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A glucose transport protein expressed predominately in insulin-responsive tissues

(membrane protein/diabetes/soleus muscle)

MAUREEN J. CHARRON*, FRANK C. BROSIUS III*†, SETH L. ALPER*†, AND HARVEY F. LODISH*‡

*Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142; †Department of Molecular Medicine and Renal Section, Beth Israel Hospital, Boston, MA 02215; and ‡Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

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ABSTRACT **Using low-stringency hybridization to the rat brain glucose transporter (GT), a 2489-base-pair cDNA clone was isolated from a rat soleus λgt10 cDNA library. It encodes a 509-amino acid protein whose sequence and predicted membrane structure is very similar to those of the rat brain and liver GTs. The muscle GT-like protein is 65% identical in amino acid sequence to the rat brain GT and 52% identical to the rat liver GT; the major differences are in the NH_2- and COOH-terminal hydrophilic segments. This GT-like mRNA is expressed predominately in tissues where glucose transport is sensitive to insulin, including striated muscle, cardiac muscle, and adipose tissue; low-level expression is also detected in smooth muscle and kidney mRNA. This GT-like cDNA is the fourth member of the mammalian GT-related gene family identified to date. We propose that it encodes an insulin-sensitive GT.**

Glucose is the primary energy source for virtually all mammalian cells and its specific transport across plasma membranes is mediated by one or more glucose transport proteins (1). The brush border of small intestine and proximal kidney absorptive epithelial cells contains a Na^+-coupled glucose transporter (GT), used to transport glucose up its concentration gradient from the lumen into the cytosol (for review, see ref. 2). These, as well as all other cells, are thought to use a facilitated diffusion GT(s) that transports glucose down its concentration gradient. The best characterized is the human erythrocyte GT (1, 3, 4). To date three distinct mammalian GT or GT-like cDNAs have been cloned and sequenced. One, called the erythroid cell/brain GT, is present in large amounts in erythroid cells and in the endothelial cells that form the blood–brain barrier (5–7). The second, the liver GT, (8, 9) is found in liver plasma membrane, in small intestine and proximal kidney tubule epithelial cells, and in the β cells of the islets of Langerhans, where it has been proposed to participate in glucose-stimulated insulin secretion (8). The third is expressed in fetal muscle and in many nonmuscle adult tissues (10).

The predicted structures of all three GT-related proteins are similar. Each is thought to contain 12 membrane-spanning α-helices, with both the NH_2 terminus and COOH terminus facing the cytoplasm. An exoplasmic loop between presumed α-helices 1 and 2 is of variable length and bears the single N-linked oligosaccharide. A long hydrophilic segment connects presumed helices 6 and 7 and, at least in the erythroid cell GT, faces the cytoplasm (11). The high-affinity GT of *Saccharomyces cerevisiae* (12) has sequence homology to, and shares a similar predicted secondary structure with, the erythroid cell/brain GT, as do several bacterial proton–sugar symport proteins (13). The Na^+–glucose cotransporter has an unrelated sequence and a different predicted membrane structure (14).

Within 10 min after the addition of insulin to rat adipocytes, there is a 20- to 30-fold increase in the V_{max} of glucose transport (for review, see refs. 15 and 16). In 3T3-L1 adipocytes insulin triggers GT translocation from intracellular vesicles, near the trans face of the Golgi apparatus, to the plasma membrane, resulting in a 2-fold increase in the number of plasma membrane GTs (17). However, this accounts for only a fraction of the increase in glucose uptake. There is also considerable activation of plasma membrane GTs, but the nature of this modification is unknown (18, 19). Phorbol esters appear to mimic insulin's action on glucose transport (20) and recent data suggest that diacylglycerol may mediate the effect of insulin-stimulated glucose transport in adipocytes (21). The nature of the modification to the plasma membrane GTs that causes an increase in transport activity is unknown, except that it apparently does not involve phosphorylation of the GT (22, 23).

Intracellular vesicles containing a GT have been purified from nonstimulated 3T3-L1 adipocytes (24) and rat epididymal fat cells (25, 26). These vesicles contain a protein immunologically related to the erythroid cell/brain GT, but controversy exists as to whether this protein is distinct from the erythroid GT (24–27). It is also disputed whether or not the K_m for import of extracellular 2-deoxyglucose or 3-methyl-*O*-glucose is affected by insulin treatment (28–33). These observations raise the possibility that the basal and insulin-stimulated transport activities may represent distinct molecular species of GTs, or different modifications of the same polypeptide.

The status of GTs has not been studied as extensively in striated skeletal muscle. Immunofluorescence microscopy of rat skeletal muscle showed that perineurial cells contain proteins that are immunoreactive with antibodies prepared against the erythroid GT. The muscle cells themselves do not contain proteins immunoreactive with the erythroid GT (34) but may contain other GT proteins (35).

Here we present the sequence§ and tissue distribution of a putative GT isolated from a rat soleus muscle cDNA library. Based upon the correlation between the tissue distribution of this putative GT and the insulin-responsive nature of these tissues, we propose that this clone may encode an insulin-responsive GT.

MATERIALS AND METHODS

DNA Library Screening. A rat soleus λgt10 cDNA library (gift of V. Mahdavi and B. Nadal-Ginard, Children's Hospital, Boston) was plated at a density of 5×10^4 plaque-forming units per 150-mm Petri dish on a lawn of Y1090 cells (36). Biotrans nylon filters with a pore size of 0.2 μm (ICN) were

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Abbreviations: GT, glucose transporter; cRNA, complementary RNA; TM, transmembrane region.

§The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04524).

used for plaque lifts. The filters were baked for 2 hr (80°C), autoclaved for 1 min at 110°C on the liquid cycle of an autoclave, washed 40 min in 0.2 M NaOH/1% $NaDodSO_4$ (50°C), and rinsed thoroughly with distilled water (8). Low-stringency hybridization of the filters was performed by prehybridizing for 3–4 hr at 42°C in 25% (vol/vol) formamide/5× SSC (1× SSC = 150 mM NaCl/15 mM sodium citrate, pH 7.0)/5× Denhardt's solution (1× Denhardt's solution = 0.02% polyvinyl pyrrolidone/0.02% Ficoll/0.02% bovine serum albumin)/50 mM sodium phosphate buffer, pH 7.0/1% $NaDodSO_4$/poly(A) (100 μg/ml)/5 mM EDTA. Filters were then hybridized at 42°C for 20 hr with ^{32}P-labeled RNA (1–2 × 10^6 cpm/ml) transcribed from the *Xho* I–*Sac* I fragment of prGT1, a segment that encodes the rat brain GT (6). The plasmid was a gift of O. Rosen (Memorial Sloan–Kettering, New York) and M. Birnbaum (Harvard Medical School, Boston). The filters were washed in 25% formamide/5× SSC/1% $NaDodSO_4$ (42°C) for four 30-min periods. They were dried on Whatman 3 paper and exposed to Kodak XAR5 film in the presence of a DuPont Cronex Lightning Plus intensifier screen for 20 hr at −70°C. All positive clones were purified by three additional rounds of hybridization screening. Seven positive clones were amplified and DNA was prepared (37). The clones, when digested with *Eco*RI, released inserts ranging in size from 500 base pairs (bp) to 2.5 kilobases (kb). These fragments were then size-fractionated on a 0.8% agarose gel, transferred to nylon in 20× SSC by the method of Southern (38), and hybridized to the ^{32}P-labeled complementary RNA (cRNA) used to screen the library; the stringency of the washes was systematically varied between 25% formamide/5× SSC/1% $NaDodSO_4$ (42°C) and 0.1× SSC/0.1% $NaDodSO_4$ (65°C).

DNA Sequencing and Computer Analysis. Overlapping restriction endonuclease fragments spanning the entire clone were subcloned into the replicative form of M13mp18 and/or M13mp19 (Pharmacia) and sequenced by the method of Sanger *et al.* (39) using the Klenow fragment of DNA polymerase I, the 17-mer universal primer (Pharmacia) or custom synthesized oligonucleotides (Research Genetics, Huntsville, AL), ^{35}S-labeled dATP (Amersham), and 7-deaza-dGTP (Boehringer). Reaction products were resolved on 6% polyacrylamide/buffer-gradient gels (40).

Percent nucleic acid sequence identity calculated was determined for the coding regions only using the ZIP program with a scanning window of 100 (41).

Predicted protein sequences were aligned using the ALNED program (Protein Identification Resource staff). Percent sequence identities were compiled based on the number of possible matches.

RNA Preparation and Northern Blots. The acid phenol/guanidinium isothiocyanate method of Chomczynski and Sacchi (42) was used to prepare total RNA from tissues of male Sprague–Dawley (CD) rats (180–200 g) perfused with ice-cold isotonic phosphate-buffered saline. RNA was separated by electrophoresis on 1.2% agarose/formaldehyde gels (43) and blotted onto Biotrans nylon membranes (ICN) using 20× SSC (44). The filters were hybridized to cRNA probes prepared using T7 RNA polymerase (Stratagene) by the method of Melton *et al.* (45). For preparation of the rat muscle GT cRNA probe, the entire *Eco*RI fragment was subcloned into the Bluescript KS vector (Stratagene) and linearized with *Sal* I prior to transcription. Hybridizations were in the same solution as described above at 60°C for 16–20 hr using 25% formamide for low stringency and 50% formamide for high stringency. Filters were washed as described above and in the figure legends.

Northern blots were probed with a mouse tubulin cDNA to normalize the amount of total RNA loaded in each lane. A 1.8-kb *Pst* I fragment containing a mouse tubulin cDNA (gift of R. Martinez, Whitehead Institute, Cambridge, MA) was labeled with [^{32}P]dCTP by the random-priming method of Feinberg and Vogelstein (46). Hybridization and washes were as described in the legend for Fig. 3. Autoradiographs were scanned using an LKB 2202 Ultrascan Laser Densitometer and the values obtained were used to calculate the relative amounts of GT mRNAs.

RESULTS

Cloning a GT-Related cDNA from Soleus Muscle. To test the hypothesis that insulin-sensitive cells, most notably adipocytes, express more than one GT, we performed Northern blot analyses. RNA from various insulin-sensitive rat tissues was hybridized at both high and low stringency using a cRNA probe transcribed from the rat brain GT cDNA. Depending on the tissue and hybridization stringency, between one and four mRNAs, ranging in size from ≈1.2 kb to 5.0 kb, were detected (see below). This suggested that indeed other GTs may be expressed in insulin-sensitive tissues. We screened a rat soleus λgt10 cDNA library with the rat brain GT cDNA; from 10^6 plaque-forming units, we isolated seven positive clones.

These cloned cDNAs were excised with *Eco*RI and subjected to Southern analysis using the rat brain GT cRNA as a probe. Also, each insert was hybridized at various stringencies to the others. Four clones were placed into a high-stringency class; these clones were very homologous to the brain cDNA and to each other. Three were put into a low-stringency class; these clones were poorly homologous to the brain probe and to the high-stringency muscle clones but were very homologous to each other (data not shown).

High-stringency clone S9A2 contained the largest insert (2.49 kb) and was chosen for further analysis. Overlapping restriction endonuclease fragments spanning the entire insert were subcloned and sequenced in two strands. S9A2 contains a 1527-bp open reading frame, encoding a 509-amino acid protein (Fig. 1). The 122-bp 5′ untranslated region contains an in-frame termination codon 9 bp upstream of the presumed initiator ATG. The 3′ untranslated region of 840 bp contains a polyadenylylation consensus site and a probable poly(A) tail.

Comparison of Rat GT and GT-Related Proteins. The amino acid squence of the S9A2 protein, called herein the muscle GT, reveals extensive similarities with both the rat brain (6) and liver GT (8) proteins (Fig. 2); 227 residues of the 522-amino acid liver GT, the longest of the three, are identical in both the brain and muscle GT. As determined by the algorithm of Kyte and Doolittle (47), the hydropathy plots of the three proteins are very similar (data not shown). All have the same number and arrangement of hydrophobic sequences that most likely form the 12 membrane-spanning α-helices that are boxed in Fig. 2. Based upon the extensive sequence similarity, it is probable that all three proteins form similar structures within the plasma membrane.

More detailed analyses show that the sequence of the muscle GT is more similar to the brain than to the liver GT. For instance, the hydrophilic exoplasmic sequence between presumed transmembrane helices 1 and 2 is longer in the liver GT (64 amino acids) than in the muscle or brain GT (37 amino acids and 33 amino acids, respectively). In this region the sequences of the muscle and brain GTs are 62% identical, and the position of the single N-linked glycosylation site is conserved (circled in Fig. 2); there is no homology in this region between either the muscle or brain GT and the liver GT. Overall, the sequences of the muscle and brain GTs are 65% identical, whereas both the muscle and brain GTs are only 52% identical with the liver GT. In addition to the 227 residues shared by all three GTs, the muscle and brain GTs are identical at 102 positions; the muscle and liver GTs share only 46 additional amino acid identities, and the brain and

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-122  GTCCCACCAGACCCGCCCTTTGCACACCACTTCCGAAGGCCGGGGTCTTCTGCCCGCCAGGCCGGGACACTATACCCTATTCATTTTTTTATTGCAGTGCCTGAGTCTT
 -13  CTTTTAAAACAAGATGCCGTCGGGTTTCCAGCAGATCGGCTCTGAAGATGGGGAACCCCCTCAGCAGCGAGTGACTGGGACACTGGTCCTTGCTGTATTCTCAGCTGTGC
  98  TTGGCTCCCTTCAGTTTGGCTATAACATTGGAGTCATCAACGCCCCACAGAAAGTGATTGAACAGAGCTACAATGCAACTTGGCTGGGTAGGCAGGGTCCTGGGGGACCG
 208  GACTCCATCCCACAAGGCACCCTCACTACCCTTTGGGCTCTCTCCGTGGCCATCTTCTCTGTGGGTGGCATGATTTCCTCCTTTCTCATTGGCATCATTTCTCAATGGTT
 318  GGGAAGGAAAAGGGCTATGCTGGCCAACAATGTCTTGGCTGTGCTGGGGGGCGCCCTCATGGGCCTAGCCAATGCCGCGGCCTCCTATGAGATACTCATTCTCGGACGGT
 428  TCCTCATTGGCGCCTACTCAGGGCTAACATCAGGGTTGGTGCCTATGTATGTGGGAGAAATCGCCCCCACTCATCTTCGGGGTGCCTTGGGAACACTCAACCAATTGGCC
 538  ATCGTCATTGGCATTCTGGTTGCCCAGGTGTTGGGTTTGGAGTCTATGCTGGGCACAGCTACCCTGTGGCCATTGCTTCTGGCTATCACAGTACTCCCTGCTCTCCTGCA
 648  GCTGCTTCTGTTGCCCTTCTGTCCTGAGAGCCCCCGATACCTCTACATCATCCGGAACCTGGAGGGGCCTGCCCGAAAGAGTCTAAAGCGCCTGACAGGCTGGGCTGATG
 758  TGTCTGATGCACTGGCTGAGCTGAAGGATGAGAAACGGAAGTTGGAAAGAGAGCGTCCACTGTCCTTGCTGCAGCTCCTGGGCAGCCGCACCCACCGGCAGCCTCTGATT
 868  ATTGCAGTGGTGCTGCAGCTGAGCCAGCAGCTCTCAGGCATCAATGCTGTTTTCTACTATTCAACCAGCATCTTTGAGTTAGCTGGGGTGGAACAGCCAGCCTACGCCAC
 978  CATAGGAGCTGGTGTGGTCAATACCGTCTTCACGTTGGTCTCGGTGCTCTTAGTAGAGCGAGCTGGGCGACGGACACTCCATCTCCTGGGCCTGGCAGGCATGTGTGGCT
1088  GTGCCATCTTGATGACGGTGGCTCTGCTGCTGCTGGAGCGGGTTCCATCCATGAGTTATGTGTCCATCGTGGCCATATTTGGCTTTGTGGCCTTCTTTGAGATTGGTCCT
1198  GGCCCCATCCCCTGGTTCATTGTGGCCGAGCTCTTCAGCCAGGGCCCCCGCCCAGCAGCCATGGCTGTAGCTGGTTTCTCCAACTGGACCTGTAACTTCATCGTTGGCAT
1308  GGGTTTCCAGTATGTTGCGGATGCTATGGGTCCCTACGTCTTCCTTCTATTTGCCGTCCTCCTGCTTGGCTTCTTCATCTTCACCTTCCTAAGAGTGCCTGAAACCAGAG
1418  GCCGGACATTTGACCAGATCTCGGCCACCTTCCGACGGACACCTTCTCTCTTAGAGCAGGAGGTGAAACCCAGTACAGAACTTGAATACTTAGGGCCAGATGAGAATGAC
1528  TGAGGGGCAAAATGGTGGGAGAGCCACCCTCCCCACCAGACTCCCTCCTTTCCTCGCAGCACTTTAGCCCTCTCTTCCCTTACCTCCAGGATGAAGGAAACAGCAGCCTG
1638  GGGAACTGGGAAGCTGGAGGGAGGGGCGGGTCACATACCCCTCATTCCCTCTGTGTGATTCTTTTGGATTATTTATGTGTTGTGGCTAGGCTGTGGCCACCTAGATGGGC
1748  TTTTCTCCCGTCCTGCCTTCCTCTTACCCCTATCCAGAGACTCAGCTCTAGAATACTTCTGTTCCCTTTAAGAGAAGGGGTCTGCTGGAGGGTGAAGCTGCCCTGAGTTC
1858  AGGGGGATGAGAAGCAGGGGGGCTGTGAGTGAGTGCTTTCCTACTATGACTCCTCCCACAAACTGGCGCTTTCACTGAACTCTTGCCACATAGACTCTGGGTGAAGGGGG
1968  TTGTCTTGACCCCTCCAGGGCAAAGGATACACCCTCCCAAAATCTAGCCTTGCCTCGCTGCTGTAGGCTCCACCCTCCAGGGCAAAGGAACACAACAGTACATCCCTGAC
2078  AGGGCAAGGATGGTAGAGCACATCAGTCTCCATTTGGGGCCCTGGCCTGTTCCCCAGGGCTGCCAAGTGTGGGTACCAACACTTTCTTGTTCCCCTCCAGGAAGGGTGCT
2188  AAACCCGAAAGCTTCTGACCAACTAAGGCAGGAGGGGATTTGAAAGGCTGCCTATAAACACTGGTTGGGAGGGAGCCTTTGGTATTTTTGTATGTACGATAGGGAGCAGA
2298  AACCTGGGGGCTGGTGTATTAAATATGTATATAGAGATTCATCCATAAAGTCACTGTATGGAGAAAAAAA
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FIG. 1. Nucleotide sequence of muscle GT clone S9A2. The initiator and stop codons are underlined as well as the putative polyadenylylation signal.

liver GTs share only 43. With respect to the nucleic acid sequence of the coding portion of the mRNAs, the brain and muscle GTs are 67% identical, whereas the brain and liver GTs are 60% identical and the muscle and liver GTs are 57% identical.

The major differences found between the muscle GT protein and those previously identified are in the NH_2- and COOH-terminal segments. The muscle GT has an NH_2 terminus 13 amino acids longer than those of the other two GTs. The COOH terminus of the muscle protein is also quite distinct from that of the other two; there are only six amino acid identities out of 24 residues to both the brain and liver GT sequences (with one gap imposed).

The muscle GT is not the rat counterpart of the human fetal skeletal muscle GT-like protein (10). The two proteins are only 55.8% identical in sequence; in contrast, the rat brain and human HepG2 cell GTs are 97.6% identical (6) and the rat and human liver GTs are 82.8% identical (8, 9). More

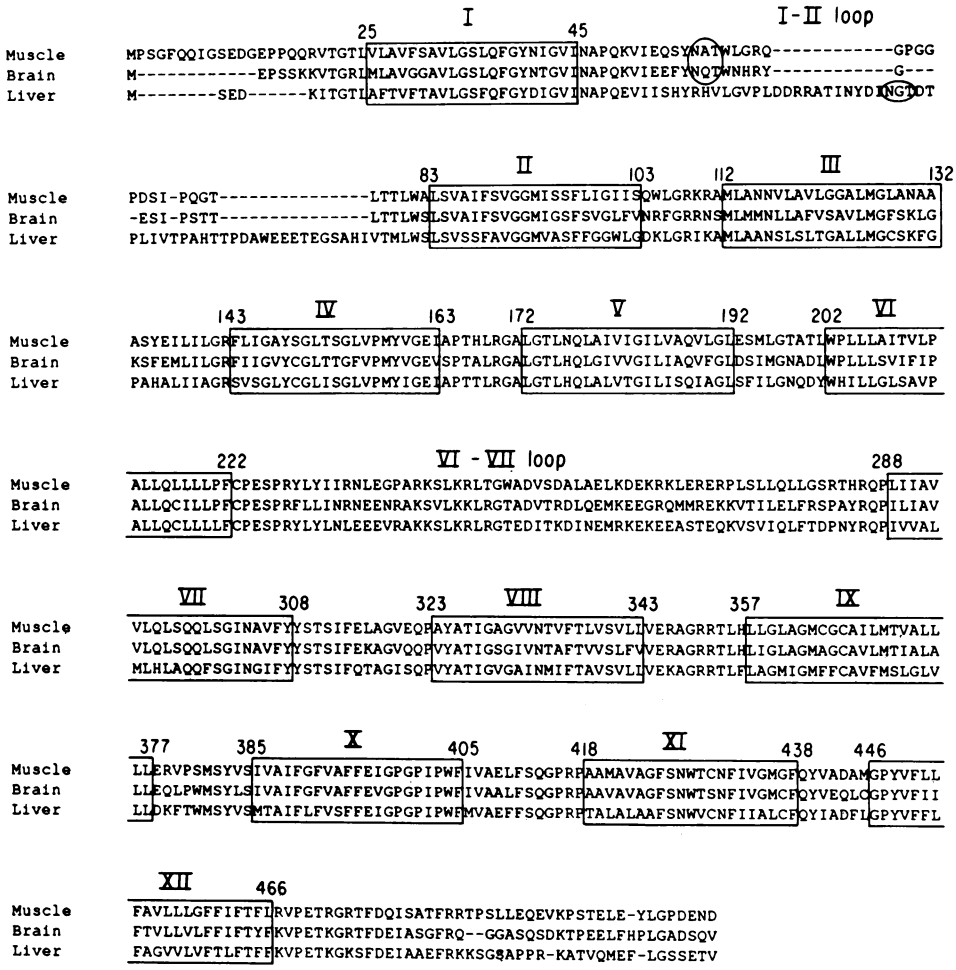

FIG. 2. Amino acid sequence comparison of the rat muscle, brain, and liver GT. Amino acids are designated by the single-letter code. The muscle sequence is shown on the top line with the brain and liver GT sequences aligned below. Sequence alignments were determined with the aid of the ALNED program (Protein Identification Resource staff). The 12 putative membrane-spanning domains are contained within boxes with Roman numerals centered above. The numbers at the ends of the boxes correspond to amino acid residues of the muscle sequence. The residues contained within the ovals designate potential N-linked glycosylation sites. Dashes represent gaps imposed for optimal sequence alignment.

importantly, the human fetal skeletal muscle GT is not expressed in adult muscle but is expressed in all other adult tissues tested; our rat muscle GT has a much different pattern of expression.

Tissue Distribution of the Presumed Muscle GT. The tissue distribution of the presumed muscle GT was determined by Northern blot analysis using total RNA isolated from various rat tissues. Blots were probed with the rat brain GT cRNA, the rat muscle GT cRNA, and also a mouse tubulin cDNA probe to normalize for the amount of RNA loaded. The most striking result is that the muscle GT cDNA clone is expressed as a 2.6- to 2.7-kb transcript in a very restricted set of tissues—only those where glucose transport is regulated by insulin. These include striated muscle (soleus, gastrocnemius, and diaphragm), cardiac muscle, and epididymal fat pad. Lower-level expression is detected in smooth muscle tissues, such as the aorta plus posterior vena cava, and urinary bladder. Although this transcript was not observed in total RNA from kidney, the mRNA was detected when poly(A) RNA was analyzed (data not shown).

By normalizing the signal with the rat muscle GT cDNA to that from tubulin cDNA, we obtain a semiquantitative measure of the abundance of the muscle GT mRNA. Relative to tubulin mRNA (whose level may differ in different tissues) the aorta and posterior vena cava contain about the same amount of muscle GT mRNA as does the atrium. Four times more is present in fat cells, and about 20-fold more is present in diaphragm, gastrocnemius, and soleus muscle. Only trace amounts are present in urinary bladder and kidney. All other tissues tested were negative within the level of sensitivity of the assay (Fig. 3 *Lower*).

In contrast, the brain GT is expressed as a 2.7-kb mRNA in most tissues analyzed, as detailed in Fig. 3 and Table 1. In many tissues both larger- and smaller-sized transcripts hybridizing to the brain GT are detected. It is interesting to note that insulin-sensitive tissues (i.e., soleus, gastrocnemius, and diaphragm) also express an abundant 1.5-kb transcript.

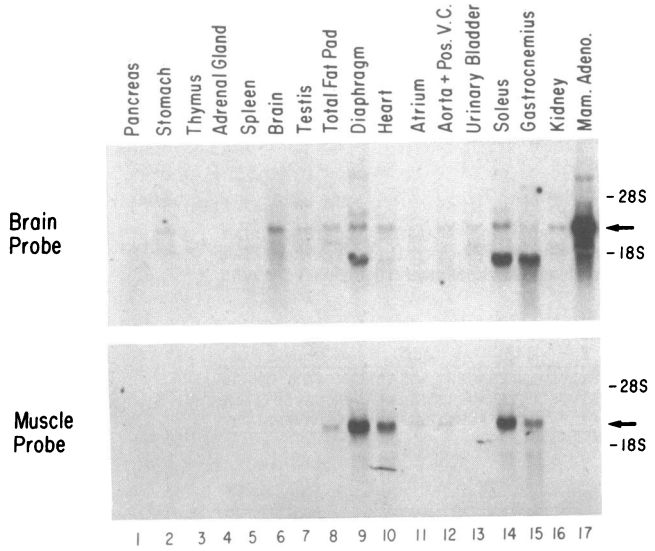

FIG. 3. Tissue distribution of the muscle and brain GT mRNAs. Northern blot analysis of 10–20 μg of total RNA from various rat tissues. Hybridizations were carried out in 50% (vol/vol) formamide at 60°C using the brain (*Upper*) and muscle (*Lower*) cRNA probes. Amount of RNA loaded in each lane was normalized by probing the blot with a mouse tubulin cDNA probe (data not shown). Filters were washed at 65°C in 0.1× SSC/0.1% $NaDodSO_4$. The position of the 28S and 18S rRNAs are shown and the arrow designates the migration of the 2.7- to 2.8-kb GT mRNA. Abbreviations are as follows: Pos. V. C., posterior vena cava; Mam. Adeno., mammary adenocarcinoma 13762N (gift of E. Katz and E. Boylan, Queens College CUNY, Flushing, NY).

Table 1. Expression of GT mRNAs

Tissue or cell line	GT probe		
	Brain	Muscle	Liver
HepG2 (human hepatoma)	+	−	−
NIH 3T3 (fibroblasts)	+	−	−
Mammary adenocarcinoma	+	−	−
Testis	+	−	−
Brain	+	−	−
Spleen	+	−	−
Stomach	+	−	−
Thymus	+	−	−
Heart	+	+	−
Soleus	+	+	−
Gastrocnemius	+	+	−
Fat pad	+	+	−
Diaphragm	+	+	−
Kidney	+	±	+
Urinary bladder	+	±	−
Aorta and posterior vena cava	+	+	±
Liver	−	−	+
Pancreatic islets	−	−	+
Small intestine	±	−	+
Adrenal gland	±	−	−
Pancreas (total)	−	−	±

High-stringency Northern blot analysis was performed as described in Fig. 3 using cRNA probes for rat brain (6), liver (8), and muscle (this report) GT. +, Expression detected; ±, low-level expression; −, inability to detect expression in the tissue/cell line assayed using described conditions.

Table 1 summarizes the distribution of the three GTs in various rat tissues. Several tissues express only the brain GT. Others, such as the liver or the β cells of the islets of Langerhans, express only the liver GT. Strikingly, the muscle GT is expressed only in those tissues that also express the brain GT.

DISCUSSION

Here we describe the isolation and partial characterization of a member of the GT gene family. Hydropathic analysis and a direct comparison of the sequences reveals that all three rat GT-related proteins have the same predicted membrane structure: 12 membrane-spanning α-helices, hydrophilic NH_2- and COOH-terminal segments, a glycosylated exoplasmic loop between transmembrane region (TM) I and TM II, and a hydrophilic cytoplasmic loop between TM VI and TM VII. Overall, there is between 50% and 64% sequence identity between these proteins. Stretches of sequence identity occur over the entire length of the proteins, except for the notable differences in the NH_2- and COOH-terminal sequences and the exoplasmic TM I–TM II loop. These unique features presumably confer differences in binding affinity for glucose or other sugars, hormone sensitivity, or subcellular localization of the GT proteins.

We have also examined the tissue distribution of the muscle GT and compared it to that of the rat brain and liver GTs. The muscle GT is expressed predominately in tissues in which glucose transport exhibits rapid changes in response to insulin. High-level expression is detected in striated muscle, cardiac muscle, and fat cells; lower levels are found in smooth muscle, such as aorta and urinary bladder. This suggests that the activity of the muscle GT is responsive to insulin. A great deal of research has been focused on the effects of insulin on glucose transport in adipocytes. Data summarized in the introduction suggest that insulin causes quantitative, as well as qualitative, changes in the GT activity present at the plasma membrane. Also, indirect evidence suggests that, in addition to the erythroid cell/brain GT,

another GT is expressed in these cells (26, 31, 35). Much less is known about the status of the GT(s) in skeletal muscle. Froehner *et al.* (34) showed that the erythroid cell/brain GT is expressed in the perineurial cells surrounding diaphragm muscle cells; the muscle cells themselves do not contain an immunoreactive protein. These data are consistent with the proposal that striated muscle, as well as adipocytes, express another GT.

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