Acute Hypoxia Increases S100 β Protein in Association with Blood Flow Redistribution away from Peripheral Circulations in Fetal Sheep

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ABSTRACT

We investigated in fetal sheep during late gestation the effects of acute hypoxemia on fetal plasma S100 β protein concentrations and how these relate to fetal redistribution of blood flow and acid-base status. Under general anesthesia, five Welsh Mountain sheep fetuses were instrumented with vascular catheters, and transit-time flow transducers were implanted around a femoral artery and an umbilical artery. At least 5 d after surgery, fetuses were subjected to 1 h of normoxia, 0.5 h of hypoxemia, and 1 h of recovery. Hypoxemia induced significant falls in fetal pH_a, arterial oxygen pressure, acid-base excess, and [HCO₃⁻], without alteration to arterial partial pressure of carbon dioxide. An increase in arterial blood pressure, a fall in heart rate, an increase in femoral vascular resistance, and a decrease in umbilical vascular resistance occurred in all fetuses. During hypoxemia, plasma S100 β increased significantly and remained elevated until the end of the protocol. Within individual fetuses, plasma S100 β correlated with femoral vascular resistance and pH. In contrast, no relationship was found between S100 β and

umbilical vascular resistance. This study reports for the first time that a controlled period of fetal hypoxemia with associated acidemia leads to persistent elevations in plasma S100 β concentrations that strongly correlate with hemodynamic changes that are known to occur during fetal blood flow redistribution. These findings open up a new role for changes in fetal S100 β concentrations as a possible early marker of fetal hypoxia with associated acidemia in perinatal medicine. (*Pediatr Res* 58: 179–184, 2005)

Abbreviations

ABE, acid-base excess
BP, blood pressure
CVO, combined ventricular output
IUGR, intrauterine growth restriction
Paco₂, arterial partial pressure of carbon dioxide
Pao₂, arterial oxygen pressure
Sat Hb, percentage saturation of Hb

The S100 β proteins are members of a multigenic family of calcium-modulated peptides characterized by a helix-loop-helix structure. They were first identified as a protein fraction detectable in brain and found to be mostly of low molecular weight of ~10,000 Da. They are called S100 because of their solubility in a 100% saturated solution with ammonium sulfate. In the nervous system, the protein is concentrated in glial cells, but it can also be present in specific neuron populations. Its half-life in the circulation is ~1 h, and 98% of the protein is excreted in urine (1–3).

An elevation in the concentration of S100 β in biologic fluids, such as cerebrospinal fluid and plasma, has been proposed to be a reliable marker of stress to the CNS (4–9). During human pregnancy, the concentration of circulating S100 β is increased in human fetuses with intrauterine growth restriction (IUGR), in particular in those who show redistribution of blood flow toward the brain (10). This is important because redistribution of blood flow away from peripheral circulations is an established cardiovascular defense response to hypoxia and asphyxia in the fetus (11). Michetti and Gazzolo (2) reported that the expression of S100 β increases in biologic fluids in the fetus during complicated intrauterine conditions, at times when other clinical assessments miss the adverse diagnosis. More recently, it was also reported that infants with moderate and severe hypoxic ischemic encephalopathy after perinatal asphyxia had significantly higher serum S100 β levels on the first 2 postnatal days relative to healthier infants (12). Thus, S100 β may be released during hypoxia/

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asphyxia, and, if so, measurement of its concentration may aid the early clinical diagnosis of hypoxic/ischemic stress and susceptibility to brain damage during the perinatal period. However, no study to date has reported changes in plasma concentrations of S100 β during actual hypoxemia in the fetus and how these may correlate with cardiovascular and metabolic function in the fetus. As a first step, this study investigated the effects of acute hypoxemia on fetal plasma concentrations of S100 β and how these relate to changes in fetal redistribution of blood flow and acid-base status, using fetal sheep surgically prepared for long-term recording.

METHODS

Surgical preparation. All procedures were performed under the UK Animals (Scientific Procedures) Act 1986 and were approved by the Ethical Review Committee of the University of Cambridge. Five Welsh Mountain sheep fetuses, between 118 and 125 d of gestation (term is ~145 d), were surgically instrumented for long-term recording using strict aseptic conditions as previously described in detail (11). In brief, food but not water was withheld from the pregnant ewes for 24 h before surgery. After induction with 20 mg/kg i.v. sodium thiopentone (Intraval Sodium; Merial Animal Health Ltd, Rhone Mérieux, Dublin, Ireland), general anesthesia (1.5–2.0% halothane in 50:50 O₂:N₂O) was maintained using positive pressure ventilation. Midline abdominal and uterine incisions were made, the fetal hind limbs were exteriorized, and on one side femoral arterial (i.d., 0.86 mm; o.d., 1.52 mm; Critchly Electrical Products, NSW, Australia) and venous (i.d., 0.56 mm; o.d., 0.96 mm) catheters were inserted. The catheter tips were advanced carefully to the ascending aorta and superior vena cava, respectively. Another catheter was anchored onto the fetal hind limb for recording of the reference amniotic pressure. In addition, transit-time flow transducers were implanted around the contralateral femoral artery (2S with silicon flange; Transonic Systems, Ithaca, NY) and one of the umbilical arteries close to the common umbilical artery, inside the fetal abdominal cavity (4SB; Transonic Systems), for continuous measurement of femoral blood flow and unilateral umbilical blood flow, respectively (11,13). The uterine incisions were closed in layers, the dead space of the catheters was filled with heparinized saline (80 IU heparin/ml in 0.9% NaCl), and the catheter ends were plugged with sterile brass pins. The catheters and flow probe leads then were exteriorized *via* a keyhole incision in the maternal flank and kept inside a plastic pouch sewn onto the maternal skin.

Postoperative care. The ewes were housed in individual pens with free access to hay and water, in rooms with a 12-h dark/12-h light cycle. They were fed concentrates twice daily (100 g; sheep nuts no. 6; H & C Beart Ltd, Kings Lynn, UK). All ewes received postoperative analgesia (10–20 mg/kg oral Phenylbutazone; Equipalozone paste; Arnolds Veterinary Products Ltd, Shropshire, UK) and antibiotics (0.20–0.25 mg/kg i.m. Depocillin; Mycofarm, Cambridge, UK). The patency of the fetal vascular catheters was maintained by a slow continuous infusion of heparinized saline (80 IU heparin/ml at 0.1 mL/h in 0.9% NaCl) that contained antibiotic (1 mg/ml benzylpenicillin; Crystapen, Schering-Plough, Animal Health Division, Welwyn Garden City, UK). In addition, the fetuses received daily antibiotics (150 mg/kg Penbritin; SmithKline Beecham Animal Health, Welwyn Garden City, Hertfordshire, UK) i.v. and into the amniotic cavity.

Experimental protocol. After at least 5 d of postoperative recovery, all fetuses were subjected to an experimental protocol that consisted of a 2.5-h period divided into 1 h of normoxia, 0.5 h of hypoxemia, and 1 h of recovery (Fig. 1). Acute hypoxemia in the fetus was induced by maternal inhalational hypoxia. In brief, a large transparent respiratory hood was placed over the

ewes' head into which air was passed at a rate of ~50 L/min for the 1-h period of normoxia. After this control period, acute fetal hypoxemia was induced for 30 min by changing the concentrations of gases breathed by the ewe to 6% O₂ in N₂ with small amounts of CO₂ (15 L/min air:35 L/min N₂:1.5–2.5 L/min CO₂). This mixture was designed to reduce fetal arterial oxygen pressure (Pao₂) to ~10 mm Hg while maintaining arterial partial pressure of carbon dioxide (Paco₂). After the 0.5-h period of hypoxemia, the ewe was returned to breathing air for the 1-h recovery period. At the end of the experimental protocol, the ewes and fetuses were killed humanely using a lethal dose of sodium pentobarbitone (200 mg/kg i.v. Pentject; Animal Ltd, York, UK), and the positions of the implanted catheters and the flow probe were confirmed and the fetuses were weighed.

Measurements. During the acute hypoxemia protocol, descending aortic blood samples (4 mL) were taken from the fetus at set intervals (arrows) to determine plasma concentrations of S100 β , arterial blood gases, and acid-base status. Values for pH (pH_a), Pao₂, Paco₂, acid-base excess (ABE), and blood concentrations of bicarbonate [HCO₃⁻] were obtained using a blood gas analyser (ABL 5; Radiometer, Copenhagen, Denmark), corrected to 39.5°C for fetal blood. Values for blood Hb concentration [Hb] and percentage saturation of Hb (Sat Hb) with oxygen were determined using a hemoximeter (OSM2; Radiometer). The remainder of the fetal blood was transferred into K⁺/EDTA-treated tubes, kept on ice, and centrifuged at 4000 rpm for 4 min at 4°C. Plasma samples were stored at -20°C until analysis. The endocrine analysis was completed within 2 mo of plasma collection.

Measurement of S100 β . Plasma concentrations of S100 β were measured in all samples by an immunoluminometric assay (Liaison Sangtec 100; AB Sangtec Medical, Bromma, Sweden). According to the manufacturer's instructions, this assay is specific for the β subunit of the S100 protein as defined by the three MAb SMST 12, SMSK 25, and SMSK 28 (10). Each measurement was performed in duplicate according to the manufacturer's recommendations, and the averages were reported. The sensitivity of the assay (B₀ $\pm$ 3 SD) was 0.02 μ g/L, and the intra- and interassay coefficients of variation were 5.5 and 10.1%, respectively, for concentrations ranging between 0.3 and 4.2 μ g/L.

Data and statistical analyses. Calibrated mean fetal arterial blood pressure (BP; corrected for amniotic pressure) and fetal heart rate (triggered from the pulsatility in arterial BP) were recorded at 1-s intervals using computerized Data Acquisition System. Femoral vascular resistance was calculated by dividing mean corrected arterial BP by the mean femoral blood flow, and umbilical vascular resistance was calculated by dividing mean corrected arterial BP by the mean umbilical blood flow. Values for all variables are expressed as mean $\pm$ SEM at 0 (N0) and 45 (N45) min of normoxia, 15 (H15) and 30 (H30) min of hypoxemia, and 30 (R30) and 60 (R60) min of recovery. All measured variables first were assessed for normality of distribution and then analyzed using one-way ANOVA with repeated measures (Sigma Chemical Co.-Stat; SPSS Inc., Chicago, IL) comparing the effect of time (normoxia *versus* hypoxemia or recovery). When a significant effect of time was indicated, the *post hoc* Tukey's test was used to isolate the statistical differences. In addition, the relationship between plasma concentrations of S100 β and cardiovascular and acid-base status in all individual fetuses was assessed using the Pearson Product moment correlation. For all comparisons, statistical significance was accepted at $p < 0.05$.

RESULTS

Fetal arterial blood gas and acid-base status during acute hypoxemia. Basal values for arterial blood gas and acid-base status were within the normal range for Welsh Mountain sheep fetuses at ~125 d of gestation. In all fetuses, acute hypoxemia induced significant falls in pH_a, Pao₂, Sat Hb, ABE, and HCO₃⁻ without any alteration to arterial Paco₂ (Table 1). During recovery, both Pao₂ and Sat Hb recovered to basal values in all fetuses. In contrast, fetal pH_a, ABE, and HCO₃⁻ remained significantly depressed from basal values until the end of the experimental protocol.

Fetal cardiovascular function during acute hypoxemia. Basal values for arterial BP, heart rate, femoral vascular resistance, and umbilical vascular resistance were similar in all fetuses and within the normal range for Welsh Mountain sheep fetuses at ~125 d of gestation. During acute hypoxemia, there was a pronounced increase in arterial BP (from 62.5 $\pm$ 1.7 to 72.0 $\pm$ 2.3 mm Hg), fall in heart rate (from 165 $\pm$ 9 to 130 $\pm$

Figure 1. The experimental protocol consisted of a 2.5-h period divided into 1 h of normoxia, 0.5 h of hypoxemia (■), and 1 h of recovery. Arrows represent time at which arterial blood samples were collected.

Table 1. Fetal arterial blood gas and acid base status

	Normoxia		Acute Hypoxemia		Recovery	
	N15	N45	H15	H30	R30	R60
pHa	7.35 $\pm$ 0.01	7.35 $\pm$ 0.01	7.29 $\pm$ 0.02*	7.22 $\pm$ 0.02*	7.24 $\pm$ 0.02*	7.28 $\pm$ 0.02*
Pa _a CO ₂ (mmHg)	54.4 $\pm$ 1.8	54.6 $\pm$ 1.9	53.3 $\pm$ 1.2	54.4 $\pm$ 1.1	52.4 $\pm$ 1.4	52.8 $\pm$ 1.7
Pa _a O ₂ (mmHg)	20.0 $\pm$ 0.7	19.8 $\pm$ 0.6	9.2 $\pm$ 0.5*	9.8 $\pm$ 1.0*	19.0 $\pm$ 2.2	18.4 $\pm$ 0.5
Sat Hb (%)	49.9 $\pm$ 4.0	51.5 $\pm$ 4.1	16.1 $\pm$ 1.1*	16.6 $\pm$ 1.8*	42.8 $\pm$ 5.2	40.6 $\pm$ 2.8
ABE (mequiv l ⁻¹)	3.2 $\pm$ 0.7	3.4 $\pm$ 0.7	-2.0 $\pm$ 1.3*	-6.8 $\pm$ 1.4*	-5.6 $\pm$ 1.5*	-3.0 $\pm$ 1.1*
[HCO ₃ ⁻]	28.6 $\pm$ 0.7	28.4 $\pm$ 0.7	23.6 $\pm$ 1.2*	20.4 $\pm$ 1.1*	20.8 $\pm$ 1.2*	23.0 $\pm$ 1.2*

* Values represent the mean $\pm$ S.E.M. of $n = 5$ at 15 (N15) and 45 (N45) min of normoxia, at 15 (H15) and 30 (H30) min of hypoxemia, and at 30 (R30) and 60 (R60) min of recovery. Significant differences: * $p < 0.05$ for normoxia vs. hypoxemia or recovery. pHa, arterial pH; Pa_aCO₂, arterial partial pressure of CO₂; PO₂, arterial partial pressure of O₂; Sat Hb, percentage saturation of oxygen with haemoglobin; ABE, acid base excess; [HCO₃], blood concentration of bicarbonate ions.

13 beats/min), increase in femoral vascular resistance (from 1.69 $\pm$ 0.15 to 12.04 $\pm$ 2.58 mm Hg $\cdot$ ml⁻¹ $\cdot$ min⁻¹), and a decrease in umbilical vascular resistance (from 0.50 $\pm$ 0.04 to 0.38 $\pm$ 0.04 mm Hg $\cdot$ ml⁻¹ $\cdot$ min⁻¹) in all fetuses (all $p < 0.05$; Fig. 2). Whereas heart rate and femoral vascular resistance returned toward basal values during recovery, arterial BP and umbilical vascular resistance remained altered from baseline ($p < 0.05$).

Plasma S100 β during acute hypoxemia. Basal fetal plasma S100 β concentrations averaged 9.6 $\pm$ 2.8 μ g/L. During acute hypoxemia, S100 β concentrations increased significantly by 24.9 $\pm$ 2.9% (Fig. 3). This elevation in S100 β concentration

Figure 2. Cardiovascular responses to acute hypoxemia in the sheep fetus. Values are mean $\pm$ SEM of $n = 5$ calculated every minute for arterial BP, heart rate, and femoral and umbilical vascular resistance during 1 h of normoxia, 0.5 h of hypoxemia (box), and 1 h of recovery.

Figure 3. Plasma S100 β concentrations in response to acute hypoxemia in the sheep fetus. Values are mean $\pm$ SEM of $n = 5$ of the percentage change from baseline during the acute hypoxemic protocol. * $p < 0.05$ for normoxia vs hypoxemia or recovery.

persisted until the end of the experimental protocol, with values remaining significantly elevated from baseline by 21.9 $\pm$ 4.1%.

Relation among plasma S100 β , cardiovascular function, and acid-base status in the fetus during acute hypoxemia. Correlation analysis for all individual fetuses during all values obtained during normoxic and hypoxemic conditions between the percentage changes in S100 β concentration and femoral vascular resistance revealed a significant positive relationship ($r = 0.71$, $n = 20$, $p < 0.001$; Fig. 4). In marked contrast, no relationship was found between the percentage change in

Figure 4. Plasma S100 β concentrations and hemodynamic changes in the sheep fetus. The data show the association between the percentage change in plasma S100 β concentration and the percentage change in femoral and umbilical vascular resistance. R, Pearson product moment correlation coefficient. Data points during normoxia and hypoxemia ($n = 20$) from all animals ($n = 5$) were correlated.

DISCUSSION

S100 β concentration and umbilical vascular resistance ($r = 0.26$, $n = 20$, $p = 0.27$; Fig. 4). Correlation analyses between the plasma S100 β concentration and fetal acid-base status was measured by assessing the relationship between the percentage changes in plasma S100 β concentration and the percentage changes in pH, ABE, and $[\text{HCO}_3^-]$ during normoxic and hypoxemic conditions in all individual fetuses. The analysis revealed significant negative relationships between S100 β and pH, ABE, and $[\text{HCO}_3^-]$ (Fig. 5).

Figure 5. Plasma S100 β concentrations and changes in acid-base status in the sheep fetus. The data show the association between the percentage change in plasma S100 β concentration and the percentage change in pH, ABE, and blood concentrations of bicarbonate ions in all individual sheep fetuses during acute hypoxemia. Data points during normoxia and hypoxemia ($n = 20$) from all animals ($n = 5$) were correlated.

For decades, clinical tools for fetal surveillance during pregnancy have relied on fetal heart rate monitoring and Doppler blood flow velocimetry. Since the introduction of these clinical tools, surprisingly, no additional techniques have been implemented routinely into perinatal clinical practice. The protein S100 β has been suggested to be a marker of stress to the CNS during the adult, newborn, and fetal periods (2–10). Therefore, an early detection of elevations in S100 β may provide the obstetrician with an additional clinical tool for detecting episodes of fetal distress. However, whether S100 β is a marker of fetal distress of the type that is associated with brain damage, a marker of fetal hypoxia, or a marker of fetal acidemia has not been addressed to date. Here, we report that a controlled period of fetal hypoxemia in chronically instrumented fetal sheep in late gestation leads to significant elevations in plasma concentrations of S100 β . The increase in the concentration of the protein during hypoxemia is rapid in onset and is sustained past 60 min after the end of the hypoxemic challenge. Furthermore, values for changes in S100 β concentrations in all individual fetuses were significantly related with hemodynamic and biochemical variables currently measured in clinical obstetric practice for the early detection of perinatal hypoxia and/or asphyxia. Specifically, changes in S100 β concentrations were positively related with hemodynamic changes indicative of redistribution of blood flow during fetal hypoxemia and negatively related with changes in fetal pH, ABE, and blood concentrations of bicarbonate, indicative of acidemia.

In the experimental situation, such as in the ovine model, the fetal cardiovascular defense to an episode of acute hypoxemia involves transient bradycardia, a gradual increase in arterial BP, and redistribution of the fetal combined ventricular output (CVO) away from peripheral circulations toward the adrenal, myocardial, cerebral, and umbilical circulations (11,14,15). The redistribution of the fetal CVO is mediated *via* vasodilation in the essential circulations and pronounced vasoconstriction in the periphery (14–16). An established index of the fetal peripheral vasoconstrictor response contributing to redistribution of the fetal CVO during hypoxemia is an increase in femoral vascular resistance (11,14). The mechanisms that mediate peripheral vasoconstriction during acute hypoxemia include well-established neural, endocrine, and local components (14–16). The initial femoral vasoconstriction is triggered by a carotid chemoreflex and is mediated *via* α -adrenergic sympathetic efferent pathways (11). As the episode of hypoxemia progresses, endocrine vasoconstrictors, such as catecholamines, are released into the fetal circulation (17), which maintain the peripheral vasoconstrictor response to acute hypoxemia. Furthermore, it has recently become appreciated that the peripheral vasoconstrictor response to acute hypoxemia may also involve local components (18,19) and that the peripheral vasomotor response to acute hypoxemia may represent the balance between neural and endocrine constrictor influences and local vasodilator actions of nitric oxide (20). In the present study, the fetal cardiovascular responses to hypoxemia were typical of fetal sheep at this stage of gestation (11,14). Furthermore, the increase in femoral vascular resistance

plasma correlated significantly with elevations in the plasma concentrations of S100 β during acute hypoxemia. In contrast, no relationship was obtained between changes in S100 β and changes in umbilical vascular resistance. In human clinical practice, redistribution of blood flow away from peripheral circulations toward essential vascular beds in the fetus is assessed by Doppler blood flow velocimetry. Typically in obstetric medicine, an increase in the ratio of the resistance indices in central circulations (*e.g.* middle cerebral artery) to those in peripheral circulations (descending aorta or femoral arteries) is representative of the "brain-sparing effect," redistributing the fetal CVO in favor of the brain at the expense of the fetal trunk (21). A previous clinical study in our laboratory reported that in human pregnancies with IUGR, the levels of S100 β in cord blood were negatively correlated with a decrease in the Doppler pulsatility index in the middle cerebral arteries (10). Combined, therefore, our experimental measurements in fetal sheep and our clinical observations in human pregnancies with IUGR convincingly support an association between elevations in S100 β and redistribution of blood flow toward the cerebral circulations during hypoxemia in the fetus. It thus follows that S100 β cannot be elevated solely during conditions of cerebral ischemia and, hence, cerebral damage, but it is better related to fetal hypoxic stress, which leads to redistribution of blood flow by triggering a chemoreflex response. As such, detection of elevations in S100 β may better serve the obstetrician as an early clinical marker of fetal hypoxia, particularly in combination with evidence of redistribution of blood flow, which if persistent may result in fetal brain damage, rather than as a marker of fetal brain damage *per se*. In this context, it was reported previously that persistent elevations in S100 β in the micromolar range can be neurotoxic and lead to apoptosis and neuronal cell death (2,22). However, it is fully acknowledged that in human clinical practice, cordocentesis will be indicated only in pregnancies that already are known to be complicated. Amniocentesis may offer a more plausible option. Assays from cord blood are clearly possible but unlikely to change management, being retrospective.

In the present article, evidence is also provided that elevations in the protein concentrations of S100 β in the fetal circulation are related not only to fetal distress in terms of fetal hypoxia but also to falls in pH, ABE, and bicarbonate concentrations, measures that are consistent with fetal metabolic acidosis. The study suggests that elevations in S100 β together with indices of redistribution of blood flow are indicative of fetal hypoxic stress, that elevations in S100 β with falls in pH, ABE, and bicarbonate are indicative of fetal acidemic stress, and that both situations are not mutually exclusive.

The source of changes in S100 β in the fetal circulation during and after the acute hypoxemic insult in the current study is unclear. One possibility is that changes in blood-brain barrier permeability as a result of fetal hypoxia itself or increased cerebral perfusion secondary to redistribution of blood flow may promote leakage of the protein from the CNS into the systemic circulation (23,24). Alternatively, S100 β may be released from the placenta during hypoxemic conditions. In the human, S100 β has been localized in villous and intermediate trophoblast cells of the normal placenta at different stages of

gestation (25). However, reports on changes in placental S100 β during adverse intrauterine conditions are inconclusive and contradicting. Whereas S100 β has been detected in pregnancies that are complicated by IUGR and preeclampsia (26), other studies did not reveal appreciable differences in S100 β expression between uncomplicated pregnancies and those with IUGR (10).

CONCLUSION

In conclusion, this study reports for the first time that a controlled period of fetal hypoxemia with associated acidemia leads to persistent elevations in plasma S100 β concentrations that strongly correlate with hemodynamic changes that are known to occur during fetal blood flow redistribution. These findings provide the first step in relating fetal plasma S100 β concentrations to actual fetal hypoxia and fetal acidemia. Future research directions should advance on these findings, creating experimental models that mimic the human clinical situation, relating both fetal plasma and amniotic fluid concentrations of the protein with fetal hemodynamic variables under conditions of chronic hypoxemia, with and without developed acidemia.

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