Glutamate Receptor Blockade Attenuates Glucose Hypermetabolism in Perihematomal Brain After Experimental Intracerebral Hemorrhage in Rat

Timothy D. Ardizzone, PhD; Aigang Lu, MD, MS; Kenneth R. Wagner, PhD; Yang Tang, MD, PhD; Ruiqiong Ran, MD, PhD; Frank R. Sharp, MD

Background and Purpose—Intracerebral hemorrhage has no effective treatment. The delayed appearance of edema, apoptosis, and inflammation in perihematomal brain suggests that these events may be targets for therapeutic intervention. To develop successful treatments, we must learn more about the effects of hemorrhage on brain tissue. In this study, we investigated the acute metabolic effects of intrastriatal hemorrhage in rat brain.

Methods—Lysed blood or saline (50 μL each) was injected into the striatum of male Sprague-Dawley rats. The rats recovered for 1 to 72 hours before injection of [14C]-2-deoxyglucose (intraperitoneally) 30 minutes before decapitation. Animals were pretreated with the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor antagonists dizolcilpine maleate (MK-801; 1 mg/kg) or 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline (NBQX; 30 mg/kg), or saline vehicle. Additional animals received intrastriatal injections of glutamate (1.0 mmol/L), NMDA (1.0 mmol/L), or AMPA (0.1 mmol/L) in the place of blood. Semiquantitative autoradiographs from the brains were analyzed to determine the effects of hemorrhage on relative glucose metabolism.

Results—We found an acute phase of increased [14C]-2-deoxyglucose uptake in the perihematomal region that peaks 3 hours after lysed blood injection. Saline injections had no effect on striatal glucose utilization. The increased [14C]-2-deoxyglucose uptake produced by the hemorrhages was blocked by pretreatment with MK-801 and NBQX. Glutamate injections alone had no effect on striatal metabolism, whereas NMDA and AMPA injections increased [14C]-2-deoxyglucose uptake.

Conclusions—The data imply that glutamate activation of NMDA or AMPA receptors increases glucose metabolism in perihematomal brain at early times after intracerebral hemorrhage. This may provide a possible target for the treatment of intracerebral hemorrhage. (Stroke. 2004;35:2587-2591.)

Key Words: intracerebral hemorrhage ■ MK-801 ■ stroke, hemorrhagic

Intracerebral hemorrhage (ICH) is a devastating disease with high mortality.¹ No current treatments decrease mortality or improve the quality of life of survivors. To develop effective treatments for ICH, more must be learned about the effects of hemorrhage in brain.

Since the introduction of computed tomography (CT), clinicians have described an area of hyperperfusion in the brain tissue surrounding the hematoma.²–⁶ The area has been described as luxury perfusion, relative focal hyperperfusion, and focal hyperemia. Although metabolic acidosis has been suggested as a potential mechanism for this phenomenon,⁶ the clinical observations of hyperperfusion after ICH remain unexplained.

The neurological damage that occurs after ICH is biphasic. The initial insult is the result of the mechanical forces from the expanding hematoma. After clot formation, edema and inflammation cause further damage to brain tissue. Events associated with clot formation appear to cause edema and inflammation.⁷,⁸ With regard to metabolism, Wagner et al⁹ showed that lactate, a metabolite of glycolysis, increased in edematous brain tissue around areas of ICH with no corresponding decrease in ATP. They suggested that the lactate increase was attributable to aerobic glycolysis, possibly stimulated by astrocytic uptake of glutamate coming from the hematoma.¹⁰

Previous animal studies of blood flow have demonstrated a short period of decreased blood flow, but not ischemia, followed by a prolonged period of hyperperfusion after ICH.¹⁰,¹¹ Ropper and Zervas¹¹ reported an acute episode of...
hypoperfusion followed by increased blood flow using a hydrogen clearance technique in a rat model of lobar hemorrhage. Similar observations were reported in a model of intrastriatal hemorrhage using [14C]-iodoantipyrine autoradiographs to measure blood flow, although mechanisms of ICH-induced hyperperfusion were not studied.

Blood flow is tightly coupled to neuronal activity. We suggest that ICH-induced hyperperfusion is the result of neuronal excitation caused by the hemorrhage. The primary excitatory neurotransmitter in the brain is glutamate. Using a rabbit model of ICH, Qureshi et al measured significant increases of glutamate in perihematomal brain. These data suggest that hyperperfusion observed after ICH could be mediated by neuronal excitation caused by glutamate receptors, with a corresponding increase in blood flow and metabolism.

The current study was undertaken to examine the effect of hemorrhage on metabolism in perihematomal rat brain. We tested the hypothesis that extracellular glutamate released after ICH causes neuronal excitation leading to areas of hyperperfusion. A rat model of ICH was studied using semiquantitative autoradiography to measure changes in [14C]-2-deoxyglucose (2DG) uptake as an indicator of relative metabolic rate. This study provides a possible explanation for the clinical observation of variable changes of cerebral blood flow (CBF), including hyperperfusion after ICH.

Methods

Animals

Sprague-Dawley rats (weighing 250 to 300 g; Harlan, Indianapolis, Ind) were used. Animals were kept 2 to a cage with free access to food and water. Animal protocols were approved by the University of Cincinnati Animal Care Committee and conform to the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Surgical Preparation

Rats were anesthetized with isoflurane and placed in a Kopf stereotaxic frame (Kopf Instruments). A heating blanket was used to maintain body temperature at 37°C. The scalp was incised to expose the skull and a burr hole drilled 1 mm anterior and 3 mm lateral to bregma. Blood lysate (50 µL) prepared by freeze-thawing whole arterial blood 4X was injected into the left striatum at a depth of 5 mm during 10 minutes through a 27-gauge needle. Additional animals (n=3) received injections of whole, unclotted, unlysed blood. Sham surgery animals were injected with saline (50 µL). Some animals were treated with glutamate receptor antagonists (1 mg/kg dizocilpine maleate [MK-801] or 30 mg/kg 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[b]zoxazol-5-yl) prepared by freeze-thawing whole arterial blood into the striatum and 30 minutes after injection of [14C]-2DG intraperitoneally.

At least 7 animals were used for each condition. In addition to the above experimental conditions, some animals received intra-striatal injections of glutamate (1 mmol/L), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; 0.1 mmol/L), or N-methyl-D-aspartate (NMDA; 1.0 mmol/L) dissolved in 50 µL sterile saline. After removal of the needle, the wound was cleaned and sutured and animals were allowed to recover in an incubator maintained at 37°C. Animals were then returned to their home cages with free access to food and water.

Autoradiography

One to 72 hours after surgery, animals were injected intraperitoneally with [14C]-2DG (1.3 mCi/kg; 55 mCi/mmoll; American Radiolabeled Chemicals). After 30 minutes, rats were anesthetized with isoflurane and decapitated. The brains were rapidly removed and frozen in 2-methylbutane, cooled with dry ice, and stored at −80°C until cryosectioning. Coronal sections 20-µm thick were cut at 1.6 mm, 0.6 mm, and −0.4 mm to bregma, as determined from a stereotaxic atlas. Slices were placed on slides and rapidly dried on a 90°C hot plate. Kodak Biomax MR-1 film was exposed to the slides in autoradiography cassettes for 7 days.

Histological Analyses

Sections cut for autoradiography were used to visualize the area of hemorrhage grossly manifested by pink or red staining. Areas of hemorrhage were compared with areas of hypometabolism and hypermetabolism observed on the autoradiographs.

Data Analysis

Films were scanned and analyzed for relative glucose metabolism using MCID Elite (version 6.0) software. The relative optical density (ROD) of the control striatum was measured and a threshold value calculated so that 90% of the RODs in the pixels in the control striatum were below that value. The areas (mm²) of the striatum above this threshold were then measured on the control and hemorrhage sides. Data were averaged for each animal at each level of the brain measured. The data presented are the hypermetabolic area (area of striatum above the threshold on the hemorrhage side minus the control side) as a percentage of the total striatal area on the control side. An ANOVA followed by a post hoc Schefé was used to determine significance between conditions.

Results

There are 3 distinct areas of altered metabolism observed on the [14C]-2DG autoradiographs of the striatum after hemorrhage. First, there is an area at the site of the injection of blood that has decreased metabolism (Figure 1, region A). This hypometabolic area was observed in every animal in the study. Comparison of the heme-stained regions on the sections used to produce the autoradiographs to the autoradiographic images confirmed that the red and pink staining areas of hemorrhage corresponded to the large area of hypometabolism in the central part of the striatum in every animal (Figure 1, region A).

Next, we observed areas of hypermetabolism in the perihematoma zone (Figure 1, region B). The hypermetabolic areas were observed in all hemorrhage animals not treated with glutamate receptor antagonists. The size of this area varied between animals and was patchy. The hypermetabolic area was confined to the striatum and did not spread to surrounding white matter or cortex. The areas of hypermetabolism were usually (although not always) at the margins of
of the hemorrhage noted as red/heme-stained regions on the cut brain sections. The hypermetabolic effect of ICH started to appear at 2 hours and peaked at 3 hours after hemorrhage (Figure 2). The hypermetabolic area gradually decreased by 8 hours after the hemorrhage (Figure 2). Beyond 8 hours, there was no detectable change in the metabolism of perihematomal brain tissue. Animals injected with whole blood had similar areas of hypermetabolism, although the increases of [14C]-2DG uptake were blunted compared with lysed blood but were prolonged beyond 8 hours.

Finally, we also determined that the relative metabolic rate of the entire striatum on the hemorrhage side was depressed compared with the contralateral striatum (Figure 1, region C). Striatal metabolism on the hemorrhage side was significantly decreased (average decrease of 10.1%; SD of 6.6%; P<0.01; ANOVA followed by post hoc Scheffe).

To determine whether the hypermetabolic effect of hemorrhage was related to glutamate-induced excitation, we tested the glutamate receptor antagonists MK-801 and NBQX. For this series of experiments, animals were injected intraperitoneally with MK-801 (1 mg/kg) or NBQX (30 mg/kg) alone or in combination 30 minutes before surgery. Control animals were injected with saline. We found that either of the glutamate receptor antagonists blocked hypermetabolism after ICH when injected alone or in combination (Figure 3).

To confirm that glutamate receptor activation could cause hypermetabolism, we injected glutamate (1 mmol/L) or NMDA (1 mmol/L) or AMPA (0.1 mmol/L) dissolved in saline (50 µL) directly into the striatum of an additional series of rats (Figure 4). We observed no change in [14C]-2DG uptake in the striatum in or near the sites of injection in all 4 of the glutamate-injected rats (Figure 4, left). However, we observed large areas of increased [14C]-2DG uptake in the striatum after NMDA injections into 3 rats and after AMPA injections in another 3 rats (Figure 4, 1 rat each shown). The importance of these findings was that glutamate alone did not increase metabolism, whereas injections of the glutamate analogues NMDA and AMPA did increase metabolism. The significance of this finding for the ICH data are discussed below.

**Discussion**

This study demonstrates that a reproducible hypermetabolic area occurs in rats after experimental ICH. The maximum effect occurs 3 hours after ICH and gradually decreases out to 8 hours. Glutamate receptor antagonists MK-801 and NBQX block the hypermetabolic effects of ICH on perihematomal brain. Glutamate injected directly into the brain had no effect on metabolism. However, direct injection of nonmetabolizable glutamate receptor agonists into the striatum did induce pronounced hypermetabolism. Our data suggest that the hypermetabolic areas observed after ICH are attributable to increased glutamate signaling in perihematomal brain. In addition, these observations may explain the relative focal hyperperfusion seen clinically after ICH.

![Figure 2. Time course of the effects of lysed blood on metabolism in striatum](image)

![Figure 3. Effects of glutamate receptor antagonists on the hypermetabolic area in rat striatum 3 hours after hemorrhage](image)

![Figure 4. [14C]-2DG autoradiographs of rat brains 3 hours after intrastriatal injections of glutamate (1.0 mmol/L), NMDA (1.0 mmol/L), and AMPA (0.1 mmol/L).](image)
Hypermetabolism

Two possible interpretations of hypermetabolism in perihematomal brain after ICH include stimulation of glycolysis by ischemia or neuronal and glial cell excitation by excitatory amino acids. During ischemia and anoxia, the cell attempts to maintain ATP levels by the production of lactate from glucose by anaerobic glycolysis. Anaerobic glycolysis is a significantly less efficient means of producing ATP from glucose. Thus, glucose transport and utilization increase to meet metabolic demand. It is important to stress that although some studies report hyperperfusion in perihematomal brain, no recent studies have demonstrated ischemia after ICH in animals or humans. The increased glycogen and glucose concentrations that occur at early times around areas of ICH may be related to the increases of [14C]-2DG uptake observed in this study. Because of the lack of cellular resolution using the [14C]-2DG technique, it is not possible to state whether the changes occur in neurons, glia, leukocytes, or other types of cells.

Excitatory amino acids stimulate brain metabolism. Repeated stimulation of glutamate receptors increases intracellular concentrations of calcium and sodium ions. Energy, in the form of ATP, is required to re-establish ionic gradients through active transport systems. The metabolic rate of the cell increases to meet the energy demands of increased transporter activity. Shiraishi et al17 showed that excitatory amino acids act on glutamate receptors to increase glucose uptake in the penumbra after middle cerebral artery occlusion in rats. Based on these data, we hypothesized that excitatory amino acids were involved with the hypermetabolic area that we observed after ICH.

Increased glutamate signaling may be caused by accumulation of extracellular glutamate or potentiation of glutamate receptors. Extracellular glutamate accumulation in brain after hemorrhage may be attributable to increased release of glutamate from damaged brain cells, glutamate release from the blood, or the decreased uptake of glutamate by astrocytes. Each of these pathways that increase glutamate signaling likely contributes to the hypermetabolic areas observed in this study because glutamate injections alone did not increase glucose metabolism.

Increased Extracellular Glutamate

Qureshi et al12 directly measured significant increases of extracellular glutamate concentrations as early as 30 minutes after hemorrhage using in vivo microdialysis. They suggested that the glutamate is increased as a consequence of an ischemic episode during hematoma growth or cell trauma caused by increased intracranial pressure. In the current study, the hypermetabolic effect was not observed until 2 hours after hemorrhage. The difference in the time course between the 2 studies may be that Qureshi measured increases in extracellular glutamate, whereas we measured changes in metabolism. As we found in this study, transient exposure of the brain to high levels of glutamate by itself was not sufficient to cause a detectable metabolic change. However, longer-term exposure to high levels of extracellular glutamate may cause metabolic stress through prolonged states of excitation and, thus, increase metabolism. Alternatively, hemorrhage may result in increased glutamate release as well as decreased glutamate reuptake by astrocytes that would result in prolonged excitation not seen with glutamate injections alone.

ICH causes necrosis and apoptosis in perihematomal brain tissue. Because extracellular glutamate increases in perihematomal brain tissue after hemorrhage, glutamate excitotoxicity may contribute to cell death after ICH. In healthy brain tissue, glutamate is rapidly cleared from the extracellular space by transport into astrocytes. During prolonged exposure to glutamate, astrocytes experience oxidative stress and start to die. The death of astrocytes would decrease the clearance of glutamate from the extracellular space. In addition, the dying cells would release their glutamate stores to affect other cells in the proximity of the dying cell. This could explain the clinical observation of persistent decreased blood flow in areas of brain that were acutely hyperperfused after ICH.

Potentiation of Glutamate Receptors

Potentiation of glutamate receptors increases the susceptibility of brain tissue to glutamate excitotoxicity. Glutamate receptor potentiation can occur in response to a stimulus that affects the Phosphorylation State of the receptor. Thrombin, a component in the blood clot cascade, is one such stimulus. Intracerebral injection of thrombin causes edema, gliosis, inflammation, apoptosis, and scar formation. One milliliter of blood can produce 260 to 360 U of active thrombin. Even a modestly sized hemorrhage results in the release of several thousand units of active thrombin. Gingrich et al24 found that hippocampal brain slices exposed to 3 U/mL thrombin displayed rapid potentiation of NMDA receptor function. This effect was mediated through the actions of thrombin on its receptor, the protease activated receptor 1. We suggest that after ICH, thrombin released in its active form permeates brain tissue and potentiates glutamate receptors leading to glutamate excitotoxicity. Further studies need to be done to address this issue.

Blood Flow

The clinical observation of “luxury perfusion” was the first indication that blood flow may be affected by ICH. Animal studies indicate that blood flow is transiently decreased after ICH, which is then followed by a period of variable hyperperfusion. These blood flow increases temporally correspond with the metabolic increase in tissue surrounding the hematoma in this study. Because local cerebral glucose metabolism and local CBF are usually tightly coupled, even in many pathological conditions, future studies would be needed to demonstrate that the regions of increased [14C]-2DG uptake observed in this study would correlate with increases of local CBF. It is important to reiterate that decreases of CBF have been observed in most recent human and animal studies, but these decreases around the ICH are not sufficient to cause ischemia.

Hypometabolism

The areas of decreased 2DG uptake in the center of the blood injection site could represent decreased glucose metabolism because of cell damage caused by the mechanical forces of
the hematoma and exposure to blood. Decreased accumulation of [14C]-2DG in this area could also be the result of compromised transport of glucose into the brain by the blood vessels in this region. The explanation for the hypometabolism in the entire striatum (Figure 1, region C) ipsilateral to the hemorrhage (outside the areas of central hypometabolism and areas of hypermetabolism) could relate to decreased glucose uptake or glucose metabolism related to the hemorrhage or edema or to decreased synaptic activity in the entire ipsilateral striatum.

Clinical Relevance
Relative focal hyperperfusion in ICH patients has been described clinically for nearly 40 years.2-6 Acute hyperperfusion was observed in 24 of 102 patients in one study using contrast-enhanced xenon CT.2 Another group, which looked at only 7 patients, observed hyperperfusion in all patients within the first 4 days of symptom onset.6 The tissue that was hyperperfused in the acute period appeared to have decreased blood flow 2 weeks later in these patients. We hypothesize that hyperperfusion and the secondary brain damage described clinically is the result of ICH-induced glutamate excitotoxicity. However, the functional significance of the hypermetabolic areas is still uncertain because MK-801 did not improve behavioral outcome in the collagenase model of ICH.25 It is possible that the glutamate antagonists were not given early enough in that previous study or that the actions of glutamate affect early edema and survival rather than behavioral outcome, possibilities that need future testing. Finally, the factors that stimulate glutamate release are unclear from this study and could include cytokines, inflammatory cells, heme itself, glutamate from blood, and other factors. In addition, the current studies do not answer which cells consume the increased glucose, nor do they answer for what cellular processes the glucose is used, such as inflammation, ion pumping, and the like.

Conclusion
The work described here provides new insight into how blood injures brain. These data suggest that in perihematoma brain, glutamate signaling is increased and may lead to metabolic stress. The increased glutamate signaling may explain the clinical observation of relative focal hyperperfusion after ICH. These data also imply that the blocking glutamate receptors after hemorrhage may be a target for decreasing mortality and improving the outcome of patients with ICH.

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