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THE BRAIN-TYPE GLUCOSE TRANSPORTER mRNA IS SPECIFICALLY EXPRESSED AT THE BLOOD-BRAIN BARRIER

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The brain-type glucose transporter (bGT) is assumed to be distributed among neurons and glial cells, but to be particularly enriched in brain microvascular endothelium, which makes up the blood-brain barrier (BBB) in vivo. However, in the course of the present studies it was found that the bGT is specifically expressed at the BBB in brain. The relative abundance of bGT and actin (used as a control) mRNAs were measured in bovine brain, brain capillaries, and capillary-depleted brain. Northern blot analysis showed that the bGT 2.9 Kb mRNA was increased 21-fold in brain capillaries as compared to total brain, and it was not detected in capillary-depleted brain even after overexposure of the film. This study demonstrates that i) the bGT is specifically expressed at the BBB in brain, ii) the bGT transcript in total brain represents only dilution of the capillary or BBB glucose transporter transcript, and iii) as yet unidentified glucose transporters are likely expressed in neurons and in glial cells.

The brain-type glucose transporter (bGT) (1, 2) is one of at least four members of the sodium-independent glucose transporter supergene family (1-8). Other members of this family are the muscle-type (5-7), the liver-type (3, 8), and the fetal muscle-type glucose transporters (4). The bGT, which is also expressed in peripheral nerve, choroid plexus epithelium, and erythrocytes (9-11), is assumed to be distributed among neurons and glial cells in brain as well as brain microvascular endothelium, which makes up the blood-brain barrier (BBB), in vivo. Moreover, the bGT mRNA is known to be highly enriched in brain capillaries as compared to total brain (12). These findings were confirmed in the course of the present studies using Northern blots under high stringency conditions and comparing bGT mRNA in total bovine brain homogenate versus isolated bovine brain capillaries. However, a quantitative analysis of our Northern blots

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showed that, after normalization for levels of an actin housekeeping gene transcript, the amount of bGT mRNA in total brain was only about 2% of that found in isolated brain capillaries. Since the brain microvasculature comprises only 1-2% of total brain volume (13), we hypothesized that the bGT may be localized exclusively at the BBB in brain, and the presence of it's mRNA in total brain represents dilution of microvascular-derived glucose transporter mRNA. If so, other as yet undefined glucose transporters may function on neuronal and glial cell membranes.

The hypothesis that the brain-type glucose transporter is localized exclusively at the brain microvasculature could be tested with in situ hybridization. However, since there is approximately 60% homology between the various members of the sodium-independent glucose transporter supergene family (1-8), there may be cross-reactivity of cDNA probes with the various glucose transporters under conditions that are not high stringency. High stringency conditions for hybridization can be assured in Northern blot analyses. Since previous studies have shown that brain homogenate can be 98% depleted of vasculature by differential centrifugation procedures (14), we developed protocols for performing Northern blots using mRNA from three different preparations: (a) isolated bovine brain microvessels; (b) total bovine brain homogenate; and (c) capillary-depleted bovine brain homogenate.

METHODS

Materials - Deoxycytidine 5'-triphosphate tetra(triethylammonium)salt [α-^{32}P], 5,000 Ci/mmol and GeneScreen Plus membranes were purchased from DuPont-NEN (Boston, MA). Guanidinium isothiocyanate was obtained from Eastman Kodak Company (Rochester, NY). Cesium chloride, phenol and EcoRI were obtained from U.S. Biochemicals (Cleveland, OH). An 0.24-9.5 Kd RNA ladder was obtained from Bethesda Research Laboratories Life Technologies, Inc. (Gaithersburg, MD). The multiprimer DNA labeling kit was obtained from Amersham Corporation (Arlington Heights, IL). The full length rat brain glucose transporter cDNA in pUC-19 was generously provided by Ora M. Rosen, M.D. (Memorial Sloane-Kettering Cancer Center, New York, NY). The mouse actin cDNA clone pAM-91 described previously (15) was generously provided by Michael J. Getz, Ph.D. (Mayo Foundation, Rochester, MN).

RNA Isolation and Northern Blotting - Microvessels were isolated from homogenate of bovine brain as described previously (16), except the homogenate was prepared with a Waring blender (7 seconds). The final microvessel pellet and an aliquot of total brain were homogenized in 4 M guanidinium isothiocyanate/25 mM sodium acetate (pH = 6.0, GIT), and total RNA was isolated by cesium chloride ultracentrifugation (17). For the preparation of microvessel-depleted brain homogenate, the homogenate was centrifuged at 1,000 g for 10 minutes at 4°C in the presence of 10 mM vanadyl-ribonucleoside complex, which resulted in quantitative depletion of vasculature from

the homogenate (see Results). The subsequent supernatant was extracted twice with one volume of saline saturated phenol:chloroform (1:1) and twice with one volume of chloroform. RNA was precipitated with one volume of 2-propranol in the presence of 0.3 M sodium acetate (pH = 6.0) and 12.5 mM EDTA for 1 hour at -70°C. The mixture was centrifuged for 15 minutes at 10,000 g at 4°C, the pellet was dried and dissolved in GIT, and RNA was purified by cesium chloride ultracentrifugation. Glassware and stainless materials were heated overnight at 230°C. All other materials were treated with 0.1% diethyldipyrocarbonate for 2 hours at room temperature and autoclaved for 30 minutes (18).

The thoroughness of the capillary-depletion step was assessed by measurements of gamma-glutamyl transpeptidase (gamma-GTP) specific activity in the brain microvessels and in the microvessel-depleted homogenate using a colorimetric procedure described previously (14). Gamma-GTP is a capillary-specific enzyme that is not found in neurons or glia.

Northern blotting was performed by applying 25 μg of RNA from the three fractions to 1.1% agarose/2.2 M formaldehyde gels followed by blotting onto GeneScreen Plus membranes (19). Following blotting to filters and baking in a vacuum oven at 80°C for 2 hours, the membranes were hybridized under high stringency conditions (50% formamide, 2x Denhardt's solution, 2x SSPE, 1% SDS, 5% dextran sulfate, 100 μg/ml denatured salmon sperm DNA; where 1x SSPE = 0.15 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH = 7.4, and 10^6 CPMs/ml of [^{32}P]-labeled bGT cDNA for 16 hours at 42°C. The filters were washed for 15 minutes with 2x, 0.5x, and 0.1x SSC containing 0.1% SDS successively for 15 minutes with a final wash of 0.1x SSC in 1% SDS for 30 minutes at 50°C. Following washing, the filters were exposed to Kodak X-Omat film using Cronex lighting plus intensifying screens at -70°C for 2-14 days. Membranes were unhybridized in hot 1.5 mM NaCl/0.15 mM Na citrate, pH = 7,/0.01% SDS for 3 minutes x 5, and hybridized with [^{32}P]-labeled α-actin as described above. Following linearization of the plasmid with EcoRI, the cDNAs were labeled with the multiprimer technique to a specific activity of 5 x 10^9 CPMs/μg (20). Autoradiograms were quantified by laser scanning densitometry (LKB Model 2202 Ultrascan Laser Densitometer, Bromma, Sweden).

RESULTS

The gamma-GTP activity in the brain microvessels and in the capillary-depleted brain homogenate was 34.0 ± 1.5 and 0.41 ± 0.03 nmol/minute/mg protein at 23°C, respectively (mean ± S.E., n = 4), indicating the homogenate is 99% depleted of vasculature [1-(0.41/34) = 0.988]. The integrity of the RNA in the capillary-depleted brain homogenate was demonstrated by ethidium bromide staining showing prominent 28S and 18S ribosomal RNA bands in all three fractions (Figure 1A). Hybridization with the actin cDNA probe showed a comparable 2.0 Kb actin transcript in all three fractions (Figure 1B). Although a prominent 2.9 Kb bGT transcript was found in the microvessel fraction and a minor band of the same size was found in the total brain homogenate fraction, there is no detectable bGT mRNA in capillary-depleted brain homogenate (Figure 1C), even after overexposure of the film (data not shown). The ratio of the bGT/actin mRNA

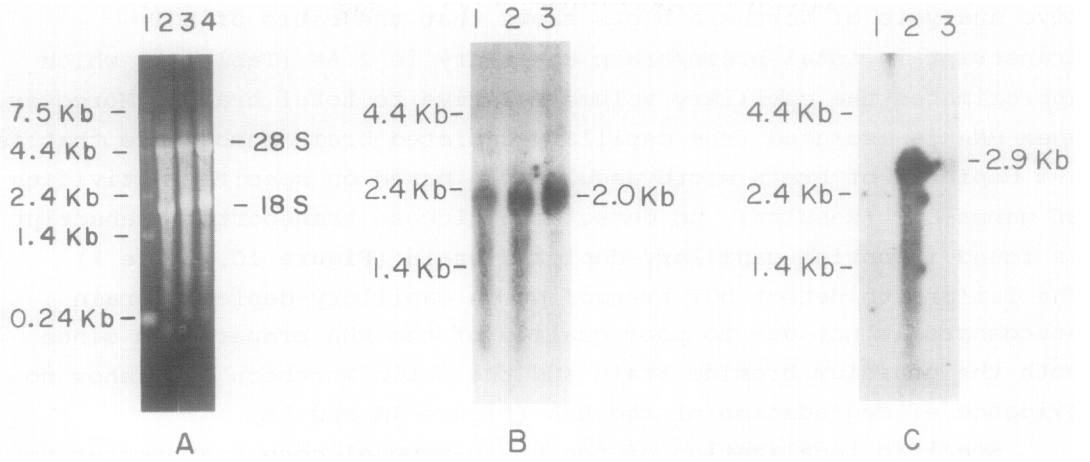

Figure 1. (A) Ethidium bromide stain of RNA ladder (lane 1); of RNA from capillary-depleted bovine brain (lane 2); of RNA from bovine brain capillaries (lane 3); and of RNA from total bovine brain (lane 4). The size of the RNA ladders is shown on the left-hand side of the figure. (B) Actin Northern blot. (C) Brain-type glucose transporter Northern blot. The RNA preparations in lanes 1, 2, and 3 of either Figures B or C are capillary-depleted bovine brain homogenate, isolated bovine brain capillaries, or total bovine brain homogenate.

in bovine brain divided by the same ratio in brain microvessels was 0.12/4.69, or 2.6% (Table 1).

DISCUSSION

The present study provides evidence that the bGT is specifically expressed in the brain microvasculature or BBB in bovine brain. Although previous studies concluded that the brain capillary was enriched in the bGT (9-12), it has been generally assumed that the bGT is also present within neurons and glial cells. However, quantita-

Table 1. Levels of Brain-Type Glucose Transporter (bGT) and Actin mRNA in Bovine Brain, Brain Microvessels, and Capillary-Depleted Brain

	mRNA (arbitrary units x 10^{-3})*		bGT / Actin Ratio
	bGT	Actin	
Bovine brain	0.34	2.79	0.12
Brain microvessels	7.07	1.51	4.69
Capillary-depleted brain	0	1.48	-

*Arbitrary densitometric units pertain to the integrated area of hybridization as shown in figure 1.

tive analysis of Northern blots shows that the ratio of bGT transcript in total brain/brain capillary is 2.6% (Table 1), which approximates the capillary volume relative to total brain. Moreover, when RNA is isolated from capillary-depleted brain homogenate that is 99% depleted of brain microvasculature, based on specific activities of gamma-GTP (Results), no detectable glucose transporter transcript is found in bovine capillary-depleted brain (Figure 1C, Table 1). The failure to detect bGT transcript in capillary-depleted brain homogenate is not due to poor quality of the RNA preparation since both the ethidium bromide stain and the actin Northern blot show no evidence of degradation of the RNA (Figure 1A and 1B).

Specific localization of the brain-type glucose transporter to the bovine BBB is not a species difference. We have recently prepared a bovine brain capillary cDNA library and have cloned from this library the bovine BBB glucose transporter cDNA (R.J. Boado and W.M. Pardridge, unpublished). Partial sequence analysis indicates the bovine brain-type glucose transporter is at least 97% identical to the rat brain glucose transporter.

The hypothesis that the bGT is localized exclusively at the BBB is not at odds with studies showing expression of the bGT in primary cultures of fetal rat brain neurons or neonatal rat brain glial cells (21). The expression of the bGT is augmented in cells in tissue culture (22, 23). Indeed, the bGT was initially cloned from a cDNA library corresponding to mRNA isolated from cultured hepatoma cells (1), although mature rat liver contains no detectable bGT (3, 8).

Finally, the hypothesis that brain-type glucose transporter is localized exclusively at the BBB suggests that other glucose transporters exist in brain on neuronal and glial cell membranes. The possibility of additional glucose transporters in brain is also supported by the observation that the level of the brain-type glucose transporter or it's transcript is decreased in postnatal rat brain, although the number of D-glucose sensitive cytochalasin B binding sites in postnatal brain membranes is unchanged (24). Moreover, pilot studies show that capillary-depleted brain membranes have abundant D-glucose sensitive cytochalasin B binding sites, but these membranes lack immunoreactive brain-type glucose transporter (R.J. Boado, C.R. Farrell, and W.M. Pardridge, unpublished observations), and capillary-depleted homogenates of brain contain no brain-type glucose transporter mRNA (Figure 1). Taken together, these findings indicate the brain-type glucose transporter is specifically localized to the BBB, and that as yet unidentified glucose transporters function at neuronal and glial cell membranes.

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