Acidotoxicity Trumps Excitotoxicity in Ischemic Brain

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P lum entitled his 1982 Wartenberg Lecture “What Causes Infarction in Ischemic Brain?”1 This question remains incompletely answered 2 decades later, during which excitatory amino acids and calcium toxicity have been central research and therapeutic targets. Recently, attention has returned to a central neurochemical feature of ischemic brain injury, acidosis, which was focused on in Plum’s lecture. Targeting this effector of injury as therapy for brain ischemia now may be closer at hand and may result in robust neuroprotection.

MORPHOLOGY OFFERS CLUES TO ISCHEMIA PATHOGENESIS

In the 1970s, Brown and Brierley2 described the prodromal morphologic features of ischemic cell change as that of microvacuolization in selectively vulnerable neurons (Figure 1). In 1984, Brian Meldrum and I,3 working at the Institute of Psychiatry in London, England, looked again at these vacuoles. We used ultrastructural studies of ischemic brain treated with the then new oxalate-pyroantimonate technique, with which calcium could be visualized as electron-dense deposits. Almost all of the microvacuoles were shown to be the result of mitochondrial dilation, and the dilated mitochondrial were further characterized by marked calcium loading (Figure 2). Thus, mitochondrial failure and intracellular calcium toxicity were demonstrated morphologically as central features of ischemic brain injury.3 This interesting result seemed similar to microvacuolization and mitochondrial calcium loading shown to occur with status epileptics by Griffiths and Meldrum.4

EXPERIMENTAL EPILEPSY SUGGESTS THERAPY FOR ISCHEMIA

Burst firing of status epilepticus can be caused by glutamate agonists acting at the N-methyl-D-aspartate (NMDA)–preferring receptor.

FAILURE OF CLINICAL TRIALS

Inasmuch as this receptor and epileptogenesis can be blocked by glutamate antagonists, Meldrum5 hypothesized that enhanced calcium entry during epileptic burst firing and ischemia may have a joint mechanism driven by NMDA function. Previous comparisons of NMDA activation in cortex or spinal cord had shown preferential blockade by APH (2-amino-7-phosphonoheptanoic acid). Thus, APH was studied by a simple experiment of injecting the drug into one hippocampus and the diluent into the contralateral hippocampus in the brain of a rat just before induction of global ischemia. Robust protection occurred in each of the pyramidal cell subsectors of the APH-infused hippocampus.6 Thus, pharmacologic blockade of the NMDA receptor protected against ischemic injury in vivo. These results were highlighted by nearly simultaneous experiments in Copenhagen, Denmark, using another technique, microdialysis, which was new at the time. Benveniste and Diemer7 showed an 8-fold increase in extracellular glutamate concentration during global ischemia in the rat brain. Thus, by 1984, the excitotoxic theory of ischemic brain injury was demonstrated in vivo.

Subsequently NMDA receptor and non-NMDA receptor structure and function were studied in detail. Using the multiple mechanisms discovered to modulate glutamate receptor function, a host of clinical trials followed. To date, none have shown efficacy in stroke. Many ideas were advanced to explain these failures. Among

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these was an observation made in a world distant from that of ischemia research—the Department of Biophysics at the University of Pennsylvania, Philadelphia. Excitatory amino acid receptor gated-channel currents in chick retinal ganglion cells were studied at varying pH levels. The NMDA receptor alone was found to be pH sensitive, with channel currents nearly abolished at pH 6.6.8

ACID-SENSING ION CHANNELS IN THE BRAIN

The role of acidotoxicity in brain ischemia lay hidden in 2 brief articles from the Ukraine published in 19809 and 198110 in which a membrane receptor for hydrogen ions was discovered in nerve cell membranes “by a chain of accidental events . . . of sheer fantasy.”11(p477) Acid-sensing ion channels (ASICs) belong to the degenerin–epithelial sodium channel superfamily of amiloride-sensitive sodium-selective cation channels. The neurotransmitter for these receptor-gated channels is the proton (hydrogen ion). There are 4 ASIC genes encoding 6 polypeptides: ASIC1a and b; ASIC2a and b; ASIC3; and ASIC4. Each exists as a quadrameric structure and has distinct pH sensitivities, kinetics, and conduction properties, which are most germane to ASIC1a and ASIC2a, inasmuch as they are the major subunits in the brain. The pH50 (half-maximum activation pH) is 6.2 for ASIC1a in its homomeric conformation and 4.4 for homomeric ASIC2a. Heteromeric channels have properties distinct from homomeric channels. The ASICs are expressed throughout the brain, where they are thought to have a role in modulation of normal synaptic neurotransmission (presynaptic vesicle pH, 5.5). Because the pH in ischemic brain is 6.0 to 6.5 with normoglycemia, the ASIC1a channel is likely activated in stroke. Further germane to ischemia, this sodium channel is also permeable to calcium.

ACIDOTOXICITY

A pH dependency can be demonstrated in mouse cortical neurons using whole-cell patch clamp. Current activated by a decrease in pH from 7.4 through 5.0 can be demonstrated, and when the dose-response curves are fit to the Hill equation, the average pH50 is 6.18, which suggests a predominance of homomeric ASIC1a channels. The current-voltage relationship shows a reversal close to the sodium equilibrium potential, as would be expected of a sodium channel. As ASICs belonging to the degenerin–epithelial sodium channel family, they are amiloride sensitive. A specific antagonist of the ASIC1a channel is the peptide PcTX1 found in the venom of a South American tarantula (*Psalmotoxin cambridgei*). PcTX1 markedly attenuates acid-induced currents at nanogram concentrations. These acid currents injure cells, as shown by lactic dehydrogenase measurements from mouse cortical cultures treated at pH 6.0 for 1 hour and observed for 24 hours. The injury occurs in the setting of blockade of NMDA, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), and voltage-dependent calcium channels. Thus, acidotoxicity is glutamate independent. The injury is calcium and ASIC1a dependent because acid injury can be blocked by low calcium concentrations in the medium, injury can be induced by transfection of Chinese hamster ovary cells with ASIC1a complementary DNA, and acid-induced injury at pH 6.0 does not occur in cortical cultures from ASIC1a knockout mice. Accordingly, acid-induced calcium toxicity is also glutamate independent. Thus, acidosis produces neuronal injury through the ASIC1a channel, which is calcium dependent but glutamate independent. But the link between ASICs and ischemic injury is even stronger because acid currents measured in the setting of modeled ischemia with oxygen and glucose deprivation have substantially increased induced current amplitude and substantially decreased desensitization (ie, the channels remain open longer) (Figure 3). Thus, the ASIC channels respond to both of the major neurochemical elements of ischemia: acidosis and substrate depletion.12

BLOCKADE OF ASICS AS A NEW THERAPY FOR STROKE

In vitro studies predict that ASIC1a gated channels may be involved in stroke. To investigate this possibility,
Figure 3. Modeled ischemia enhances activity of acid-sensing ion channels. A and B, Whole-cell currents from mixed cortical neurons exposed to pH 6.0 in control medium or in oxygen- and glucose-deprived (OGD) medium. C, Superimposition shows decreased current desensitization in setting of oxygen- and glucose-deprived medium.

Figure 4. Neuroprotective time window of efficacy using acid-sensing ion channel blockade. Infarct volume 24 hours after middle cerebral artery occlusion (MCAO) in mice treated with PcTX venom administered intracerebroventricularly at 1, 3, and 5 hours after middle cerebral artery occlusion; control is denatured PcTX venom administered intracerebroventricularly. Representative triphenyltetrazolium chloride–stained brain sections are shown above each bar. Asterisks indicate $P<.005$ (analysis of variance and Newman-Keuls post hoc tests).
middle cerebral artery occlusion was used. In rats, stroke volume was substantially reduced by amiloride and markedly reduced (approximately 60%) by the ASIC1a blocker PcTX1 administered intracerebroventricularly. When middle cerebral artery occlusion is induced in ASIC1a knockout mice, stroke size is reduced by approximately 60%. How does ASIC channel blockade compare with NMDA blockade? The ASIC1a blockade is more potent than, but additive to, maximally effective doses of the NMDA antagonist memantine. Further, memantine produces more than twice the protection against middle cerebral artery occlusion in ASIC1a knockout mice compared with wild-type mice. Most remarkable, however, is the time window of effectiveness of ASIC blockade in stroke. While NMDA antagonists have a time window of approximately 1 hour for a protective effect after middle cerebral artery occlusion, the time window of protection by ASIC1a blockade is 5 hours, with little change in effectiveness within the first 3 hours (Figure 4). Thus, acidotoxicity trumps excitotoxicity in ischemic stroke.

CODA

What about the ASIC2a channel protein? With a pH$_{0.5}$ of 4.4, can it have a role in stroke? To answer this question, we used global ischemia in rats and showed an increase in ASIC2a protein during the 72 hours of reperfusion. We then stained hippocampal sections for ASIC2a-like immunoreactivity and double stained the sections with a marker for single- or double-stranded DNA breaks. No ASIC2a-positive cell also labeled with the DNA damage marker. Thus, up-regulation of the ASIC2a subunit may confer protection after ischemia, probably by binding with ASIC1a protein subunits to form heteromeric ASIC1a-ASIC2a channels. Whole-cell patch of such heteromeric channels shows marked attenuation of acid-induced currents in glucose-free media.

CONCLUSIONS

Thus, ASICs, which are abundant in brain, flux calcium and are activated by hydrogen ion, and oxygen and glucose deprivation. They cause injury via the ASIC1a subunit and may modulate injury via the ASIC2a subunit. Hence, ASICs are new, glutamate-independent targets for neuroprotection in stroke.

Accepted for Publication: May 11, 2006.
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Funding/Support: This study was supported by grants NS24728, NS42799, and NS50619 from the National Institutes of Health.
Previous Presentation: This study was presented as the Soriano Lecture at the 130th Annual Meeting of the American Neurological Association; September 26, 2005; San Diego, Calif, as part of a symposium on stroke in honor of Fred Plum, MD.

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