Regulation of GLUT-3 glucose transporter in the hippocampus of diabetic rats subjected to stress

Lawrence P. Reagan, Ana Maria Magarínó, Louis R. Lucas, and Bruce S. McEwen. Regulation of GLUT-3 glucose transporter in the hippocampus of diabetic rats subjected to stress. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E879–E886, 1999.—Previous studies from our laboratory have demonstrated that chronic stress produces molecular, morphological, and ultrastructural changes in the rat hippocampus that are accompanied by cognitive deficits. Glucocorticoid attenuation of glucose utilization is proposed to be one of the causative factors involved in stress-induced changes in the hippocampus, producing an energy-compromised environment that may make hippocampal neuronal populations more vulnerable to neurotoxic insults. Similarly, diabetes potentiates neuronal damage in acute neurotoxic events, such as ischemia and stroke. Accordingly, the current study examined the regulation of the neuron-specific glucose transporter, GLUT-3, in the hippocampus of streptozotocin-induced diabetic rats subjected to restraint stress. Diabetes leads to significant increases in GLUT-3 mRNA and protein expression in the hippocampus, increases that are not affected by stress. Collectively, these results suggest that streptozotocin-induced increases in GLUT-3 mRNA and protein expression in the hippocampus may represent a compensatory mechanism to increase glucose utilization during diabetes and also suggest that modulation of GLUT-3 expression is not responsible for glucocorticoid impairment of glucose utilization.

The neurological consequences of diabetes mellitus have most recently been receiving greater attention in both clinical and experimental settings. Hyperglycemia is known to potentiate neurological damage in stroke and ischemia in both human and animal studies (17). In addition, diabetes contributes to cerebrovascular complications, disruption of the blood-brain barrier, and cerebral edema (23). Streptozotocin (STZ), an N-nitroso derivative of glucosamine, has been shown to possess potent diabetogenic properties (29). Administration of STZ leads to the selective destruction of insulin-producing β-cells of the pancreas, resulting in the hallmark clinical features of diabetes, such as hyperglycemia, polyuria, polydipsia, and polyphagia (5). Accordingly, STZ-induced hyperglycemia has been used extensively to examine the physiological and pathophysiological consequences of diabetes. Among them, greater sensitivity to both acute and chronic stressful events is reflected by exaggerated rises in plasma corticosterone (CORT) (9, 36). Indeed, sustained elevations in basal CORT concentrations in STZ-induced diabetes (27) have supported the use of the STZ-treated rat as a paradigm of chronic stress (35). Previous studies from our laboratory have shown that chronic stress or chronic exposure to stress levels of glucocorticoids (GCs) produces atrophy of the apical dendrites of CA1 pyramidal neurons in the hippocampus (14, 44, 45). However, unlike the stress-mediated dendritic remodeling, which is reversible, GCs have also been shown to potentiate neuronal damage in the hippocampus produced by ischemia and excitotoxicity (30). GCs are believed to potentiate neuronal damage during neurotoxic events by decreasing neuronal glucose utilization (21), thereby placing neurons in an energy-compromised environment and increasing neuronal allostatic load (20). Indeed, GCs have been shown to decrease glucose utilization in both experimental and clinical settings (1, 4, 6, 43).

The family of facilitative glucose transporter (GLUT) proteins is responsible for the entry of glucose into cells throughout the periphery and the brain (16, 42). The expression, regulation, and activity of glucose transporters play an essential role in neuronal homeostasis, because glucose represents the primary energy source for the brain (13, 28). Although many isoforms of glucose transporters have been identified in the brain, GLUT-3, the neuron-specific glucose transporter, is solely responsible for the delivery of glucose into neurons in the central nervous system. GLUT-3 mRNA is widely expressed in the brain, including the pyramidal neurons of the hippocampus and the granule neurons of the dentate gyrus (18, 24, 26), and immunohistochemical analysis has demonstrated that GLUT-3 protein expression also exhibits a widespread distribution in the brain (19, 26, 46). In the hippocampus, GLUT-3 immunoreactivity has been identified in mossy fibers, the stratum radiatum and stratum oriens of Ammon’s horn, and the molecular layer of the dentate gyrus (2, 19). Ultrastructural studies have determined that GLUT-3 immunoreactivity is associated with pre- and postsynaptic neuronal processes, nonmyelinated nerves, and small neuronal processes, vesicles, and mitochondria (10).

In an attempt to further define the interaction between GCs and glucose utilization during conditions in which neuronal homeostasis is compromised, we have...
examined the mRNA and protein expression of the neuron-specific glucose transporter GLUT-3 in the hippocampus of STZ diabetic rats subjected to stress. Unlike our previous studies, which examined the effects of 21 days of stress under normal allostatic load, the current study examines the molecular and cellular changes initiated in the hippocampus by 7 days of restraint stress in rats with a preexisting strain on their homeostatic load. The results of this study demonstrate that diabetes leads to a significant increase in GLUT-3 mRNA levels in the rat hippocampus, an effect that does not appear to be affected by restraint stress. Moreover, diabetes also increases GLUT-3 protein in the hippocampus, increases which may be modulated by stress.

MATERIALS AND METHODS

Experimental animals. Adult male Sprague-Dawley rats (CD strain, Charles River, MA) weighing 200–250 g were housed in groups of three with ad libitum access to food and water. Animals were housed in accordance with all guidelines and regulations of The Rockefeller University Animal Care and Use Committee. Animals were maintained in a temperature-controlled room, with a 12:12-h light-dark cycle (lights on at 0700), and were handled daily for 1 wk before being randomly assigned to experimental groups. Control (n = 16) and STZ-treated rats (n = 16) were deprived of food the night before drug administration. The following morning, rats were anesthetized with Ketamine/PromAce (10:1.7; 0.1 ml/100 g) by intravenous injection. STZ (Sigma Chemical, St. Louis, MO; 70 mg/kg) dissolved in citrate buffer (0.1 M, pH 4.2) was delivered through the femoral vein. Control animals were injected with vehicle only. When the development of diabetes had been confirmed by measuring fasting serum glucose concentrations, the 7-day restraint stress paradigm was initiated. Vehicle control rats (n = 8) and diabetic rats (n = 8) were subjected to 6 h of restraint stress daily for 7 days in wire mesh restrainers secured at the head and tail ends with clips. The rats were returned to their home cages during the stress session. Experimental groups now consisted of a vehicle nonstressed group (control, n = 16) and STZ-treated rats (n = 16) who were deprived of food the night before drug administration. The following morning, rats were anesthetized with Ketamine/PromAce (10:1.7; 0.1 ml/100 g). A single intravenous injection of STZ (Sigma Chemical, St. Louis, MO; 70 mg/kg) dissolved in citrate buffer (0.1 M, pH = 4.2) was delivered through the femoral vein. Control animals were injected with vehicle only. When the development of diabetes had been confirmed by measuring fasting serum glucose concentrations, the 7-day restraint stress paradigm was initiated. Vehicle control rats (n = 8) and diabetic rats (n = 8) were subjected to 6 h of restraint stress daily for 7 days in wire mesh restrainers secured at the head and tail ends with clips. The rats were returned to their home cages during the stress session. Experimental groups now consisted of a vehicle nonstressed group (control, n = 8), a diabetes nonstressed group (STZ, n = 8), a vehicle stress group (stress, n = 8), and a diabetic group subjected to stress (STZ/stress, n = 8). On completion of the stress session on day 7, rats were given access to food for several hours before an overnight fast. Eighteen hours after the final stress session, rats were weighed and blood was collected for plasma glucose analysis to confirm that diabetic parameters had been maintained in the STZ-treated rats.

Plasma glucose analysis. The development of diabetes was verified 48 h after STZ administration by measuring fasting glucose concentrations with the glucose oxidase method (Glucose (trinder) kit, Sigma). Blood samples were collected from the tip of the tail in heparinized tubes. Rats were considered diabetic and included in the study if they had plasma glucose levels >350 mg/dl. In addition, fasting serum glucose concentrations were determined 18 h after the final restraint stress session to confirm that hyperglycemia was maintained during the 7-day stress paradigm.

In situ hybridization histochemistry. Coronal 16-µm sections were prepared on a cryostat microtome, collected on gelatin-coated diethylpyrocarbonate-treated slides, and stored at −70°C until hybridization. Before hybridization, sections were fixed in 4% formaldehyde in PBS, treated with 0.1 M triethanolamine-HCl, and then acetylated in a solution of 0.25% acetic anhydride in 0.1 M triethanolamine-HCl. Sections were then rinsed twice in 2× standard sodium citrate (SSC), dehydrated with increasing ethanol washes, and allowed to air dry at room temperature. Antisense and sense riboprobes were generated from pGEM vector corresponding to nucleotides 1040 to 1353 of the rat GLUT-3 gene, graciously provided by Dr. Graeme Bell. Antisense riboprobes were labeled with [35S]UTP using SP6 RNA polymerase. Sense probes were labeled with [35S]UTP using T7 RNA polymerase. Hybridization mix ([50% formamide, 600 mM NaCl, 10 mM Tris·HCl (pH 7.4), 1× Denhardt’s solution, 1 mM EDTA, 10 mM dithiothreitol, 10% dextran sulfate, 100 µg/ml denatured salmon sperm DNA, 250 µg/ml tRNA, and 3× 106 counts/min of radiolabeled probe] was added at 0.120 ml per slide, and slides were coveredslipped and incubated overnight at 55°C in a humidified environment. After hybridization, coverslips were removed and sections were washed twice in 2× SSC. Sections were treated with RNase A (20 µg/ml) in RNase digestion buffer [0.5 M NaCl, 10 mM Tris·HCl (pH 7.4), and 1 mM EDTA] for 30 min at 37°C, washed in RNase buffer without RNase A for 10 min at room temperature, and washed in 2× SSC for 5 min, followed by a 2× SSC wash for 20 min at room temperature. Sections were then washed twice in 0.2× SSC at 55°C for 30 min, dehydrated in increasing ethanol washes, and allowed to air dry. Dried sections were exposed to Kodak X-OMAT film for autoradiography and subsequently dipped in Kodak NTB-2 emulsion. Emulsion slides were developed with Kodak D-19 developer at 15°C and counterstained with cresyl violet. All autoradiographic films and emulsion slides were developed and processed for analysis at identical time points in the study.

Radioimmunocytochemistry. Radioimmunocytochemistry (RIC) was performed using previously established protocols in our laboratory (12). Nonetheless, all parameters were reevaluated to optimize the RIC protocol for the GLUT-3 antisera. For example, concentration curves were performed for both [35S]-labeled secondary antisera and primary antisera (see Fig. 3). Postfixing rat brain sections with 4% paraformaldehyde or 4% formaldehyde produced identical results. The addition of Triton-X 100 to the assay did not affect optical density signals and was therefore omitted from the protocol. Finally, washes performed in the presence of Tween 20 produced similar results as washes performed in the absence of Tween 20. After these preliminary assays, the RIC protocol was performed in the following manner. Rat brain sections were cut on a cryostat microtome at a thickness of 16 µm on gelatin-coated diethylpyrocarbonate-treated slides and stored at −70°C. Slides were removed from the freezer and air dried at room temperature for 15 min. Sections were fixed with 4% formaldehyde in 0.05 M PBS (pH 7.4) for 30 min at room temperature with gentle shaking. Sections were washed three times for 5 min each at room temperature with gentle shaking in 0.05 M PBS and were incubated with 1% BSA in 0.05 M PBS at room temperature for 30 min with gentle shaking to reduce nonspecific binding of primary antibody. Sections were incubated with previously characterized polyclonal antisera directed against GLUT-3 ([ALM-SCC, see Ref. 15]) in 0.05 M PBS plus 1% BSA for 2 h at room temperature with gentle shaking. Sections were washed three times for 5 min each with PBS plus 1% BSA and incubated with [35S]-labeled goat anti-rabbit secondary antisera (Amersham) at a dilution of 1:400 in 0.05 M PBS plus 1% BSA at room temperature with gentle shaking for 2 h. Sections were rinsed several times with 0.05 M PBS and washed three times for 5 min with gentle shaking. Slides were air dried under a fan overnight and exposed to Kodak X-OMAT. Because saturat-
Table 1. Physiological parameters of control rats, streptozotocin diabetic rats, rats subjected to restraint stress, and diabetic rats subjected to restraint stress

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<th>Control</th>
<th>STZ</th>
<th>Stress</th>
<th>STZ Stress</th>
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<tr>
<td>Plasma glucose, mg/dl</td>
<td>117 ± 0.003</td>
<td>402 ± 0.031*</td>
<td>116 ± 0.003</td>
<td>419 ± 0.007*</td>
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<tr>
<td>Body weight gain, g</td>
<td>65.75 ± 4.03</td>
<td>11.38 ± 3.5*</td>
<td>42.25 ± 3.15†</td>
<td>-2.75 ± 4.13‡</td>
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Values represent means ± SE. Fasting plasma glucose concentrations measured 24 h after final restraint stress session. Changes in body weight calculated as difference in weight on final day of paradigm vs. body weight on day of STZ administration. See MATERIALS AND METHODS for description of groups. Significantly different (P < 0.001): * from control; † from STZ; ‡ from stress. Minus sign, loss of body weight relative to initial body weight.

Results

Two days after STZ administration, rats exhibited the expected manifestations of diabetes, including polyuria, polydipsia, and polyphagia. The effectiveness of STZ administration to induce diabetes in rats was confirmed by the determination of fasting serum glucose concentrations. In this regard, serum glucose concentrations were increased 2 days after STZ administration (data not shown) and remained elevated until completion of the 7-day stress paradigm (Table 1). Restraint stress did not affect fasting serum glucose concentrations in either vehicle or STZ-treated rats. However, restraint stress did produce the expected attenuation in body weight gain. For example, rats subjected to stress alone exhibited significant reductions in weight gain compared with vehicle controls. Diabetes alone produced even greater reductions in weight gain, whereas diabetic rats subjected to stress exhibited weight loss during the diabetes/stress paradigm (Table 1). In addition, we have previously demonstrated that diabetic rats exhibit increased basal levels of GCs compared with control rats, as well as potentiated stress-induced increases in GCs compared with nondiabetic stressed rats (15). On completion of the stress paradigm and physiological confirmation of diabetes, the stress/diabetes regulation of GLUT-3 expression in the hippocampus was examined by in situ hybridization histochemistry and RIC.

GLUT-3 in situ hybridization. In agreement with previous reports (24, 26), GLUT-3 mRNA was detected in the pyramidal neurons of the hippocampus and the granule neurons of the dentate gyrus (Fig. 1A). The specificity of the antisense-labeled probe was demonstrated with the use of a 35S-labeled sense probe that did not detect GLUT-3 mRNA in rat brain (Fig. 1B). Emulsion autoradiographic analysis revealed that GLUT-3 mRNA was expressed exclusively in the hippocampal pyramidal neurons and granule neurons of the dentate gyrus; GLUT-3 mRNA was not detected in hippocampal interneurons (data not shown). The regulation of GLUT-3 mRNA expression was analyzed in CA1, CA2, and CA3 pyramidal neurons, as well as granule neurons of the dentate gyrus (see Fig. 1A). Subsequent statistical analysis revealed that the 7-day stress paradigm produced no change in GLUT-3 mRNA levels in hippocampal pyramidal neurons relative to controls. However, GLUT-3 mRNA expression was significantly increased in hippocampal pyramidal neurons of diabetic rats compared with control rats, increases that were not altered by daily restraint stress (Fig. 2A). Similar results were observed in granule neurons of the superior blade of the dentate gyrus (DGs), in that diabetes increased the expression of GLUT-3 mRNA,

Fig. 1. Autoradiographic analysis of GLUT-3 mRNA expression in rat brain. A: GLUT-3 expression in rat brain as detected by use of 35S-labeled antisense riboprobe. In agreement with previous reports, high expression of GLUT-3 mRNA is observed in the hippocampus, in particular in the pyramidal cell layer (CA1, CA2, CA3) and the dentate gyrus (DG). B: adjacent section incubated with 35S-labeled sense riboprobe fails to detect GLUT-3 mRNA in rat brain.
an effect not altered by stress (Fig. 2B). Diabetic rats not subjected to stress also exhibited significant increases in GLUT-3 mRNA expression in the inferior blade of the dentate gyrus (DGi). The regulation of GLUT-3 mRNA expression in the cerebral cortex of diabetic rats, stressed rats, and diabetic rats subjected to stress was also examined. However, unlike the hippocampus, the cortex did not exhibit changes in GLUT-3 mRNA levels in any of these groups compared with control animals (data not shown).

RIC for GLUT-3. The regulation of GLUT-3 protein expression in the hippocampus of diabetic rats was examined using previously characterized antisera selective for this glucose transporter isoform (2, 10, 19) and 35S-labeled goat anti-rabbit secondary antisera. When combined with in situ hybridization histochemistry, RIC allows for the examination of both protein and mRNA expression in adjacent sections from the same animal. Moreover, the use of radiolabeled secondary antisera provides a distinct advantage over traditional immunohistochemical techniques, namely, the ability to quantify changes in protein expression. Finally, RIC allows for the determination of changes in protein expression in individual neuronal populations, analysis that cannot be performed using quantitative immunoblot techniques. Indeed, this technique has successfully been used to compare GLUT-3 protein expression with glucose utilization in rat brain (46). Previous studies with GLUT-3 antisera have established 1:5,000 as the appropriate dilution for nonisotopic immunohistochemistry using diaminobenzidine as the substrate for the peroxidase reaction (19). To identify changes in protein expression mediated by the stress/diabetes paradigm, saturating concentrations of antisera must be utilized.

In an attempt to determine the optimal primary antibody dilution, RIC was performed using GLUT-3 antisera at increasing concentrations ranging from 1:20,000 to 1:3,000. The percent specific optical density signal was determined by autoradiographic analysis and revealed that antibody dilutions in the range of 1:3,000–1:5,000 provided saturating concentrations of antisera (Fig. 3). Therefore, a dilution factor of 1:5,000 was used in subsequent RIC analysis. GLUT-3 RIC was performed in adjacent sections that were used for in situ hybridization histochemical analysis in vehicle controls, diabetic rats, stressed rats, and diabetic rats subjected to stress.
In agreement with previous studies (2, 10, 19), GLUT-3 immunolabeling was present within the neuropil, whereas the cell bodies of principal cells in the hippocampus did not exhibit GLUT-3 immunoreactivity (Fig. 4A). The specificity of GLUT-3 immunolabeling was confirmed by omitting the primary antisera from the reaction (Fig. 4B). The distribution of GLUT-3 immunolabeling using the RIC protocol was identical to previously performed immunohistochemical analysis (2, 10, 19). Autoradiographic image analysis of GLUT-3 immunolabeling was performed in the following neural populations of the hippocampus: stratum oriens and stratum radiatum in CA1, CA2, and CA3, the molecular layer of the superior blade of the dentate gyrus (DGs-mol), the molecular layer of the inferior blade of the dentate gyrus (DGi-mol), and hilus (see Fig. 4A). Subsequent statistical analysis revealed that GLUT-3 protein expression was increased in the oriens and radiatum of Ammon’s horn (Fig. 5A) as well as the molecular layers of the dentate gyrus and the hilus (Fig. 5B) in diabetic rats subjected to restraint stress compared with vehicle-treated nonstressed rats. Diabetic rats also exhibited trends for increased expression of GLUT-3 protein, although with the exception of CA2 radiatum, these increases in GLUT-3 protein expression did not achieve statistical significance. Conversely, daily restraint stress had no effect on GLUT-3 protein expression compared with controls. Collectively, these results demonstrate that, whereas restraint stress alone had no effect on GLUT-3 protein expression compared with controls, these results suggest that restraint stress administered to vehicle-treated rats or STZ-treated rats did not produce changes in GLUT-3 mRNA expression as determined by in situ hybridization histochemistry. The results of the current study also revealed that GLUT-3 protein levels were increased in the hippocampus of diabetic rats subjected to restraint stress, suggesting that under conditions of increased neuronal allostatic load, stress may contribute to increased expression of GLUT-3 protein in the

DISCUSSION

The results of the current study demonstrate that hyperglycemia induced by STZ administration results in increased expression of GLUT-3 mRNA levels in the pyramidal neurons of the hippocampus and the granule neurons of the dentate gyrus. Conversely, daily restraint stress administered to vehicle-treated rats or STZ-treated rats did not produce changes in GLUT-3 mRNA expression as determined by in situ hybridization histochemistry. The results of the current study also revealed that GLUT-3 protein levels were increased in the hippocampus of diabetic rats subjected to restraint stress, suggesting that under conditions of increased neuronal allostatic load, stress may contribute to increased expression of GLUT-3 protein in the
hippocampus of diabetic rats. These findings are relevant to previous studies that have examined the regulation of GLUT-3 expression during exposure to stress levels of GCs, as occurs during acute neurotoxic events (30).

Regulation of glucose transporter expression in diabetes. Previous studies examining the regulation of GLUT-3 expression in the brain during different glycemic conditions have provided equivocal results. For example, chronic hypoglycemia has been shown to increase GLUT-3 mRNA in mouse hippocampus (25) as well as to increase GLUT-3 protein levels in rat brain (39). GLUT-3 expression has also been examined after STZ administration. Kainulainen et al. (7) reported that, 3 wk after STZ administration, GLUT-3 protein levels were unchanged in diabetic rats compared with control animals as determined by immunoblot analysis of crude brain membranes. Such results suggest that GLUT-3 protein levels are unchanged in response to chronic hyperglycemia. Similarly, GLUT-3 mRNA levels were unchanged 2 wk and 3 wk after STZ administration in mouse brain, as determined by Northern analysis (25). However, immunoblot and Northern analysis using crude brain preparations may conceal regional changes in GLUT-3 expression in response to STZ administration. Indeed, in the current study, GLUT-3 mRNA levels were increased in the hippocampus of STZ diabetic rats, increases not observed in the cortex.

The STZ-treated rat is used as a model of type I (insulin-dependent) diabetes. Several genetic models of type II (non-insulin-dependent) diabetes have also been utilized to examine the consequences of hyperglycemia on GLUT-3 expression. For example, Vannucci et al. (41) examined the developmental regulation of GLUT-3 mRNA in several brain regions of the db/db mouse. In particular, GLUT-3 mRNA levels were increased in the thalamus of the db/db mouse at 5 wk, whereas GLUT-3 mRNA levels were decreased in the frontal cortex in the 10-wk-old db/db mouse. Accordingly, it would be interesting to examine the regulation of GLUT-3 mRNA and protein expression in the hippocampus of db/db mice. Such results would allow for a direct comparison of the regulation of GLUT-3 expression in experimental models of both type I and type II diabetes.

Glucose transporter expression, neurotoxicity, and damage. The regulation of glucose transporter expression and activity may also play an important role during acute neurotoxic events such as ischemia. Indeed, numerous studies have demonstrated that hyperglycemia or experimentally induced diabetes exacerbates neuronal damage produced by ischemia (17, 23). In this regard, Lee and Bondy (8) demonstrated that middle cerebral artery occlusion increased GLUT-3 mRNA expression. These results were confirmed and extended by subsequent studies, which reported that GLUT-3 mRNA expression increases after ischemia and reperfusion (3, 40). Conversely, McCaill et al. (18) demonstrated that GLUT-3 mRNA levels were unaffected 24 h after global forebrain ischemia but were reduced 4–7 days after the ischemic episode in CA1 pyramidal neurons. Similarly, GLUT-3 immunoreactivity was reduced in the CA1 region 4–7 days after ischemia. The decrease in GLUT-3 mRNA and protein levels in CA1 pyramidal neurons is expected, because this neuronal population is particularly vulnerable to ischemic insults (30). The expression and activity of GLUT-3, therefore, may play an important role in determining neuronal survival after an ischemic episode. The questions that remain to be addressed are what is the relationship between glucose transporter activity and expression during ischemia and how does the modulation of GLUT-3 expression serve to protect or endanger vulnerable neuronal populations during acute neurotoxic events. For example, because GLUT-3 mRNA and protein expression are unaffected by restraint stress, it would be interesting to examine the stress regulation of other glucose transporters expressed in the brain, such as GLUT-1, particularly because neuronal expression of GLUT-1 is observed during ischemia (8), which is associated with increased levels of GCs.

Effects of stress and GCs. Previous studies have clearly established that, unlike the relationship between glucose transporter expression and neuronal damage, GCs make hippocampal neurons more vulnerable to neurotoxic events. For example, Sapolsky and Pulsinelli (34) reported that stress levels of GCs enhanced neuronal damage produced by ischemia, whereas adrenalectomized rats exhibited attenuated damage. Subsequent investigations revealed that GCs potentiated excitotoxicity-induced neuronal damage (31–33, 37). The ability of GCs to inhibit glucose utilization in hippocampal neurons provides a potential mechanism through which GCs may potentiate neuronal damage during acute neurotoxic events (1, 4, 6, 43). Decreases in glucose uptake mediated by stress levels of GCs could place neurons in an energy-compromised environment, which could detrimentally affect neuronal responsiveness to pathophysiological events. Indeed, stress has been shown to lead to increased concentrations of glutamate in the hippocampus (11, 22, 38), an effect that is proposed to result from compromised activity of the energy-dependent excitatory amino acid transporters (21, 30). The results of the current study demonstrate that a 7-day period of restraint stress, however, does not modulate GLUT-3 mRNA or protein expression in the rat hippocampus. Moreover, GLUT-3 mRNA levels are unchanged 24 h after acute administration of stress levels of CORT (L. P. Reagan and B. S. McEwen, unpublished observations). Such results suggest that the GC-mediated decreases in glucose utilization in the hippocampus do not involve decreases in GLUT-3 mRNA or GLUT-3 protein expression and therefore may instead involve a stress-mediated impairment of GLUT-3 activity.

In conclusion, the results of the current study demonstrate that GLUT-3 mRNA levels are increased in the hippocampus of diabetic rats. In addition, GLUT-3 protein expression is increased in the hippocampus of...
STZ-treated rats subjected to restraint stress. The upregulation of GLUT-3 mRNA and protein in the hippocampus of STZ-treated rats may represent a compensatory mechanism to increase glucose uptake and utilization, because glucose utilization and uptake have been shown to be decreased in experimentally induced diabetes (17, 23). However, GLUT-3 mRNA levels or protein expression was not changed in rats subjected to restraint stress. Therefore, the results of this study suggest that the impairment of glucose utilization by stress levels of GCs does not occur at the transcriptional or translational levels for GLUT-3.

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Address for reprint requests and other correspondence: L. P. Reagan, The Rockefeller Univ, Box 165, 1230 York Ave, New York, NY 10021 (e-mail: reagani@rockvax.rockefeller.edu).

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REFERENCES


36. Scribner, K. A., C. D. Walker, C. S. Cascio, and M. F. Dellman. Chronic streptozotocin diabetes in rats facilitates the...


