Experimental Diabetes Attenuates Cerebral Cortical—Evoked Forelimb Motor Responses

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Poorly controlled diabetes leads to debilitating peripheral complications, including retinopathy, nephropathy, and neuropathy. Chronic diabetes also impairs the central nervous system (CNS), leading to measurable deficits in cognition, somatosensory, and motor function. The cause of diabetes-associated CNS impairment is unknown. In this study, sustained hyperglycemia resulting from insulin deficiency was shown to contribute to CNS motor dysfunction. Experimental diabetes was induced in rats by streptozotocin (STZ) injection. CNS motor function was assessed by intracortical microstimulation of the sensorimotor cortex. Experimental diabetes significantly \((P < 0.01; n = 14)\) attenuated the number of motor cortical sites eliciting forelimb movements. The net area of the motor cortex representing the forelimb in diabetic rats was significantly reduced \((4.0 \pm 0.5 \text{ [control]} \text{ vs. } 2.4 \pm 0.4 \text{ [STZ]} \text{ mm}^2; P < 0.05)\). Experimental diabetes attenuated the activation of some, but not all, forelimb motor cortical neurons. Insulin treatment of diabetic rats prevented the attenuation of cortical-evoked forelimb responses. Peripheral nerve–evoked responses were unaffected by this short period of diabetes, suggesting the absence of peripheral nerve dysfunction. This study showed that metabolic imbalance resulting from insulin deficiency elicits a marked attenuation of cortical-evoked motor function. Uncontrolled hyperglycemia, deficiencies of central insulin, or both may contribute to corticospinal motor dysfunction. *Diabetes* 54:2764–2771, 2005

Diabetes and its chronic complications lead to extensive quality of life and economic burdens that are shared across the world (1,2). The etiology and pathogenesis of diabetes and its complications remain unclear. An imbalance in blood glucose regulation is the clinical hallmark of the diabetic syndrome and occurs as a result of deficiencies in insulin secretion, insulin action, or both. Sustained periods of hyperglycemia are considered a contributing factor in the development of diabetic complications, including retinopathy, nephropathy, and neuropathy. Diabetic patients are also at increased risk for developing central nervous system (CNS) dysfunction (3–5), including impaired central motor conduction (6,7) and, on rare occasions, hemichorea-hemiballismus associated with nonketotic hyperglycemia (8).

The adult brain, historically considered an insulin-insensitive tissue, is uniquely dependent on the availability of glucose for energy homoeostasis. The entry of glucose into the brain occurs by facilitative transport. Brain GLUT proteins exhibit both cell type (e.g., GLUT1, microvessel endothelial cells/astroglia; GLUT3, neurons) and region-specific localization (rev. in 9). The insulin-sensitive GLUTs (GLUT4 and GLUT8) are expressed in the brain and may participate in the centrally mediated actions of insulin and glucose. The insulin receptor is also expressed in discrete neuronal populations in the CNS (9,10) and is now recognized as serving a critical role in neuronal growth, differentiation, and function (rev. in 11). Insulin delivery to the brain may occur either through circumventricular brain regions or by active transport using a saturable insulin receptor–mediated transporter (12). De novo synthesis of central insulin is also suggested by the presence of insulin gene expression in discrete brain regions (13,14); modest levels of insulin are expressed within the hippocampus, hypothalamus, olfactory bulb, cerebral cortex, and Purkinje cells of the cerebellar cortex. Although previous studies have implicated central insulin in the control of energy expenditure and ingestive and appetite behaviors (15), more recent studies suggest that insulin may even participate in strengthening synaptic efficacy (16–18). The functional significance of central insulin in the healthy and diabetic subjects, however, remains unclear.

In the present study, we examined the effects of uncontrolled insulin-dependent diabetes on cerebral cortical topography and the excitability of evoked forelimb motor...
responses in SD rats. We report that a short duration (8 weeks) of sustained hyperglycemia/hypoinsulinemia produced a marked attenuation of cerebral cortical—evoked forelimb motor responses with a significant reduction of motor area topography. This novel finding is consistent with previous reports of decreased hippocampal synaptic plasticity (19,20) and disruption of neuronal function (6,7,21,22) in diabetes. This study extends these observations and suggests that dysregulation of central glucose and insulin in patients with poorly controlled diabetes may result in altered cerebral corticospinal motor function.

RESEARCH DESIGN AND METHODS

This study was conducted using protocols approved by the Institutional Animal Care and Use Committee in accordance with the principles of laboratory animal care (National Institutes of Health Publ. 86-23, 1985). All animals were housed in pairs, allowed standard rat diet and water ad libitum, and maintained on a 10/14-h light/dark cycle. Adult male SD rats (initial body weight 300 g) were divided into three experimental groups: nondiabetic vehicle-treated control animals (n = 14), untreated diabetic animals (n = 14), and insulin-treated diabetic animals (n = 6). Rats from each group were randomized to undergo a 45-day period of vehicle or streptozotocin administration. The rationale for this short period of assessment was to minimize the time of potential confounders secondary to diabetes-induced nutritional deficiencies.

Experimental diabetes was induced in nonfasted rats (initial body weight 300 g) by a single intraperitoneal injection of freshly prepared STZ (60 mg/kg body wt; Sigma, St. Louis, MO) dissolved in citrate buffer (100 mmol/l; pH 4.5). For the insulin-treated group, two sustained-release insulin pellets (~2 units · 24 h⁻¹ · implant for >40 days; Lin Shin Canada, Ontario, Canada) were implanted subcutaneously 4 weeks after STZ injection to normalize and maintain blood glucose levels near physiological concentrations of 100 mg/dl. Control rats received an equal volume of intraperitoneally injected, freshly prepared citrate buffer (100 mmol/l; pH 4.5). Nonfasting blood glucose levels were determined before vehicle or STZ was administered and at regular weekly intervals throughout the study. Blood samples were obtained by tail prick, and glucose content was quantitated using a commercial glucometer (Glucosemeter Dex; Bayer, Elkhart, IN). Animals were decapitated, and their brains were rapidly removed, snap frozen at −80°C, and stored at −70°C until used. Thawed aliquots of cortical homogenates were diluted, and protein concentrations (~10 mg/ml protein) were quantitated by the method of Lowry et al. (26) with BSA as the standard. Cortical proteins (30 μg) were resuspended with 5 μl lysis buffer (1% SDS, 10% β-mercaptoethanol, 0.1 M Tris, pH 7.4) and electrophoresed on 15% sodium dodecyl sulfate-polyacrylamide gels. Densitometric analysis of Coomassie blue-stained gels was performed using GS-670 and Molecular Imager (Bio-Rad, Hercules, CA). Protein concentrations were estimated with a BCA protein assay kit (Pierce, Rockford, IL).

STZ-induced diabetes. Administration of STZ to SD rats (n = 14) produced a marked elevation of nonfasting blood glucose levels (~400 mg/dl) that was sustained throughout this 8-week study (Fig. 1). Subcutaneous implantation of insulin pellets at 4 weeks (n = 6) reversed the observed hyperglycemic effects of STZ and normalized blood glucose levels to near physiological concentrations, consistent with the experimental induction of insulin-dependent diabetes (28,29). By comparison, nondiabetic control rats (n = 14) receiving an equal volume of citrate buffer (100 mmol/l; pH 4.5) maintained nonfasting blood glucose levels near 100 mg/dl (Fig. 1A). The body weight of the nondiabetic rats steadily increased over the course of study. The STZ-induced diabetic rats, while consistently demonstrating sustained hyperglycemia, maintained their starting body weight (~300 g) and failed to gain weight over the course of the study. In contrast, diabetic rats receiving insulin treatment experienced a rapid and steady weight gain that was nearly identical to that of the nondiabetic control rats at the time they were killed (Fig. 1B). The diminished capacity of the STZ-induced diabetic rat to gain body weight and the reversal of this with insulin treatment provided further evidence of the induction of clinically relevant diabetes in these animals. At no time throughout the course of this study did our STZ-induced diabetic rats lose body weight, suggesting that complications secondary to diabetes-induced nutritional deficiencies were negligible. Cortical-evoked functional responses in STZ-induced diabetic rats. Microstimulation of the right sensorimotor
cortex of ketamine-xylazine–anesthetized rats (Fig. 2A) elicited reproducible contralateral forelimb and hindlimb motor responses that were visually recorded by two independent observers. In a previous mapping study using nondiabetic Long-Evans hooded rats, Neafsey et al. (23) demonstrated the presence of at least two discontinuous cortical forelimb areas: a large caudal primary motor area beginning at the bregma and a small rostral supplementary area located near the frontal pole. In contrast, the topographical boundaries of the forelimb motor cortical areas in the nondiabetic SD rats were found to extend uniformly over an area 1.0–4.0 mm lateral from the midline (Fig. 2B) and from 4.0 mm rostral to 2.0 mm caudal of the bregma (Fig. 2C), suggesting possible strain-related differences in cortical forelimb topographical boundaries. The majority of cortical sites eliciting forelimb motor movements were located 3.0–0.0 mm rostral and 1.5–3.5 mm lateral to the bregma. Stimulation along the topographical areas bordering the forelimb motor cortex domain in these rats elicited various types of motor movements. Rostral borders produced jaw and tongue movements whereas medial limits caused vibrissae to move. The caudal borders showed tail, hindlimb, and toe representation.

After 8 weeks of untreated diabetes, affected rats showed a significant attenuation in the number of individual motor cortical sites eliciting forelimb motor movements (Figs. 2B and C). In addition, the net area of the motor cortex that represented the forelimb was also significantly reduced compared with nondiabetic control rats (Fig. 3). The overall topographical organization of the forelimb cortical region was reduced in diabetic rats. These findings demonstrated that an 8-week course of STZ-induced uncontrolled diabetes reduces but does not shift the motor cortical topographical map in the SD rat. Cortical neurons in STZ-induced diabetic rats that were capable of responding to stimuli exhibited near normal activation thresholds (Table 1). The mean activation thresholds for vehicle-treated (20.8 ± 1.5 μA; n = 13), STZ-induced diabetic (24.8 ± 2.0 μA; n = 14), and STZ-induced diabetic and insulin-treated (23.5 ± 1.4 μA; n = 6) rats were not significantly different. These findings suggest that uncontrolled hyperglycemia can significantly attenu-
FIG. 2. Experimental diabetes attenuates cerebral cortical–evoked forelimb motor responses. Cerebral cortical sites eliciting contralateral forelimb movements in the same vehicle-treated (□; n = 13), STZ-administered (■; n = 14), or STZ-administered and insulin-implanted (▲; n = 6) rats used in Fig. 1 were identified by ICMS after 8 weeks of treatment. For each animal, ~80 separate cortical coordinates were stimulated. All cortical points were screened for movement using an initial current of 50 μA. Data shown are the cortical areas that, when stimulated, elicited a contralateral forelimb movement. Stimulated cortical areas that did not elicit contralateral forelimb movements are not shown. A: Dorsal aspect of a rat brain showing representative sites of cerebral cortical stimulation in the right hemisphere. b, bregma. B: Medial to lateral quantitation of evoked forelimb movements. Data are means ± SE. *P < 0.05, **P < 0.01 vs. vehicle-treated controls (by one-way ANOVA with a Bonferroni multiple comparison post hoc test).
ate the activation of some but not all forelimb motor cortical neurons. Cerebral cortical forelimb motor neurons that were responsive in STZ-induced diabetic rats exhibited normal activation thresholds, suggesting that these neurons are relatively resistant to the effects of uncontrolled hyperglycemia.

Diabetic rats treated at 4 weeks with insulin implants exhibited cortical-evoked forelimb motor responses that were nearly identical to those of nondiabetic control rats (Figs. 2B and C). The net cortical area representing the rat forelimb was also spared or restored by insulin therapy (Fig. 3), suggesting that central insulin/euglycemia is important for the maintenance of motor cortical responsiveness.

Peripheral-evoked physiological responses in STZ-induced diabetic rats. Peripheral nerve disease is a chronic complication of human and experimental diabetes (30) that may confound observational or quantitative measurements of evoked motor responses. To determine the degree to which peripheral nerve disease influenced our measurements of evoked motor responses, we quantitated peripheral nerve function in vehicle- and STZ-administered animals. The conduction velocity of the sciatic nerves in STZ-induced diabetic rats in this study was found not to differ significantly from those of nondiabetic vehicle-treated control rats (43 ± 6 [n = 8] vs. 44 ± 6 m/s [n = 10]; P > 0.05). In addition, evoked compound

**FIG. 3.** STZ-induced diabetes reduces, but does not shift, cerebral cortical-evoked forelimb motor topography. Shown are representative data from Fig. 2 topographically demonstrating (A) and quantitatively summatting (B) cerebral cortical-evoked forelimb motor regions in vehicle-treated (vehicle; n = 13), STZ-administered (STZ; n = 14), or STZ-administered and insulin-implanted (STZ + Ins; n = 6) rats. Note that the mean area (mm$^2$) of the forelimb motor cortex is significantly reduced in rats administered STZ compared with control rats receiving vehicle only or diabetic rats with insulin implants. Data are means ± SE. *P < 0.05 vs. vehicle-treated controls (by one-way ANOVA with a Bonferroni multiple comparison post hoc test).
muscle action potential amplitudes, quantitated as the ratio of proximal (sciatic notch) to distal (ankle) responses (27), were similarly unaffected by the relatively short-term (8-week) duration of diabetes \(0.80 \pm 0.04 [n = 8] \) vs. \(0.89 \pm 0.07 [n = 10]; \ P > 0.05\) used in this study. From these data, we concluded that 8 weeks of uncontrolled diabetes was insufficient to produce statistically significant changes in peripheral nerve function. These findings do not, however, address the possibility of the occurrence of remote, and thus diabetes-dependent effects on peripheral and central vascular morphology were not quantitated.

An alternative explanation for the attenuated cortical-evoked responses in our STZ-induced diabetic rats could have been the indirect effects of altered electrolyte balance. Although we did not measure osmolarity or electrolyte levels, sustained plasma glucose levels >500 mg/dl, as observed in this study, would be expected to produce notable osmotic diuresis, with possible changes in electrolyte homeostasis. Egleton et al. (43), however, reported that electrolyte levels within the plasma and CSF of hyperglycemic rats with STZ-induced diabetes of 4 weeks’ duration were similar to those of nondiabetic euglycemic controls. Although ion transporter dysfunction was seen in their study, Egleton et al. (43) reported that the net concentration of electrolytes in the rat CSF was clearly not affected during acute STZ-induced diabetes. Extended periods of uncontrolled hyperglycemia, however, may adversely affect CSF electrolyte levels to a degree that ultimately influences CNS function.

A direct effect of elevated blood glucose (possibly ketogenesis/hypoinsulinemia on CNS function must also be considered. In this regard, changes in local glucose concentrations can alter the firing rate of some types of CNS neurons (i.e., hypothalamic). However, in one study, elevating local glucose concentrations from 5 to 30 mmol/l had no effect on long-term potentiation in healthy CA1 hippocampal neurons, suggesting that hyperglycemia alone may be insufficient to initiate cortical decline in the diabetic patient (44). Decreases in central insulin content or compensatory changes of cortical insulin (or other) neotrophic receptor expression may contribute to CNS decline in the insulin-dependent diabetic patient. Differential regulation of insulin receptor expression in retina from diabetic patients (45) and STZ-induced diabetic rats (46) has been reported. Earlier studies by Pacold and Blackard (47), however, found no change in insulin receptor expression in brain homogenates from STZ-induced diabetic rats when compared with vehicle-treated controls. In our hands, insulin therapy was effective at preventing cerebral cortical-evoked forelimb motor response deficits, possibly because of changes in central insulin receptor expression or signaling. We found that the relative content of the insulin receptor protein expressed in cerebral cortical homogenates of STZ-induced diabetic rats was not, however, altered when compared with vehicle-treated animals and quantitated by Western immunoblot (data not shown).

The cerebral cortical expression of low-affinity p75 neurotrophic receptors, recognized as a 75-kDa doublet on immunoblot, was similarly unaffected by STZ-induced diabetes (data not shown). These findings contrast with the well-established changes of neurotrophic factors implicated in the pathogenesis of diabetic neuropathy (48), a common peripheral complication of chronic diabetes. We suggest that cerebral cortical-evoked forelimb motor response deficits in diabetes occur through a mechanism(s) that does not involve changes in the expression of cerebral cortical insulin or p75 neurotrophic receptors. Altered expression of other growth factor receptors, including...
brain-derived neurotrophic factor, remains an intriguing possibility. Diabetes-induced deficits in cerebral corticospinal motor function involving altered postsynaptic receptor signaling (49) or neurotransmitter metabolism/receptor dysfunction (50) remain to be established.

In summary, we have reported that uncontrolled hyperglycemia/hypoinsulinemia secondary to STZ-induced diabetes produces a marked attenuation of cerebral cortical—evoked forelimb motor responses in SD rats. Deficits in evoked motor responses were preventable with insulin therapy. We propose that CNS deficits related to insulin-dependent diabetes, including impairment of motor responses, may be preventable with appropriate glycemic control.

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