Effects of acute hypoxemia on insulin-like growth factors and their binding proteins in fetal sheep

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IWAMOTO, Harriet S., Mary A. Murray, and Steven D. Chernausek. Effects of acute hypoxemia on insulin-like growth factors and their binding proteins in fetal sheep. Am. J. Physiol. 263 (Endocrinol. Metab. 26): E1151-E1156, 1992.—It has been proposed that insulin-like growth factor I (IGF-I) regulates fetal growth and differentiation. Plasma IGF-I concentrations correlate positively with fetal nutrient availability and newborn birth weights. To explore the hypothesis that hypoxemia decreases fetal growth by decreasing fetal IGF-I availability, we instrumented 14 fetal sheep with vascular catheters. At least 4 days after surgery, 10 fetuses were made acutely hypoxic by infusing nitrogen into the maternal trachea for 3 h. Fetal blood oxyhemoglobin saturation decreased from 53 ± 6 (SD) to 31 ± 9%. Concomitantly, plasma IGF-I concentrations decreased from 91 ± 11 to 67 ± 10 ng/ml and IGF-I binding protein-1 concentration increased significantly, as assessed by ligand and Western blot analysis. Fetal IGF-I concentrations remained below control values throughout a subsequent recovery period (68 ± 12 ng/ml at 6 h). In four control fetuses and in the ewes, plasma IGF-I concentrations were not significantly different from control values (97 ± 18 and 181 ± 18 ng/ml, respectively). These data support the hypothesis that decreases in fetal oxygen availability may decrease fetal growth by decreasing IGF-I production and availability.

Fetal hypoxia; fetal growth; intrauterine growth retardation; growth factors

INSULIN-LIKE GROWTH FACTOR I (IGF-I) is a peptide growth factor that promotes postnatal growth by acting as an intermediate factor in growth hormone action. Recent evidence suggests that IGF-I may play an important role in regulating fetal growth. IGF-I promotes growth and differentiation in many types of fetal cells in vitro (13, 16, 22, 37), promotes growth of immature animals (29), is synthesized by virtually every fetal tissue (10, 20, 28), and has specific receptors located in nearly every cell type in fetuses of many species (7, 12, 32). Because cells that contain IGF-I immunoreactivity are found in close relation to cells that express specific IGF-I mRNA, it has been proposed that IGF-I in the fetus acts primarily as an autocrine or paracrine growth factor (11). However, plasma IGF-I concentrations in the fetus correlate positively with nutritional status and birth weight (3, 4, 9, 19, 24, 25, 40). This could mean that plasma IGF-I concentrations simply reflect cellular IGF-I production in aggregate but also could indicate an endocrine role for IGF-I in fetal growth regulation.

In the adult, nutritional status is an important regulator of plasma IGF-I concentrations (36). Similarly, fetal plasma IGF-I concentrations decrease when nutrient supply to the fetus is reduced by uterine artery ligation or reduction in placental size (24, 40). However, these procedures also reduce oxygen supply. Oxygen has been shown to be an important independent regulator of fetal growth (5, 23), but the mechanism by which oxygen regulates fetal growth is unknown. To examine the hypothesis that hypoxemia reduces fetal growth by first reducing IGF-I availability in the fetus, we measured the effects of moderate hypoxemia on fetal plasma IGF-I concentrations in chronically instrumented fetal sheep. We also measured the effects of moderate hypoxemia on IGF-I specific binding proteins (IGFBP) because IGFBP have been shown to modify the actions of IGF-I, and growth retardation in fetal and neonatal rats is associated with changes in IGFBP concentration and expression (9, 34, 38).

MATERIALS AND METHODS

Animals and surgical preparation. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Cincinnati. Fourteen ewes pregnant with singleton fetuses were obtained at 115—124 days gestational age (term = 145 days). After a 36- to 48-h fast, the ewe was sedated with intravenous pentobarbital sodium (15 mg/kg), ventilated with 2% isoflurane in oxygen, and secured in a supine position. With the use of established procedures (31), catheters were placed into the maternal descending aorta and inferior vena cava, amniotic fluid cavity, and fetal descending aorta, inferior vena cava, and umbilical vein via a cotyledary tributary. All surgical incisions were sutured, and the catheters were exteriorized to the ewe’s left flank. Vascular catheters were filled with heparin saline solution (1,000 U/ml) and sealed. A 12-Fr polyvinyl catheter was placed in the maternal trachea and protected in a cloth sleeve secured around the neck. This catheter did not interfere with ventilation and had no significant effects on maternal oxygenation. The animals were housed postoperatively in mobile carts and had free access to food and water. Ampicillin (1 g) was administered to the ewe and fetus daily. Studies were performed no earlier than the fourth postoperative day to ensure recovery from anesthesia and surgical stress.

Experimental procedures. Baseline blood samples were obtained from the maternal and fetal aorta for the determination of pH and blood gases, blood glucose and lactate concentrations, and plasma IGF and IGFBP concentrations. After obtaining duplicate baseline samples, hypoxemia was produced in one group of 10 fetuses by infusing humidified nitrogen into the trachea of the ewe at a rate that was adjusted to maintain fetal arterial blood oxygen saturation of hemoglobin at 30% for 180 min. A 180-min recovery period followed, during which the ewes inhaled room air. Blood samples were obtained at 30, 60, 120, 180, 240, 300, and 360 min after initiation of the nitrogen infusion. In five of these fetuses, additional blood samples were obtained at 24 h. A control group of four fetuses was also studied. Blood samples were obtained as for the hypoxic group, and the ewes inhaled room air throughout the 6-h sampling period. To maintain blood volume in the fetus, an equivalent amount of maternal blood was infused at each sampling time. Arterial pH, blood gases, oxygen content, and glucose and lactate concentrations were determined using a blood gas analyzer (Corning model 170, Ciba Corning Diagnostics, Medfield, MA), hemoximeter (model OSM2; Radiometer, West Lake, OH), and a glucose/lactate analyzer (model 2300, Yellow Springs Instruments, Yellow Springs, OH).
Plasma insulin, IGF-I, and IGF-II concentrations were determined by radioimmunoassay (8). To measure total IGF concentration, plasma samples were processed to remove IGFBP by lyophilization, and assayed. Although the antibody used in the IGF-I assay was raised against human IGF-I, it can be used to reliably detect ovine IGF-I because human and ovine IGF-I differ by only one amino acid residue (15), the antibody readily detects ovine IGF-I, and ovine IGFBP-2 is specific for ovine IGF-II. The antibody used in the IGF-II assay was a monoclonal antibody (clone no. S1-F2, Amano International Enzyme, Troy, VA) prepared against human IGF-II. This antibody readily detects ovine IGF-II, and cross-reaction with IGF-I of ~3%. All samples from a single animal were analyzed in the same assay.

IGFBP in fetal and maternal plasma were examined by a ligand blot technique developed by Hossenlopp (see Ref. 21). Plasma (10 μl) was mixed with nonreducing sample buffer [90 μl of 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.1% bromphenol blue in 625 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 6.8] and boiled for 3 min. Twenty-five microliters of this mixture (2.5 μl of plasma) was fractionated by size by SDS-polyacrylamide gel electrophoresis (12% total acrylamide) overnight at 20 V in 25 mM Tris, 250 mM glycine, and 0.1% SDS, pH 8.3 (model SE 700, Hoefer Scientific Instruments, San Francisco, CA). The proteins were electroblotted to nitrocellulose membranes at a constant 100 V overnight at 4°C in 25 mM Tris, 192 mM glycine buffer containing 20% methanol (Transphor II, Hoefer). The nitrocellulose membrane was washed sequentially in 8 mM Tris-buffered saline containing 1% bovine serum albumin and 0.10% Tween 20, then in Tris-buffered saline alone. The membranes were incubated with the latter buffer containing 400,000 counts/min 125I-labeled IGF-I (cDNA-derived human [Thr52]IGF-I, Amgen Biologicals, Thousand Oaks, CA), iodinated by chloramine-T method to a specific activity of 200 μCi/μg overnight at 4°C with gentle agitation. Excess radioactivity was washed from the membrane in Tris-buffered saline containing 0.10% Tween 20, then in Tris-buffered saline alone. The membranes were exposed to Hyperfilm-MP (Amersham, Arlington Heights, II) in cassettes with intensifier screens at -70°C. Areas of specific binding were detected, and relative changes in binding proteins were assessed by densitometric analysis in triplicate (model 620 video densitometer, Bio-Rad, Richmond, CA).

Western immunoblots were performed to detect plasma IGFBP-1 and IGFBP-2. SDS-polyacrylamide gel electrophoresis and transfer of 2.5-μl aliquots of plasma were performed as described above. Membranes were washed in 50 mM Tris-buffered saline, pH 7.4, containing 0.05% Tween 20 and 3% nonfat dry milk, then incubated overnight at room temperature in the same buffer containing either rabbit anti-human IGFBP-1 (rabbit no. 4, bleed no. 3 diluted 1:1,000) or rabbit anti-bovine IGFBP-2 (rabbit no. K52, bleed no. 5 diluted 1:2,000). The membranes were washed, incubated with biotinylated goat anti-rabbit antiserum (1:3,000) for 60 min, and washed. These particular antisera, generous gifts from Dr. D. R. Clemmons of the University of North Carolina, were suitable for detecting ovine binding proteins. Antibody detection was achieved with the Vectastain ABC-AP kit (Vector Laboratories, Burlingame, CA).

Statistical analysis of data. Data were analyzed by analysis of variance for repeated measures. Differences from control values were compared by Dunnett’s t test. Differences between control and hypoxic groups were analyzed by two-way analysis of variance for repeated measures. The level of significance of differences were as indicated.

RESULTS

Maternal hypoxemia produced expected changes in the fetus. Fetal arterial blood oxygen saturation decreased from 53 ± 6 (SD) to 31 ± 9% (P < 0.01) by 30 min. After 180 min, when the ewes were allowed to breathe room air, fetal arterial hemoglobin saturation increased to 47 ± 8% and remained at that level throughout the recovery period. Arterial pH, blood gases, and glucose and lactate concentrations in the fetuses were within the normal range during the control period (Table 1). Acute hypoxemia decreased fetal arterial pH, P02, Pco2, and oxygen content, and increased blood glucose and lactate concentrations significantly (Table 1 and Fig. 1). The changes in pH, P02, Pco2, glucose, and lactate persisted in the recovery period. However, 24 h later, pH, P02, Pco2, oxygen content, and glucose and lactate concentrations had returned to normal in the five animals studied at this time period and were 7.36 ± 0.01, 21 ± 1 Torr, 54 ± 2 Torr, 3.05 ± 0.22 mM, 1.05 ± 0.24 mM, and 1.42 ± 0.25 mM, respectively. Plasma insulin concentration in the fetus was 8.2 ± 1.9 μU/ml during the control period, and was not altered significantly by moderate hypoxemia.

During the control period, fetal plasma IGF-I concentrations were 91 ± 11 ng/ml. They decreased significantly during hypoxemia and remained low throughout the recovery period even though arterial blood oxygen content had returned to control values (Fig. 1). The average of the lowest value for IGF-I was 58 ± 8 ng/ml, which is 62 ± 14% of the control value. There was variability in the IGF-I response with respect to the time at which the lowest concentration was achieved in each animal. The decline in plasma IGF-I concentrations occurred as early

| Table 1. Effect of acute hypoxemia and recovery on fetal arterial blood |
|----------------|----------------|----------------|----------------|----------------|
|                | Control | Hypoxemia | Recovery       |
|                | time 0  | 30 min    | 60 min         | 120 min        | 180 min        | 240 min        | 380 min        |
| pH             | 7.37±0.02 | 7.38±0.03 | 7.35±0.06      | 7.30±0.14       | 7.27±0.16       | 7.30±0.11       | 7.35±0.05      |
| P02 Torr       | 22±2   | 15±3*     | 16±3*          | 15±3*          | 16±3*          | 21±3           | 20±2           |
| Pco2 Torr      | 50±5   | 44±5*     | 42±7*          | 42±6*          | 45±6*          | 44±7*          | 45±6           |
| O2 content, mM | 2.78±0.45 | 1.64±0.35 | 1.49±0.44      | 1.63±0.60       | 1.59±0.45       | 2.42±0.61       | 2.40±0.30      |
| Glucose, mM    | 0.96±0.17 | 1.11±0.18 | 1.14±0.23      | 1.29±0.40       | 1.21±0.40       | 1.26±0.35       | 1.18±0.37      |
| Lactate, mM    | 1.36±0.25 | 3.02±1.10 | 4.50±2.74      | 6.4±1.90        | 7.24±2.75       | 6.46±3.80       | 4.47±2.68      |
| IGF-II ng/ml   | 994±157 | 992±219   | 952±138        | 941±151        | 873±171        | 715±260        | 820±256        |

Values are means ± SD; insulin-like growth factor II (IGF-II), n = 7; n = 10 for all other variables. Data were analyzed by one-way analysis of variance for repeated measures. Differences from control values were determined by Dunnett's t test: * P < 0.05; † P < 0.01.
As 30 min in some animals but as late as 240 min after the onset of hypoxemia in others. Values measured in five fetuses after 24 h had returned to baseline (105 ± 19 ng/ml), indicating that recovery occurred within 24 h. In contrast to the effect on fetal IGF-I concentrations, acute hypoxemia had no significant effect on plasma IGF-II concentrations in control group. Acute hypoxemia significantly decreased plasma IGF-I concentrations in fetus. Significant differences from control values: * P < 0.05; † P < 0.01. Data are means ± SE.

DISCUSSION

An adequate supply of oxygen and nutrients from the mother is essential for normal fetal growth and development. When the fetal supply of oxygen or nutrients or both is reduced, fetal growth rate decreases and intrauterine growth retardation may result (6, 21, 23). The factors that regulate fetal growth, particularly in close relation to oxygen and nutrient availability, are not known. Growth hormone is not an important regulator of growth before birth (27). Anencephalic human fetuses and fetal animals that develop in the absence of growth hormone grow normally before birth. Placental lactogen has been proposed as a possible regulator of fetal growth; however, some fetuses that lack placental lactogen apparently grow at normal rates (17). Insulin has been proposed as a regulator of fetal growth because an excess or deficiency of insulin has been associated with increased and decreased intrauterine growth rates, respectively, in a number of species (14). However, the effects of insulin concentrations within the physiological range on fetal growth have not been clearly defined, and it is not clear whether insulin exerts effects as a permissive factor or as a growth-promoting factor that works through its own receptor or through its interaction with the type I IGF-I receptor. IGF-I is potentially an important regulator of fetal growth. In addition to the many trophic and mitogenic effects that IGF-I exerts in vitro, IGF-I has been shown to modulate growth rate in vivo (29). Nutrient deficiency decreases fetal growth rate and decreases plasma IGF-I concentrations in the fetus (3, 9, 24, 40).

The results from the present investigation support the concept that IGF-I may be an important regulator of fetal growth in relation to oxygen availability. Acute hypoxemia significantly decreased plasma IGF-I concentrations in fetal sheep. The mechanisms responsible for this
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Fig. 2. Ligand and Western immunoblots of plasma from 2 fetal sheep made hypoxic from 0 to 180 min. Top: binding of $^{125}$I-IGF-I to 5 components of fetal sheep plasma with molecular weights that ranged from 24,000 to 45,000. An increase in intensity of $^{125}$I-IGF-I binding is evident in the 29,000 mol wt component during hypoxemia. This component is identified as IGF binding protein-1 (IGFBP-1) in the Western immunoblots for IGFBP-1 (bottom). Antibody for IGFBP-2 identified the 36,000 mol wt binding component as IGFBP-2 (data not shown).

response were not determined, but there are several possibilities. It is possible that acute hypoxemia decreased the rate of IGF-I synthesis. The mediator of this response is unclear and is probably not related to a local reduction in oxygen delivery to all sites of IGF-I synthesis. This is because the degree of hypoxemia is not the same in every vascular bed. In response to acute hypoxemia of a degree comparable to the one in this study, blood flow and oxygen delivery are preferentially redistributed so that some areas of the circulation do not become hypoxic (30). Blood flow to the brain and heart increases so that oxygen delivery to these areas is maintained. In other areas, such as the musculoskeletal, renal, and splanchnic vascular beds, oxygen extraction increases and adequate oxygen delivery is maintained. Although anaerobic metabolism must have occurred in some tissues because blood lactate concentration increased, it seems unlikely that all tissues decreased IGF-I synthesis as a direct consequence of tissue hypoxia.

It is possible that synthesis of IGF-I by the fetal liver could have decreased. The liver is believed to be the principle site from which plasma IGF-I is derived. It is expected that oxygen delivery to the liver decreased in these studies, secondary to decreases in umbilical venous PO$_2$ and oxygen content. However, we did not measure hepatic production of IGF-I, and will need to determine hepatic secretion rates in additional studies.

A decrease in IGF-I synthesis cannot completely explain the results obtained in this study. It is possible that the clearance rate of IGF-I may have increased. In normal fetal sheep the clearance rate of injected $^{125}$I-IGF-I has several components, with estimated half-lives of 30, 202, and 412 min (2). Initial distribution of injected $^{125}$I-IGF-I accounts for the 30-min half-life component, and thus should not be a factor in the present study, in which we measured changes in endogenous IGF-I. If a decrease in IGF-I synthesis were the only response to hypoxemia, one would expect the kinetics of the decrease in plasma IGF-I concentration to be a function of the remaining clearance components. It is apparent that a decrease in synthesis alone cannot account for the observation that plasma IGF-I decreased significantly as early as 30 min after the initiation of hypoxemia. Thus the time course of the response suggests that IGF-I clearance increased in response to hypoxemia. One possible site of increased clearance is the placenta, because the proportion of total blood flow distribution to the placenta generally increases during hypoxemia, and the placenta has been shown to remove $^{125}$I-IGF-I from the fetal circulation (2).

It is possible that neither synthesis or clearance rates changed but, rather, the volume of distribution for IGF-I increased in the present study. Using ligand and Western blot analyses, we identified five proteins in fetal sheep plasma that bind IGF-I. These are identified as IGFBP-1 (mol wt 29,000) and IGFBP-2 (36,000) by size and immunoreactivity. IGFBP-3 (40,000–44,000) and IGFBP-4 (24,000) are identified by size, relative activity in fetal and maternal plasma, and by analogy with previous reports (18). Binding proteins have been reported to prolong the circulating half-life of the IGFs, increase or decrease the biological activity of the IGFs, and have inherent biological activity. In the present study, acute hypoxemia significantly increased plasma concentrations of IGFBP-1 in every animal, although later than the decrease in plasma IGF-I concentrations. If the function of plasma IGFBP-1 in the fetus were to protect IGF-I from being cleared, we would have observed an increase in plasma IGF-I concentrations. It is possible that the increases in plasma IGFBP-1 concentrations occurred secondary to an increase in IGFBP-1 content in certain
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Fig. 3. Effect of acute hypoxemia on plasma IGF-I (●) and IGFBP-1 (○) concentrations, assessed by radioimmunoassay and densitometric analysis, expressed as a percentage of baseline in 2 fetal sheep. Identification of IGFBP-1 was made on the basis of immunoreactivity and size, as described in text.

tissues and represents spillover of the protein from tissue stores. If that were the case, the decrease in plasma IGF-I concentrations could have resulted from sequestration of plasma IGF-I in tissues producing increased amounts of IGFBP-1 or from increased IGFBP-1-mediated transendothelial transport of IGF-I (1). We did not measure tissue concentrations of IGFBP-1 in this study, and additional studies are necessary to resolve this issue.

Results from previous studies indicate that nutritional status and insulin are important regulators of IGF-I and IGFBP-1 synthesis and plasma concentrations. Diminished nutrient supply to maternal starvation in rats and sheep or uterine artery ligation in rats decreases IGF-I and increases IGFBP-1 plasma concentrations (9, 18, 34, 38). Inhibition of glucose uptake by fetal liver explants stimulates and insulin inhibits a cyclic nucleotide-dependent pathway believed to increase IGFBP-1 production (26). Insulin treatment of insulin-dependent diabetes mellitus patients or insulin administration to animals decreases IGFBP-1 concentrations (35, 39). The concept has developed that glucose and insulin are major regulators of IGF-I and IGFBP-1 in the fetus. However, we have shown that EGF treatment of newborn rats retards growth, decreases metabolic rate, and decreases plasma IGF-I and increases plasma IGFBP-1 concentrations (8; M. A. Murray, unpublished observation), and in the present study we showed that acute hypoxemia increased IGFBP-1 and decreased IGF-I plasma concentrations, but did not decrease blood glucose or alter plasma insulin concentrations. Taken together, the data are consistent with the concept that when the intracellular supply of nutrients, including oxygen, is diminished, IGF-I availability decreases and the abundance of IGFBP-1 increases. This may serve to sequester IGF-I from exerting anabolic actions in target cells until nutrient supplies are restored.

In summary, we observed that a brief hypoxemic stimulus was transduced into a prolonged decrease in plasma IGF-I concentrations, which is perhaps a signal to slow growth rate. Thus intermittent fetal stressors, such as cord compression or oscillations in uterine blood flow due to shifts in maternal position, could have marked effects on fetal IGF-I and IGFBP-1 concentrations over prolonged periods of time. These changes in IGF-I and IGFBP-1 concentrations may be an important mechanism by which fetal growth changes appropriately in relation to nutrient and oxygen supply.

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REFERENCES


