High concentration of glucose decreases glucose transporter-1 expression in mouse placenta in vitro and in vivo

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Abstract

Facilitative glucose transporter-1 (GLUT1) is expressed abundantly and has an important role in glucose transfer in placentas. However, little is known about the regulation of GLUT1 expression in placental cells. We studied the changes in placental GLUT1 levels in relation to changes in glucose concentration in vitro and in vivo. In in vitro experiments, dispersed mouse placental cells were incubated under control (5·5 mM) and moderately high (22 mM) glucose concentrations, and 2-deoxyglucose uptake into cells was studied on days 1–5 of culture. After 4 days of incubation under both conditions, GLUT1 mRNA and protein levels were examined by Northern and immunoblot analyses. Treatment of cells with 22 mM glucose resulted in a significant decrease in 2-deoxyglucose uptake compared with control, from day 2 to day 5 of culture. Moreover, GLUT1 mRNA and protein levels on day 4 of culture were significantly reduced in cells incubated with 22 mM glucose compared with control. Next, we rendered mice diabetic by administering 200 µg/g body weight streptozotocin (STZ) on day 8 of pregnancy. Animals were killed on day 12 of pregnancy and placental tissues were obtained. [3H]Cytochalasin B binding study was carried out to assess total GLUTs, and GLUT1 mRNA and protein were measured as above. [3H]Cytochalasin B binding sites in placentas from STZ-treated mice were significantly less than those in control mice. Northern and immunoblot analyses revealed a significant decrease in GLUT1 mRNA and protein levels in diabetic mice compared with the controls. These findings suggest that the glucose concentration may regulate the expression of placental GLUT1.

Introduction

Transfer of glucose from the maternal circulation to the fetus is fundamental in the utero–placental–fetal transfer system (Danics & Schneider 1975). Fetal plasma glucose concentrations have been shown to correlate with maternal glucose concentrations (Economides & Nicolaides 1989). The glucose transfer across the placenta is facilitated, and is independent of the Na⁺ gradient (Johnson & Smith 1980). The expression of glucose transporters (GLUTs) shows a tissue-specific distribution. For example, GLUT1 has been found in erythrocytes, kidney and blood–tissue barriers, including those in the brain and placenta (Mueckler et al. 1985, Asano et al. 1988). GLUT3 has been shown to be abundant in organs with high glucose requirements, such as the brain (Haber et al. 1993), and to be expressed in the placenta in rodents (Shepherd et al. 1992, Haber et al. 1993, Zhou & Bondy 1993, Boileau et al. 1995). Although GLUT3 mRNA has been reported to be expressed in many tissues, including human and rat placentas (Kayano et al. 1990), it was not easy to detect GLUT3 protein by immunoblot analysis (Haber et al. 1993, Jansson et al. 1993) because of the low level expression of GLUT3 in human placenta (Shepherd et al. 1992). These observations suggest that GLUT1, not GLUT3, is the major GLUT isoform in human placenta.

Two reports in humans (Jansson et al. 1993, Sakata et al. 1995), a report in rats (Zhou & Bondy 1993), and a report in mice (Yamaguchi et al. 1996) describe the changes in placental GLUT gene expression during pregnancy. In the human placenta, GLUT1 is a dominantly expressed glucose transporter and its levels increase during pregnancy (Jansson et al. 1993, Sakata et al. 1995). The data in rats (Zhou & Bondy 1993) showed that placental GLUT1 mRNA decreased throughout pregnancy, whereas placental GLUT3 mRNA showed little change after midpregnancy. However, in mice, we have shown that the level of GLUT1 mRNA increased after midpregnancy, not only in the placenta, but also in the decidua (Yamaguchi et al. 1996). An increase in the level of GLUT1 in the utero–placental unit during pregnancy may be helpful in the increased requirement of glucose in the fetus, especially after midpregnancy.

It is known also that the fetal plasma glucose concentration increases when the mother is diabetic and hyperglycemic (Pedersen 1977). Despite the importance of
GLUTs, the changes in the level of placental GLUT in the presence of a high glucose concentration have not been clearly elucidated. In this study we analyzed the effect of high glucose concentration on placental glucose transport and the levels of GLUT1 protein and mRNA, using a primary mouse placental cell culture and the placental tissues from pregnant mice with streptozotocin-induced diabetes.

Materials and Methods

Chemicals

Cytochalasin B, d- and l-glucose, EDTA, aprotinin, leupeptin, pepstatin A, antipain, soybean trypsin inhibitor and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma (St Louis, MO, USA). 2-[1,2-3H]Deoxy-d-glucose (30 Ci/mmol) and 5-[1,2-3H]hydroxytryptamine (serotonin) (28 Ci/mmol) were purchased from DuPont New England Nuclear (Boston, MA, USA). Glucose (30 Ci/mmol) and 5-[1,2-3H]hydroxytryptamine were purchased from DuPont New England Nuclear (Boston, MA, USA) and 5% fetal calf serum (FCS). Glucose concentrations were adjusted and the cells were incubated for 5 days. The medium was changed daily. The day on which the cells were plated was considered as day 0.

Animals and placental cell culture

The Institute of Cancer Research (ICR) pregnant mice (10 weeks old), which originated from Charles River Laboratories Inc., (Wilmington, MA, USA), were purchased from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan). Tissue for cell culture was obtained on day 12 of gestation. Mice were killed by spinal dislocation on day 12, and placentas and fetuses were collected, frozen in liquid nitrogen, and kept at −80 °C until required for use. Diabetes was ascertained by a blood glucose concentration greater than 250 mg/dl. All subsequent procedures for placental membrane preparation were performed at 4 °C. Pieces of placentas were homogenized using a Dounce homogenizer in a buffer containing 250 mM sucrose, 10 mM Tris–HCl (pH 7.4), 5 mM EDTA, 10 mg/ml of a mixture of leupeptin, pepstatin A, aprotinin and antipain, 50 µg/ml soybean trypsin inhibitor and benzamide and 1 mM PMSF as described previously (Sakata et al. 1992). The homogenate was centrifuged at 45 000 g for 1 h and the resultant supernatant was centrifuged at 45 000 g for 1 h (Sakata et al. 1992). The final pellet was resuspended in a buffer containing 20 mM Tris–HCl, 1 mM EDTA, 100 mM NaCl and 4 mM MgCl2, to determine protein concentration (Sakata et al. 1992).

Measurement of 2-deoxyglucose uptake

Uptake of 2-[3H]deoxyglucose, a non-metabolizable analog of glucose, was measured in 12 wells per group in each experiment on mouse placental cells plated in 24-well plates as described previously (Kitagawa et al. 1991), with modifications. Each experiment was repeated twice with similar results; representative results are shown. After the medium was removed, the cells were washed three times with 1·0 ml prewarmed PBS and incubated in 1·0 ml prewarmed transport buffer containing 25 mM HEPES, pH 7·4, 0·8 mM MgSO4, 140 mM NaCl, 5·4 mM KCl, 1·8 mM CaCl2, and 1 µCi/ml 2-[3H]deoxyglucose (1 µM) at 37 °C for 10 min. To terminate 2-[3H]deoxyglucose uptake, the radioactive buffer containing 2-[3H]deoxyglucose was removed and rapidly washed three times with ice-cold transport buffer. Incorporation of the radioactivity into the cells was linear during this period (data not shown). The cells were solubilized in

Cells were plated in multiwell plates at a density of $1 \times 10^3$/cm² and were allowed to attach for 2 h under an atmosphere of 95% air/5% CO₂ at 37 °C. Then the medium was exchanged for a fresh one containing 2% FCS. Glucose concentrations were adjusted and the cells were incubated for 5 days. The medium was changed daily. The day on which the cells were plated was considered as day 0.

Animal models for diabetes melitus and preparation of placental membrane

Time-pregnant female ICR mice were housed under standard conditions and allowed free access to standard food and water. Diabetes was induced by intraperitoneal (i.p.) injection of 200 µg/g body weight of streptozotocin (STZ) dissolved in 50 mM sodium citrated buffer, pH 4·5 on day 8 of gestation. Mice were killed by spinal dislocation on day 12, and placentas and fetuses were collected, frozen in liquid nitrogen, and kept at −80 °C until required for use. Diabetes was ascertained by a blood glucose concentration greater than 250 mg/dl. All subsequent procedures for placental membrane preparation were performed at 4 °C. Pieces of placentas were homogenized using a Dounce homogenizer in a buffer containing 250 mM sucrose, 10 mM Tris–HCl (pH 7·4), 5 mM EDTA, 10 mg/ml of a mixture of leupeptin, pepstatin A, aprotinin and antipain, 50 µg/ml soybean trypsin inhibitor and benzamide and 1 mM PMSF as described previously (Sakata et al. 1992). The homogenate was centrifuged at 2600 g for 10 min and the resultant supernatant was centrifuged at 45 000 g for 1 h (Sakata et al. 1992). The final pellet was resuspended in a buffer containing 20 mM Tris–HCl, 1 mM EDTA, 100 mM NaCl and 4 mM MgCl2, to determine protein concentration (Sakata et al. 1992).

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0.6 ml 0.03% sodium dodecyl sulfate (SDS). A 0.4-ml aliquot was counted in a liquid scintillation counter. A 0.1-ml aliquot was used to determine protein concentration using a DC protein assay kit (Bio-Rad, Richmond, CA, USA). Non-carrier-mediated uptake of 2-deoxyglucose was determined in parallel wells containing either 10 μM cytochalasin B or 100 mM d-glucose.

Measurement of serotonin and leucine uptake

Serotonin uptake was studied as described previously (Cool et al. 1991). Cells were incubated for 45 min at room temperature before the measurement of serotonin uptake, in a buffer containing 25 mM HEPES, pH 7.5, 0.8 mM MgSO₄, 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 5 mM d-glucose and 0.1 mM iproniazid (transport buffer). After the incubation, serotonin uptake was studied by adding 1 ml transport buffer containing 50 nM radiolabeled serotonin. After 20 min of incubation, the radioactive buffer was removed and each well was rapidly washed three times with transport buffer containing 0.1 mM imipramine. Cells were solubilized and the radioactivity incorporated into the cells was counted as described above. Leucine uptake was studied using a procedure similar to that described above, except that the preincubation with iproniazid was omitted and the washing buffer did not contain imipramine. Uptake of serotonin and leucine was measured in 12 wells per group in each experiment on mouse placental cells plated in 24-well plates. Each experiment was repeated twice.

Northern blot analysis

Total RNA was prepared from cells cultured with 5.5 mM or 22 mM glucose for 4 days. Total RNA was also prepared from placentas of pregnant mice with or without the STZ treatment. Twenty micrograms total RNA was denatured and electrophoresed on a 1% agarose–0.66 M formaldehyde gel and blotted onto a nylon membrane filter (Zeta-Probe; Bio-Rad). The filter was hybridized for 18 h at 43 °C in a hybridizing buffer containing 2 × 10⁶ c.p.m./ml 32P-labeled rabbit GLUT1 cDNA probe (Asano et al. 1988). The coding region of this rabbit cDNA (Asano et al. 1988) shared 93.2% and 89.4% nucleotide homology with that of human (Mueckler et al. 1985) and rat (Birnbaum et al. 1986) glucose transporter respectively. The EcoRI-digested insert of this cDNA can be used as a probe for Northern blot analysis in mouse (Asano et al. 1988). The filter was washed and autoradiographed for 3 days at −80 °C. The filter was stripped, rehybridized with 32P-labeled β-actin (Cleveland et al. 1980) probes and autoradiographed for 1 day at −80 °C. The band intensities were analyzed with a densitometer (Imaging Research Inc., St Catharines, Ontario, Canada) and the amounts of GLUT1 mRNA were divided by those of β-actin mRNA.

Immunoblot analysis

Cells incubated with 5.5 mM or 22 mM glucose for 5 days were washed with NCTC–135 medium and treated as described previously (Sakata et al. 1992). After treatment with detachment buffer (250 mM sucrose, 10 mM Tris, pH 7.4, 1 mM EDTA, 0.3 mM PMSF, 1 μg/ml each pepstatin and leupeptin), cells were scraped off the plates, collected by centrifugation at 12 000 g at 4 °C for 10 min, and solubilized with 1% Triton X-100. After preparation of placental membranes, aliquots were denatured with an equal amount of buffer containing 200 mM dithiothreitol, 20% glycerol, 0.04% bromophenol blue, 10% SDS and 120 mM Tris–HCl (pH 6.8) at 60 °C for 15 min to avoid aggregation of GLUT proteins (Morita et al. 1992). In in vivo experiments, placentas were homogenized in a ice-cold sucrose buffer (250 mM sucrose, 10 mM Tris, pH 7.4, 5 mM EDTA, 0.3 mM PMSF, 1 μg/ml each aprotinin and leupeptin) (Sakata et al. 1992), then centrifuged at 2600 g for 10 min at 4 °C. The supernatant was centrifuged at 45 000 g for 60 min at 4 °C. The crude membrane pellet was resuspended in a cold resuspension buffer (20 mM Tris, pH 7.4, 1 mM EDTA, 100 mM NaCl, and 4 mM MgCl₂) (Sakata et al. 1992). The protein concentration was determined using the Bradford method. Twenty-five micrograms denatured samples were subjected to 10% SDS–PAGE (Laemmli 1970), and transferred to a nitrocellulose filter (Wilson et al. 1984) (Bio-Rad). The filter was incubated with a rabbit anti-GLUT1 antiserum (Oka et al. 1988) at a dilution of 1:8000, followed by a second incubation with goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase (Bio-Rad) at a dilution of 1:16 000. Then the filter was stained with an enhanced chemiluminescence (ECL) system (Amersham, Arlington Heights, IL, USA) and exposed to a Kodak X-Omat film (Kodak, Rochester, NY, USA) for 15 s. The band intensities were analyzed as described above.

[^]H[cytochalasin B binding assay]

D-Glucose-protectable binding of cytochalasin B is established as a reliable assessment of the total number of glucose transporters (Gorge & Lienhard 1981).[^]H]Cytochalasin B binding assay was performed as described previously (Gorge & Lienhard 1981). To determine[^]H]cytochalasin B-specific binding simultaneously in all the membrane preparations from placentas of control and STZ treated mice, 400 nM[^]H]cytochalasin B was used in the binding assay as described previously (Gorge & Lienhard 1981). Membrane-bound and free[^]H]cytochalasin B were separated by a rapid filtration system using GF/B glass fiber filters (Whatman International Ltd,
Maidstone, UK). The filters were dried and counted for radioactivity in 10 ml liquid scintillation fluid.

Statistical methods

Data were expressed as the mean ± s.e., and were statistically analyzed using analysis of variance followed by unpaired t-test or Scheffe’s multiple range test. Statistical significance was determined at P<0.05.

Results

Incubation of mouse placental cells with 22 mM glucose significantly (P<0.01) reduced the 2-deoxyglucose uptake from day 2 to day 5 of culture (Fig. 1a). This inhibitory effect of 22 mM glucose on 2-deoxyglucose uptake was not observed on day 1. Figure 1b shows that the inhibitory effect of glucose was dose-dependent.

To rule out the possibility that the inhibitory effect of 22 mM glucose on 2-deoxyglucose uptake may not be the result of glucose toxicity.

To study whether the GLUT1 synthesis is decreased by 22 mM glucose, we analyzed the changes in steady-state GLUT1 mRNA levels by Northern blot analysis. After the mouse placental cells had been incubated with 5·5 mM (control) or 22 mM glucose for 4 days, total RNA samples were obtained. Hybridization with a 32P-labeled GLUT1 cDNA probe (Asano et al. 1988) yielded a single band of approximately 2·8 kb (data not shown). The amounts of GLUT1 mRNA were normalized with reference to those of β-actin mRNA. The GLUT1 band intensity in cells treated with 22 mM glucose was significantly (P<0.01) decreased compared with control (normalized band intensity (arbitrary units): control, 2·3±0·3; 22 mM, 1·5±0·1) (Fig. 3). The filter was rehybridized with a 32P-labeled GLUT3 cDNA. GLUT3 mRNA could not be detected, even in an autoradiograph exposed for more than 10 days at −80 °C (data not shown).

To examine the effect of 22 mM glucose on the GLUT1 protein level, immunoblot analysis was performed. Mouse placental cells were incubated with 5·5 mM (control) or with 22 mM glucose for 4 days and solubilized with 1% Triton X-100. Amounts of 49 000-Da protein detected by a rabbit anti-GLUT1 antiserum were also decreased by 22 mM glucose (Fig. 4a), consistent with the results from 2-deoxyglucose uptake and Northern blot analysis. GLUT1 protein band intensity in cells treated...
with 22 mM glucose was significantly (P<0.01) decreased compared with control (normalized band intensity (arbitrary units): control, 0.42±0.05; 22 mM, 0.23±0.04) (Fig. 4b). A significant (P<0.01) time-dependent decrease in GLUT1 protein in response to 22 mM glucose treatment compared with that in controls (5.5 mM glucose) was observed on days 3 and 4 of culture (Fig. 4c).

Placental glucose transport has been suggested to be independent of insulin stimulation (Challier et al. 1986), in spite of the presence of large amounts of placental insulin receptors (Jones et al. 1993). Therefore, we investigated the effect of 1 and 5 µg/ml insulin on glucose uptake by placental cells. Treatment of mouse placental cells with insulin for 4 days had no significant effect on the level of 2-[3H]deoxy-d-glucose uptake (Fig. 5), consistent with the results in previous reports (Challier et al. 1986). STZ-treated pregnant mice were sacrificed on day 12 of pregnancy. Maternal and fetal weight, crown–rump length (CRL) of the fetuses, maternal and fetal blood glucose concentrations, and maternal insulin concentrations are shown in Table 1. STZ-treated pregnant mice were significantly (P<0.01) hyperglycemic and hypoinsulinemic compared with controls. Fetal blood glucose concentrations of STZ-treated pregnant mice were significantly (P<0.01) greater than those of controls. Fetuses showed significant (P<0.01) growth retardation under these conditions (Table 1). Figure 6 shows the levels of [3H]cytochalasin B specific binding was determined in placentas from control and STZ-treated mice on days 10, 12, 16, and 18 of pregnancy. On day 10 of pregnancy, we failed to find a significant difference in [3H]cytochalasin B binding levels between control and STZ-treated mice (data not shown). On day 12 of pregnancy, the levels of [3H]cytochalasin B binding to placental tissues in STZ-treated mice (1.83±0.45 pmol/mg protein) were significantly (P<0.05) lower than those in the controls (3.52±0.48 pmol/mg protein) (Fig. 6). On day 16 and 18 of pregnancy, the levels of [3H]cytochalasin B binding to placental tissues in STZ-treated mice were substantially lower than those in the controls, but not statistically significant, because of a large variation in the [3H]cytochalasin B binding level. This may have resulted from the difficulty in separating placental tissues from decidua, which was easily performed on day 12 of pregnancy. Therefore, we performed the [3H]cytochalasin B binding assay on day 12 of pregnancy. The mothers' brain levels of [3H]cytochalasin B binding in STZ-treated mice (1.32±0.48 pmol/mg protein) were also significantly (P<0.05) lower than those in the controls (2.55±0.13 pmol/mg protein) (Fig. 6).

[3H]Cytochalasin B recognizes not only GLUT1, but also other GLUTs (Mueckler et al. 1985, Kayano et al. 1990). Therefore, the results observed in Fig. 6 are the changes in levels of total GLUTs, and may not specifically reflect the changes in GLUT1. To determine the effect of...
maternal diabetes on placental GLUT1 mRNA expression, we analyzed the amounts of the steady-state GLUT1 mRNA in placentas from STZ-treated and control mice. GLUT1 mRNA was observed at 2·8 kb in all RNA samples examined (data not shown). The GLUT1 band intensity of placentas from STZ-treated mice was significantly \( (P<0·01) \) decreased compared with control (normalized band intensity (arbitrary units): control, 1·26 ± 0·12; STZ-treated, 0·76 ± 0·23) (Fig. 7). The filter was rehybridized with a \(^{32}\)P-labeled GLUT3 cDNA. GLUT3 mRNA could not be detected, even in an autoradiograph exposed for more than 10 days at \(-80\) °C (data not shown).

To determine the effect of maternal diabetes on placental GLUT1 protein level, amounts of GLUT1 protein were determined in placentas from diabetic mice compared with control mice by immunoblot analysis using a specific antiserum against GLUT1 (Oka et al. 1988). When the filter was incubated with a non-immune serum, no specific bands were observed (data not shown). GLUT1 protein level in placentas from diabetic mice was significantly \( (P<0·01) \) decreased compared with that in controls (normalized band intensity (arbitrary units): control, 5·66 ± 0·35; STZ-treated, 3·23 ± 0·20) (Fig. 8), consistent with the results in the \([^{3}H]\)cytochalasin B binding assay and Northern blot analysis.

Discussion

In mouse placentas, immunohistochemical studies reveal that GLUT1 protein is observed mainly in the membranes found on both sides of the trilaminar epithelial cells that predominantly line the labyrinthine maternal vascular spaces, and less abundantly in those of trophoblastic giant...
cells (Devaskar et al. 1994). These findings suggest that GLUT1 expression in mouse placenta is specifically localized to cells responsible for transplacental transport of glucose. GLUT3 immunoreactivity is observed in the trilaminar epithelial and basal trophoblastic cells (Devaskar et al. 1994). The GLUT1 mRNA level in the mouse placenta increases as gestation proceeds, whereas the amount of GLUT3 mRNA is unchanged throughout gestation (Yamaguchi et al. 1996). These observations suggest that GLUT1 is a main glucose transporter in mouse placenta, from midpregnancy through to term.

In this study, we failed to detect GLUT3 mRNA in mouse placenta on day 12 of pregnancy. Although a previous study using 2 µg poly(A)+ RNA demonstrated the presence of GLUT3 mRNA in mouse placenta, GLUT3 mRNA expression on day 13 of pregnancy was faint (Yamaguchi et al. 1996). These results suggest that the amount of GLUT3 mRNA expression in mouse placenta was insufficient to be detected by Northern blot analysis using 20 µg total RNA.

Table 1 Physiological parameters in control and STZ-treated pregnant mice on day 12 of gestation. (STZ was injected i.p. on day 8 of gestation)

<table>
<thead>
<tr>
<th>Group</th>
<th>Placental weight (mg)</th>
<th>Fetal weight (mg)</th>
<th>CRL (mm)</th>
<th>Maternal blood glucose (mg/dl)</th>
<th>Maternal serum insulin (ng/ml)</th>
<th>Fetal blood glucose (mg/dl)</th>
</tr>
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<tr>
<td>Control (n=17)</td>
<td>81.6 ± 8.5</td>
<td>214.4 ± 28.1</td>
<td>10.9 ± 0.64</td>
<td>156.4 ± 28.9</td>
<td>1303.9 ± 347.5</td>
<td>40.9 ± 16.4</td>
</tr>
<tr>
<td>STZ-treated (n=11)</td>
<td>59.9 ± 12.2**</td>
<td>153.4 ± 44.5**</td>
<td>9.6 ± 1.0**</td>
<td>436.9 ± 169.3**</td>
<td>425.2 ± 172.6**</td>
<td>232.2 ± 56.3**</td>
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CRL, crown–rump length.
**P<0.01 compared with control.

To date, the regulation of placental GLUT1 has not been extensively investigated. In the mouse placental cell culture, we have demonstrated that cAMP inhibited glucose transport activity (Sakata et al. 1996), suggesting that cAMP was a potent modulator of mouse placental GLUT1. In this report, incubation with a moderately high concentration (22 mM=400 mg/dl) of glucose decreased mouse placental 2-[3H]deoxy-β-glucose uptake (Fig. 1a, b) and expression of GLUT1 protein (Fig. 4) and mRNA (Fig. 3). GLUT1 protein level in mouse placental cells on day 4 of culture in 22 mM glucose medium was diminished by 45% (Fig. 4b), but 2-deoxyglucose uptake was diminished by only 30% compared with control (Fig. 1a). Although it remains unclear why the changes observed in immunoblot analysis were larger than those in glucose uptake experiments, this phenomenon has been observed previously (Sakata et al. 1996).

Figure 6 Levels of [3H]cytochalasin B binding to placental (n=13) and brain (n=3) membranes in the control and the STZ-treated mice on day 12 of gestation. Results are expressed as mean ± S.E. of three separate experiments. **P<0.01: statistically significant difference from control (unpaired t-test).

Figure 7 Northern blot analysis of GLUT1 mRNA in mouse placenta from the control and the STZ-treated mice on day 12 of gestation. Total RNA (20 µg) was electrophoresed and hybridized with [32P]-labeled GLUT1 or with β-actin cDNA probes. Band intensities were quantitated by densitometry; that of GLUT1 mRNA was normalized with respect to that of β-actin. Densitometry data obtained from three individual Northern blots are shown in arbitrary units. Results are expressed as mean ± S.E. of three separate experiments. **P<0.01: statistically significant difference from control (unpaired t-test, n=3).
A previous study demonstrated that incubation with extremely low concentrations of D-glucose (0–18.2 mg/dl) enhanced 2-[14C]deoxy-D-glucose uptake in human first-trimester trophoblast-like cells, and that the effects of D-glucose on GLUT1 mRNA level paralleled the uptake of 2-[14C]deoxy-D-glucose (Gordon et al. 1995). Other studies, and our data, suggest that the glucose concentration may be a potent regulator of GLUT1 expression in the placental cells.

In this study, we observed an increase in glucose uptake and in GLUT1 levels from day 1 to day 4 of culture in the presence of a 5.5-mM glucose concentration (Figs 1a, 4c). The mechanisms responsible for this time-dependent increase are not fully understood. A previous study using cultured human trophoblast cells showed that the level of GLUT1 mRNA was significantly increased during 3 h of incubation with 20% FCS, and declined to basal values after 20 h of culture (Mouzon et al. 1994). We examined the acute effect of a high concentration of glucose on glucose uptake in mouse placenta. Within 24 h, treatment with 22 mM glucose had no significant effects on glucose uptake compared with controls (data not shown). Another investigation using an HRP.1 rat placental cell line revealed that treatment of 0 and 5 mM glucose did not affect GLUT1 protein levels during 48 h of incubation (Das et al. 1998). The differences between these data and ours may derive from the fact that different species and cell types were used in these studies. Our previous report (Sakata et al. 1996) demonstrated a time-dependent increase in glucose uptake in mouse placental cells that was consistent with the data in the present Fig. 1a and with those from human first-trimester chorionic villi (Gordon et al. 1995).

Insulin is an important modulator of GLUT4 in muscle and adipose tissues. Although placentas have abundant insulin binding sites (Jones et al. 1993), it has been accepted that the placenta is insulin-insensitive for glucose transport (Challier et al. 1986), which is consistent with our data (Fig. 5).

STZ, a nitrosourea derivative of Streptomyces achromogenes that destroys β-cells in pancreas (Schein et al. 1967), has been used for induction of diabetes in rats (Younes et al. 1980) and mice (Schein et al. 1967). A previous study demonstrated that brain expression of GLUT1 mRNA in STZ–treated adult rats was significantly decreased compared with that in controls, which is similar with our data (Fig. 6), whereas liver and kidney GLUT1 levels were not changed by STZ treatment (Koranyi et al. 1991). These findings suggest that the concentration of GLUT1 in the brain was decreased to protect the brain – an organ indispensable for life – from the toxicity of hyperglycemia. It is known that placental glucose transport was closely related to maternal plasma glucose concentration (Battaglia & Meschia 1978) and that glucose transport from the mother to the fetus is enhanced in diabetic rats (Thomas et al. 1990). Our in vitro finding that the placental GLUT1 level was decreased by a moderately high glucose concentration may enable us to speculate that placental GLUT1 expression was down-regulated by a hyperglycemia, to protect fetuses from the glucose toxicity during pregnancy. To confirm this speculation, we investigated the effect of hyperglycemia on placental GLUT1 expression, fetal growth and fetal plasma glucose concentration, using an STZ–treated pregnant mouse model. We found that placental GLUT1 levels decreased in STZ–treated pregnant mice compared with those in controls, which was consistent with our speculation. However, this protective mechanism may not be established within the fetal tissues because the levels of GLUT1 in the fetal muscle of STZ–treated pregnant mice (36.1 mM blood glucose) were greater than those of the controls (Atkins et al. 1994). These results suggest that the placenta, but not the fetus, might have a mechanism of protection from maternal hyperglycemia.

A previous study in rats demonstrated that GLUT1 mRNA and protein levels in placentas from STZ–treated animals remained unchanged compared with controls, whereas GLUT3 mRNA and protein expression in placentas from diabetic rats increased compared with controls (Boileau et al. 1995). The findings in another report (Das et al. 1998), that there was no significant change in rat placental GLUT1 protein levels after STZ treatment, were different from our data. This may be attributable to the facts that the STZ–treated rats did not
exhibit intraterine fetal growth retardation, and that rat placentas from day 20 of pregnancy were used in the previous report, whereas the fetuses were growth-retarded and mouse placentas from day 12 of pregnancy were used in our study.

Finally, these results may enable us to accept the concept that the placental expression of GLUT1 in the diabetic mouse may be down-regulated to inhibit further excessive glucose supply from the mother to the fetus. Further investigation will be necessary to clarify the regulatory mechanisms of placental glucose transport.

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