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BLOOD-BRAIN BARRIER GLUCOSE TRANSPORTER mRNA IS INCREASED IN EXPERIMENTAL DIABETES MELLITUS

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The blood-brain barrier (BBB) glucose transporter activity in vivo is known to be down-regulated in experimental diabetes mellitus. To determine whether parallel changes in BBB glucose transporter mRNA levels occur in experimental diabetes we isolated brain microvessels, which make up the BBB in vivo, from both control and experimental diabetic rats. Microvessel RNA fractions were isolated by cesium chloride density gradient centrifugation and were applied to 1.1% agarose gels for Northern blotting. The blots were probed with [^{32}P]-labeled cDNAs corresponding to the rat brain glucose transporter and a cDNA to α-actin was used to monitor the transcript level of a typical housekeeping gene. The study was repeated three times and, in all cases, the BBB glucose transporter mRNA level was increased in experimental diabetes relative to control rats. These studies suggest that factors associated with experimental diabetes mellitus in rats lead to either an increased transcription or a decreased degradation of brain capillary glucose transporter mRNA.

Patients with diabetes mellitus and poor glycemic control are known to have symptoms of hypoglycemia at relatively normal blood glucose concentrations following the institution of insulin therapy (1, 2). These clinical observations suggest that the rate of glucose transport into brain of the poorly controlled diabetic is down-regulated. The rate limiting step of sugar transport from blood to brain is at the brain capillary endothelial wall, which makes up the blood-brain barrier (BBB) in vivo (3). Moreover, previous studies have shown that the BBB glucose transporter activity is down-regulated in experimental diabetes in vivo (4, 5), and that the number of brain microvessel cytochalasin B binding sites is decreased in experimental diabetes (6). The site of down-regulation of the BBB

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glucose transporter in chronic hyperglycemia is unknown. One such site may be at the level of glucose transporter mRNA, and these levels may be changed owing either to altered transcription or mRNA degradation rates. Therefore, the present studies were undertaken to determine whether there are any changes in the level of brain capillary glucose transporter mRNA in experimental diabetes employing a cDNA probe of the rat brain glucose transporter (7).

METHODS

Materials - Deoxycytidine 5'-triphosphate tetra(triethylammonium) salt, [α-^{32}P], 5,000 Ci/mmol, was purchased from DuPont-NEN (Boston, MA). Guanidinium isothiocynate was obtained from Eastman-Kodak Company (Rochester, NY). Cesium chloride and phenol were obtained from U.S. Biochemicals (Cleveland, OH). A 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 pUC19 was generously provided by Ora M. Rosen, M.D. (Memorial Sloane-Kettering Cancer Center, New York, NY). A mouse α-actin cDNA clone in pAM91 described previously (8) was generously provided by Michael J. Getz, Ph.D. (Mayo Foundation, Rochester MN). Streptozotocin and all other chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Treatment of Animals and Brain Microvessel Isolation - Experimental diabetes mellitus was induced in male, Sprague-Dawley rats (250 g) with intraperitoneal streptozotocin (65 mg/kg) dissolved in citrate buffer as described previously (9). Diabetes was verified in the streptozotocin-treated group by monitoring urine glucose throughout the study and the urine of the diabetic animals was always in excess of 1 g/100 ml without ketonuria. All animals were allowed Purina rat chow and water ad libitum for 6 days prior to isolation of microvessels. At this time, animals were sacrificed by decapitation, trunk blood was collected for measurement of serum glucose by an enzymatic fluorometric method as described previously (10), and the brains were immediately removed and placed in cold buffer (0.10 M NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, 1 mM pyruvate, 0.5% bovine albumin). The choroid plexus were carefully removed from the brains and the cerebral microvessels were prepared as described previously (9) from groups of 20 rats with experimental diabetes and 20 control rats. The experiment was repeated three times with a total of 60 rats in both the control and the diabetic groups.

RNA Isolation and Northern Blotting - The microvessels from 20 rat brains were pooled and suspended in 5 ml of 4 M guanidinium isothiocynate and homogenized with a polytron PCU-2 homogenizer for 15 seconds at ½ speed. The homogenate was centrifuged at 1,000 g for ten minutes and the supernatant was applied to 4.5 ml of 5.7 M cesium chloride and centrifuged overnight at room temperature at 32,000 rpm in a swinging bucket (SW41Ti) in a Beckman L8-70M ultracentrifuge (11). The RNA pellet was dissolved in 0.3 M sodium acetate (pH = 6) and extracted with saline-saturated phenol and chloroform. The RNA was precipitated with 70% ethanol, dried, and suspended in RNAse-free distilled water. Aliquots were diluted in water for measurements of A_{260} and A_{280}, and these ratios ranged from 1.8 to 2.2. A typical yield of total brain microvessel RNA from 20 rat brains was 20 μg. Equal 5 or 10 μg aliquots of RNA from either the control or the

diabetic group were applied to 1.1% agarose/2.2 M formaldehyde gels and, following ethidium bromide staining, the 18S and 28S ribosomal RNA bands were of equal intensity in the diabetic and control groups. Following blotting to Gene-screen nylon filters and baking in a vacuum oven at 80°C for 2 hours, the filters were hybridized under high stringency conditions with either [^{32}P]-labeled glucose transporter cDNA or [^{32}P]-labeled α-actin cDNA. Blots were hybridized in 50% formamide, 2x Denhardt's solution, 2x SSPE (1x SSPE = 0.15 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH = 7.4), 1% SDS, 100 μg/ml salmon sperm DNA, and 5% dextran sulfate at 42°C overnight. The filters were washed for 15 minutes with 2x, 0.5x, and 0.1x standard saline citrate (SSC) containing 0.1% SDS successively for 15 minutes, with a final wash of 0.1x SSC and 1% SDS for 30 minutes at 50°C. Filters were unhybridized in hot 0.01x SSC containing 0.01% SDS. Autoradiography was performed with Kodak X-Omat AR film using intensifying screens and autoradiograms were quantified by scanning densitometry (LKB Model 2202 Ultrascan Laser Densitometer, Bromma, Sweden).

RESULTS

The serum glucose of the diabetic animals six days following streptozotocin was 422 ± 23 mg% (mean ± S.E., n = 24) versus the blood sugar of the control animals, 98 ± 4 mg% (mean ± S.E., n = 12). In all studies, the level of brain microvessel glucose transporter mRNA was increased in the diabetic group as compared to the controls, and a representative study is shown in Figure 1. Conversely, the level of α-actin mRNA in the control and diabetic rat brain microvessels was unchanged as shown in Figure 2. The glucose transporter/actin intensity ratio in arbitrary units was 3.0 in the diabetic group and 1.2 in the controls, which represents a 152% increase in glucose transporter mRNA levels in the diabetic microvessels as compared to the controls.

DISCUSSION

These studies report the unexpected finding of an up-regulation of the BBB glucose transporter mRNA level in experimental diabetes mellitus. This represents a specific increase in the glucose transporter mRNA levels since α-actin messages were unchanged in experimental diabetes (Figure 2). Whether the increased BBB glucose transporter mRNA levels in experimental diabetes reflect increased transcription rates or decreased mRNA degradation cannot be determined at the present time. Nevertheless, these studies suggest that factors in the metabolic milieu of experimental diabetes mellitus augment levels of glucose transporter mRNA in brain microvessels.

The increased mRNA levels, however, do not lead to accelerated BBB glucose transport in experimental diabetes. Two recent studies

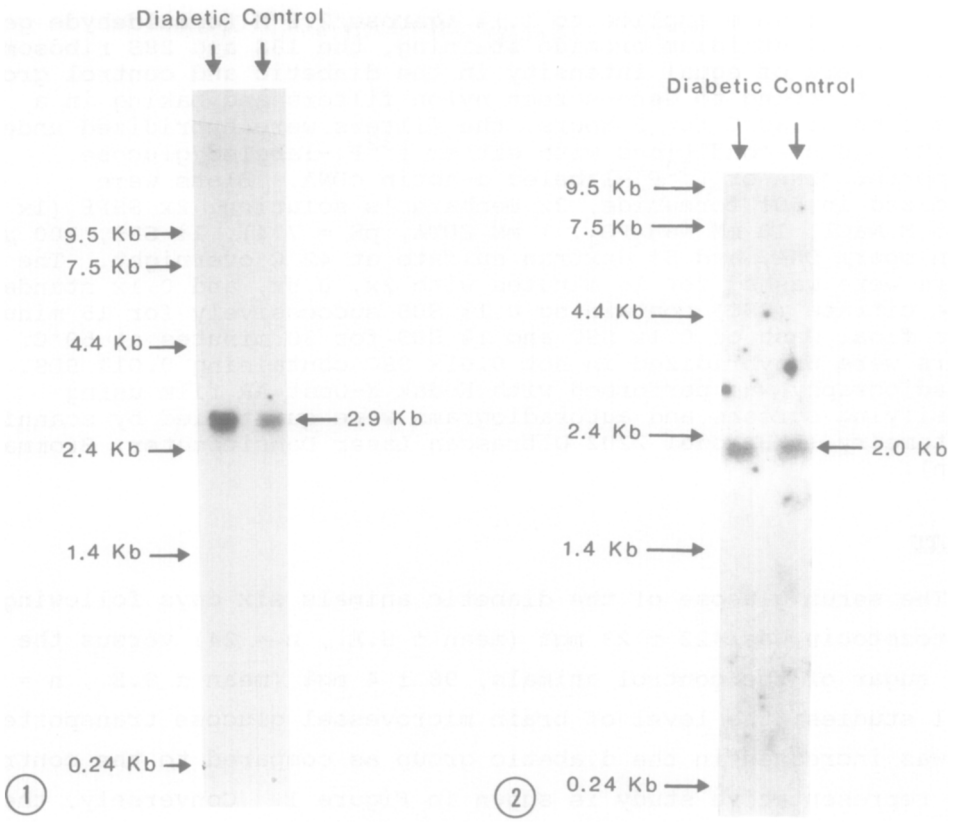

Figure 1. Rat brain glucose transporter (rGT) Northern blot of RNA from rat brain capillaries isolated from either control or streptozotocin-induced diabetic animals. The rGT cDNA hybridized with a 2.9 Kb transcript in both control and diabetics and this transcript was increased 150% in the diabetic group as compared to controls, based on scanning laser densitometry. The molecular weights of an RNA ladder are shown on the left-hand side of the figure.

Figure 2. α-Actin Northern blot of RNA from brain capillaries obtained from either control or diabetic rats. Hybridization with the cDNA showed a specific 2.0 Kb transcript that was present in the control and diabetic animals at equal amounts based on scanning laser densitometry. The molecular weight of the RNA ladder is shown on the left-hand side of the figure.

(12, 13) fail to confirm two earlier reports showing a down-regulation of the BBB glucose transporter activity in experimental diabetes (4, 5). However, our recent studies using the internal carotid artery perfusion technique (14) indicate there is a 54% decrease in the BBB permeability·surface area product to glucose in experimental diabetes (D. Triguero and W.M. Pardridge, manuscript in preparation). Therefore, the increased BBB glucose transporter mRNA level in experimental diabetes is not associated with enhanced glucose transporter activity at the BBB in vivo. The actual mechanism underlying this discordance remains to be determined.

The results of the present studies in conjunction with the recent report by Garvey et al. (15) reveal differential regulation of the brain-type (7) and muscle-type (16) glucose transporters in experimental diabetes. For example, mRNA levels corresponding to the muscle-type glucose transporter are decreased 30-60% in fat and muscle of animals with experimental diabetes, whereas there is no decrease in mRNA levels corresponding to the brain-type glucose transporter in fat tissues of animals with experimental diabetes (14). Thus, the muscle- and brain-type type glucose transporter mRNA levels are regulated in opposite directions in muscle and blood-brain barrier tissues, respectively, in experimental diabetes. Whether insulin deficiency, glucose excess, or some other factor is responsible for the increased BBB glucose transporter mRNA levels in experimental diabetes remains to be determined. Serum growth factors such as fibroblastic growth factor (FGF), or platelet derived growth factor (PDGF), which are also expressed in endothelial cells (17, 18) are known to increase the mRNA levels secondary to enhanced transcription of the brain-type glucose transporter in cultured fibroblasts (19, 20). A putative third factor (i.e., a factor other than glucose or insulin) regulating the level of BBB glucose transporter mRNA levels may be operative in experimental diabetes since the increased mRNA levels (Figure 1) are changed in a way that is opposite of what would be expected on the basis of the high glucose and low insulin levels in experimental diabetes. For example, recent studies show that either glucose deprivation (21) or insulin treatment (22) result in increased levels in cultured cells of the brain-type glucose transporter mRNA.

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REFERENCES

1. DeFronzo, R.A., Hendler, R., and Christensen, N. (1980) Diabetes 29:125-131.
2. Boyle, P.J., Schwartz, N.S., Shah, S.D., Clutter, W.E., and Cryer, P.E. (1988) N. Engl. J. Med. 318:1487-1492.
3. Pardridge, W.M. (1983) Physiol. Rev. 63:1481-1535.
4. Gjedde, A. and Crone, C. (1981) Science 214:456-457.
5. McCall, A.L., Millington, W.R., and Wurtman, R.J. (1982) Proc. Natl. Acad. Sci. USA 79:5406-5410.

6. Matthaei, S., Horuk, R., and Olefsky, J.M. (1986) Diabetes 35: 1181-1184.
7. Birnbaum, M.J., Haspel, H.C., and Rosen, O.M. (1986) Proc. Natl. Acad. Sci. USA 83:5784-5788.
8. Minty, A.J., Caravatti, M., Robert, B., Cohen, A., Daubas, P., Weydert, A., Gros, F., and Buckingham, M. (1981) J. Biol. Chem. 256(2):1008-1014.
9. Frank, H.J.L., Pardridge, W.M., Jankovic-Vokes, T., Vinters, H.V., and Morris, W.L. (1986) J. Neurochem. 47:405-411.
10. Pardridge, W.M., Crane, P.D., Mietus, L.J., and Oldendorf, W.H. (1982) J. Neurochem. 38:560-568.
11. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., and Rutter, W.J. Biochemistry 18:5294-5299.
12. Harik, S.I., and LaManna, J.C. (1988) J. Neurochem. 51:1924-1929.
13. Duckrow, R.B. (1988) Metabol. Brain Dis 3:201-209.
14. Takasato, Y., Rapoport, S.I., and Smith, Q.R. (1984) Am. J. Physiol. 247:H484-H493.
15. Garvey, W.T., Hueckstead, T.P., and Birnbaum, M.J. (1989) Science 245:60-63.
16. Birnbaum, M.J. (1989) Cell 57:305-315.
17. Schweigerer, L., Neufeld, G., Friedman, J., Abraham, J.A., Fiddes, J.C., and Gospodarowicz, D. (1987) Nature 325:257-259.
18. Starksen, N.F., Harsh, G.R., Gibbs, V.C., and Williams, L.T. (1987) J. Biol. Chem. 262(30):14381-14384.
19. Hiraki, Y., Rosen, O.M., and Birnbaum, M.J. (1988) J. Biol. Chem. 263(27):13655-13662.
20. Rollins, B.J., Morrison, E.D., Usher, P., and Flier, J.S. (1988) J. Biol. Chem. 263(32):16523-16526.
21. Walker, P.S., Donovan, J.A., Van Ness, B.G., Fellows. R.E., and Pessin, J.E. (1988) J. Biol. Chem. 263(30):15594-15601.
22. Garcia de Herreros, A. and Birnbaum, M.J. (1989) J. Biol. Chem. 264(17):9855-9890.