Time-dependent physiological regulation of rodent and ovine placental glucose transporter (GLUT-1) protein

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Das, Utpala G., H. Farouk Sadiq, Michael J. Soares, William W. Hay, Jr., and Sherin U. Devaskar. Time-dependent physiological regulation of rodent and ovine placental glucose transporter (GLUT-1) protein. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R339–R347, 1998.—To examine the in vivo and in vitro time-dependent effects of glucose on placental glucose transporter (GLUT-1) protein levels, we employed Western blot analysis using placenta from the short-term streptozotocin-induced diabetic pregnancy (STZ-D), uterine artery ligation-intrauterine growth restriction (IUGR) rat models, pregnant sheep exposed to chronic maternal glucose and insulin infusions, and the HRP-1 rat trophoblastic cell line exposed to differing concentrations of glucose. In the rat, 6 days of STZ-D with maternal and fetal hyperglycemia caused no substantive change, whereas 72 h of IUGR with fetal hypoglycemia and ischemic hypoxia resulted in a 50% decline in placental GLUT-1 levels (P < 0.05). In late-gestation ewes, maternal and fetal hyperglycemia caused an initial threefold increase at 48 h (P < 0.05), with a persistent decline between 10 to 21 days, whereas maternal and fetal hypoglycemia led to a 30–50% decline in placental GLUT-1 levels (P < 0.05). Studies in vitro demonstrated no effect of 0 mM, whereas 100 mM glucose caused a 60% decline (P < 0.05; 48 h) in HRP-1 GLUT-1 levels compared with 5 mM of glucose. The added effect of hypoxia on 0 and 100 mM glucose concentrations appeared to increase GLUT-1 concentrations compared with normoxic cells (P < 0.05; 100 mM at 18 h). We conclude that abnormal glucose concentrations alter rodent and ovine placental GLUT-1 levels in a time- and concentration-dependent manner; hypoxia may upregulate this effect. The changes in placental GLUT-1 concentrations may contribute toward the process of altered maternoplacentofetal transport of glucose, thereby regulating placental and fetal growth.

intrauterine growth restriction; maternal diabetes; maternal hyperglycemia; maternal hypoglycemia

UNDER BASAL CONDITIONS, glucose supply to the fetus is derived solely from the maternal circulation via placental transport of the substrate (16). Placental glucose transport is a stereospecific, saturable, and carrier-mediated process of facilitated diffusion (5, 7, 24, 32). This process is mediated by a family of closely related, membrane-spanning, heterogeneously glycosylated proteins called glucose transporters (3, 13). In previous studies we and others have demonstrated the expression of two major facilitative glucose transporter isoforms in mammalian placenta (2, 12, 33). The predominant form is GLUT-1, the tissue-barrier isofrom noted in trophoblast cell plasma membranes (12, 28), and is highly conserved across various mammalian species, demonstrating >95% amino acid homology (3, 12). Previous studies employing in vivo tracer methodology have demonstrated significant changes in the rate of placental glucose transport with perturbations in maternal and/or fetal glucose concentrations specific to conditions of a diabetic pregnancy or intrauterine growth restriction (20, 29). We hypothesized that changes in circulating glucose concentrations affecting placental glucose transfer would affect placental GLUT-1 concentrations. The studies done to date in rat, mouse, and human have not been conclusive (6, 12, 18). To test the above-stated hypothesis, we undertook the present study to comprehensively investigate the time-dependent effects of hyperglycemia and hypoglycemia on placental GLUT-1 concentrations in differing mammalian species, i.e., the rat and sheep. We initially examined the in vivo effect of maternal and fetal hyperglycemia as seen in a diabetic pregnancy and fetal hypoglycemia with uteroplacental insufficiency, as associated with intrauterine growth restriction (IUGR), on rat placental GLUT-1 protein levels. Furthermore, we studied the time-dependent separate effects of maternal and fetal hyperglycemia or hypoglycemia on ovine placental GLUT-1 concentrations. Subsequently, we determined in vitro the time-dependent separate and combined effects of glucose and hypoxia on cultured rat trophoblastic cell GLUT-1 concentrations, attempting to mimic the in vivo condition of ischemic hypoxia that is encountered in uteroplacental insufficiency of maternal diabetes (11) and on uterine artery ligation (22). Furthermore, we studied the time-dependent separate effects of maternal and fetal hyperglycemia or hypoglycemia on ovine placental GLUT-1 concentrations. Our studies demonstrate that changes in placental glucose transporter protein levels are time dependent and glucose and hypoxia dependent, allowing reconciliation of the in vivo and in vitro observations in the rat and sheep.

MATERIALS AND METHODS

In Vivo Experiments

Animals: rats. Gestationally timed Sprague-Dawley rats (Taconic Farms, Germantown, NY) were housed in individual...
cages and were exposed to 12:12-h light-dark cycles. As approved by the St. Louis University School of Medicine's Animal Care Committee, the National Institutes of Health (NIH) guidelines for the care and use of animals were followed. The pregnant animals were allowed at least 1 day of aclimatization before experimental manipulation.

Animals: sheep. Columbia-Rambouillet mixed-breed pregnant ewes, each carrying a single fetus, were obtained from Nebeker Ranch, Santa Monica, CA. Each sheep was kept in a separate cart, but two sheep were kept in the same room for company. All studies, animal surgery, and animal care procedures were in accordance with NIH guidelines and were approved by the University of Colorado Health Sciences Center Institutional Animal Care and Use Committee. The animals were cared for and studied in the University of Colorado Health Sciences Center Perinatal Research Facility which is NIH, US Department of Agriculture, and Association for the Assessment and Accreditation of Laboratory Animal Care approved.

Animal Model: Rat

Intrauterine growth restriction. Eighteen-day timed pregnant Sprague-Dawley rats (term 21 ± 22 days with date of mating = day 0) were allowed free access to food and water 2 h before surgery. Under methoxyflurane inhalational anesthesia, a laparotomy was performed on day 18 of gestation, the uterus was exteriorized, and complete bilateral uterine artery ligation was performed (n = 6), as reported previously (30). Sham-operated control animals underwent the same procedure, except that uterine artery ligation was not performed (n = 4). The animals recovered within 20–30 min of the procedure, after which they were returned to their respective cages and were allowed free access to standard rat chow (Purina, St. Louis, MO) and water. On day 21, pregnant rats were anesthetized with pentobarbital sodium, subjected to hysterotomy, and the fetuses delivered.

Diabetic pregnancy. On day 12 of gestation, rats received either 65 mg/kg of freshly reconstituted streptozotocin (Sigma) in 0.5 ml of vehicle, which was maintained on ice, or an equal volume of the vehicle. Maternal urine was checked for ketonuria using ketostix strips (Ames, Indianapolis, IN). Maternal tail vein glucose concentrations were monitored with a One Touch glucose analyzer (Lifescan, Milpitas, CA), and the animals were divided into three groups based on the treatment and glucose values. The vehicle-injected animals demonstrated blood glucose concentrations of <10 mM and were assigned to the control group (Con; n = 5). The streptozotocin-injected animals (n = 10) were further divided into two groups. The animals that did not develop overt diabetes were assigned to the streptozotocin nondiabetic group (STZ-N; n = 5) and had glucose concentrations <10 mM. This group served as an additional intermediate group that helped distinguish between the effects of streptozotocin and those of overt maternal diabetes. The rest of the animals that received streptozotocin were found to have glucose concentrations >13.5 mM and were designated to the severely diabetic group (STZ-D; n = 5). This maternal diabetic model has been established and previously reported (26). On day 20 of an expected 21-day gestation (day of mating = day 0), pregnant rats were anesthetized with pentobarbital sodium, subjected to hysterotomy, and the fetuses were delivered.

Plasma assays. Pooled jugular fetal blood from a single litter was collected, and the plasma was separated by centrifugation at 4°C and stored in aliquots at −20°C for further analyses. Fetal plasma glucose concentrations were assayed by the standard glucose oxidase method using a Beckman glucose analyzer (Fullerton, CA). Plasma insulin concentrations were measured by radioimmunoassays, as described previously (26).

Animal Model: Sheep

The late-gestation (125–140 day; term 145 days) ovine fetus, which has classically been used for glucose kinetic measurements, was studied. Surgery was done at ~115 days of gestation to place maternal and fetal infusion and blood sampling catheters (0–20 days). In some animals the maternal and fetal infusion and sampling catheters were placed at 90 days gestation to undertake longer term experiments (~25 days). The ewes were fasted for 2 days before surgery. Surgery was conducted under ketamine anesthesia (12–15 mg/kg bolus induction followed by 0.3–0.5 mg·kg⁻¹·min⁻¹ constant infusion) and intramuscular acepromazine (2 mg/kg) and intramuscular diazepam (10 mg) for muscle relaxation and sedation. Each ewe and fetus also received 1% lidocaine local anesthesia into all wounds. A maternal sampling catheter was placed into a femoral artery, and maternal infusion catheters were placed into a femoral vein, all through a single groin incision. The ewe's abdomen was opened through a midline laparotomy, and fetal hindlimbs were extracted through a uterine incision. Fetal sampling catheters were placed into the abdominal aorta via hindlimb pedal arteries, and fetal infusion catheters were placed into the femoral veins via hindlimb saphenous veins. After the uterine and abdominal wounds were closed, the catheters were tunneled subcutaneously through a skin incision and kept in a plastic pouch attached to the ewe's flank. Each ewe received intramuscular gentamicin (80 mg) and procaine penicillin G (600,000 U), and the fetus was treated with intra-amniotic ampicillin (500 mg) at the time of surgery. Postoperatively, each ewe was maintained in its own cart and allowed ad libitum access to water, alfalfa pellets, and a mineral block (10, 14, 21). The ewes were paired in the same room to decrease psychological stress, given the flocking nature of sheep, and maintained under environmental conditions of 18 ± 3°C with 18 h of light and 6 h of darkness daily. Weekly intramuscular injections of a multivitamin preparation were given (B-complex Vitamins, Vedco, St. Joseph, MO). The catheters were flushed daily with 1.5 (fetal) or 3.0 ml (maternal) of a heparinized saline solution (150 U heparin/ml 0.9% wt/vol NaCl in H₂O). Each animal was allowed at least 4 days to recover from surgery before beginning the study of chronic infusions. At the end of the studies, each ewe and fetus were killed with a rapid intravenous infusion of T-61 euthanasia solution (Taylor Pharmaceutical, Decatur, IL) (10, 14, 21). The fetal tissue samples were snap-frozen in liquid nitrogen and stored at −70°C until further analyses.

Glucose infusions. The pregnant ewes were made chronically and markedly hyperglycemic by receiving a continuous 50% dextrose (wt/vol in H₂O) intravenous infusion at a variable rate that was adjusted in response to twice daily measurements of blood glucose concentrations (Yellow Springs model 2300 glucose analyzer) to maintain maternal arterial blood glucose concentration at relatively high and constant levels (Δglucose concentration above euglycemia = 2 mM) (8). A control group consisted of normal, euglycemic (~2.8 mM) pregnant ewes. The hyperglycemic group of animals was hyperinsulinemic during the first 48–72 h of glucose infusions, but by the end of the first week, despite persistent hyperglycemia, the fetal insulin concentrations returned to the normal range (12 ± 2 μU/ml). Fetal oxygen saturations and contents were no different in the hyperglycemic group; there were no changes in pH or in catecholamine levels (8, 9).

Insulin infusions. Insulin was infused into the ewes to produce acute and/or chronic hypoglycemia (14). Insulin
counts · min$^{-1}$ at 30–60 pmol (5–10 nM) maternal weight, as previously described (14). The insulin infusion rate was adjusted daily in response to measurements of maternal arterial blood glucose concentrations, attempting to maintain maternal glucose concentrations that were 50% of normal (1.4–1.5 mM). Maternal insulin concentrations averaged 40–45 µU/ml or about twice normal. Fetal insulin concentrations averaged 4–5 µU/ml or about 50% of normal.

In Vitro Experiments

Placental trophoblastic cell culture. In vitro studies were conducted using an HRP.1 rat placental cell line. The HRP.1 cell line is an untransformed trophoblastic cell line derived from midgestation rat chorioallantoic placenta (17). Cells in 75-cm$^2$ flasks were grown to confluence in RPMI 1640 containing 50 µM 2-mercaptoethanol (Bio-Rad, Richmond, CA), 1 mM sodium pyruvate, 100 mg/ml penicillin, 100 µM streptomycin, and 20% fetal bovine serum (JRH Biosciences). Cells were maintained at 37°C in a humidified atmosphere of 21% oxygen and 3% carbon dioxide. Media was changed every 2 days. Confluent placental cells were then subjected to varying glucose concentrations and/or hypoxia. To mimic the in vivo status of hypoglycemia, euglycemia (5 mM or 90 mg/dl), and hyperglycemia, cells were grown to confluence in RPMI 1640 media as above but containing varying concentrations of glucose, namely 0, 10, or 100 mM glucose, with 5 mM concentration serving as the control. Cellular hypoxia was attained by maintaining the cells in an atmosphere of 18% oxygen and 3% carbon dioxide. Cells were harvested by using a rubber policeman after 6, 18, or 48-h exposure to the experimental conditions. The cells were collected by centrifugation at 4°C and resuspended in cold phosphate-buffered saline (PBS). This wash cycle was repeated three times. Cells were stored in PBS at ~70°C until further analyses.

Glucose transporter protein assays. Thoroughly washed placental tissue (rodent or ovine) or harvested trophoblastic cells were mechanically homogenized using a Tekmar tissue homogenizer (Cincinnati, OH). The rodent and sheep placental samples were then sonicated (60 sonic, Dismembrator, Fisher Scientific, Pittsburgh, PA) using two 50-s cycles of 5–7 W to ensure adequate homogenization of tissue. Protein content was determined by the BioRad protein dye binding assay (BioRad, Richmond, CA). Seventy-five micrograms of rat placental tissue homogenates, 100 µg of ovine placental, or 75 µg of HRP.1 cell homogenates were subjected to discontinuous 10–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by electroblot transfer to nitrocellulose (Nytran, Schleicher & Schuell, Keene, NH). Protein content was initially ensured. The OD of each protein band was assessed and standardized against the OD of an internal control protein band using densitometry to control for interlane loading variability. This ratio was then expressed as a percent of the mean of the corresponding control samples, which then represented the changes in glucose transporter protein levels (26). In certain rat studies (in vivo and in vitro) and in the sheep studies, both the radioactivity and OD quantitation techniques were used, and the trends in change were noted to be similar within each group.

Data analysis. All results are expressed as means ± SE. Differences when comparing more than two groups or time points were determined by the one-way analysis of variance and the intergroup differences validated by the Newman–Keuls test. Nonparametric testing was also conducted using Kruskal-Wallis with tied ranks followed by the Dunn’s test to ensure that the conclusions drawn were not due to either small numbers or a heteroscedastic distribution of observations. With the use of both parametric and nonparametric testing, statistically significant differences between the groups/time points were similar. When only two groups were compared, as in the IUGR versus the sham-operated control, the Student’s t-test was used at a P < 0.05 level of significance.

RESULTS

Rat Studies

Uteroplacental insufficiency studies. Table 1 demonstrates the fetal characteristics of maternal uterine artery ligation. A distinct decline in fetal glucose concentrations was present with a trend toward a decrease in fetal insulin concentrations. Associated with these changes, a decline in placental and fetal body weights was observed with fetal brain weight sparing. Placental GLUT-1 concentrations decreased by 50% in the IUGR group compared with the sham-operated control group, which is denoted as 100% (Fig. 1, A and C).

Diabetic pregnancy studies. Table 2 depicts the characteristics of the streptozotocin-induced diabetic model. As reported previously and seen here, there were no changes in fetal body weights and placental weights secondary to the state of maternal diabetes (26). Diabetes was evidenced by maternal hyperglycemia with

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<tr>
<th>Table 1. IUGR study: fetal body, brain, and placental weights and fetal glucose and insulin concentrations</th>
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<tr>
<td><strong>IUGR</strong></td>
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<tr>
<td>Fetal body wt, g</td>
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<tr>
<td>Fetal brain wt, mg</td>
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<tr>
<td>Fetal glucose, mM</td>
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<td>Fetal insulin, nM</td>
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<td>Placental wt, g</td>
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Values are means ± SE. IUGR, intrauterine growth restriction.
*P < 0.05 vs. Sham.
maternal hypoinsulinemia as previously reported (26). The concomitant fetal hyperglycemia with no change in fetal insulin levels is depicted in Table 2. In contrast, streptozotocin treatment alone in the absence of diabetes led to no change in both the fetal glucose and insulin levels (Table 2). Western blot analysis, although demonstrating a trend toward a decline, revealed no change in placental GLUT-1 protein (~50 kDa) levels in both the streptozotocin-diabetic and streptozotocin-nondiabetic groups compared with the vehicle-treated group (Fig. 1, B and C); the latter is represented as 100%.

Table 2. Maternal diabetes study: fetal body, placental weights, and fetal glucose and insulin concentrations

<table>
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<tr>
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<th>Control</th>
<th>STZ-ND</th>
<th>STZ-D</th>
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<tr>
<td>Fetal body wt, g</td>
<td>3.6 ± 0.8</td>
<td>3.1 ± 0.4</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>Placental wt, g</td>
<td>0.57 ± 0.12</td>
<td>0.65 ± 0.04</td>
<td>0.69 ± 0.03</td>
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<tr>
<td>Fetal glucose, mM</td>
<td>3.4 ± 0.3</td>
<td>3.1 ± 0.4</td>
<td>12 ± 0.7*</td>
</tr>
<tr>
<td>Fetal insulin, nM</td>
<td>0.55 ± 0.06</td>
<td>0.50 ± 0.07</td>
<td>0.46 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE. STZ-ND, streptozotocin nondiabetic group; STZ-D, streptozotocin-induced diabetic group. *P < 0.05 vs. control or STZ-ND.

maternal hypoinsulinemia as previously reported (26). The concomitant fetal hyperglycemia with no change in fetal insulin levels is depicted in Table 2. In contrast, streptozotocin treatment alone in the absence of diabetes led to no change in both the fetal glucose and insulin levels (Table 2). Western blot analysis, although demonstrating a trend toward a decline, revealed no change in placental GLUT-1 protein (~50 kDa) levels in both the streptozotocin-diabetic and streptozotocin-nondiabetic groups compared with the vehicle-treated group (Fig. 1, B and C); the latter is represented as 100%.
In vitro trophoblastic cell study. As evidenced by Western blot analysis and quantitation (Fig. 2, A and C), absence of glucose in the culture medium did not demonstrate a time-dependent change in GLUT-1 protein levels at 6, 18, or 48 h compared with 5 mM glucose, which served as the control glucose concentration. On the other hand, high glucose concentration of 10 and 100 mM did cause a time-dependent and concentration-dependent trend toward a decline in GLUT-1 levels compared with control, with only the 100 mM concentration achieving statistical significance at 48 h (Fig. 2, A and C). The combined exposure of these cells to varying concentrations of glucose and 18% oxygen, mimicking a hypoxic environment, resulted in a time-dependent and concentration-dependent trend toward an increase in GLUT-1 levels in both zero and high (100 mM) glucose concentrations, as seen in Western blot analysis and by quantitation (Fig. 2, B and C). In the absence of glucose (0 mM), the hypoxia-induced increase in GLUT-1 levels was 2.5-fold in magnitude at 48 h only (Fig. 2, B and C) compared with the hypoxic 5 mM control and the normoxic time- and glucose-matched control (Fig. 2C).

**Sheep Studies**

Chronic maternal hyperglycemia caused an initial threefold increase by 48 h in placental GLUT-1 levels.
followed by a persistent 40–50% decline in GLUT-1 levels from 10 to 20 days (Fig. 3, A and B). Chronic maternal hypoglycemia caused a decline in placental GLUT-1 levels as seen by the Western blot analysis and by quantitation (Fig. 3, C and D). This decrease in GLUT-1 is similar to that seen in the rat uteroplacental insufficiency model. The 30% decline in ovine placental GLUT-1 began at 24–48 h followed by a 60% decline at 15–25 days and back to a 30% decline by 33–41 days (Fig. 3D).

**DISCUSSION**

The predominant placental glucose transporter isoform is GLUT-1 (2). This isoform is expressed by trophoblastic cells distributed in the junctional and labyrinthine regions of the mouse and rat placenta (12, 33), which are hemochorial in nature. Thus, by way of its localization, GLUT-1 appears to mediate glucose transport both into the placenta and to the fetus (12, 33). We examined this isoform exclusively in the present study and noted no effect of maternal diabetes on placental GLUT-1 concentrations. This lack of effect secondary to a maternal diabetic state of 6 days duration is similar to previously reported studies when streptozotocin-induced maternal diabetes of 20 days was associated with uteroplacental insufficiency, as evidenced by a decline in placental and fetal weights (6, 11). In both cases of short-term and long-term (6) maternal diabetes, regardless of the placental and fetal growth characteristics, high circulating maternal and fetal glucose concentrations did not alter the net placen-
tional GLUT-1 protein levels. Similar observations have been made in human hemochorial diabetic placentas as well (23). These observations both in the human and rat placentas are different from the time-dependent and glucose concentration-dependent decline in GLUT-1 protein levels noted in our current in vitro rat trophoblastic studies and in the ovine epitheliochorial placenta. In both cases, ongoing exposure of trophoblasts to high concentrations of glucose results in a diminution of the GLUT-1 protein concentrations. Evidence in vitro supports the role of hypoxia in modifying the regulatory influence of high glucose concentrations on trophoblastic GLUT-1 amounts. In a disease state of maternal diabetes, where other associated changes besides hyperglycemia occur, the concomitant presence of placental ischemia-induced hypoxia in both the rat (6, 11, 23) and human may mask the time-dependent expected hyperglycemia-induced decline in placental GLUT-1 levels.

Despite the decline in ovine placental GLUT-1 when exposed to hyperglycemia and no change in the rat diabetic placental GLUT-1 levels, the presence of fetal hyperglycemia suggests a change in the intrinsic activity of the GLUT-1 protein leading to an increased affinity for transporting glucose. This change in the intrinsic activity of a glucose transporter protein is reliant on a conformational change in the tertiary or quaternary structure of the protein, including isomerization (3, 34). The decline in ovine placental GLUT-1 concentrations secondary to hyperglycemia is preceded by a short-term (48 h) increase in the protein levels. This increase reflects an immediate attempt to handle the increased maternal glucose load. However, during this early period of maternal and fetal hyperglycemia there is a concomitant increase in fetal insulin concentrations, which subsequently normalize. Whether circulating fetal insulin concentrations can regulate placental GLUT-1 concentrations independently of hyperglycemia, particularly when insulin receptors have previously been localized to the maternal surface of the placenta (31), remains to be investigated.

Low maternal circulating glucose concentrations due to an insulin infusion led to concomitant fetal hypoglycemia with hypoinsulinemia. In this ovine model, there was a persistent decline in placental GLUT-1 concentrations. These observations are identical to that noted in a 72-h uteroplacental insufficiency rat model, where placental GLUT-1 levels were also diminished. Particularly in the rat, maternal uterine artery ligation leads to fetoplacental ischemic hypoxia, as evidenced by adverse effects on fetoplacental weight (22, 30). The presence of concomitant placental cellular ischemic hypoxia could potentially alter the degree of the resultant GLUT-1 decline observed. Furthermore, the added presence of fetal hypoinsulinemia may also regulate the placental GLUT-1 concentrations. The degree of ischemic hypoxia for a longer duration than 48 h and the levels of fetal circulating insulin concentrations may have been responsible for the absence of a change in human placental GLUT-1 concentrations when associated with IUGR secondary to preedampsia or maternal hypertension (18). In vitro isolated trophoblastic cells did not demonstrate any change in the GLUT-1 levels under normoxic conditions in the absence of extracellular glucose, a protective mechanism whereby the cell perhaps maintains its viability in an isolated state (19). The added presence of hypoxia, however, tended toward an increase in the trophoblastic GLUT-1 protein concentrations in a time-dependent manner. The effect of hypoxia has been noted in vivo to increase GLUT-1 concentrations in tissues other than the placenta and more recently the cis elements involved in mediating hypoxia-induced GLUT-1 transcription have been identified (15, 27). Our present in vivo investigations in the rat and sheep support the concept that a lowered maternal glucose load leads to an adaptive decline in placental GLUT-1 concentrations in the face of limited substrate availability. This, in turn, may reduce the transport of substrate to both the placenta and fetus, further potentiating diminished growth.

The other glucose transporter isoform that is expressed by the rat placenta is GLUT-3 (6, 33). Placental expression of GLUT-3 has been more variable in the human (1, 2). GLUT-3 is the most efficient transporter and functions in the face of diminished substrate availability (3). We did not address GLUT-3 in our present report, because previous studies in the 20-day streptozotocin-induced maternal diabetic state demonstrated an increase in placental GLUT-3 concentrations (6). Preliminary studies by us suggest no change in the rat UGR placental GLUT-3 compared with sham-operated controls. Our attempts at detecting ovine placental GLUT-3 with an anti-mouse GLUT-3 antibody (12, 25) revealed absence of cross-reactivity, attesting to the species specificity of the anti-murine GLUT-3 antibody, unlike that of GLUT-1. The need exists for generating an ovine-specific anti-GLUT-3 antibody based on the published partial sequence information (4) before undertaking comparative rodent and ovine placental GLUT-3 studies similar to our present investigation with GLUT-1.

In conclusion, changes in placental GLUT-1 concentrations are not species specific, being similar in rodent (hemochorial) and ovine (epitheliochorial) species, are time dependent, and are isoform specific. Conditions causing fetal hypoglycemia lead to a diminution in placental GLUT-1 concentrations. Hyperglycemia (maternal and fetal) causes an increase in placental GLUT-1 within 48 h followed subsequently by a decline in placental GLUT-1 concentrations with the development of fetal hyperglycemia. The presence of fetal hyperglycemia despite a decline in placental GLUT-1 levels attests to a conformational change in the protein, resulting in an increased intrinsic activity or the contribution of another glucose transporter isoform (GLUT-3). Hypoxia independently augments the levels of placental GLUT-1, thereby modifying the effect of glucose beyond physiological concentrations on placental GLUT-1 concentrations. The concomitant presence of ischemic hypoxia may reconcile the differences between the in vitro and in vivo effects of glucose on trophoblastic GLUT-1 concentrations. The in vivo changes in
placental GLUT-1 concentrations secondary to various perturbations may play a role in substrate delivery and the ultimate growth and size of the fetoplacental unit.

Perspectives

GLUT-1, the major facilitative glucose transporter of tissue barriers, such as the trophoblastic cell layer of mammalian placenta, mediates maternoplacentofetal glucose transport, thereby influencing fetal metabolism and growth. Perturbations in maternal circulating glucose concentrations affect placentofetal delivery of glucose by regulating the expression of placental GLUT-1 in a time-dependent manner. Acute changes reflect adjustments necessary for immediate handling of the altered glucose load presented to the placenta, whereas chronic aberrations reflect placental attempts of the altered glucose load presented to the placenta.

Future studies should focus on the in vivo effects of placental GLUT-1 levels. One such alteration is the ischemic hypoxia encountered in uteroplacental insufficiency due to either maternal diabetes or maternal uterine artery ligation, where placental GLUT-1 levels may be increased when extracellular or circulating glucose concentrations are perturbed. This is suggestive of the need to ensure adequate glucose delivery for placental cellular oxidation in an energy-deprived or toxic state. Future studies should focus on the in vivo effects of hypoxia alone independent of placental ischemia in a time-dependent manner on placental GLUT-1 expression. It is also important to determine the interplay between placental GLUT-1 and GLUT-3, the second facilitative glucose transporter isoform known to be expressed in mammalian placenta.

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