Neurogenesis after brain injury not only leads to the replacement of damaged cells but might also contribute to functional recovery, suggesting the possibility of endogenous neural repair. We investigated the extent of hippocampal neural regeneration in a rat model of hypoglycemia. Two weeks after 30 min of insulin-induced isoelectric electroencephalogram, extensive neuronal loss was observed in the hippocampus, including area CA1 and dentate gyrus (DG). A transient increase in progenitor cell proliferation in the DG subgranular zone (SGZ) was detected, leading to an increase of immature neuroblasts 1–2 weeks after hypoglycemic insult. Most of the surviving newborn cells assumed a neuronal phenotype within 1 month in DG, a few cells near the site of granule-cell death becoming astroglia or microglia. No neuronal regeneration was observed in the CA1 after hypoglycemia, although dividing cells appeared to be astroglia or microglia in CA1 and dentate hilus. At 4 weeks after hypoglycemia, proliferative activity in the SGZ diminished below baseline in experimental versus control rats, with a subsequent reduction of neuroblasts. Morphological findings (doublecortin staining) suggest permanent progenitor cell loss in some areas of SGZ. Reduced neurogenesis in DG and lack of neuronal regeneration in CA1 may impede cognitive recovery after severe hypoglycemia injury. *Diabetes* 54:500–509, 2005

Neural stem cells have been implicated in processes that lead to neural regeneration following central nervous system diseases and injuries, raising the possibility that it might be feasible to ameliorate brain injury by boosting endogenous neural regeneration (1–4). Neurogenesis occurs in the hippocampus of all mammals, including humans (5,6). Recent data show that newly born cells become functionally integrated into the dentate gyrus (DG) and have passive membrane properties, action potentials, and functional synaptic inputs similar to those found in mature dentate granule cells (7). Most important are findings that newly generated neurons play a significant role in synaptic plasticity (8) and that a reduction in the number of these cells impairs learning and memory (9). Our recent evidence suggests that neurogenesis following ischemic brain injury not only leads to the replacement of damaged cells, but might also contribute to functional recovery (10,11), thereby further supporting the notion that strategies for augmenting endogenous neurogenesis may hold clues for the development of restorative therapy.

Hypoglycemic brain injury is a common and serious complication of insulin therapy that may also occur during tight management of blood glucose levels with other hypoglycemic agents. Similar to cerebral ischemia, increased aspartate and glutamate levels during and after hypoglycemia cause a sustained activation of glutamate receptors that results in an excitotoxic neuropathology in which certain neurons are selectively killed (12). Pathological studies in humans and animals show that hypoglycemia-induced neuronal death occurs preferentially in the CA1, subiculum, and DG of the hippocampus, superficial layers of the cortex, and striatum (12). Because of the extensive neuronal loss, one of the neurological sequelae associated with hypoglycemia is cognitive decline. According to clinical studies, significant learning and memory deficits correlate with the frequency of hypoglycemia not only in patients with type 1 diabetes, but also in the relatively younger group among the population with type 2 diabetes (13). The prevalence of severe hypoglycemia, including hypoglycemic coma, could be as high as 40.5% in patients with type 1 diabetes alone (14). In support of such clinical observations are studies in which rats had significant deficits in spatial learning and memory with associated hippocampal neuronal loss in an experimental model of hypoglycemia (15). As there is no effective treatment for hypoglycemia-caused cognitive impairment, enhancing neurogenesis might provide a novel therapeutic strategy for treating patients with hypoglycemia-induced brain damage and functional impairment.

Despite a wealth of literature on the neurochemistry and neuropathology of hypoglycemia, little is known about the
potential for neurological repair and regeneration following hypoglycemic brain damage. A recent experimental model of repetitive hypoglycemia with mild damage to the parasagittal cortex failed to show neurogenesis in the DG (16), suggesting that the extent and location of brain injury might determine whether regeneration occurs. Granule cell death, seen in both global ischemia and epilepsy, is associated with increased progenitor cell proliferation in the subgranular zone (SGZ), further supporting our hypothesis that hippocampal cell loss might be required to induce neurogenesis in the DG. Here we investigate whether progenitor cell proliferation and neurogenesis occur in the hippocampus in a rat model of hypoglycemia. Our results demonstrate a transient increase in progenitor cell proliferation in the SGZ and neurogenesis in the granule cell layer (GCL) during the first 2 weeks after hypoglycemia, with subsequent loss of progenitor cells in the SGZ beginning 4 weeks after the insult. Unlike findings reported in regard to cerebral global ischemia (17), no neuronal regeneration was detected in the CA1 proper after hypoglycemia. Apart from extensive cell loss in many hippocampal regions, including the CA1, reduced neuronal replacement due to progenitor cell loss in the DG might limit cognitive recovery after hypoglycemic brain injury.

RESEARCH DESIGNS AND METHODS

Animal surgery and hypoglycemia induction. Two-month-old male Sprague-Dawley rats (Charles River, CA) were housed and cared for according to federal and institutional guidelines. Hypoglycemia induction was performed as described (15); sham-treated control rats underwent all procedures except insulin injection. Rats were kept under anesthesia following the induction of hypoglycemia until 3 h after the onset of glucose infusion to permit monitoring and control of physiological parameters. A femoral artery line was placed for blood pressure monitoring (BIOPAC System, Santa Barbara, CA) and blood sampling. Blood gas was measured at 1-h intervals by I-STAT (Princeton, NJ). Tidal volume and oxygen flow were adjusted via a small animal respirator (Harvard Apparatus, South Natick, MA) to keep PaCO₂ between 35 and 45 mmHg and PaO₂/H11022100 mmHg. For electroencephalogram (EEG) monitoring (BIOPAC), burr holes were made in the skull bilaterally over parietal cortex and monopolar electrodes placed beneath the dura. A

![FIG. 1. Hypoglycemia induces EEG, heart rate, and blood pressure changes and subsequent widespread neuronal loss. A: Hypoglycemia induces isoelectric EEG and increased blood pressure and heart rate. a., Normal resting EEG pattern. b., EEG pattern slowed down with increased amplitude after the onset of hypoglycemia. Blood pressure and heart rate were still within the normal range. c., EEG showed isoelectric pattern. Mean arterial blood pressure and heart rate increased up to 180 mmHg and 400/min, respectively. d., EEG, blood pressure, and heart rate completely normalized 60 min after glucose infusion. B: Hypoglycemia (HG)-induced wide spread neuronal loss was detected by NeuN immunohistochemistry at 2 weeks following 30 min of isoelectric EEG and sham treatment. Representative photomicrographs detected apparent neuronal loss (indicated by arrowheads) in the subiculum, CA1 pyramidal layer, tip and upper blade of the DG, striatum (ST), and superficial layers of the cerebral cortex (CTX). Scale bars, 250 μm.](image)

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>HG-2W</th>
<th>HG-4W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell number (×10⁶)</td>
<td>20.5 ± 0.09</td>
<td>5.19 ± 1.52*</td>
<td>5.95 ± 1.27*</td>
</tr>
<tr>
<td>Volume (mm³)</td>
<td>0.66 ± 0.03</td>
<td>0.27 ± 0.03†</td>
<td>0.39 ± 0.02†</td>
</tr>
<tr>
<td>Cell density (×10⁵ cells/mm³)</td>
<td>3.14 ± 0.03</td>
<td>1.76 ± 0.26†</td>
<td>1.48 ± 0.24†</td>
</tr>
</tbody>
</table>

Data are means ± SD per hemisphere. n = 4 in sham-operated control group (Sham) and n = 7 in each hypoglycemia group (HG). An optical fractionator was used to estimate the total numbers of NeuN immunoreactive cells in the septal CA1. Significant difference in cell number, volume, and density was found at 2 weeks (HG-2W) and 4 weeks (HG-4W) after hypoglycemia when compared with controls, as indicated. *P < 0.001, †P < 0.005.
reference needle was placed in neck muscle. A heating blanket/rectal probe servo-loop was used to maintain core temperature at 36.5–37.5°C until the rats had recovered from anesthesia, at which time they were euthermic. Hypoglycemia was induced by intraperitoneal injection of 15 units/kg insulin (Novo-

lin-R; Novo Nordisk). Blood glucose was measured (YSI 2700 glucose analyzer; YSI, Yellow Springs, OH) at 30-min intervals during the induction of hypoglycemia and at 60-min intervals during the recovery from hypoglycemia. Mean arterial blood pressure was maintained between 160 and 200 mmHg during the entire isoelectric period by adjusting isoflurane concentration, and bradycardia during hypoglycemia was prevented with atropine (1 mg/kg; American Pharmaceutical Partners, Los Angeles, CA). Hypoglycemia was terminated by intravenous injection of 0.2 ml of 50% glucose via the femoral vein, followed by continuous infusion of 1:1 solution of 50% glucose and Krebs-Henseleit buffer (1.5 ml/h for 3 h). No seizure activity was observed in >200 rats subjected to 30 min of isoelectric EEG.

Cell proliferation and survival and phenotype determination. Ki67 immunohistochemistry was used to determine the amount of cell proliferation in the SGZ at 4 days and at 1, 2, and 4 weeks after the induction of hypoglycemia. To quantify hypoglycemia-induced cell proliferation at the subventricular zone (SVZ), a separate group of rats received three intraperitoneal injections of BrdU (50 mg/kg; Sigma, St. Louis, MO) 6 h apart at 4 days after the induction of hypoglycemia and were killed 24 h later. To investigate the phenotype and survival of newborn cells at the GCL, rats received intraperitoneal injections three times daily of BrdU for 4 consecutive days from 3 to 6 days following hypoglycemia and were killed 2 and 4 weeks later.

Immunohistochemistry and immunofluorescence staining. Rats were anesthetized and transcardially perfused with 4% paraformaldehyde as described (10). Free floating coronal sections (40-μm) were immunostained as described (10) using the following reagents: mouse anti-BrdU (0.25 μg/ml; Roche, Indianapolis, IN), rat anti-BrdU (2 μg/ml; Accurate Chemicals, Westbury, NY), rabbit anti-calbindin-D28k (0.1 μg/ml; SWANT, Bellinzona, Switzerland), rabbit anti-Ki67 (recognizing nuclear antigen expressed during all proliferative stages of the cell cycle except G0 [18], 0.1 μg/ml; Novocastra, Newcastle upon Tyne, U.K.), mouse anti-NeuN (1 μg/ml; Chemicon, Temecula, CA), goat anti–double cortin (DCX) (recognizing neuroblasts [19], 0.8 μg/ml; Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti–TuJ-1 (1 μg/ml; BABCO, Berkeley, CA), mouse anti–CD11b (1 μg/ml; Serotec, Raleigh, NC) or mouse anti–glial fibrillary acidic protein (GFAP) (1 μg/ml; Ameri-

sham, Cleveland, OH), ABC solution (Vector laboratories, Burlingame, CA), and Streptavidine Alexa Fluor 488 and Alexa Fluor 594 goat anti-mouse or anti-rabbit IgG conjugates (5 μg/ml; Molecular Probes, Eugene, OR).

Confocal microscopy. Fluorescence signals were detected using the Zeiss LSM 510 confocal imaging system (Zeiss, Thornwood, NY) with a sequential scanning mode for Alexa 488 and 594. Stacks of images (1,024 × 1,024 pixels) from consecutive slices of 0.66–0.7 μm in thickness were obtained by averaging four scans per slice and processed with Adobe Photoshop (Adobe Systems, Mountain View, CA).

Cell counting. The number of NeuN-stained CA1 neurons was determined in every 12th coronal section spanning the septal hippocampus (Bregma level

![FIG. 2. Hypoglycemia induces progenitor cell proliferation and increases the amount of immature neurons in the SGZ. The numbers of proliferating cells (A and B) and immature neurons (C) transiently increased in the SGZ during the first week after 30 min of isoelectric EEG. A: The number of Ki67 immunoreactive cells were estimated by unbiased stereology per hemisphere spanning the septal hippocampus of rats killed at the time indicated. One-way ANOVA was performed on the log-transformed Ki67 counts. Significant differences were detected between sham-operated controls and hypoglycemic animals at 4 days and at 1 and 4 weeks after the induction of hypoglycemia. **P < 0.01. Representative photomicrographs showing Ki67 (B) and DCX (C) immunostaining of proliferating cells and immature neuroblasts 1 (HG-1) and 4 (HG-4) weeks after sham and hypoglycemia (HG) treatment. At 4 weeks after hypoglycemia, diminished immature neuroblasts are likely due to loss of progenitor cells (arrows). Scale bar, 250 μm.](image-url)
-1.8 to -3.8) using unbiased stereology (20) (Stereo Investigator; Micro-Brightfield, Williston, VT). Counting frames (15 × 15 × 20 μm) were placed at the intersection of a matrix (30 × 30 μm for Ki67 and 200 × 200 μm for NeuN and BrdU estimation) randomly superimposed onto the region of interest by the program. Ki67 and BrdU immunoreactive cells were counted in the SGZ and GCL, respectively, in the same fashion. The volume of the dentate granule layer was determined using Cavalieri’s principal (21) with a 60 × 60 μm grid matrix. For quantification of BrdU immunostaining at the SVZ, the mean area of BrdU immunoreactivity was determined by tracing the regions of interest according to the method of Parent et al. (22).

**RESULTS**

**Hypoglycemia induces isoelectric EEG and widespread neuronal loss.** A rat model of hypoglycemia was produced as described (15). EEG was used to monitor brain activity and to generate a reproducible injury pattern. The EEG patterns passed through progressive stage characteristics of severe hypoglycemia (Fig. 1A). The isoelectric EEG pattern generally developed after blood glucose concentrations had fallen to <0.5 mmol/l for several minutes, simulating hypoglycemic coma. A concurrent increase in blood pressure and heart rate also occurred during isoelectric EEG. EEG, blood pressure, and heart rate recovery began 12–15 min after the onset of glucose infusion and completely returned to baseline level 60 min after glucose infusion.

Two weeks after rats underwent 30-min insulin-induced isoelectric EEG, neuronal loss was seen in the cerebral cortex, striatum, subiculum, CA1, and DG (Fig. 1B). There is no apparent progressive cell loss in the CA1 during the period of 2–4 weeks after the insult, as indicated by the cell number and volume in this region (Table 1). Unbiased stereology estimated a near 30% reduction in the volume of the DG at 4 weeks after hypoglycemia brain injury (sham 3.76 ± 1.56 × 10^8 μm^3, n = 6, and hypoglycemia 2.61 ± 1.84 × 10^8 μm^3, n = 6). A more extensive cell loss of 70–75% occurred in the CA1 (Table 1).

**Hypoglycemia induces a transient increase in progenitor cell proliferation and permanent progenitor cell loss in the DG.** To investigate how hypoglycemia-induced neuronal loss in the DG affected the SGZ progenitor cell proliferation and neurogenesis, rats were injected with thymidine analog BrdU as described and were killed at 4 days and at 1, 2, and 4 weeks after the induction of hypoglycemia. Perfusion-fixed coronal rat brain slices were assessed by Ki67, DCX, and BrdU immunohistochemistry. Increased cell proliferation occurred in the SGZ of rats as early as 4 days after 30-min insulin-induced isoelec-
cerebral global ischemia, no colocalization between BrdU proliferating cells in the CA1. In contrast to findings with BrdU and neuronal/glial markers to reveal the identity of newborn cells in the DG after hypoglycemic insult, double immunofluorescence staining with BrdU and neuronal/glial markers, including Tuj1, NeuN, Calbindin28kd, and GFAP, were performed, and signals were detected by confocal microscopy (Fig. 4). At 2 weeks after hypoglycemia, a high percentage of newborn cells expressed immature neuronal markers Tuj1 and DCX (Fig. 4A and B). At 4 weeks after hypoglycemia, significantly more new neurons were found in the GCL of rats subjected to hypoglycemia (40,948 BrdU/NeuN double-labeled cells per DG) compared with sham-treated rats (11,860 BrdU/NeuN double-labeled cells per DG). However, hypoglycemia did not significantly alter the extent of neuronal differentiation, with 68.2 ± 1.51% of BrdU-labeled cells expressing NeuN at 4 weeks after the induction of hypoglycemia compared with 66.5 ± 1.79% in the sham group (P = 0.53). In the DG, cells positively labeled for both BrdU and GFAP or for both BrdU and CD11b were only detected near the site of injury within the GCL (Fig. 4A); they were otherwise seen in the dentate hilus (data not shown).

No neuronal regeneration in the CA1 after hypoglycemia. Despite substantial cell loss in the CA1, there was no significant increase in the number of CA1 neurons between 2 and 4 weeks after the induction of hypoglycemia (Table 1), unlike the increase observed after cerebral global ischemia (17). We again deployed double immunofluorescence staining with BrdU and neuronal/glial markers to reveal the identity of proliferating cells in the CA1. In contrast to findings with cerebral global ischemia, no colocalization between BrdU and NeuN or BrdU and Tuj1 was ever detected (Fig. 5A). Most of the cells incorporating BrdU were smaller than the CA1 pyramidal neurons and were colocalized with microglial marker CD11b (Fig. 5B). Some BrdU cells in the CA1 were also positive for GFAP marker, suggesting gliogenesis rather than neurogenesis in this region.

DISCUSSION

The morphologic and functional sequelae following hypoglycemic brain injury are well established, but little is
FIG. 5. No neuronal regeneration has been detected in the CA1 region of the hippocampus after hypoglycemia. A: Consecutive images from confocal partial z stack (nos. 1–5) (X630) in BrdU (green), NeuN and Tuj1 (red), and merged channel showing that the remaining NeuN and Tuj1 immunoreactive cells in the CA1 at 4 weeks following hypoglycemia did not colocalize with BrdU (arrows). Despite in close proximity, BrdU-labeled cells (arrows) are on different focal planes from corresponding NeuN- and Tuj1-labeled cells. B: Whereas many of the BrdU-labeled cells were colocalized with either GFAP or CD11b immunoreactive cells (arrowheads) shown in consecutive confocal partial z-stack images (nos. 1–6), suggesting that most newborn cells in the CA1 after hypoglycemic brain injury are of glial origin. Cells immunoreactive only for BrdU are labeled with arrows. Scale bars, 10 μm.
known about the potential for brain repair and regeneration after a hypoglycemic insult. The principal finding of this study was that a transient increase in both progenitor cell proliferation and neurogenesis occurred in the SGZ and GCL after severe hypoglycemia, followed by a reduced production of new neurons below baseline level several weeks later. In addition to the diminished neurogenesis induced by hypoglycemia, afterward, the early transient increase in neurogenesis was insufficient to replace the neuronal loss in the DG, thus resulting in permanent structural damage. Moreover, unlike findings in rats undergoing cerebral global ischemia, no neuronal regeneration was detected in the CA1 in the rats subjected to hypoglycemia. Our finding suggests that the pattern of neuronal regeneration in the hippocampus following hypoglycemic injury differs from that of cerebral global ischemia, perhaps reflecting the extent and location of neuronal loss in these two different types of insults.

Neuronal loss was found in the several brain regions vulnerable to both hypoglycemia and cerebral ischemia (12). However, within each region the damage was not distributed the same way in the rats subjected to hypoglycemia as it was in those with cerebral ischemia (12). Infarction or cavitation generally did not occur after hypoglycemia, whereas they frequently occurred following ischemia. After hypoglycemia, the caudateputamen was involved more heavily near the white matter, and a superficial-to-deep gradient in the density of neuronal loss was commonly found in the cortex. The hippocampus also showed distinct patterns of neuronal loss at the crest of the DG and a gradient of increasing selective neuronal loss medially in CA1, with marked loss in the subiculum (Fig. 2). The unique distribution of neuronal loss following hypoglycemia is probably related to fluid-born toxin (12).

Adult hippocampal progenitor cells divide at the DG SGZ and produce thousands of new neurons every day in the GCL (4,5). These new neurons develop the morphological and functional properties of dentate granule cells and become integrated into existing neuronal circuitries (7,8). Although the function of hippocampal neurogenesis in adult is not yet clear, inhibition of hippocampal neurogenesis is associated with reduced learning and memory capacities (9), and impairment of hippocampal neurogenesis may be linked to cognitive decline in aging, Alzheimer’s disease, and major depression (23–25). The factors involved in the regulation of hippocampal neurogenesis in adults are not well defined, but hormones, neurotransmitters, environmental stimuli, and growth factors have been identified as mediators (26). Pathological conditions including cerebral ischemia (10,27), seizure (28), Alzheimer’s disease (29), and traumatic brain injury (30) are known to temporarily increase neurogenesis in the DG. Exposure to an enriched environment is linked to improved brain function and increased neurogenesis in the hippocampus (31) but not in the olfactory bulb (32), which further supports the unique functional role of hippocampal progenitor cells. We found that hypoglycemic brain injury stimulated a transitory neurogenesis at the DG (Fig. 3 and 4), whereas no significant change in the extent of proliferation in the progenitor cells at the SVZ was observed ($P = 0.063$; mean area of BrdU immunoreactivity: $136294.74 \mu m^2$ in sham vs. $148397.62 \mu m^2$ in hypoglycemia). The difference in the response of progenitor cells to hypoglycemia between the SVZ and SGZ is not well understood. It might be due to the differences in the neurogenic environment between two locations resulting from hypoglycemic brain injury.

Recurrent severe hypoglycemia results in permanent cognitive impairment in humans as a consequence of damage to the medial temporal lobe (33,34) including the hippocampus (33,35,36). Numerous clinical studies suggest that intensive insulin treatment of type 1 diabetes is associated with an increased frequency of hypoglycemic coma (37–39) and cognitive impairment (40,41). Young children who have diabetes frequently have memory and cognitive deficits (37), and infants of diabetic mothers also showed significant memory deficits, as evaluated by event-related potential (42,43). These relationships suggest that the developing brain is especially vulnerable to hypoglycemic damage. One possible cause for this phenomenon is that hypoglycemia damages progenitor cells that are required for brain development. Although there are no available data to prove progenitor cell loss in the young who have suffered from hypoglycemic brain injury, our results in the adult rats with severe hypoglycemia provided convincing evidence to support such a notion (Fig. 2A and B). Another mechanism that quite likely contributes to hypoglycemia-associated cognitive dysfunction in both young and adult human beings is hippocampal neuronal loss and insufficient regeneration. Although a recent report speculated that impaired synaptic plasticity plays a role in cognitive deficits in young rats (16), the basis for the proposed hypothesis was a model of mild repetitive hypoglycemia that produced no cell death in the hippocampus. Nevertheless, the transient increase in neurogenesis observed in this study was insufficient to compensate for the neuronal loss that took place in multiple brain regions, including the DG. In contrast to observations after cerebral ischemia, no regeneration of hippocampal CA1 pyramidal neurons was observed in our model of hypoglycemia. Our stereological evaluation showed that the number of CA1 neurons did not increase over a period of 4 weeks, as compared with the number extant after ischemia (17). Confocal Z-stack analysis revealed that there were no neuronal markers colocalized with BrdU labeling in the CA1 (Fig. 5A), and all proliferating cells in the CA1 proper were either microglia or astroglia (Fig. 5B). The difference in the regenerative potential of CA1 neurons between our model of hypoglycemic brain injury and the reported ischemic model might be due to the difference in the severity of the CA1 injuries. A <1% survival of neurons in the CA1 after severe ischemic insult probably triggered the recruitment of progenitor cells from the posterior periventricular wall to participate in CA1 repair (17).

Reduced neurogenesis in the DG seen 4 weeks after hypoglycemia in this study could be due to loss of progenitor cells as a result of direct injury to these cells. It could also be attributed to a reduced survival of newly formed neuroblasts resulting from an unfavorable microenvironment induced by hypoglycemic injury. Hypoglycemia causes inflammation and microglia activation (44). Enhanced CD11b expression started as early as 2 days, peaked at ~1 week, and lasted up to 2 months after the
induction of hypoglycemia. The spatial distribution of CD11b expression coincided with areas of neuronal loss, including layer II/III of the cortex, striatum, subiculum, CA1–3, and DG. Activated microglia were also observed in corpus callosum (data not shown). Activated microglia are cytotoxic by releasing reactive oxygen species, proteolytic enzymes, arachidonic acid metabolites, and inflammatory cytokines (45). Neuroinflammation and microglial pathology are associated with many diseases of cognition, including Alzheimer’s disease, Lewy body dementia, and AIDS dementia complex (46–48). Lipopolysaccharide-induced activated microglia in vitro also produce soluble antiinflammatory factors that reduce neuronal differentiation into DCX-expressing cells from cocultured neural progenitor cells (49). Activated microglia are localized in close proximity to the newly formed cells, and the impairment of neurogenesis depends on the degree of microglia activation (50), suggesting that inflammation compromises the survival of the new hippocampal neurons. The nonsteroidal anti-inflammatory drug indomethacin restores newborn neurons and reduces activated microglia by 35% in the DG after cranial irradiation (49), supporting a role for inflammation in inhibiting neurogenesis. Moreover, another anti-inflammatory drug, minocycline, also restored neurogenesis after generalized seizure (50). Based on our finding, we speculate that there is a link between chronic inflammation after repeated hypoglycemic injury and failed neurogenesis. Reduced progenitor cell proliferation and lack of neuronal replacement in many brain regions may limit the recovery of cognitive function in patients with type 1 diabetes. Our results also suggest that it might be necessary to augment neurogenesis by prolonging newborn neuron survival with anti-inflammatory agents in addition to boosting endogenous repair by growth factor infusion or behavioral manipulation to combat hypoglycemia-induced cognitive impairment.

ACKNOWLEDGMENTS
This work was supported by National Institutes of Health Grant R01 NS40469 (to J.L.), the Department of Veterans Affairs Merit Program (to J.L.), American Heart Association Grant SDG003007 (to J.L.), Juvenile Diabetes Research Foundation Grant 3-2004-298 (to S.W.S.), Department of Veterans Affairs REAP Grant (to R.A.S.), and Department of Veterans Affairs Merit program (to P.R.W.).

REFERENCES
   *Ann Rev Pharmacol Toxicol* 44:399–421, 2004
   *Cell* 110:399–402, 2002
4. Cameron HA, McKay RD: Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. 
5. Gage FH: Neurogenesis in the adult brain. 
7. Song HJ, Stevens CF, Gage FH: Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. 
    *Baillieres Clin Endocrinol Metab* 7:611–625, 1993
    *Diabetes Care* 23:1467–1471, 2000
    *Cell* 110:429–441, 2002
21. West MJ, Slomianka L, Gundersen HJ: Unbiased stereological estimation of the total number of neurons in subdivisions of the rat hippocampus using the optical fractionator. 
    *Ann Neurol* 52:802–813, 2002
23. Zitnik G, Martin GM: Age-related decline in neurogenesis: old cells or old environment? 
    *J Neurosci Res* 70:258–263, 2002
    *Brain Behav Immun* 16:602–609, 2002
    *J Neurochem* 83:1509–1524, 2001
    *Eur Arch Psychiatry Clin Neurosci* 251:152–158, 2001
    *Proc Natl Acad Sci USA* 101:343–347, 2004
28. Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH: Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. 
    *J Neurosci* 17:3727–3738, 1997
    *Proc Natl Acad Sci USA* 101:343–347, 2004
31. van Praag H, Kempermann G, Gage FH: Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. 
    *Nat Neurosci* 2:296–297, 1999


