Hyperglycemia Enhances Extracellular Glutamate Accumulation in Rats Subjected to Forebrain Ischemia

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**Background and Purpose**—An increase in serum glucose at the time of acute ischemia has been shown to adversely affect prognosis. The mechanisms for the hyperglycemia-exacerbated damage are not fully understood. The objective of this study was to determine whether hyperglycemia leads to enhanced accumulation of extracellular concentrations of excitatory amino acids and whether such increases correlate with the histopathological outcome.

**Methods**—Rats fasted overnight were infused with either glucose or saline 45 minutes before the induction of 15 minutes of forebrain ischemia. Extracellular glutamate, glutamine, glycine, taurine, alanine, and serine concentrations were measured before, during, and after ischemia in both the hippocampus and the neocortex in both control and hyperglycemic animals. The histopathological outcome was evaluated by light microscopy.

**Results**—There was a significant increase in extracellular glutamate levels in the hippocampus and cerebral cortex in normoglycemic ischemic animals. The increase in glutamate levels in the cerebral cortex, but not in the hippocampus, was significantly higher in hyperglycemic animals than in controls. Correspondingly, exaggerated neuronal damage was observed in neocortical regions in hyperglycemic animals.

**Conclusions**—The present results demonstrate that, at least in the neocortex, preischemic hyperglycemia enhances the accumulation of extracellular glutamate during ischemia, providing a tentative explanation for why neuronal damage is exaggerated. (*Stroke*. 2000;31:183-192.)

**Key Words:** cerebral ischemia ■ excitatory amino acids ■ glutamates ■ hyperglycemia ■ pathology ■ rats

It has been known for 2 decades that preischemic hyperglycemia aggravates brain damage due to transient global or forebrain ischemia in experimental animals. Clinical studies on diabetic and hyperglycemic patients support the notion that raised plasma glucose concentrations augment brain lesions associated with stroke. The aggravation in global ischemia has the following features: (1) the neuronal necrosis evolves more rapidly, (2) additional structures are recruited, (3) selective neuronal necrosis is transformed into pannecrosis, and (4) postischemic seizures develop and then end in fatal status epilepticus (for reviews, see References 2 and 3).

The mechanisms involved have not been adequately defined. Hyperglycemia probably acts by enhancing accumulation of lactate plus H⁺, thereby exaggerating the reduction of intracellular and extracellular pH (pHᵢ and pHₑ, respectively). In support of this notion are the results reported by Smith et al. (1986) showing that hyperglycemic subjects have more profound decreases in pHᵢ and pHₑ during ischemia, and those of Katsura et al. (1992), demonstrating an almost linear correlation between the increase in lactate during ischemia and the fall in pHᵢ and pHₑ. Furthermore, Li et al. (1994, 1995) reported a very narrow threshold of plasma glucose concentration (10 to 14 mmol/L) and of pHᵢ (6.3 to 6.5), above and below which brain damage was exaggerated and postischemic seizures were triggered, supporting the notion of a critical pH range.

It has been established that excitatory amino acids (EAAs), notably glutamate, play a pivotal role in neuronal death. Recent studies demonstrate that mitochondria are a primary target of glutamate toxicity. Glutamate-mediated massive influx of calcium triggers opening of a mitochondrial permeability transition pore, which may contribute to cell death. The possibility that hyperglycemia exaggerates brain damage by increasing the efflux of glutamate has not been established. In the present study we used in vivo microdialysis coupled with high-performance liquid chromatography (HPLC) techniques to detect the pattern of changes in extracellular glutamate, glutamine, glycine, taurine, alanine, and serine concentrations in brain tissue of rats subjected to 15 minutes of forebrain ischemia. We also evaluated the...
pathological outcome in separate groups of animals. The hypothesis tested was whether hyperglycemia enhances the rise in extracellular glutamate concentration and whether it correlates with exaggerated tissue damage.

Materials and Methods
Male Wistar rats of a specific-pathogen-free strain (Charles River Animals Facility, Quebec, Canada), weighing 300 to 390 g, were used in the present experiments. All animal use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of the University of Saskatchewan. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to use alternatives to in vivo techniques, if available.

Implantation of Guide Cannulas
Five to 7 days before microdialysis experiments, the animals were anesthetized with Equithesin (a mixture of pentobarbital, propylene glycol, ethanol, MgSO₄, and chloral hydrate). The hair on the skull was shaved, and the skin was treated with an antiseptic. The animals were placed in a stereotaxic frame (David Kopf) for positioning of the guide cannula of the microdialysis probes in the hippocampus and cortex. The skull was exposed and treated with 3% hydrogen peroxide. Three metal screws were firmly connected to the skull for fixation of a guide cannula on the skull. Two burr holes were made in the cranium. One was made overlying the left dorsal hippocampus 3.8 mm posterior to bregma, 2.5 mm lateral to midline, and 1.5 mm ventral to dura. The other was drilled over the right parietal cortex 3.0 mm posterior to bregma, 5.0 mm lateral to midline, and 2.0 mm ventral to dura. The probe length was 2 mm longer than the guide cannula, resulting in a final depth, after inserting the probe, of 3.5 and 4.0 mm in the hippocampus and neocortex, respectively. After insertion, the guide cannulas were fixed to the skull by placing dental acrylic around the cannula. The scalp was then closed surgically, and the animal was returned to its home cage, being individually housed.

Operative Procedures
The animals were fasted overnight before surgery with free access to water. Anesthesia was induced by inhalation of 3.5% halothane in a mixture of N₂O and O₂ (60:40) and was maintained with 1.0% to 1.5% halothane in N₂O and O₂ (60:40) during the operation. A 1.5% glucose saline solution was determined by an Ames Glucometer II/Glucostix system (model 7D polygraph; Grass Instrument Co.). Blood glucose concentrations were measured with a micro sample blood gas monitor (ABL 300, Radiometer). Blood pressure was monitored and recorded on a Grass Model 7D polygraph (Grass Instrument Co.).

Induction of Ischemia
Reversible forebrain ischemia of 15 minutes’ duration was induced by a combination of bilateral carotid artery clamping and hypothermia, the latter being achieved by withdrawal of blood through the catheter inserted into the jugular vein.15 Blood pressure was maintained at 40 to 50 mm Hg during the ischemic period. The EEG was monitored before, during, and after ischemia. A flat EEG was considered to constitute the onset of ischemia. Ischemia was terminated by reinfusion of the shed blood and removal of the carotid clamps.

Experimental Groups
Twenty animals were divided into 3 groups for microdialysis studies. Experimental groups included 16 animals subjected to 15 minutes of transient forebrain ischemia with preischemic saline or glucose infusion (normoglycemia and hyperglycemia; n=8 in each). Four sham-operated rats served as control (n=4). In another series, 20 animals were used for the evaluation of histopathological outcome in normoglycemic and hyperglycemic animals. Hyperglycemic animals were infused with a 25% glucose solution (2.5 to 3.0 mL/h) for 45 minutes before ischemia to yield a plasma glucose concentration of ~370 g/100 mL, while normoglycemic rats were infused with the same amount of normal saline solution.

In Vivo Microdialysis Procedure
After the operation was finished, a microdialysis probe with a 2.0-mm membrane length (CMA 12, Carnegie Medicine AB) was inserted through the preimplanted guide cannula into the left dorsal hippocampus and the right parietal cortex. The probe was perfused with a modified Ringer’s solution (mmol: NaCl 145, KCl 2.7, CaCl₂ 1.2, MgCl₂ 1.2, pH 7.4) at a flow rate of 2 μL/min by means of a microinfusion pump (CMA/microdialysis, 100 microinjection pump, Carnegie Medicine AB). Transient increases in amino acids and other neurotransmitters from probe insertion damage were avoided by a 90-minute stabilization period. Microdialysis sampling was performed at 15-minute intervals for 180 minutes. Three samples were collected before ischemia, 1 sample during ischemia, and 8 samples during the reperfusion period. All samples were collected on an ice bath and then stored at −20°C until analysis. At the end of each experiment, the microdialysis probes were removed, and the recovery rate for each probe was determined with the use of a 100 pg/10 μL standard solution in vitro. The recovery rate of interstitial amino acids was 8% to 13%. In 6 animals, the location of the microdialysis probe was verified on brain sections after the experiment.

Detection and Quantification of EAAs
Analysis of extracellular concentrations of glutamate, glutamine, glycine, taurine, alanine, and serine in the microdialysate was performed by HPLC (model 510 Liquid Pump equipped with a 10-μm filter; Millipore Corporation, Waters Chromatography Division) with electrochemical detection after precolumn derivatization by methods described previously in detail.13 A 5.0-μm Delta-Pak Cartridge filter was installed between the pump and a 715 Ultra Wisp Sampler/Precolumn Processor (Millipore Corporation). The precolumn was preceded by a reverse-phase Nova-Pak steel column (Waters) (3.9×150 mm) (4.0 particle size), which in turn, was connected to a model 460 High Sensitivity Waters Electrochemical Detector. Precolumn derivatization of amino acids with o-phthalaldehyde/β-mercaptoethanol was done before electrochemical detection. Using the 715 Ultra Wisp Sampler Processor (Millipore Corporation), we employed a fully automated system for the derivatization procedure to minimize the degradation of amino acids. This sample processor dispenses and mixes the reagent thoroughly into the microdialysis perfusate in a 200-μL loop. The injection was made precisely 2 minutes after the reaction time. Amino acid concentrations were measured and stored in the computer for future analysis with the Baseline 810 Chromatography Work Station Processing System (Waters). The mobile phase consisted of 0.01 mol/L disodium hydrogen orthophosphate, 0.13 mmol ethylenediaminetetraacetonic acid–sodium salt, and 20% methanol. External standards of 0.625, 1.25, 2.5, 25, 100, 300, and 1200 pg/10 μL were used for postexperiment probe calibration for all the amino acids.

Quantification of Brain Damage
None of the normoglycemic animals subjected to 15 minutes of forebrain ischemia at 37°C body and brain temperature developed postischemic seizures, and all survived for 7 days, allowing conven-
tional histopathological evaluation of brain damage. In hyperglycemic animals, however, survival was severely restricted. Thus, when 6 animals in a pilot study were allowed to wake up after the ischemia, all developed seizures during the first 1 to 3 hours and subsequently died in status epilepticus. For that reason, brain damage in hyperglycemic animals was evaluated after only 3 hours of reperfusion and was compared with that incurred in normoglycemic animals.

For perfusion-fixation, animals were reanesthetized with 3% halothane, tracheotomized, and artificially ventilated. The thorax was opened, and a perfusion needle was transcardially inserted into the ascending aorta. The brains were first rinsed with saline for 30 seconds and then perfusion-fixed with 4% formaldehyde buffered to pH 7.35. The brains were cut coronally in 2- to 3-mm-thick slices, dehydrated, embedded in paraffin, sectioned in 5 μm with a Reichert sledge microtome, and then stained with acid fuchsin and celestine blue, as described by Auer et al (1984). The sections were examined in a blinded fashion at a magnification of ×400. Any necrotic neurons of both hemispheres were counted in one coronal section at the level of bregma −3.8 mm in the hippocampal CA1 sector, and the percentage of necrotic neurons was calculated by dividing the number of necrotic neurons by the total number of existing neurons at the identical brain section level in nonoperated normal animals. Damage in the neocortex was too extensive to count through the whole section in hyperglycemic animals. Therefore, the damage was assessed by counting dead neurons in a strip, 400 μm wide, through layers 1 to 6 at a site just between the midline and the entorhinal fissure at the level of bregma −3.8 mm. The average total number of neurons in this area was 450 per stripe in nonoperated normal animals. The percentage of necrotic neurons was calculated.

Statistical Analysis
EAA concentrations are presented as mean±SEM. Paired t test (for chronological data versus preischemic baseline) and unpaired t test (for hyperglycemia versus normoglycemia at identical sampling time) were applied for statistical analysis. All tests were 2-sided. P <0.05 was considered statistically significant.

Results
Physiological Parameters and Seizure Incidence
Blood glucose concentrations were 90.0±14.4 and 372.6±27.2 g/100 mL in normoglycemic and hyperglycemic animals, respectively. Both head and body temperatures were maintained at 37.2±0.2°C. Mean arterial blood pressure was maintained at 120±13 mm Hg. Ventilation was adjusted to give an arterial pH of 7.4±0.2, Pco2 of 40.2±2.2 mm Hg, and Po2 of 104±18 mm Hg. There were no statistically significant differences between normoglycemic and hyperglycemic groups. EEG was continuously monitored during the entire microdialysis periods, and no subclinical seizure activities were observed in either normoglycemic or hyperglycemic rats. In the series for histopathological study, no animal developed postsischemic clinical seizures in normoglycemic subjects, while 4 of 6 showed seizure in hyperglycemic animals. Pathological evaluation showed that the 2 seizure-free rats had the same extent of damage as rats with seizures.

Histopathological Outcome
Predictably, normoglycemic animals did not have any damage in the hippocampal CA1 sector after 3 hours of recovery, and only a few scattered necrotic neurons were found in the neocortex. When the recirculation was prolonged for 7 days, the ischemia gave rise to nearly 100% damage in the CA1 sector and 30% damage in the neocortex. In the hyperglycemic animals, only a few necrotic neurons could be observed in the CA1 sector (5% damage) after the 3-hour recovery period. The small amount of CA1 damage in both normoglycemic and hyperglycemic animals after 3 hours of reperfusion was probably due to the fact that such a short period of reperfusion did not allow the CA1 damage to mature. Damage in the neocortex was dramatically exaggerated in hyperglycemic rats (P <0.01 versus normoglycemic controls). Pannecrotic lesions were observed in 5 of 6 hyperglycemic animals; additionally, status spongiosus suggested edema. Figure 1 contains representative microphotographs showing neuronal damage after 3 hours of reperfusion in the neocortical area in both normoglycemic and hyperglycemic rats. Adverse effects of hyperglycemia on tissue damage could also be observed in other brain areas. Thus, while only mild to moderate damage was observed in the striatum and thalamus in normoglycemic animals after 7 days of recovery, pannecrosis occurred in these structures of hyperglycemic animals (data not shown) as early as after 3 hours of recovery. In addition, damage to the cingulate cortex and the substantia nigra was not observed unless hyperglycemia was instituted (data not shown).

Extracellular Amino Acids
Three microdialysis samples were collected at 15-minute intervals to obtain a stable preischemic EAA baseline. Since
there were no differences among them, the results of these 3 samples were pooled and served as baseline values.

Glutamate Concentrations
Preischemic extracellular glutamate baseline concentrations in both the dorsal hippocampal region and the neocortex were \( \approx 20 \text{ pg/10 } \mu\text{L} \), with no differences among sham, normoglycemic, or hyperglycemic subjects.

The changes in extracellular glutamate concentrations in the hippocampal CA1 subregion before, during, and after ischemia are summarized in Figure 2, top panel. In sham-operated animals, the extracellular glutamate concentrations were constant throughout the 180-minute sampling period. In normoglycemic ischemic animals, extracellular glutamate concentration increased from \( 20.3 \pm 2.2 \text{ to } 284.8 \pm 24.6 \text{ pg/10 } \mu\text{L} \) (mean \( \pm \) SEM; 14.0-fold) after the induction of ischemia \( (P<0.0001) \). The glutamate concentrations remained elevated after 15 minutes of recirculation \( (150.8 \pm 34.7 \text{ pg/10 } \mu\text{L}; P=0.01) \) and returned to near-control levels thereafter. Changes in glutamate of hyperglycemic rats were similar to those recorded in normoglycemic ones. Thus, glutamate content increased from \( 19.4 \pm 2.2 \) to \( 295.5 \pm 72.3 \text{ pg/10 } \mu\text{L} \) during ischemia and remained elevated after 15 minutes of recirculation \( (178.4 \pm 42.6 \text{ pg/10 } \mu\text{L}; P<0.01) \), subsequently returning to baseline.

The glutamate transients were different in the neocortex compared with the hippocampus. First, the elevation of glutamate concentration during ischemia in normoglycemic animals was less than that in the hippocampus \( (91.9 \pm 26.1 \text{ versus } 284.8 \pm 24.6 \text{ pg/10 } \mu\text{L}; P<0.01) \). Second, and most importantly, hyperglycemic animals showed a more pronounced increase in glutamate content than normoglycemic ones. As shown in Figure 2, bottom panel, glutamate levels increased from \( 24.3 \pm 1.7 \) to \( 91.9 \pm 26.1 \text{ pg/10 } \mu\text{L} \) (3.8-fold; \( P<0.01 \)) in normoglycemic ischemic animals, whereas the increase in glutamate was from \( 19.6 \pm 2.4 \) to \( 200.5 \pm 39.8 \text{ pg/10 } \mu\text{L} \) (10.2-fold; \( P<0.001 \)) in hyperglycemic animals. The peak value in hyperglycemic subjects was twice that in normoglycemic animals \( (P<0.05) \).

Glutamine
Figure 3, top panel, illustrates changes in hippocampal glutamine concentrations. Extracellular glutamine concentration decreased slightly in the normoglycemic group after the induction of ischemia, and there was a further decrease at 15 and 30 minutes after recirculation \( (P<0.05) \). Afterward, glutamine gradually recovered to baseline.

The baseline resting levels of glutamine were similar to those recorded in normoglycemic ones. Thus, glutamate content increased from \( 19.4 \pm 2.2 \) to \( 295.5 \pm 72.3 \text{ pg/10 } \mu\text{L} \) during ischemia and remained elevated after 15 minutes of recirculation \( (178.4 \pm 42.6 \text{ pg/10 } \mu\text{L}; P<0.01) \), subsequently returning to baseline.

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In the cortex, glutamine changes were not as pronounced as in the hippocampus (Figure 3, bottom panel). Significant decreases of glutamine concentration could only be detected at 15 minutes of recirculation in normoglycemic animals \((P<0.05)\) and during the first 45 minutes of reperfusion in hyperglycemic animals. However, the results reinforce the impression that hyperglycemia prolongs the reduction in glutamine concentration.

Glycine

The alterations in the extracellular glycine concentration in the hippocampus are shown in Figure 4, top panel. Ischemia induced an abrupt increase in extracellular glycine concentration in both normoglycemic and hyperglycemic groups. Similar to the changes in glutamate, the peak concentrations were reached after the induction of ischemia, and increased levels were maintained during the immediate reperfusion period. Unlike glutamate, however, the increase in glycine was sustained in the posts ischemic period, and such a sustained increase lasted longer in hyperglycemic animals. In normoglycemic animals the glycine concentration returned to baseline level after 45 minutes of recirculation \((P<0.05)\); however, it was persistently increased (except in the 120-minute sample) in hyperglycemic animals \((P<0.05)\).

In the neocortex (Figure 4, bottom panel), glycine concentrations were moderately raised immediately after reperfusion and declined to baseline values at 60 minutes of recovery in the normoglycemic group. The increase in glycine concentration appeared somewhat delayed in the hyperglycemic rats, but it was sustained for a longer period than in the normoglycemic animals.

Taurine

Figure 5 shows the alterations of extracellular taurine concentration in the hippocampal and cortical regions. Transient increases of taurine in microdialysates were observed in both normoglycemic and hyperglycemic subjects, and increased values persisted for the first 30-minute period of recirculation \((P<0.01)\). The peak values appeared slightly lower in hyperglycemic than in normoglycemic animals.

Alanine

Extracellular alanine concentrations in the hippocampal CA1 subregion and cortex are presented in Figure 6. In the hippocampus, alanine concentrations increased after ischemia in the saline-infused rats. Unlike other amino acids, alanine did not increase in concentration during ischemia and the immediate reperfusion phase, but an increase occurred 45 to 90 minutes after reperfusion. There was no major change in glucose-infused animals. Alanine changes in the cortex were similar to those in the hippocampus.

Serine

Extracellular serine concentrations are given in Figure 7. In both the cortex and the hippocampus, sham-operated animals had a higher serine level than the 2 ischemic groups. Thus, the neocortical values in hyperglycemic animals and hippocampal values in both normoglycemic and hyperglycemic groups
were significantly lower than the values in the sham-operated group. Extracellular serine concentrations were not changed after ischemia and reperfusion in saline-infused rats. In animals infused with glucose, a decrease in serine concentration could be observed in the hippocampus and the cortex during ischemia. The decrease was also evident in samples collected after 15 to 60 minutes of reperfusion.

Discussion

When the present results are discussed, it should be kept in mind that even in normoglycemic animals, 15 minutes of ischemia at a brain temperature of 37°C is a relatively severe insult that, apart from giving close to 100% CA1 neuronal necrosis, kills ~30% of the neurons in the neocortical strip examined. Damage to both of these structures is delayed, the rate of evolution being faster in the neocortex than in the CA1 sector. It is thus possible to find reperfusion times when neocortical but not CA1 damage is observed.

With this model, hyperglycemia results in such a severe insult that survival is no longer possible, mainly because intractable seizures develop. However, the ischemia does not compromise reflow since the bioenergetic state recovers upon reperfusion and capillary patency remains intact. It is thus possible to use the first hour of reperfusion to study events that prime the tissue for seizures and secondary bioenergetic failure. In one respect, it would have been advantageous to employ 10 minutes of ischemia and to use a lower plasma glucose concentration since this would allow hyperglycemic animals to survive. However, that model carries the potential disadvantage of large interanimal variations.

Another consideration is the difference in pH_e (and pH_i) between the normoglycemic and hyperglycemic animals. As measured under comparable ischemic conditions, the differences in pH_e and pH_i are ~0.5 pH units, e.g., pH_e falls from 7.3 to 6.8 in normoglycemic animals and from 7.3 to 6.3 in hyperglycemic animals. It is not known whether such a difference could affect amino acid release during ischemia. An additional consideration is the effect of anesthesia on the measurements of EEs. Equithesin contains magnesium, which is a noncompetitive NMDA antagonist, and therefore it would possibly have influenced the EEA levels. However, since equithesin was used for implantation of cannulas 5 to 7 days before microdialysis samples were collected, it is unlikely to have influenced the measurements of EEs conducted 5 to 7 days after the use of this anesthetic agent.

The rats that were kept under anesthesia for use in microdialysis experiments showed no subclinical seizure activity. Given the fact that the anesthesia obviously prevented seizure activity from occurring, it might influence the measurements of EEs. However, increases of extracellular EEs, including glutamate, were observed during ischemia and in the immediate reperfusion period (15 minutes). It is not likely that subclinical seizure activity develops at such an early stage in reperfusion. In support, Nakashima and Todd reported that anesthetic agents such as isoflurane and nitrous oxide are not

Figure 6. Extracellular alanine concentrations in dialysate (mean ± SEM) over the course of the experiment for 3 groups in the hippocampal CA1 region and in the neocortex. The microdialysis alanine levels rose slightly in response to the ischemic insult in normoglycemic but not in hyperglycemic animals. *P < 0.05 vs baseline. Abbreviations are as defined in Figure 2.

Figure 7. Extracellular serine concentrations in dialysate (mean ± SEM) over the course of the experiment for 3 groups in the hippocampal CA1 region and in the neocortex. The microdialysis serine levels rose in response to the ischemic insult in both normoglycemic and hyperglycemic animals. *P < 0.05 vs baseline; †P < 0.05 vs normoglycemic group at identical times. Abbreviations are as defined in Figure 2.
pentobarbital do not influence the glutamate transient in rats subjected to complete global ischemia.

**Effect of Hyperglycemia on Brain Damage**

Preischemic hyperglycemia worsens the ischemic injury (for reviews, see References 2 and 3) Previous results have demonstrated that while ischemia of 10 to 15 minutes’ duration results in maximal CA1 damage in normoglycemic subjects, hyperglycemia aggravates damage in the parietal cortex, striatum, and thalamus and recruits the cingulate cortex, the CA3 sector of the hippocampus, and the substantia nigra in the damage process. The present results confirm these findings and provide an interesting observation of extensive neocortical injury already present after 3 hours of reperfusion. Predictably, damage to CA3 neurons was delayed to a later reperfusion period (see above). The mechanisms by which hyperglycemia exaggerates ischemic brain damage have not been clarified. Acidosis may be one of the key factors in hyperglycemia-related damage. This contention is supported by the findings that acidosis induced by superimposed hypercapnia augments selective neuronal necrosis in normoglycemic ischemic animals and in rats with hypoglycemic coma.

**Excitotoxic Theory and the Link Between Glucose and Increased Glutamate**

Numerous studies have shown that glutamate, when present in excessive amounts extracellularly, is neurotoxic. Glutamate and related EAAs activate postsynaptic glutamate receptors. Activation of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) type of glutamate receptor allows influx of Na⁺, Cl⁻, and water (and a small amount of Ca²⁺) by way of receptor-gated channels. This leads to swelling of the neurons. Activation of the N-methyl-D-aspartate (NMDA) type of glutamate receptor opens a channel permeable to calcium, leading to intracellular calcium overload. The resulting massive increase in intracellular calcium has a number of consequences. The crucial event may be the assembly of a mitochondrial permeability transition pore, which then triggers release of cytochrome c from mitochondria, and a burst of production of reactive oxygen species.

Our results demonstrate that the rise in neocortical extracellular glutamate concentration during ischemia and in the immediate reperfusion period is more pronounced in hyperglycemic animals. Choi and colleagues previously tried to assess the effect of hyperglycemia on extracellular glutamate concentrations in rats subjected to brief periods of ischemia. Their samples were taken from the hippocampus. This may be why they did not observe an increased extracellular glutamate concentration in hyperglycemic rats.

More than a decade ago, Gaitonde et al (1987) described the pathway of glucose metabolism into EAAs. Approximately 70% of the 14C-labeled glutamate was formed from acetyl coenzyme and 30% by 14CO₂ fixation of pyruvate after injection of [3,4-14C]glucose. Thus, glutamate in the brain is formed by metabolism of glucose via the hexose monophosphate shunt as well as via the Embden-Meyerhof pathway. It cannot be argued that hyperglycemia enhances glutamate synthesis. However, we cannot anticipate a relationship between the total amount of glutamate formed and the amount available for release during ischemia. Hyperglycemia may also influence other factors determining the uptake of amino acids. As will be discussed below, changes in glial metabolism may explain some of the changes in glutamate and glutamine concentrations.

Glia cells carry the main responsibility of rapid postsischemic removal of EAAs accumulated in extracellular space. Increases in glutamate and decreases in glutamine could be best explained by assuming that while some glial uptake of glutamate may persist during ischemia, at least initially, the subsequent metabolism of glutamate to glutamine is impeded. The reduced metabolism of glutamate by glial cells may be responsible for part of the extracellular glutamate overflow during ischemia. However, an increased neuronal efflux of glutamate is also likely to contribute. It seems likely that postsynaptic neuronal elements as well as glial cells contribute to the extracellular overflow of EAAs during an ischemic event.

The additional increase of glutamate concentration in hyperglycemic subjects may either reflect the fact that glucose enhances the formation and release of glutamate from presynaptic endings, as described by Gaitonde et al (1987), or reflect the fact that acidosis inhibits glutamate uptake by glial cells (astrocytes). A reduction in pH from 7.4 to 5.8 in primary rat astrocyte cultures causes a significant inhibition of glutamate uptake, and this inhibition is more pronounced when acidosis is combined with hypoxia. A similar situation may have existed in our experiments, in which severe acidosis is combined with cellular hypoxia.

It has been reported by Dietrich and colleagues (1993) that hyperglycemia causes breakdown of the blood-brain barrier after reperfusion following 20 minutes of global ischemia, and it may therefore account for the increase in the extracellular glutamate. However, since extracellular glutamate already increased during ischemia, it is unlikely that the extra increase in extracellular glutamate resulted from the breakdown of the blood-brain barrier.

It is not clear that an increased accumulation of glutamate in extracellular fluid, above that observed in normoglycemic animals, can explain the exacerbated cell damage in hyperglycemic subjects. However, since hyperglycemia-enhanced glutamate release is correlated with aggravated damage observed in the neocortical areas, it is therefore possible that the additional increase of glutamate concentrations in the extracellular space may be responsible, at least partially, for the aggravated damage in hyperglycemic animals. It is not clear why hyperglycemia enhances glutamate release in the neocortex but not in the hippocampus. Probably, maximal accumulation of glutamate in extracellular fluid already occurs in normoglycemic rats; therefore, hyperglycemia cannot increase it further. It has been established that the hippocampus has an abundance of glutamatergic inputs. This agrees with our findings that the absolute values of extracellular glutamate were much higher in the hippocampus than in the neocortex. The failure of hyperglycemia to increase extracellular glutamate levels in the hippocampus is not compatible with any general hypothesis of a coupling between glutamate...
concentration and hyperglycemia-exaggerated damage; in fact, the data could be used to prove that such a coupling does not exist. However, because of its vulnerability to ischemic insults, the hippocampus could be a special case. Thus, although the CA1 damage matures more rapidly in hyperglycemic subjects (present data and Reference 4), the ultimate damage is already maximal in normoglycemic subjects. It remains to be shown that a coupling exists between glutamate release and hippocampal (CA1) damage when the duration of ischemia is reduced, for example, to 5 minutes.

Changes in Glutamine

Our results demonstrate that a large increase in glutamate efflux is coupled with a corresponding decline in glutamine efflux. The decrease of glutamine is sustained for a longer period in hyperglycemic animals. Glutamine is a major metabolic precursor of glutamate. The reduction of extracellular glutamine may reflect utilization of intracellular glutamine for synthesis of glutamate or inhibition of synthesis due to depletion of energy stores.37 However, the decrease in extracellular glutamine could also be linked to an increased activity of the phosphate-activated glutaminase, a key enzyme in the synthesis of neurotransmitter glutamate, since tissue inorganic phosphate content increases markedly during ischemia, and calcium promotes phosphate activation of glutaminase. Inhibition of the glutamic enzyme glutamine synthetase, which converts glutamate to glutamine, has been reported during reperfusion after cerebral ischemia.39 Suppression of activity of this enzyme could result in both a reduction in glutamine efflux and synaptic glutamate accumulation.

Prolonged Release of Glycine by Hyperglycemia

Glycine is located in presynaptic terminals. There are 2 types of glycine receptors, Gly 1 (the classic strychnine-sensitive inhibitory site) and Gly 2 (the strychnine-insensitive site). Gly 1 acts as an inhibitory neurotransmitter because of its ability to cause postsynaptic hyperpolarization. Gly 2 is associated with excitatory transmission since it potentiates NMDA activity. It has been assumed by some investigators that glycine levels are probably already saturated under physiological conditions and that changes in glycine are unlikely to make any difference with respect to NMDA receptor activation; however, many in vivo and in vitro experiments provide convincing data that NMDA-associated glycine sites are not saturated at physiological levels of glycine (for review and literature, see Reference 40). Delayed treatment with glycine site antagonists attenuates infarct size in focal ischemia.41 In the present study glycine concentrations increased during ischemia, and the elevated levels were sustained into the postischemic period. Such changes in concentrations of glycine have been previously reported.28 The sustained increase may help to explain the ongoing toxicity despite the return of glutamate to baseline levels. In the present study, when compared with normoglycemic animals, hyperglycemic animals had a much more prolonged elevation in glycine levels in both hippocampal and neocortical areas after ischemia. It is tempting to postulate that the rise in glycine modulates the action of glutamate in vivo during and after ischemia and may be responsible, at least partially, for the detrimental effect of hyperglycemia on neurological outcome.

In summary, the present results demonstrate that periischemic hyperglycemia enhances the accumulation of extracellular glutamate in the neocortex and that the enhanced increase of glutamate is correlated with exaggerated cell damage. The increase was observed during ischemia and in the immediate (15-minute) posts ischemic period. Hyperglycemia also prolonged the postischemic decrease in glutamate concentration normally seen during the initial 15 to 30 minutes of recirculation and gave rise to a sustained increase in glycine concentrations after recirculation. Changes in taurine, alanine, and serine concentrations were inconclusive. Thus, although extracellular taurine and alanine concentrations rose during ischemia and after reperfusion, no clear effects of hyperglycemia were observed.

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References

It is clearly established that preischemic hyperglycemia worsens ischemic outcome in laboratory animals. There is also sufficient correlative human data for most clinicians to accept the conclusion that glucose infusion should be withheld in patients at risk for ischemic brain damage. This perhaps has greatest relevance to surgical patients, in whom ischemic events can often be presaged, and is also relevant to patients undergoing cardiopulmonary resuscitation (CPR). For these reasons, dextrose-containing solutions are usually withheld from surgical patients, and the Advance Cardiac Life Support algorithm has been modified to recommend administration of normal saline as the principal intravenous solution during CPR.1,2

From a scientific perspective, it remains a paradox that giving glucose can worsen damage in ischemic tissue that is by definition deprived of sufficient substrate to maintain normal metabolic function. One likely mechanism for this is the enhanced lactic acidosis resulting from anaerobic metabolism of glucose, which is disproportionately more available than oxygen. However, in vitro studies have found that acidosis actually provides an advantage to cultured neurons deprived of metabolic substrate.3 Accordingly, although lactic acidosis, in part, explains hyperglycemia-augmented ischemic brain damage, other mechanisms are also likely involved.

It is widely accepted that disregulated glutamate metabolism during ischemia, and the concordant increase in extracellular glutamate concentration, contribute to ischemic brain injury. It has been known that hyperglycemia augments extracellular glutamate increases during ischemia.4 What is novel in the current work by Li et al is the attempt to correlate these changes with histological outcome. Rats were subjected to severe forebrain ischemia while either normoglycemic or hyperglycemic brain damage.

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ever, because hyperglycemic animals typically died within the first few hours after ischemia, histological information could not be obtained when full maturation of hippocampal injury would be expected to occur. Because normoglycemic rats subjected to the same insult survived 7 days and were found to have 100% hippocampal CA1 damage, it is likely that the ischemic insult alone was sufficient to cause maximal glutamate accumulation, disallowing any further augmentation by hyperglycemia. This could be confirmed in future studies by repeating the experimental protocol with a shorter duration of ischemia, which would result in only moderate damage in the CA1 sector in normoglycemic rats.

The results of this work by Li et al are consistent with the hypothesis that hyperglycemia worsens ischemic outcome, at least in part, by augmenting extracellular glutamate accumulation, which in turn upregulates $N$-methyl-$D$-aspartate receptor activation and conductivity to calcium. However, data to date remain only correlative. The importance of this limitation can be demonstrated by examining effects of hypothermia on glutamate concentrations during ischemia. It is known that hypothermia nearly abolishes any increase in glutamate concentration. However, recent evidence provided by Yamamoto et al questions the importance of this effect. When hypothermic gerbils underwent microinjection of glutamate into hippocampal CA1 in sufficient quantity to cause intrischemic extracellular glutamate concentrations to be similar to those observed in normothermic gerbils, hypothermia still caused a profound reduction in histological damage. Accordingly, additional work is required to confirm a causal relationship between hyperglycemia and glutamatergic excitotoxicity. Because NMDA receptor activation is associated with increased permeability of calcium, assessment of calcium transients in normoglycemic versus hyperglycemic animals would be important. Also, because glutamate excitotoxicity has been linked to opening of the mitochondrial permeability transition pore, investigation of mitochondrial responses to hyperglycemic versus normoglycemic ischemia should be examined. The results of Li et al justify further pursuit of these questions.

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