

# Streptozotocin-Induced Diabetes Causes Astrocyte Death After Ischemia and Reperfusion Injury

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**Diabetes exacerbates neuronal cell death induced by cerebral ischemia. One contributing factor is enhanced acidosis during ischemia. Astrocytes are vulnerable to hypoxia under acidic conditions in vitro and may be targets of ischemia under diabetic conditions. The objective of this study was to determine whether diabetes would cause damage to astrocytes after an ischemic brain injury in vivo. Diabetic and nondiabetic rats were subjected to 5 min of forebrain ischemia and followed by 30 min, 6 h, or 1 or 3 days of recovery. The results showed that ischemia caused activation of astrocytes in nondiabetic rats. In contrast, diabetes caused astrocyte activation in early stage of reperfusion and astrocyte death in late stage of reperfusion. Remarkable astrocyte death was preceded by increased DNA oxidation. Further studies revealed that increased astrocyte damage coincided with enhanced production of free radicals. These data suggest that hyperglycemic ischemia worsens outcome in astrocytes, as it does in neurons. *Diabetes* 55:349–355, 2006**

It has been documented that hyperglycemia occurring during ischemic stroke is associated with a poorer prognosis, irrespective of patient's age, severity of the condition, or stroke subtype in both animal and human studies (1). Hyperglycemia exacerbates neuronal damage and has been associated with an increase in the brain edema and augmentation in the size of the infarct (2,3). The exact cellular and molecular mechanisms that lead to this poor prognosis remain to be elucidated.

Astrocytes represent the most abundant cell type in the central nervous system (CNS). Astrocytes provide structural, trophic, and metabolic support to neurons and modulate synaptic activity. Impairment of astrocytes can critically influence homeostasis of neuronal cells. Astro-

cyte functions that are known to influence neuronal survival include glutamate uptake, glutamate release, free radical scavenging, and the production of cytokines and nitric oxide (NO) (4). However, whether prolonged reactive astrocytic response is beneficial to neuronal recovery remains controversial. Significant astrocyte death occurs after reactive astrocytosis (5), and dying astrocytes engage neighbor cells into cell death by stander after transient ischemia (6).

It has been shown that enhanced acidosis is one of the contributing factors responsible for aggravation of delayed neuronal damage caused by preischemic hyperglycemia. During experimental global ischemia, cerebral blood flow is reduced to <5% of control values, and oxygen delivery is dramatically decreased. The anaerobic metabolism of glucose (and glycogen) produces H<sup>+</sup> and lactate (7,8). This led to the concept that hyperglycemia exacerbates the acidosis occurring during ischemia because it allows more lactate and H<sup>+</sup> to be formed (8). Astrocytes are highly sensitive to acidic conditions. Sustained reduction of extracellular pH from 7.4 to 6.4 destroys cultured glia and increases the vulnerability of the glia to injury induced by combined oxygen and glucose deprivation, suggesting that acidosis that accompanies ischemia may contribute to glial injury. This concept was further supported by studies showing that reduction of intercellular pH values from 7.4 to values in the range of 6.8–6.2 in cultured astrocytes led to swelling (9) and that acidosis induced both necrotic and apoptotic cell death in cultured hippocampal slices (10). Moreover, acidosis, when combined with hypoxia in an environment mimicking in vivo ion shift, caused rapid death of astrocytes (11). It is not known, however, whether hyperglycemia causes astrocytic damage in vivo after a transient cerebral ischemic insult. The objective of the present study was to determine whether hyperglycemia causes damage to astrocytes after an ischemic injury in vivo.

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8-OHdG, 8-hydroxy-2-deoxyguanosine; CNS, central nervous system; GFAP, glial fibrillary acidic protein; HET, hydroethidine; iNOS, inducible NO synthase; ONOO<sup>-</sup>, peroxynitrite; RNS, reactive nitrogen species; ROS, reactive oxygen species.

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## RESEARCH DESIGN AND METHODS

Male Wistar rats (Simonsen Laboratory, Gilroy, CA) weighing 280–350 g were used in the experiments. Animal surgical procedures were performed in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee, University of Hawaii. A total of 56 animals were used.

Diabetes was induced by a single subcutaneous injection of streptozotocin (Sigma Chemical, St. Louis, MO) freshly dissolved in 0.1 mol/l citrate buffer at pH 4.5 and delivered at a dose of 40 mg/kg body wt. Before induction of brain ischemia, blood glucose concentrations were measured, and those rats with blood glucose levels exceeding 20 mmol/l were included in the diabetic group. Age-matched, nondiabetic rats injected with an equal volume of citrate buffer served as controls. These rats were used 4 weeks after streptozotocin or citrate buffer injections.

Rats were fasted the night before the induction of ischemia but had free access to water. Anesthesia was induced by inhalation of 3.5% halothane in a mixture of N<sub>2</sub>O:O<sub>2</sub> (70:30) and maintained at 1.5% halothane with use of a

facemask during surgical procedures. A tail artery and a vein were cannulated for blood sampling; monitoring of mean arterial blood pressure, blood glucose, and blood gases (i-Stat, Princeton, NJ); and administration of heparin (30 IU/kg) before inaugural blood sampling. Rectal and skull temperatures were both maintained at  $\sim 37^{\circ}\text{C}$  by a combination of a homeothermic blanket control unit and a heating lamp.

Forebrain ischemia of 5-min duration was induced in both diabetic and nondiabetic rats by clamping bilateral common carotid arteries and bleeding hypotension to 45–50 mmHg (12–14). Reperfusion was introduced by reinfusing the shed blood and by releasing the clamps placed around the carotid arteries. Animals were killed after 30 min, 6 h, or 1, 3, or 7 days of reperfusion. Sham-operated diabetic and nondiabetic rats were used as controls.

**Immunohistochemistry.** Brains ( $n = 4$ , per group) were perfused with 4% paraformaldehyde, postfixed, and sectioned at 30  $\mu\text{m}$  thicknesses using a vibratome (VT 1000S; Leica). Vibratome sections were washed, and nonspecific binding sites were blocked with 3% BSA. The sections were incubated overnight with primary antibody for anti-glial fibrillary acidic protein (GFAP) (mouse monoclonal antibody, clone G-A-5; Sigma) at a dilution of 1:400. The sections were washed and then incubated with rhodamine red-conjugated secondary antibody (1:300; Jackson ImmunoResearch Laboratories, West Grove, PA). Double labeling of GFAP with 8-hydroxy-2-deoxyguanosine (8-OHdG), a marker for DNA oxidative damage, was performed by incubating the sections with primary antibody for 8-OHdG (rabbit polyclonal antibody, 1:100 dilution; Abcam, Cambridge, MA). Sections were mounted on glass slides and sealed under coverslips using Gelvatol. Three microscopic fields at a magnification  $\times 400$  were captured from each side of the cortex using a Zeiss LSM5 laser-scanning confocal microscope (Zeiss SMT, Oberkochen, Germany). Number of GFAP-positive astrocytes, diameter of astrocytic cell bodies, number of astrocytic processes, and length of the processes were measured using Zeiss LSM5 Image Examiner software.

**Electron microscopic studies.** Brains of the sham-operated controls and those subjected to ischemia with 1 day of reperfusion in both nondiabetic and diabetic groups ( $n = 2$  per group) were perfused with 2.5% glutaraldehyde. Coronal brain sections (200  $\mu\text{m}$  thick) at the level of Bregma  $-3.8$  mm were postfixed with 4% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4). Sections were then soaked in 1% osmium tetroxide in 0.1 mol/l cacodylate buffers for 2 h, rinsed in distilled water, and stained with 1% aqueous uranyl acetate overnight. Tissue sections were dehydrated in ascending series of ethanol to 100% followed by dry acetone, and embedded in epoxy resin. Ultra-thin sections were counterstained with lead citrate before examination by LEO 912 EFTEM transmission electron microscope (Zeiss SMT).

**Western blot analysis.** Forty rats were used for Western blot analyses ( $n = 4$  per group) that were carried out using 8 or 10% SDS-PAGE methods described previously (14). Briefly, an equal amount of protein (30  $\mu\text{g}$ ) was applied to each lane in a slab gel of SDS-PAGE. After electrophoresis, proteins were transferred to an Immobilon-P membrane (Millipore, Billerica, MA). The membrane was incubated with primary antibodies against inducible NO synthase (iNOS) (1:500; Santa Cruz Biotechnology, Santa Cruz, CA) or nitrotyrosine, a marker for peroxynitrite ( $\text{ONOO}^-$ ) (1:500; Upstate Cell Signaling Solutions, Lake Placid, NY) overnight at  $4^{\circ}\text{C}$ . The membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. The blots were then developed using the ECL reagents (Amersham, Arlington Heights, IL).

**In vivo detection of superoxide anion.** Hydroethidine (HET) was prepared in dimethyl sulfoxide (Sigma) to a concentration of 100 mg/ml and then diluted in 0.9% saline to a final concentration of 1 mg/ml (1:100). Thirty minutes before ischemic onset, HET (2 ml) was injected intravenously into animals under halothane anesthesia. Animals ( $n = 4$  per group) were perfusion-fixed with 4% paraformaldehyde after 3 days of recirculation, and brains were sectioned using the Leica vibratome. To determine the fluorescent intensity of oxidized HET, three microscopic fields at the magnification  $\times 400$  from the cortex and CA1 regions of each hemisphere were captured using the Zeiss LSM5 confocal laser-scanning microscope at excitation of 480 nm and emission of 567 nm. Fluorescence intensity of the oxidized HET was measured using Zeiss LSM5 Image Examiner.

**Colocalization of superoxide with cellular markers.** Brain sections collected from the HET-injected rats were washed in PBS containing 0.2% Triton X-100 at room temperature, and nonspecific binding sites were blocked with 3% BSA. These sections were incubated overnight with primary antibodies against NeuN (1:300, mouse monoclonal; Chemicon, Temecula, CA), CD11b (1:50, mouse monoclonal; Chemicon), GFAP (1:400, rabbit polyclonal; DakoCytomation, Glostrup, Denmark), RECA-1 (1:50, mouse monoclonal; Chemicon), and MitoTracker Green (Molecular Probes, Eugene, OR), which are cell markers for neurons, macrophage, astrocytes, endothelial cells, and mitochondria, respectively. These sections were washed and then incubated with fluorochrome isothiocyanate-conjugated secondary antibody (Jackson ImmunoResearch Laboratories). Sections were mounted on glass slides and

sealed with coverslips using Gelvatol. The slides were analyzed on the Zeiss LSM5 laser-scanning confocal microscope.

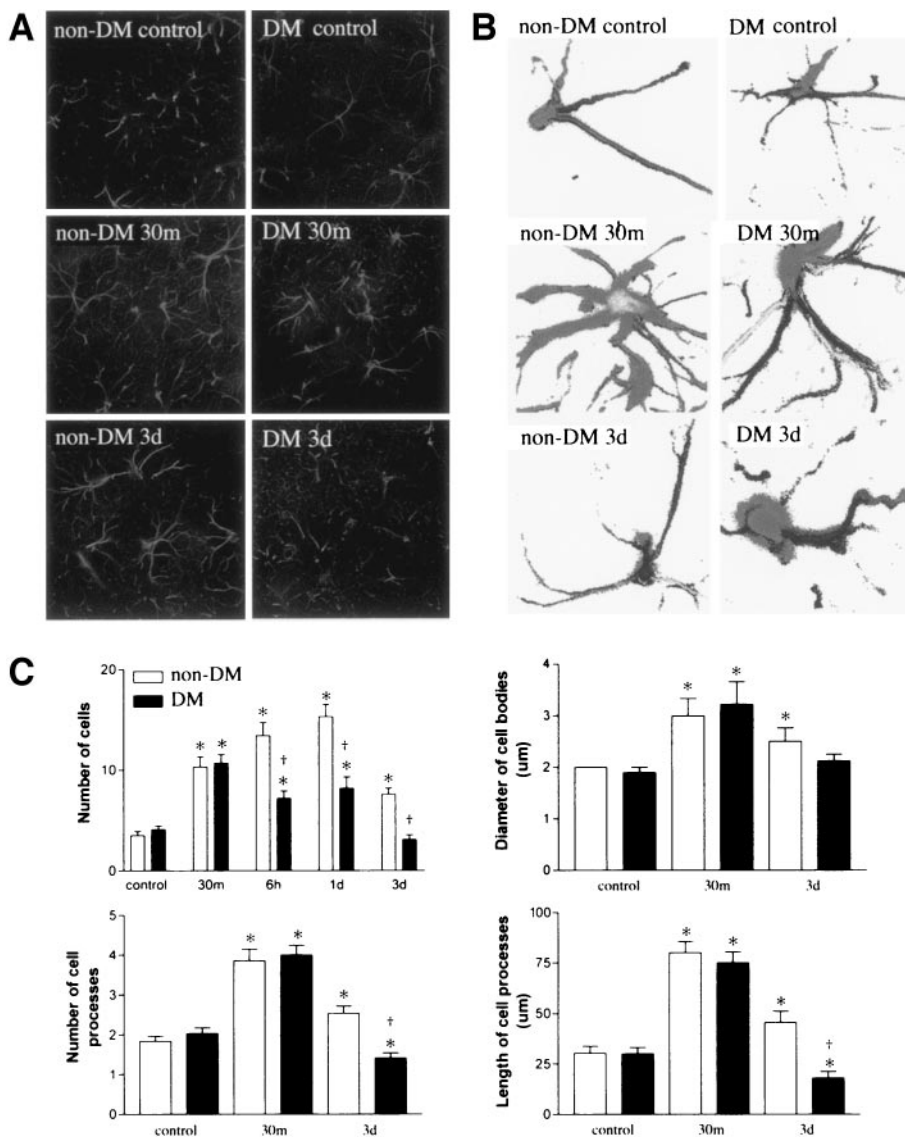
**Statistics.** Optical densities of Western blots and fluorescent intensities of oxidized HET were measured using a Photoshop 7.0 image program (Adobe) and Zeiss LSM5 Image Examiner, respectively. Comparisons of means between diabetic and nondiabetic animals were made by ANOVA followed by post-Scheffe's test.  $P < 0.05$  was regarded as statistically significant.

## RESULTS

**Physiological variables.** Physiological variables were well controlled in the experiments. Physiological variables were measured at 5 min before the induction of ischemia. Mean  $\text{CO}_2$  was maintained at 35–44 mmHg,  $\text{PaO}_2$  at 106–123 mmHg, arterial pH at 7.40–7.46, blood pressure at 100–130 mmHg, and body temperature at 36.9–37.4 $^{\circ}\text{C}$ . No significant differences were found for the above parameters between diabetic and nondiabetic groups. As expected, plasma glucose concentrations were significantly higher in diabetic animals (20–24 mmol/l) than in nondiabetic animals (4–5 mmol/l) ( $P < 0.001$ ).

**Astrocytes in nondiabetic and diabetic samples.** To examine the effects of diabetic ischemia on astrocytic morphology, we performed GFAP immunohistochemistry on brain sections. GFAP detects both normal and pathological astrocytes. Increases in cell body size, number of processes and length of the processes are considered signs of activated astrocytes. For this reason, the number of GFAP-positive astrocytes, diameter of astrocytic cell bodies, number of astrocytic processes, and length of the processes were counted and measured. The results showed that the number of GFAP-positive astrocytes increased significantly in both the cortex and CA1 areas of the nondiabetic rats after 30 min of reperfusion and persisted up to 1 day. At 3 days of reperfusion, the number of astrocytes remained higher than the nonischemic controls but to a lesser extent. The diameters of astrocyte cell bodies were found to be enlarged, and the number and length of astrocyte foot processes were increased. In diabetic rats, a biphasic change was observed in astrocytes. In the early reperfusion phase (30 min of reperfusion), the number of activated astrocytes increased. However, at late reperfusion phase (3 days of recovery), the number of astrocytes significantly decreased. This may reflect increased astrocyte death. Similarly, although the diameter of the cell bodies and the number and length of the astrocytic foot processes were significantly increased after 30 min of reperfusion, the number and the length of the astrocytic foot processes were significantly decreased after 3 days of reperfusion compared with 3 days of recovery in nondiabetic rats and with diabetic sham-control rats. Figure 1 illustrates the changes of the number and diameter of GFAP-positive cells as well as the number of astrocytic foot processes and the length of the processes in the cortex after 30 min and 3 days of reperfusion.

**Ultrastructural alterations of the astrocytic nuclei and mitochondria.** Astrocytes in the cortex were examined by electron microscopy. In the cortex, the nuclear chromatin was homogenous, nuclei were visible, and the nuclear membrane was intact up to 1 day of recovery (Fig. 2, nondiabetes). Mitochondrial morphology was normal up to 1 day of recovery in nondiabetic rats (Fig. 3, nondiabetes). In contrast to nondiabetic rats, the nuclear and mitochondrial morphological alterations were prominent in diabetic rats after 1 day of recovery. Thus, nuclear shrinkage, chromatin condensation, and void space were evident (Fig. 2, diabetes) and disarray of mitochondrial



**FIG. 1.** Astrocyte activation after diabetic ischemia. **A:** Representative micrograms of GFAP-stained astrocytes in control, nondiabetic (non-DM) ischemic, and diabetic (DM) ischemic animals. Magnification  $\times 400$ . **B:** High magnification ( $\times 1,000$ ) of astrocytic morphology in control, non-DM ischemic, and DM ischemic animals. **C:** Numbers of astrocytes, diameters of the astrocytic cell bodies, numbers of astrocyte foot processes, and the length of the processes. Data are means  $\pm$  SD ( $n = 4$  per time point). \* $P < 0.05$  vs. sham-operated control and † $P < 0.05$  vs. non-DM samples at an identical time point. ANOVA followed by Scheffe's test. Ctr, control.

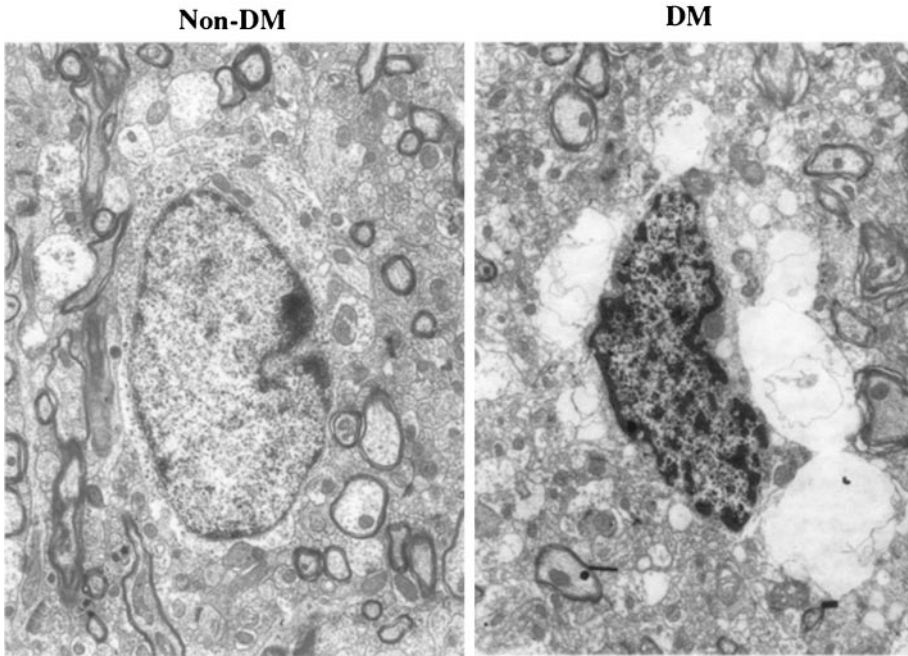
cristae, lucency, and swelling were observed in astrocytes after 1 day of recovery (Fig. 3, diabetes).

**Changes of iNOS and NO metabolites.** Western blot analyses demonstrated that iNOS increased at 6 h of reperfusion and was further elevated at 1 and 3 days of recovery in nondiabetic samples. Similarly, iNOS increased to the same extent in diabetic samples compared with the nondiabetic samples. In contrast, ONOO<sup>-</sup> was significantly increased in diabetic rats after 3 days of reperfusion but was not changed in nondiabetic rats (Fig. 4). Semiquantitation of immunoblots showed that the percent change of relative density was significantly higher in diabetic than in nondiabetic animals ( $P < 0.01$ ).

**Superoxide production in nondiabetic and diabetic rat brain.** In vivo detection of oxidized HET revealed that nondiabetic ischemia increased the production of superoxide in the cortex and CA1 regions, compared with sham-operated control. Diabetic ischemia further enhanced the superoxide production in these two examined structures after 3 days of recirculation. Interestingly, in the CA1 region, diabetes alone (without ischemia) provoked the same strong reaction as that resulting from nondiabetic ischemia (Fig. 5).

**Superoxide production in neurons and invaded macrophages.** To determine which cell type was the source of superoxide production, we performed double labeling of oxidized HET with NeuN, CD11b, GFAP, and RECA-1, which are markers for neurons, macrophages, astrocytes, and endothelia, respectively. The results showed that superoxide production was colocalized with NeuN, suggesting neuronal production of superoxide (Fig. 6A). Moreover, we found colocalization of superoxide with CD11b-positive macrophages situated around the vessels (Fig. 6B) but not the ones located in the parenchyma, suggesting that the superoxide-producing cells were invading macrophages. The superoxide fluorescence did not colocalize with endothelial and astrocytic markers (data not shown). Further studies revealed that oxidized HET appeared as punctuate fluorescence in the cytosol, suggesting mitochondrial production of superoxide. This was confirmed by double labeling of oxidized HET with Mito-Tracker Green, a mitochondrial marker (Fig. 6C).

**Astrocyte DNA oxidative damage.** Double labeling of GFAP with DNA oxidation marker, 8-OHdG on sections obtained after 1 and 3 days of recovery from ischemia in diabetic and nondiabetic rats showed that the number of



**FIG. 2.** Electron micrographs of osmium-uranyl-lead-stained astrocytes in nondiabetic (non-DM) and diabetic (DM) rats subject to 5 min of global ischemia followed by 1 day of recirculation. Magnification  $\times 2,000$ .

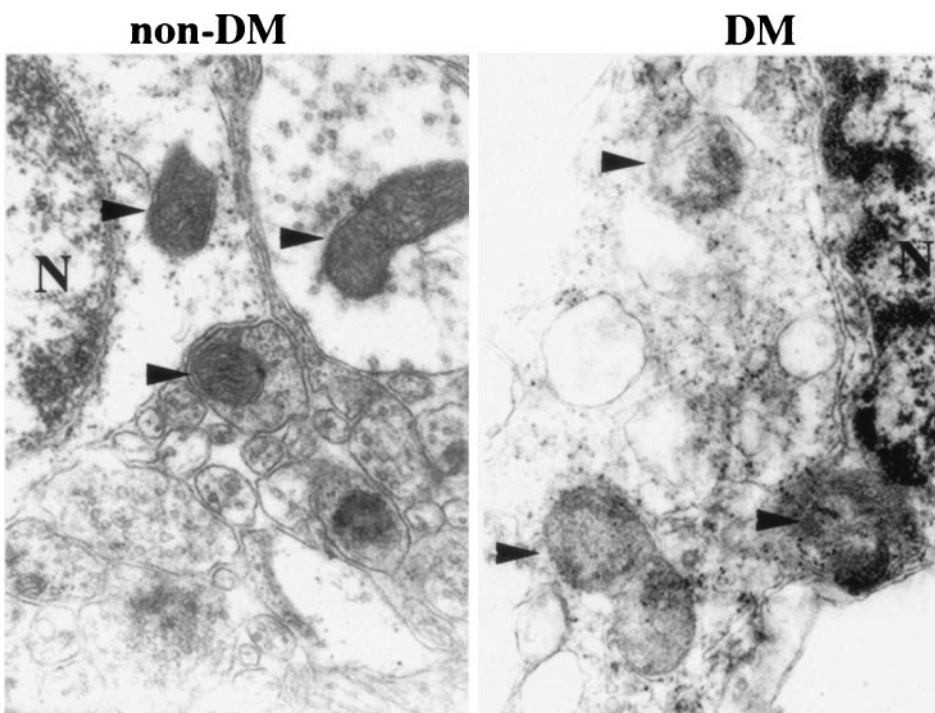
8-OHdG-positive astrocytes increased after 1 day of recovery (Fig. 7), a time point before massive astrocyte loss. After 3 days of recovery, only a few 8-OHdG-positive cells were identified as astrocytes, and most of them morphologically established as neurons (data not shown). The fact that 8-OHdG-positive astrocytes were found to have decreasing numbers at 3 days of recovery may reflect massive astrocyte loss at this reperfusion time.

#### DISCUSSION

Our previous studies using specimens obtained from the same animals used for this study demonstrated that a brief period of cerebral ischemia resulted in mild damage to the hippocampal CA1 and cortex areas (15). Pre-existing hy-

perglycemia accompanied by diabetic conditions significantly enhanced the damage in both the CA1 and cortex (15). The present study further revealed that diabetic hyperglycemia caused early damage to astrocytes and increased production of reactive nitrogen species (RNS) and reactive oxygen species (ROS).

In the present study, we observed that astrocytes were activated after 30 min of recovery and persisted up to 1 day in nondiabetic animals. This is consistent with previous findings showing that ischemia causes activation of astrocytes (16). It is generally believed that reactive astrocytes, as defined by elevated GFAP immunoreactivity, enlarged cell body, and increased cell processes, at the early stage of CNS injury have beneficial effects on neu-



**FIG. 3.** Electron micrographs of osmium-uranyl-lead-stained astrocytes in nondiabetic (non-DM) and diabetic (DM) rats subject to 5 min of global ischemia followed by 1 day of recirculation. Arrows denote mitochondria with disarrayed cristae, mild lucency, and swelling. Magnification  $\times 40,000$ .

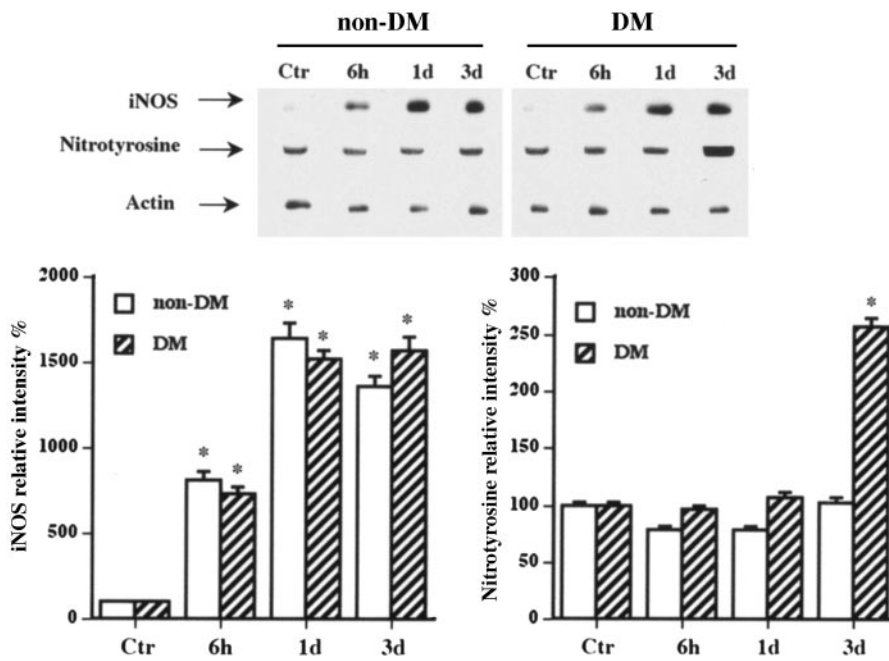


FIG. 4. *A*: Western blot analysis of iNOS and nitrotyrosine in cytosolic fractions from the neocortical tissues after 6 h and 1 and 3 days of recovery in nondiabetic (non-DM) and diabetic (DM) animals. *B*: Semiquantitative changes of iNOS and nitrotyrosine. Data are means  $\pm$  SD ( $n = 4$  per time point). \* $P < 0.05$  vs. control and † $P < 0.05$  vs. non-DM. ANOVA followed by Scheffes' test. Ctr, control.

rons by participating in several biological processes such as the regulation of extracellular ions and neurotransmitter levels, repair of the extracellular matrix, control of the blood-CNS interface, and trophic support of neurons. However, prolonged reactive astrocytic response may upregulate the synthesis of iNOS that leads to increase in RNS and ROS production.

The novel finding of the current study was that astrocytes died quickly after ischemia and reperfusion in diabetic rats. This was determined using two different readouts. First, the number and length of astrocyte processes were significantly decreased in diabetic rats at 3 days of recovery compared with diabetic sham controls

and with nondiabetic and ischemic rats at the identical reperfusion stage. Second, ultrastructural studies showed evident nuclear shrinkage, chromatin condensation, and mitochondrial swelling in diabetic rats after 1 day of recovery. These novel findings indicate that astrocytes, similar to neurons, serve as targets in hyperglycemic ischemia.

Mechanisms by which astrocytes are irreversibly injured from brain ischemia remain incompletely defined. Friede and van Houten (17) showed that astrocytes lose their processes (clasmotodendrosis) when irreversibly injured by energy failure and acidosis. Astrocytes in culture are more sensitive than neurons to extended periods of mild acidosis (pH 6.8) (18). This sensitivity to acidosis may

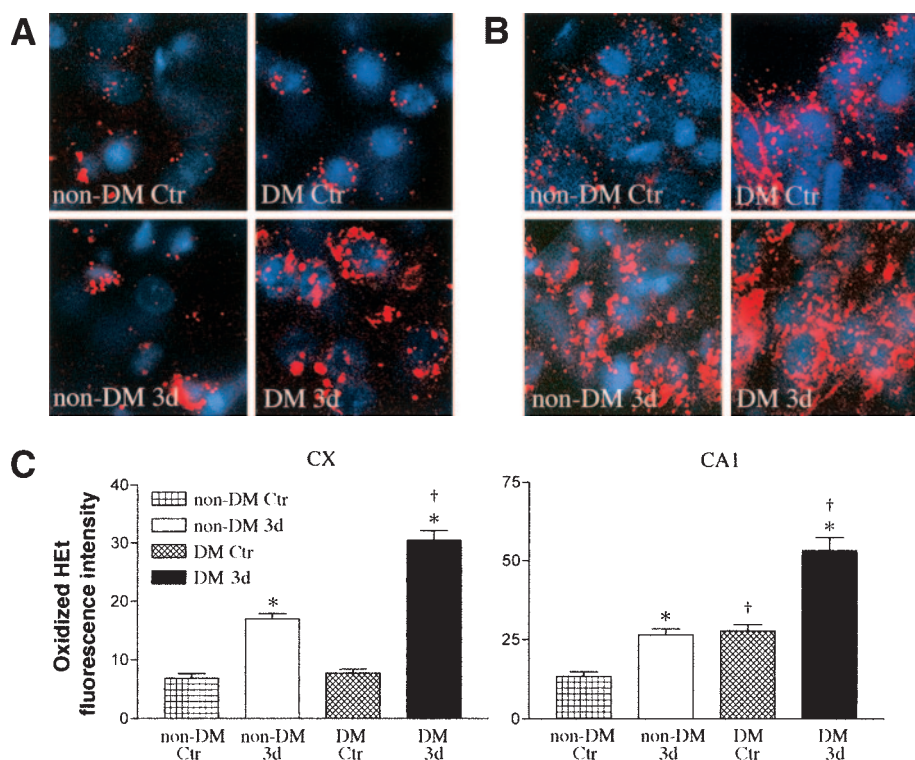
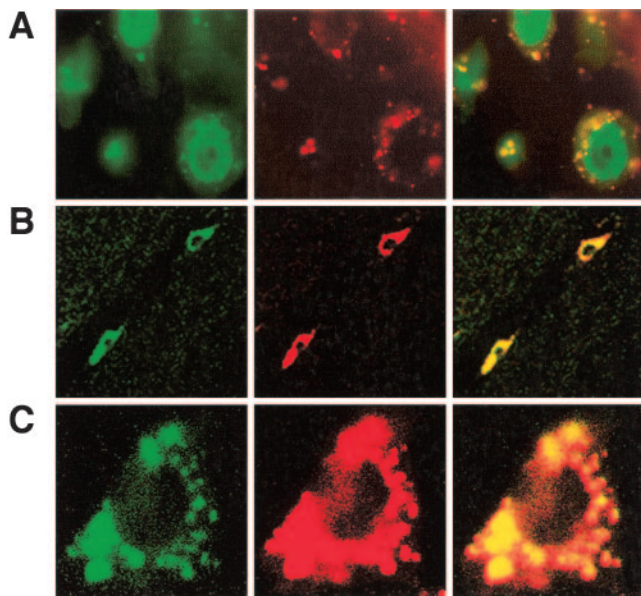
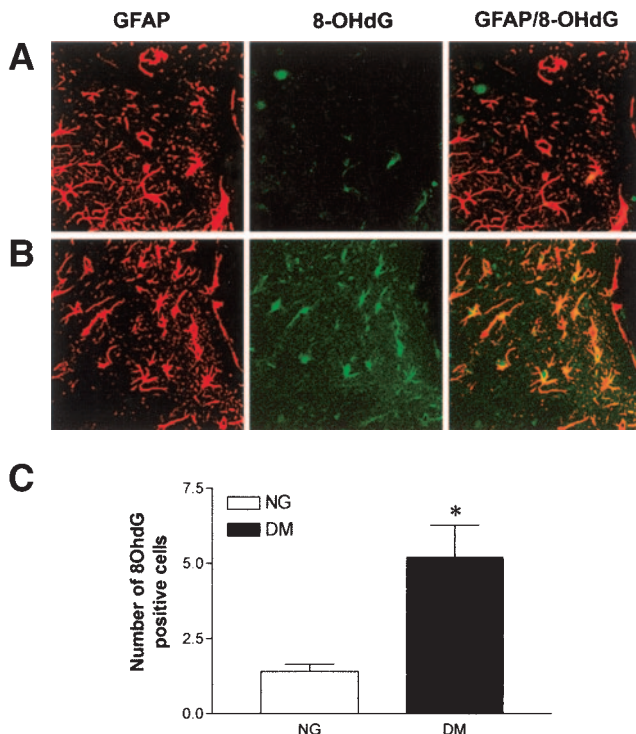


FIG. 5. Superoxide production in the cortex and CA1 region. Diabetic (DM) ischemia caused increase of oxidized HET in cortical (*A*) and hippocampal (*B*) CA1 region after 3 days (3d) of recirculation. Blue represents diamidinophenylindole staining. Magnification  $\times 630$ . *C*: Quantitative analysis of oxidized HET intensity in CX and CA1. Data are means  $\pm$  SD ( $n = 100$  per time point in the cortex and  $n = 50$  in the CA1). \* $P < 0.05$  vs. control and † $P < 0.05$  vs. nondiabetic (non-DM) samples at an identical time point (ANOVA followed by Scheffe's test). Ctr, control.



**FIG. 6.** Superoxide production in neurons and macrophages. Oxidized HET-positive cells (red) colocalized with NeuN (A), CD11b (B), and MitoTracker Green (C). Magnifications are  $\times 630$ ,  $\times 400$ , and  $\times 1,000$  for A, B, and C, respectively.

significantly contribute to astrocyte death during ischemia (19). Astrocytes exposed for as short as 15 min to acidosis (pH 6.6) in conjunction with hypoxia and ion-shifted media are irreversibly injured (11,20). During ischemia, the extracellular and intracellular pH values in hyperglycemic animals dropped to 6.3–6.4 and 6.0–5.9, respectively



**FIG. 7.** DNA oxidative damage in astrocytes as detected by anti-8-OHdG antibody. A: Occasional 8-OHdG-positive stained cells were observed in normoglycemic (NG) animals. B: Number of 8-OHdG-positive cells increased remarkably in diabetic animals, and they were colocalized with GFAP-positive cells. C: Number of 8-OHdG cells increased more significantly in diabetic than in nondiabetic animals. \* $P < 0.05$  (Student's *t* test).

(21,22). Thus, it is likely that hyperglycemia may cause astrocytic cell damage by enhancing tissue acidosis.

The present results revealed that ischemia led to increased synthesis of iNOS, probably by astrocytes, as reflected by the colocalization of iNOS with GFAP. Enhanced synthesis of iNOS could cause increased formation of nitric oxide (NO), which has been shown to impair mitochondrial function (23). Inhibition of NO production protected cell death induced by anoxia in cultured neurons and ameliorated brain damage caused by cerebral ischemia under normoglycemic conditions (24). Furthermore, pretreatment with a NOS inhibitor eliminated the no-flow zone, decreased brain edema, and reduced infarct volume in both normo- and hyperglycemic rats subjected to a transient ischemia. These results suggest that NO is an important mediator in the postischemic brain injury in both normoglycemic and hyperglycemic rats (25–28).

NO is not a stable product. It rapidly diffuses into neurons where it reacts with superoxide to form  $\text{ONOO}^-$ ; the latter causes lipid peroxidation and mitochondrial dysfunction (29–31). It was an interesting observation that, although iNOS levels increased after 30 min and peaked at 3 days of reperfusion in both diabetic and nondiabetic animals,  $\text{ONOO}^-$  increased only in diabetic rats. Because iNOS elevated to the same extent in both groups after reperfusion, one might predict that the same amount of NO would be produced. Given this, why do only the diabetic rats show increased production of  $\text{ONOO}^-$ ? A likely explanation that we tested was that diabetic hyperglycemia increases  $\text{ONOO}^-$  production by enhancing the formation of  $\cdot\text{O}_2$  because  $\text{ONOO}^-$  is derived from the reaction of NO with  $\cdot\text{O}_2$ . This proved to be the case in our studies using HET as a marker for in vivo detection of  $\cdot\text{O}_2$  (32). HET enters cells freely and is oxidized to a red fluorescent product when it reacts with  $\cdot\text{O}_2$ . Using this method, we detected a moderate enhancement of superoxide production in nondiabetic ischemia after 3 days of recovery, and the production was markedly enhanced in diabetic ischemia. These results suggest that hyperglycemia enhances  $\text{ONOO}^-$  production by increasing the generation of  $\cdot\text{O}_2$ .

DNA oxidative damage was observed in astrocytes after 1 day of recovery and shifted to neurons after 3 days of reflow. DNA oxidative damage in astrocytes was observed before remarkable astrocytic cell loss, suggesting that oxidative damage may be responsible for astrocytic cell death. Massive astrocytic and neuronal cell death coincided with increased ROS and RNS production, suggesting that increased free radical production may be associated with cell death.

In summary, our study indicates that diabetic hyperglycemia causes rapid damage to astrocytes, probably due to the oxidative damage of DNA. Our data suggest that hyperglycemic ischemia increases damage to astrocytes, similar to that observed in neurons.

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