by observing an immediate sharp rise in mean arterial blood pressure (MAP) and a rapid fall in FHR. In both groups, fetal arterial blood samples were taken from the axillary artery catheter 15 min before asphyxia, 5 and 25 min during asphyxia, and 1, 24, 48, and 72 h after asphyxia for pH and blood gas determination (Ciba-Corning Diagnostics, 845 blood gas analyzer and co-oximeter, East Walpole, MA) and for glucose and lactate measurements (YSI model 2300, Yellow Springs, OH). Blood samples (2.8 ml) were also taken from the catheter 1 h before asphyxia, and 3 min and 72 h after asphyxia, for measurement of plasma renin and angiotensinogen levels. Blood was transferred immediately upon collection to chilled test tubes and spun at 4°C (3,000 rpm) for 10 min. Plasma was stored at −20°C for subsequent analysis.

At 72 h, the ewes and fetuses were killed by intravenous pentobarbital sodium overdose (7.5 g; Chemstok Animal Health, Christchurch, NZ) to the ewe. At postmortem, fetuses were weighed, and fetal kidney, liver, and brain were removed and weighed. Both kidneys were frozen in liquid nitrogen and stored at −80°C. Tissue edema was measured by calipers at the same place at the shoulder and rump. Hearts were also taken for histological analysis. Ten milliliters of cold (4°C) 15% potassium citrate were injected into the left atrium. The heart was quickly rinsed and frozen. The fetal aorta was clamped distal to the brachiocephalic artery, and the left atrium was opened to facilitate coronary flow and ensure optimal perfusion of the myocardium. Thirty milliliters of cold cardioplegic solution (0.4% NaCl, 0.25% KCl, 0.2% glucose, and 0.1% NaHCO3) were perfused into the coronary circulation through the brachiocephalic artery, followed by 200 ml of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) over 30 min. The hearts were removed and immersed in the 2.5% glutaraldehyde solution. Several days later, they were sectioned by transverse division through the ventricle midway between the apex and the atrioventricular sulcus. Standard, 2-mm-thick transverse sections were cut along the axis of symmetry. Systematic light microscopic examination of these transmural myocardial sections was performed by a cardiac histopathologist who was blind to the experimental group.

In addition, three blocks of tissue (1 mm3) were excised from the subendocardial regions of the right and left ventricles and placed in 2.5% glutaraldehyde. These blocks were later washed in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide, and embedded in freshly prepared 100% epoxy resin. Sections 1.5 μm thick were stained with 1% toluidine blue and examined with a light microscope to identify areas of interest. Thin sections (90 nm) of selected regions were then cut and stained with uranyl acetate and lead citrate and were then examined with a transmission electron microscope (E.M. 410 Philips, Eindhoven, The Netherlands) operated at an accelerating voltage of 100 kV.

**Renin and angiotensinogen assays.** Plasma renin concentration (PRC) was measured as the rate of formation of ANG I at pH 7.4 and 37°C after addition of extra homologous substrate (nephrectomized sheep plasma (NSP)). For measurement of renal renin concentration, 0.5 g of fetal kidney was homogenized in 4 ml of phosphate buffer (pH 7.4). Renin levels were measured in the supernatant obtained after centrifugation, dilution to 1:400, and addition of 100 μl of NSP. Plasma angiotensinogen levels were measured by incubation of 100 μl of fetal plasma with an excess of human renal renin (0.5 mU, Calbiochem, La Jolla, CA) at 37°C for 1 h. At this time, the rate of formation of ANG I had reached a plateau (33). ANG I was measured by radioimmunoassay based on methods described previously (23). Renal protein levels were measured using the Lowry method (22).

**Measurement of renin mRNA.** Total RNA was extracted from ~300 mg of kidney using acid guanidinium-phenol-chloroform (10). An aliquot of each sample of RNA was
diluted in 20× standard sodium citrate (3 M NaCl, 0.3 M sodium citrate) and denatured in formaldehyde and formamide. RNA samples (20 μg) were dotted onto a nylon membrane by means of a dot-blot apparatus (Bio-Rad, Hercules, CA). The membrane was dried at room temperature for 1 h and baked at 80°C for 2 h. As described previously (32), a 30-mer oligonucleotide probe for sheep kidney renin (supplied by Dr. R. Fernley, Howard Florey Institute, Melbourne, Australia) was labeled at the 5’ end with [γ-32P]ATP (Promega, Madison, WI) with the use of T4 polynucleotide kinase (Promega). The probe corresponds to amino acids 73–82 of sheep renin (Thr-Asn-Tyr-Leu-Asp-Thr-Gln-Tyr-Tyr-Gly). The membrane blots were then hybridized with the sheep renin probe. The procedures of autoradiography and quantification of mRNA were the same as described previously (32). After the renin probe was stripped from the membrane by using boiling 0.5% SDS solution, the membrane was then rehybridized with a β-actin probe (supplied by Prof. B. Morris, University of Sydney, Australia) at 42°C overnight. The ratio of renin to β-actin probe mRNA was determined.

**Measurement of angiotensinogen mRNA by ribonuclease protection assay.** A 380-base PstI fragment containing exon II of the sheep angiotensinogen gene and flanking 5’ and 3’ sequences was inserted into the PstI site of the plasmid vector, pBluescript KS. The DNA template, when linearized with Smal endonuclease, yields the antisense RNA upon transcription from the T3 promoter. The riboprobe used to detect renal angiotensinogen mRNA was prepared by in vitro transcription in the presence of NTPs and T3 RNA polymerase (Promega) by use of [α-32P]CTP (Du Pont, North Sydney, NSW, Australia). An 18 S rRNA (Ambion, Austin, TX) was transcribed using [α-32P]CTP in the presence of T7 polymerase and NTPs (Ambion). The procedures of purification of the probe were the same as for the angiotensinogen probe. The 32P-labeled antisense RNA probes of angiotensinogen and 18 S rRNA were mixed with the RNA samples extracted from fetal kidneys. The mixture was denatured at 90°C for 1 min. Hybridization of the probes and their complementary RNAs was carried out overnight at 45°C. Samples were then digested by adding RNase A/T1 (1:100 dilution, Ambion). The protected renal angiotensinogen mRNA and 18 S rRNA were detected by autoradiography after a 5% polyacrylamide gel was run. Quantitative estimation was performed using a laser densitometer (Bio-Rad). The densitometric readings are mean values of the densities of angiotensinogen mRNA and 18 S rRNA bands on the film. Results were expressed as the ratio of angiotensinogen mRNA to 18 S rRNA in each sample (32, 33).

**Data analysis.** Data are presented as means ± SE. PRC is expressed as the rate of formation of ANG I in plasma in nanograms per milliliter per hour after addition of NSP. Tissue renin concentration is expressed as nanograms per milligram of protein. Plasma angiotensinogen levels were expressed as nanograms per milligram of plasma. To determine the effects of asphyxia on plasma renin and angiotensinogen, analysis of variance was used, with time as a repeated measure and the baseline period as a covariate. Because a significant effect between groups was found for the renin measurements, post hoc comparisons at individual time points were performed using the Mann-Whitney test, with significance corrected for the number of comparisons by the Bonferroni method. To determine whether asphyxia affected renal renin levels and renin-to-β-actin and angiotensinogen-to-18 S rRNA ratios, values obtained were compared with those found in the sham group by the Mann-Whitney test. The cardiovascular parameters and blood gas data were analyzed by two-way analysis of variance, with time as a repeated measure. When a significant effect of group or intergroup and time was found, analysis of covariance was used to compare individual time points by using the baseline period before occlusion as the covariate. For intragroup comparisons over time, data were analyzed by one-way analysis of variance for repeated measures.

**RESULTS**

**Blood composition measurements.** Values and statistical comparisons for arterial pH, blood gases, lactate, and Hb for the control and asphyxia groups are presented in Table 1. All fetuses were considered healthy according to their blood gas, acid-base, and glucose and lactate status before each experiment.

**Cardiovascular measurements.** There were no significant differences in MAP, mean venous pressure (MVP), or FHR between the control and asphyxia groups during the baseline period and in the control group during or after sham occlusion compared with baseline. In the asphyxia group, occlusion was associated with an initial rapid rise in MAP, which peaked by 3 min. MAP then progressively fell to 10.1 ± 1.4 mmHg by 30 min of occlusion (vs. 33.5 ± 1.2 mmHg in the control group, P < 0.001). In the 1 h postocclusion, there was a brief significant rebound hypertension between 5 and 16 min, with MAP peaking at 7 min (P < 0.001). MVP initially rose at the onset of occlusion to 2.8 ± 0.4 mmHg by 4 min (vs. 1.0 ± 0.4 mmHg in the control group, P < 0.05) and returned to control values by 6 min. Immediately postocclusion, MVP transiently

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Values are means ± SE at 15 min before (control), during (at 25 min of occlusion) and after [1 and 72 h postocclusion (Post)] either 30 min of sham (S) or complete umbilical cord occlusion (O). *P < 0.001 between group comparisons.
increased during the first 4 min, peaking in the first min (3.2 ± 0.9 vs. 1.7 ± 0.2 mmHg in the control group, \( P < 0.05 \)) and thereafter returned to control. In the asphyxia group, FHR fell rapidly at the onset of occlusion, and bradycardia was sustained throughout the occlusion period, reaching a nadir of 49.8 ± 3.0 beats/min by the end of occlusion compared with 195.3 ± 2.3 beats/min in the control group (\( P < 0.001 \)). After the end of occlusion, there was a brief rebound tachycardia, peaking by 6 min at 249.0 ± 8.4 beats/min (\( P < 0.001 \)).

During the remainder of the 72-h recovery period, MAP in the control group rose over the course of the study and was significantly greater than baseline by 72 h (\( P < 0.01 \)). In the asphyxia group, MAP had a tendency to be elevated between 6 and 12 h and was significantly higher than control group values at 6 and 8 h (\( P < 0.05 \)). MAP was significantly lower than control group values between 36 and 72 h (\( P < 0.05 \)).

There was no significant difference in MVP between the occlusion and control groups postocclusion. FHR in the occlusion group tended to be elevated during the first 4 h postocclusion, but this was not significant. FHR was significantly lower at 10 and 12 h compared with the control group (\( P < 0.05 \)), but thereafter FHR returned to control group values.

**Body and tissue weight comparison.** The fetuses in the asphyxia group tended to be heavier than those in the control group, but the differences were not significant (1,015 ± 73 vs. 884 ± 46 g). All fetuses in the asphyxia group had generalized tissue edema (upper body edema, 2.2 ± 0.3 mm; lower, 5.8 ± 0.8 mm, \( P < 0.001 \)), ascites, and pleural effusions. No edema, ascites, or pleural effusions were seen in the control group (outer skin thickness 0.03 ± 0.0 mm in the upper body; 0.04 ± 0.0 mm in the lower body). The asphyxia group fetuses had significantly smaller brains (18.7 ± 0.6 vs. 21.0 ± 0.7 g, \( P < 0.05 \)). Heart weight also tended to be less, but this difference was not significant (7.8 ± 0.7 vs. 9.1 ± 0.4 g). There was no significant difference between groups for combined kidney weights (9.8 ± 0.2 vs. 9.1 ± 0.3 g) or liver weight (61.0 ± 4.8 vs. 60.8 ± 3.0 g).

**Effect of asphyxia on renal and plasma renin and angiotensinogen.** There was no significant difference in PRCs between the groups before occlusion (3.7 ± 1.2 ng·ml\(^{-1}·h\(^{-1}\) in the control group, vs. 4.1 ± 1.1 ng·ml\(^{-1}·h\(^{-1}\) in the asphyxia group). Postocclusion PRC levels were significantly greater in the asphyxia group than in controls (\( P < 0.005 \)), with no significant effect of time. Plasma renin levels did not change over time in the control group. Three minutes after the end of occlusion, plasma renin levels in the asphyxia group had risen markedly (16.3 ± 5.3 ng·ml\(^{-1}·h\(^{-1}\), \( P < 0.01 \) vs. controls; Fig. 1A). After 72 h, plasma renin levels were still significantly elevated, tending to be higher than at 3 min, 30.6 ± 6.3 ng·ml\(^{-1}·h\(^{-1}\) (\( P < 0.002 \), vs. controls). Plasma angiotensinogen levels were not significantly different in the baseline period (983 ± 63 ng/ml in controls vs. 1,061 ± 33 ng/ml in the asphyxia group). There was no significant effect of asphyxia on postocclusion angiotensinogen levels (Fig. 1B).

Levels of renin in the kidney after 72 h of recovery were significantly greater in the asphyxia group than in the control group (2.0 ± 0.2 vs. 1.4 ± 0.1 µg/mg protein, \( P < 0.01 \); Fig. 2A). The ratio of renin to β-actin mRNA was also significantly increased in the asphyxia group (\( P < 0.002 \); Fig. 2B). There was no significant difference in the angiotensinogen mRNA-to-18 S rRNA ratio between the two groups (1.1 ± 0.1 in the occlusion group vs. 1.3 ± 0.1 in the control group).

**Cardiac histology.** The light microscopic and ultrastructural appearances of the myocardium of the control fetuses and the asphyxia fetuses were indistinguishable and were consistent with previous descriptions of normal fetal heart muscle, with fewer contractile elements and proportionally more cellular organelles than in adult hearts (7, 12). The myocytes were usually fully contracted, the sarclemma scalloped, nuclear membrane indented, and chromatin evenly dispersed. The mitochondria contained closely packed cristae and dense matrix.

**DISCUSSION**

This study reports for the first time that a severe asphyxial insult at 0.6 gestation can lead to the development of hydrops fetalis. The development of hydrops was accompanied by increased PRC. The initial release of renin was very rapid, as higher levels were already present 3 min after release of cord occlusion. The key
observations were that PRC was still elevated after 72 h of recovery and that this sustained increase was associated with increased synthesis of renin in the fetal kidney, probably due to increased transcription of the renin gene.

At postmortem, all asphyxiated fetuses were edematous, with increased subcutaneous skin thickness and visible pleural effusions and ascites. No sham control animals were affected in this way. Fundamentally, the development of extracellular edema results from an imbalance between the transcapillary hydrostatic and oncotic pressure gradients. Classic causes of hydrops fetalis include diseases leading to cardiac failure (e.g., severe anemia), which results in increased venous pressure and thus increased transcapillary hydrostatic pressure. Other causes include conditions in which the lymphatic drainage is impaired, such as in Turner’s syndrome and conditions leading to leakage of protein into the perivascular space (e.g., with infection).

In the present case, it is unlikely that asphyxia led to continued cardiac failure, because the central venous pressure was not elevated after asphyxia and the myocardium was histologically normal after 72 h of recovery. Furthermore, there were no marked changes in fetal blood pressure or heart rate after asphyxia, and anemia did not develop. Hydrops after asphyxia has not been reported in the late gestation fetus, although it has been reported in association with other mechanisms (15). It is likely that its occurrence in the pre-mature fetus reflects the much greater duration of asphyxia and profound hypotension than the premature fetus was able to tolerate (4). A further contributory factor may have been an increase in capillary permeability to water and colloids after asphyxia (20).

Recent studies, however, have implicated a combination of renal impairment and high levels of angiotensin in the etiology of hydrops fetalis.

Briefly, fetal nephrectomy is associated with a fall in both interstitial and blood volume (16). Infusions of nonpressor doses of angiotensin prevent this reduction in extracellular volume (16). These data strongly implicate the fetal RAS in the control of placental fluid balance. The mechanism by which angiotensin exerts this effect is probably related to its action on fetal precapillary resistance in the placental vasculature, leading to a reduction in fetal capillary hydrostatic pressure, which in turn promotes transplacental fluid and electrolyte transfer to the fetus (1, 14). Data from our laboratory (E. R. Lumbers and J. B. Burrell, unpublished data) and from Cox and Rosenfeld (11) show that the umbilical artery has mainly AT1 receptors. These are the receptors through which angiotensin mediates its vasoconstrictor action.

Consistent with this hypothesis are the findings that pressor doses of ANG I given to the intact fetus cause polyhydramnios (2) but in nephrectomized fetuses lead to hydrops fetalis (13). Similarly, fetuses in which the ureters are bilaterally ligated also develop hydrops, a further indication that the kidneys play a key role in removing angiotensin-stimulated fluid loading of the fetus (15). Unfortunately, renal histology is not available for the present study; however, reversible renal dysfunction is universally reported after comparably severe episodes of asphyxia clinically (28) and experimentally (18). Thus we speculate that, in the first 72 h after asphyxia, the fetuses were oliguric and unable to excrete the excess fluid accumulated within their extracellular space as a result of angiotensin-stimulated fluid transport across the placenta. The effect of this on amniotic fluid volume was not quantified in the present study; however, it would be expected to lead to either a relative or an absolute reduction in amniotic fluid production, depending on the severity of oliguria.

Renal dysfunction after asphyxia may be due to either vasomotor nephropathy or acute tubular necrosis, with associated interstitial edema (28). The premature fetus and infant have a very low glomerular filtration rate and thus are extremely susceptible to vasomotor nephropathy (prerenal failure) related to hypoperfusion and hypotension (28). In the present study, there was mild impairment of MAP compared with controls in the latter half of the recovery period (4). Furthermore, we have previously demonstrated in the present model a postasphyxial decrease in femoral blood flow and an increase in femoral vascular resistance that persisted throughout 72 h of recovery (4).

We speculate, therefore, that there was persistent renal hypoperfusion related to relative hypotension and to a secondary postasphyxial increase in vascular resistance. This, in turn, would cause sustained stimu-
lation of renin synthesis and its release, possibly through alterations in pressure gradients across the afferent arteriolar wall and/or lack of fluid flow past the macula densa.

In the present study, we did not measure ANG II levels, in view of the large sample volumes that would be required in relation to the limited fetal blood volumes at this gestation. However, the substantial changes in plasma renin (Fig. 1A) are highly likely to have been associated with a rise in angiotensin levels, especially because plasma angiotensinogen levels were stable (Fig. 1B). Although ANG II is a pressor agent, it is not surprising that elevated ANG II levels would not be associated with hypertension in the context of recovery from severe asphyxia. Clinically, for example, infants exposed to asphyxia frequently develop hypertension, requiring intensive inotrope support (26, 29).

In the present study, fetuses were subjected to an acute, severe period of circulatory compromise with bradycardia, hypoperfusion, and hypotension. The profound hypotension and hypoperfusion (4) probably caused the initial rise in PRC that was present within 3 min of the end of asphyxia (Fig. 1A). Stepwise reductions in renal blood flow caused by occluding the fetal aorta above the renal arteries cause renin to be released in an incremental fashion (5). As well, high levels of catecholamines can stimulate renin release from the kidney through β-adrenoceptors in both fetal and adult animals (27). Plasma renin levels were still elevated after 72 h. Because both renin-to-β-actin mRNA ratios and renal renin content were increased in fetuses recovering from asphyxia compared with control animals, it is highly likely that this was due to upregulation of renin synthesis. Other factors that might also contribute to increased circulating renin are increased conversion from prorenin to renin or altered clearance of renin from the circulation.

The reasons that renin synthesis was upregulated and that renin continued to be released into the circulation for the 72-h period after asphyxia are not clear, although it could be related to sustained postasphyxial depression of renal blood flow, as aforementioned (4). This induction of renin mRNA was specific, as neither renal angiotensinogen synthesis nor hepatic angiotensinogen synthesis were stimulated, since renal mRNA levels for angiotensinogen and plasma angiotensinogen levels did not change. Plasma angiotensinogen levels reflect the amount of angiotensinogen produced by the fetal liver. The fetal kidney, however, also has high levels of angiotensinogen and its mRNA (33).

Finally, there are some clinical data that are consistent with the findings of the present study. A review of 14 cases of nonimmune hydrops fetalis showed that 13 were preterm and asphyxia was a “frequent occurrence” (21). Furthermore, elevated plasma renin activity has been reported in newborn infants with nonimmune hydrops fetalis (30). A significant question for future studies is whether recovery from the acute renal injury would be associated with reduced activation of the RAS and resolution of hydrops. Such a mechanism may be associated with some cases of transient hydrops fetalis in midgestation (20).

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