Glucose-dependent Regulation of Glucose Transport Activity, Protein, and mRNA in Primary Cultures of Rat Brain Glial Cells*

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The facilitative D-glucose transporter is a $M_\text{r} = 54,000$ integral membrane glycoprotein containing multiple membrane spanning domains (1–3) and is responsible for the movement of D-glucose across the cell surface membrane (2, 4). This transport system is found almost universally in animal tissues (4, 5) and is especially abundant in human erythrocytes, placenta, and brain (4–15). The regulation of D-glucose transport activity in response to altered metabolic states (high and low D-glucose concentrations) has been studied in a variety of tissue culture cell types (4, 16–20). These studies have demonstrated that maintenance of these cells in the absence of D-glucose (replacement with D-fructose, D-xylose, or uridine) for 24 h resulted in the specific elevation in the level of D-glucose transport activity compared to cells maintained under typical (25 mM D-glucose) culture conditions. This D-glucose starvation-induced increase in D-glucose transport activity occurred with an increase in the $V_{\text{max}}$ for transport with no significant effect on the D-glucose $K_m$ (17, 21–25). More recently, the starvation induction of D-glucose transport activity has been observed to result from an increase in the number of D-glucose transporter molecules (20, 26–29).

The molecular mechanism responsible for the D-glucose-dependent regulation of D-glucose transport activity has not been established to date. Several studies have suggested that the D-glucose starvation-induced increase in D-glucose transport activity is protein (16, 18, 21, 27, 36–41) and/or RNA (17, 42) synthesis-dependent. In contrast, several reports have also indicated that the increase in D-glucose transport activity induced by D-glucose deprivation is independent of protein (22, 43, 44) and/or RNA synthesis (21, 43–45) and thus have been interpreted to suggest an inhibition of D-glucose transporter protein degradation. These conflicting results underscore the necessity for detailed analysis of this transport system under clearly defined cellular and experimental conditions. For this purpose, we have recently developed the methodology to isolate and culture homogeneous primary rat brain neuronal and glial cell populations (46). This avoids complications of in vitro studies due to the presence of the endothelial cell barrier and in vitro effects due to cell immortalization. Further, the brain is relatively enriched in the D-glucose transporter protein and in the D-glucose transporter mRNA compared to several other tissues such as kidney and heart (13). This tissue is also highly dependent upon circulating D-glucose for its extrinsic metabolic energy source and is metabolically responsive to altered states of hyper- and hypoglycemia (90, 31, 35, 56, 47–49).

In this manuscript, we have examined the relationship...

EXPERIMENTAL PROCEDURES

Materials—Timed pregnant Sprague-Dawley rats and tissue culture supplies were obtained from BioLab and GibCO, respectively. Soybean trypsin inhibitor, poly-D-lysine, and cytochalasin B were obtained from Sigma. DNase I, 2-deoxy[3H]glucose, and [32P]-labeled protein A were purchased from Boehringer Mannheim, Du Pont-New England Nuclear, and Amersham, respectively. The full-length 2.8-kb rat brain glucose transporter cDNA (prGT-1) was a generous gift of Drs. Ora Rosen and Morris Birnbaum (Memorial Sloan-Kettering Cancer Center). A rabbit polyclonal anti-human erythrocyte D-glucose transporter antibody (aGT-8) was kindly provided by Dr. Michaelfef (Ningxia).

Primary Rat Brain Neuronal Cell Cultures—Primary cultures of rat brain neuronal cells were prepared essentially as previously described by Ahmed et al. (46). Timed 18–19-day pregnant Sprague-Dawley rats were anesthetized with diethyl ether, and the fetuses were removed by laparotomy. Whole brains were removed from each fetus and placed in isotonic buffer containing 100 milliliters/ml penicillin, 100 mg/ml streptomycin, and 250 mg/ml amphotericin B. The brain tissue (10–20 fetuses) was cleaned of meninges and blood vessels, minced with an iris scissors, and enzymatically dissociated in isotonic buffer containing 50 mg/ml trypsin plus 16 mg/ml DNase I at 37 °C. The dissociated cells were collected by centrifugation at 100 × g for 10 min in the presence of 50 mg/ml soybean trypsin inhibitor followed by resuspension and filtration through cheesecloth. Under these conditions, greater than 98% of the cells remained viable based on trypan blue exclusion. The cells were plated at 9 × 10^5 cells/cm² on poly-D-lysine-coated tissue culture dishes in Dulbecco’s modified Eagle’s medium in the absence of serum as described previously (46). The cultures were maintained for 4 days in culture.

Primary Rat Brain Glial Cell Cultures—Primary rat brain glial cells were prepared from 1-day-old Sprague-Dawley rats essentially as described by Clark et al. (50). Dissociated cells, pooled from 20 newborn rat cerebral cortices, were obtained as described for the neuronal cell cultures above. The dissociated cells were resuspended and plated in Dulbecco’s modified Eagle’s medium supplemented with 10% FBS on poly-D-lysine-coated tissue culture dishes at a density of 1.8 × 10^6 cells/cm². The cultures were incubated at 37 °C, and the medium was initially changed after 30 min and again in 3 days. Following 7 days in culture, the confluent monolayers were washed twice with Hanks’ balanced salt solution followed by enzymatic dissociation with 0.25% trypsin and 0.1% EDTA. The dissociated cells were pooled, resuspended, and replated (2:1) in the same medium and grown to confluency (approximately 7 days) before use.

Immunofluorescence Staining—The homogeneity of the neuronal and glial cell populations were defined by immunocytochemical analysis. Indirect immunofluorescent staining was performed, as previously described (46, 51), using antibodies directed against the astrocyte- and oligodendrocyte-specific marker glial fibrillary acidic protein (GFAP) and the neuron-surface-specific ganglioside binding protein tetanus toxin (52). Gial and neuronal cell cultures contained with rhodamine-conjugated goat anti-rabbit GFAP antibody and fluorescein-conjugated anti-tetanus toxin antibody demonstrated greater than 95% homogeneity of neuronal and glial cell populations (46, 51).

2-Deoxyglucose Uptake—2-Deoxyglucose transport experiments were performed on glial and neuronal cell seeded in 24-well tissue culture plates, 24 h prior to uptake measurements, both the neuronal and glial cell cultures were incubated in serum-free medium containing either 25 mm D-glucose or 25 mm D-fructose. The initial rate of 2-deoxyglucose uptake was determined as described by Klip and Ramal (53) and in the individual figure legends. The cell monolayers were washed twice with glucose-free HEPES-buffered saline solution (140 mm NaCl, 20 mM HEPES, 1 mM CaCl₂, 5 mM KCl, and 2.5 mM MgSO₄, pH 7.4). The rate of glucose uptake was then determined by incubations with 2-deoxy[14C]glucose (10 μM, 0.2 μCi/μM) at 23 °C for the indicated times. Sugar uptake was terminated by aspiration, followed by three rapid washes with ice-cold phosphate-buffered saline (150 mm NaCl, 5 mm NaH₂PO₄, pH 7.4). The cells were solubilized in 0.05 N NaOH and subjected to scintillation counting. Noncarrier-mediated uptake was determined in parallel wells containing 10 μM cytochalasin B. The extent of specific uptake was normalized for the protein content/well determined by the method of Lowry (64). The protein content/well was found not to significantly decrease by D-glucose starvation for 5 h but decreased by approximately 40% after 24 h compared to the control cells. In addition, the recovery of total cellular RNA/well was identical under all experimental conditions employed (data not shown). Under these conditions, the initial rate of 2-deoxyglucose uptake is presumed to be rate-limited (55) and has been previously used to examine the insulin stimulation of glucose transport activity in primary rat brain glial cultures (50).

Isolation of Cellular RNA—Total cellular RNA was isolated from 4 × 10⁶ cells previously incubated under the appropriate experimental conditions. The cells were harvested and lysed in GIT buffer containing 4 μM guanidine isothiocyanate, 25 mM NaCl, pH 6.0, 120 μM ionic-marcaptoethanol, 0.5 mg/ml poly-D-lysine, 1 mg/ml cytochalasin B, 5.0 mg/ml soybean trypsin inhibitor, 1 mg/ml DNase I, 2-deoxy[3H]glucose, and [32P]-labeled protein A were purchased from Boehringer Mannheim, Du Pont-New England Nuclear, and Amersham, respectively. The full-length 2.8-kb rat brain glucose transporter cDNA (prGT-1) was a generous gift of Drs. Ora Rosen and Morris Birnbaum (Memorial Sloan-Kettering Cancer Center). A rabbit polyclonal anti-human erythrocyte D-glucose transporter antibody (aGT-8) was kindly provided by Dr. Michaelfef (Ningxia).

RESULTS

In this study, we have used primary rat neuronal and glial cell cultures as in vitro model systems to examine the D-glucose-dependent regulation of the D-glucose transporter protein in the brain. The cellular homogeneity of the primary neuronal and glial cell culture populations have been previously established using the criteria of immunofluorescent staining of specific neuronal and glial cell markers (46, 51). Both neuronal and glial cell cultures were shown to be >95% homoge-
neous by these criteria (data not shown).

To determine whether D-glucose deprivation had any effect on the rate of D-glucose transport activity, neuronal and glial cell cultures were maintained for 24 h in medium with either 25 mM D-glucose or 25 mM D-fructose. Under these experimental conditions, the time course of 2-deoxy[3H]glucose uptake was observed to be linear for at least 15 min at 23 °C in both cell types (Fig. 1). In four independent experiments, D-glucose deprivation for 24 h in the primary rat brain glial cultures resulted in a 4–5-fold increase in the initial rate of cytochalasin B-specific D-glucose transport activity. D-Glucose deprivation of the glial cell cultures increased the initial rate of 2-deoxyglucose uptake from 123 to 580 pmol/min/mg, respectively. Parallel experiments in primary rat brain neuronal cultures demonstrated only a small increase (2-fold) in the initial rate of 2-deoxyglucose uptake from 16 to 33 pmol/min/mg by D-glucose deprivation (Fig. 1). In addition, the relative amount of D-glucose transport activity in the glial cells was observed to be 7-fold greater compared to the neuronal cells under hyperglycemic culture conditions and 17-fold greater under hypoglycemic culture conditions, respectively. These data further indicate that the magnitude of D-glucose transport stimulation by D-glucose deprivation occurs in a brain cell type-specific manner.

The effect of D-glucose deprivation on the regulation of the D-glucose transporter mRNA in the rat brain glial and neuronal cell cultures was determined by Northern blot analysis. Glial cell cultures maintained in D-glucose free medium (25 mM D-fructose) for 24 h had a 4–6-fold increase in the steady-state level of the 2.8-kb D-glucose transporter mRNA compared to cells maintained in the presence of 25 mM D-glucose (Fig. 2A). Ethidium bromide staining of the total cellular RNA demonstrated equal loading of the samples under all conditions and showed that the method used to isolate the RNA did not result in any appreciable degradation (Fig. 2B). To insure that the effect of D-glucose deprivation was specific for the D-glucose transporter mRNA, the samples were probed with the cDNA for the housekeeping marker glyceraldehyde-3-phosphate dehydrogenase (Fig. 2C). In contrast to the 4–6-fold increase in the D-glucose transporter mRNA, the amount of the glyceraldehyde-3-phosphate dehydrogenase mRNA (1.8 kb) was observed to be slightly decreased by approximately 30%. Similar results were also obtained when the blots were probed with the housekeeping cDNA marker β-actin (data not shown).

D-Glucose deprivation for 24 h in rat brain neuronal cell cultures did not significantly alter the steady-state level of the D-glucose transporter mRNA (Fig. 3A). This is consistent with the relatively small effect of D-glucose deprivation on D-glucose transport activity in the neuronal cultures (Fig. 1). Ethidium bromide staining (Fig. 3B) and Northern blot analysis with the control glyceraldehyde-3-phosphate dehydrogenase cDNA (Fig. 3C) demonstrated equal loading of the RNA.

Since the D-glucose transporter in the neuronal cell cultures was only marginally responsive to regulation by D-glucose, we next examined the D-glucose concentration dependence of the D-glucose transporter mRNA induction in the glial cell cultures (Fig. 4). Northern blot analysis of total cellular RNA isolated from the glial cells maintained for 24 h at various concentrations of D-glucose is shown in Fig. 4A. The relative amount of the D-glucose transporter mRNA was observed to increase as the initial concentration of D-glucose in the culture medium decreased. Quantitative laser scanning densitometry of the Northern blot indicated that the half-maximal increase in the D-glucose transporter mRNA occurred at 3.5 mM D-glucose, whereas the maximal induction occurred at approximately 0.5 mM D-glucose (Fig. 4B).

The time dependence of D-glucose deprivation on the induction of the D-glucose transporter mRNA in the primary glial cell cultures was next determined (Fig. 5). Northern blot analysis revealed that deprivation of D-glucose for 30 min in the glial cell cultures significantly induced the D-glucose transporter mRNA above control levels (Fig. 5A). Quantitation by laser scanning densitometry indicated that half-maximal induction of the D-glucose transporter mRNA occurred by 2 h with maximal increase in the D-glucose transporter mRNA within 6–12 h (Fig. 5B).

In order to directly correlate the alteration in the D-glucose transporter mRNA with D-glucose transport activity, a time course of glial cell D-glucose deprivation on the initial rate of D-glucose transport activity was determined (Fig. 6). Glial cells, which were maintained in the presence of 25 mM D-glucose for up to 24 h, had no significant alteration in the initial rate of 2-deoxyglucose uptake. Similar to results shown in Fig. 1, D-glucose deprivation for 24 h resulted in an approximate 5-fold increase in D-glucose transport activity. Half-maximal induction of D-glucose transport activity occurred by 2–3 h, and maximal increase in the D-glucose transport activity was observed within 12 h. Under these conditions, there was a reasonable close correlation between the extent of D-glucose transporter mRNA induction and the observed increase in cytochalasin B inhibitable D-glucose transport activity (Figs. 5 and 6).

Since intact cell D-glucose transport activity may not necessarily reflect the number of D-glucose transporter proteins present, Western blot analysis was performed to specifically determine the relative amount of membrane associated D-glucose transporters (Fig. 7). Glial cells were maintained in the presence of 25 mM D-glucose or 25 mM D-fructose for various periods of time, and total particulate cellular membranes were prepared. The anti-human erythrocyte D-glucose transporter antibody (αGT-8) identified a closely spaced protein doublet with Mr = 43,000 and 46,000. D-Glucose deprivation for 1.5 h resulted in a small but significant increase in the relative amount of immunoreactive D-
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FIG. 2. Northern blot analysis of the D-glucose transporter mRNA from primary rat brain glial cells. A, total cellular RNA (20 μg, in triplicate), isolated from glial cells maintained in the presence of 25 mM D-glucose or 25 mM D-fructose for 24 h, was probed with the 2.8-kb nick-translated full-length rat brain D-glucose transporter cDNA (prGT-1) as described under "Experimental Procedures." B, ethidium bromide staining (EthBr) of the total cellular RNA used in A. C, rehybridization of the Northern blot used in A with the nick-translated 0.7-kb CDNA for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This was a representative experiment independently performed three times.

FIG. 3. Northern blot analysis of the D-glucose transporter mRNA from primary rat brain neuronal cells. A, total cellular RNA (20 μg, in triplicate), isolated from neuronal cells maintained in the presence of 25 mM D-glucose or 25 mM D-fructose for 24 h, was probed with the 2.8-kb nick-translated full-length rat brain D-glucose transporter cDNA as described under "Experimental Procedures." B, ethidium bromide staining of the total cellular RNA used in A. C, rehybridization of the Northern blot used in A with the nick-translated 0.7-kb CDNA for glyceraldehyde-3-phosphate dehydrogenase. This was a representative experiment independently performed two times.

glucose transporter protein compared to the control cells. Maximal induction of the D-glucose transporter protein occurred by 12 h with half-maximal increase at approximately 6 h. The time frame for the D-glucose starvation increase in the D-glucose transport protein (Fig. 7) is consistent with the increase in D-glucose transport activity (Fig. 6) and D-glucose transporter mRNA (Fig. 5). It should also be noted that the particulate membranes isolated at each time point represent an independent experiment such that comparisons can only be made between the control and D-fructose-treated cells.

The induction of the D-glucose transport activity and D-glucose transporter mRNA by D-glucose deprivation was also observed to be fully reversible upon the readdition of D-glucose to the culture medium (Fig. 8). Northern blot analysis revealed that, after 6 h of D-glucose deprivation (5-fold increase in the D-glucose transporter mRNA), readdition of 25 mM D-glucose resulted in a significant decrease in the amount of the D-glucose transporter mRNA by 4 h (Fig. 8A). The half-maximal time for reversibility was observed by approximately 12 h with complete return to the basal mRNA levels within 24 h (Fig. 8, A and B). Similarly, after the 24-h readdition of D-glucose to the culture medium, the cytochalasin B inhibited D-glucose transport activity was also returned to the basal state (Fig. 8C).

DISCUSSION

Altered metabolic states of hyperglycemia and hypoglycemia in diabetes mellitus can result in significant alterations in the availability of D-glucose to various tissues (30-34). Rats maintained under chronic hypoglycemic conditions by insulin therapy have been reported to increase the total number of D-glucose transporters in adipocytes (32, 57, 58) and brain tissues (35, 47). Similarly, rats rendered hyperglycemic by streptozotocin treatment have a decrease in the total number of D-glucose transporters in adipocyte and brain tissues (30, 32, 34). However, it has been very difficult to study the response of the brain to altered metabolic states in vivo due to the complications imposed by the blood brain barrier. These difficulties have led us to examine the effects of the tissue culture equivalent to hyperglycemia (25 mM D-glucose) and hypoglycemia (25 mM D-fructose) on isolated rat brain primary cultures of neuronal and glial cell populations. The glial and neuronal cell cultures have been previously established as an excellent in vitro brain cell model system. These primary cultures have the appropriate morphological and phenotypic characteristics of the in vivo cell types without the additional problems associated with the endothelial cell blood brain barrier (46). In addition, these cells do not have the complications of immortalization which may alter the regulation of D-glucose transport system. For example, it has been well established that cell transformation can result in an increase in D-glucose transport activity similar to the overall effect observed by D-glucose deprivation (23-25, 45, 59-64). Further, these neuronal and glial cell cultures can be maintained in serum-free defined medium, eliminating possible uncontrolled effects due to the presence of serum factors.

Using these in vitro model cell systems, we have demonstrated a brain cell type-specific increase in D-glucose transport activity, transporter protein, and transporter mRNA induction following D-glucose deprivation. Primary rat brain glial cells maintained for 24 h in D-glucose-deficient medium (25 mM D-fructose) have a 4-5-fold increase in D-glucose transport activity (Fig. 1) and a 3-4-fold increase in D-glucose transporter protein (Fig. 7) associated with a 4-6-fold increase in D-glucose transporter mRNA (Fig. 2). In contrast, primary rat neuronal cell cultures maintained in the absence of D-glucose for 24 h had a relatively small increase in D-glucose transport activity (Fig. 1) without any significant effect on the amount of the D-glucose transporter mRNA (Fig. 3). This cell type-specific differential response is supported by studies of Roeder et al. (65) which demonstrated the presence of two distinct carrier-mediated uptake systems for D-glucose trans-
FIG. 4. Effect of various D-glucose concentrations on the amount of D-glucose transporter mRNA. Primary rat brain glial cells were maintained with the various initial concentrations of D-glucose indicated (plus the appropriate concentration of D-fructose to equal 25 mM monosaccharide) for 24 h as described under "Experimental Procedures." The final concentration of D-glucose in the medium after the 24-h incubation was determined to be 0, 0, 0, 3, 8, and 25 mM when the initial starting D-glucose concentration was 0, 0.5, 1, 2, 5, 10, and 25 mM, respectively. A, total cellular RNA was isolated (20 µg/lane) and subjected to Northern blot analysis using the nick-translated 2.8-kb full-length rat brain D-glucose transporter cDNA. B, quantitative laser scanning densitometry of the Northern blot presented in A. This was a representative experiment independently performed four times.

The D-glucose concentration dependence of the D-glucose transporter mRNA induction in glial cell cultures demonstrated a half-maximal increase at 3.5 mM D-glucose, with maximal induction at 0.5 mM D-glucose based upon the initial starting concentrations of D-glucose (Fig. 4). Normal physiologic circulating concentrations of D-glucose in rat blood is approximately 5.6 mM which is somewhat higher than the IC₅₀ for the apparent inhibition of the D-glucose transporter mRNA observed in these cell cultures. However, glial cells in vivo are not directly exposed to the circulation, and the local concentration of D-glucose is determined by the transport across the endothelial cells of the blood brain barrier. It should be noted that, during the 24 h time frame of this experiment, the glial cells preferentially metabolized the D-glucose over the D-fructose available, such that, when the initial concentration of D-glucose was less than 2 mM, essentially all the D-glucose was depleted from the culture medium (Fig. 4).

The time dependence of the induction of glial cell D-glucose transporter mRNA is also relatively rapid with half-maximal induction by 2 h and maximal stimulation within 6–12 h (Fig. 5). The increased levels of the D-glucose transporter mRNA was found to correlate with the increase in cytochalasin B-inhibitable 2-deoxy-[¹H]glucose transport (Fig. 6) and total cellular membrane D-glucose transport protein (Fig. 7). This time course of D-glucose transporter mRNA induction is consistent with the time frame observed by others for the D-glucose starvation-induced increases in D-glucose transport activity (17, 22, 36, 44). In addition, Fig. 8 demonstrated that, in these glial cell cultures, induction of D-glucose transport activity and mRNA were fully reversible within 24 h upon the readdition of D-glucose. Reversibility of the D-glucose deprivation-induced increase in D-glucose transport activity in other cell
culture systems has also been reported (21, 27, 36, 38, 44). Our data clearly establish that hypoglycemic culture conditions induce a glial cell type-specific increase in D-glucose transport activity and a concomitant increase in the steady-state level of the D-glucose transporter mRNA and D-glucose transporter protein.

Several previous studies have suggested that different mechanisms may account for the observed D-glucose deprivation-induced increase in D-glucose transport activity, including inhibition of D-glucose transporter degradation (20, 40, 44) and/or an increase in D-glucose transporter protein and RNA synthesis (39, 42, 59). The molecular basis for the D-glucose-dependent regulation of the D-glucose transport system may be different in the various cell types examined. For example, several reports have provided indirect evidence suggesting transcriptional regulation of D-glucose transporter in chick embryo fibroblasts by D-glucose starvation (17, 42) as well as by directly measuring an increased rate of D-glucose transporter biosynthesis (40). Induction of D-glucose transport activity by D-glucose starvation in cultured human skin fibroblasts appeared to require protein synthesis but not RNA synthesis (21). Similarly, D-glucose deprivation in Chinese hamster fibroblasts resulted in a 4-8-fold stimulation of D-glucose uptake which was apparently regulated by a balance between transporter synthesis and inactivation (27, 36, 37, 41). On the other hand, D-glucose starvation of mouse 3T3C2 cells in culture induced an increase in the number of D-glucose transporters without any detectable change in D-glucose transporter mRNA (20). Consistent with these results, the differential effects of low and high cycloheximide concentrations in chicken embryo fibroblasts have been interpreted to indicate a D-glucose starvation-induced increase in D-glucose transporter protein stabilization (27). In contrast, the present study provides direct evidence for the regulation of D-glucose transporter activity by the control of D-glucose transporter mRNA and protein levels in D-glucose-starved rat brain glial but not neuronal cells. In addition, we have not observed any increase in D-glucose transporter mRNA in the C6 glioma and BC3H-1 cell lines by D-glucose starvation.

The apparent divergence in the molecular mechanism of D-glucose transporter regulation may be a consequence of multiple D-glucose transporter species. Recent studies have indicated that insulin-sensitive tissues express a D-glucose trans-

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2-DG uptake (pmol/min/mg protein)
glucose transporter based upon [3H]cytochalasin B photoaffinity labeling (68) and display different kinetic properties of D-glucose transport compared to human erythrocytes (69, 70). Recent studies in the L6 rat skeletal muscle cell line have also indicated the presence of high and low affinity D-glucose transporter systems (71). In addition, rat liver D-glucose transporters have a substantially reduced binding affinity for cytochalasin B (72) and do not cross-react in Northern blots with the rat brain or human Hep G2 D-glucose transporter cDNA under stringent conditions (3, 12). Thus, it is possible that these postulated multiple forms of the D-glucose transporter may respond to D-glucose deprivation in a protein subtype as well as in a cell type-specific manner.

In summary, we have examined the effect of D-glucose deprivation on D-glucose transport activity, protein, and mRNA using an antibody (aGT-8) specific for the same gene product encoded by the rat brain cDNA (prGT-1). This has been accomplished using a primary isolated cell culture system which should more closely correlate with the regulatory events involved in D-glucose transporter activity in vivo. These results provide evidence for the regulation of at least one D-glucose transporter species by the control of D-glucose transporter protein and mRNA expression in D-glucose-starved primary rat brain glial cells. Consequently, we have begun to examine whether the D-glucose starvation induction of D-glucose transporter mRNA is due to an increase in mRNA synthesis (transcriptional regulation) and/or due to the specific inhibition of D-glucose transporter mRNA degradation. In this regard, the genomic clone of the human placenta D-glucose transporter has recently demonstrated the presence of consensatory regulatory sequences for both AP-1 and Sp1 transcription factor binding (73). The apparent complexity of D-glucose transporter regulation is perhaps consonant with its central role in cellular metabolism in all tissues and strongly emphasizes the need for further detailed analysis in homogenous cell systems using defined probes.

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