Differences in Ischemia-Induced Accumulation of Amino Acids in the Cat Cortex

Nobumitsu Shimada, MD, Rudolf Graf, PhD, Gerd Rosner, PhD, and Wolf-Dieter Heiss, MD

It is well established that excitatory amino acid neurotransmitters are extensively liberated during ischemia and that they have neurotoxic properties contributing to neuronal injury. To study changes in the liberation of excitatory and other amino acids during cerebral ischemia, we measured their extracellular concentrations and related them to blood flow levels and electrophysiologic activity (electrocorticogram and auditory evoked potentials) before and for up to 2 hours after multiple cerebral vessel occlusion in 14 anesthetized cats. Blood flow levels between 0 and 43 ml/100 g/min were reached. Concentrations of the excitatory amino acid neurotransmitters increased most (aspartate 10-fold, glutamate 30-fold, and γ-aminobutyric acid 300-fold compared with control values) below a blood flow threshold of 20 ml/100 g/min. The total power of the electrocorticogram and the amplitude of the auditory evoked potentials were affected below the same blood flow threshold. In contrast, concentrations of the nontransmitter amino acids taurine, alanine, asparagine, serine, and glutamine increased 1.5-5-fold as blood flow decreased, while concentrations of the essential amino acids phenylalanine, valine, leucine, and isoleucine did not change during cerebral ischemia. The great increases in concentrations of the excitatory amino acid neurotransmitters below a blood flow threshold close to that for functional disturbance is in accordance with the role of these amino acids in ischemic cell damage. Their release at blood flow levels compatible with cell survival and the increase in their concentrations with severity and duration of cerebral ischemia imply that excitotoxic antagonists may have potential as therapeutic agents. (Stroke 1990;21:1445-1451)

In vitro studies on brain slices and cell cultures and in vivo investigations applying intracerebral microdialysis have shown that excitatory amino acid (EAA) neurotransmitters, especially L-glutamate (Glu), are extensively liberated during hypoxia and ischemia. By their activating effect on kainate receptors, EAAs induce ionic changes, with excessive entry of Na+, Cl−, and water and exit of K+ leading to early cell injury by osmolysis. Additionally, delayed cell death can be triggered by Ca2+ influx primarily through the action of EAAs on the N-methyl-D-aspartate (NMDA) receptor-operated calcium channel and through voltage-dependent calcium channels opened during depolarization. Together with the subsequent cascade of pathophysiological events, “excitotoxicity” is postulated as a new mechanism of cell damage in ischemia and epilepsy and as indicating the potential of therapeutic strategies through the inhibition of EAA effects.

While microdialysis has demonstrated increases in the concentrations of EAA and inhibitory amino acid neurotransmitters, dopamine, and adenosine in the hippocampus and basal ganglia, studies on changes in the concentrations of amino acids in the cerebral cortex during ischemia are scarce and correlations of such changes with the severity of cerebral ischemia and functional impairment have not been obtained. In a preliminary study, we established a threshold-like relation between the severity of cerebral ischemia and the accumulation of extracellular Glu; the cerebral blood flow (CBF) level below which extracellular Glu accumulates (20 ml/100 g/min) corresponds well with the CBF threshold for impairment of auditory evoked potentials (AEFs). In the present study, we compared changes in the concentrations of other EAA neurotransmitters and nontransmitter amino acids with CBF decreases and electrophysiologic impairment.
Materials and Methods

We used 14 adult cats of either sex weighing 2.5–5.2 kg. Anesthesia was induced with 25 mg/kg i.m. ketamine hydrochloride. After catheterization of the left femoral artery and vein, the cats were tracheotomized, immobilized with 0.2 mg/kg i.v. pancuronium bromide, and ventilated artificially. To avoid the protective effect of ketamine against excitotoxic and ischemic damage, anesthesia was continued with 0.8–1.5% halothane in a 70%:30% N2O:O2 gas mixture. Intravenous infusion of 2 ml/kg/hr Ringer’s solution containing 5 mg/kg/hr gallamine triethiodide for muscle relaxation was maintained throughout the experiment. Arterial blood pressure was monitored continuously; arterial blood gases were monitored intermittently. Paco2 was kept between 30 and 35 mm Hg. Body temperature, measured with a rectal probe, was kept at 37.5–38.5°C by means of a heating blanket.

Both common carotid arteries were exposed proximal to the origin of the superior thyroid artery near the level of the thyroid gland. The vertebral arteries, the superficial cervical arteries, and the costocervical trunks (Figure 1) were approached through a transverse skin incision along upper margins of pectoral muscles for cat.

Steady-state levels of CBF and extracellular amino acid concentrations were reached 2 hours after probe/electrode assembly implantation. Thereafter, dialysate was collected at 10-minute intervals. Following 30 minutes of control measurements in all 14 cats, mild to moderate brain ischemia was induced in nine cats by occluding the common carotid and vertebral arteries for 2 hours. To generate more severe ischemia in the other five cats, the superficial...
Shimada et al  Cortical Amino Acids in Ischemia  1447

CBF (ml/100g/min)

-10 0 10 20 30 40 50 60 70 80 90 100 110 120
time after occlusion (min)

FIGURE 2. Graph of cerebral blood flow (CBF) in 14 cats during 2 hours of cerebral ischemia produced by occlusion of multiple extracranial vessels.

cervical arteries and the costocervical trunks were also occluded for 2 hours. After vascular occlusion systemic blood pressure increased from the control level of 111.7±21.9 mm Hg (mean±SD, n=14) to 156.9±40.5 mm Hg and then gradually returned to the control value within approximately 1 hour.

Results

During the control period, mean±SD CBF was 55.8±10.7 ml/100 g/min (n=14) in the auditory cortex. After vascular occlusion, CBF fell immediately and (depending on the cat and on the extent of occlusion) individual CBF values ranged from 0 (no saturation of H2) to 43 ml/100 g/min (Figure 2). Within a cat, CBF was maintained at a fairly constant level during the 2 hours of ischemia.

Mean±SEM extracellular concentrations of 12 amino acids in the dialysate during the control period are listed in Table 1. Changes in the extracellular concentrations of Glu, y-aminobutyric acid (GABA), taurine (Tau), and leucine (Leu) for each cat during the 2 hours of ischemia are illustrated in Figure 3. Depending on the severity of ischemia, the concentrations of Glu and GABA increased by up to 30-fold and 300-fold the control value, respectively, while the concentration of Tau increased by only about fivefold and that of Leu remained nearly unchanged. The relations between CBF reduction and extracellular concentrations of the 12 amino acids 50–60 minutes after occlusion are plotted for each cat in Figure 4. A threshold-type relation was obtained for the EAA neurotransmitters aspartate and Glu and the inhibitory neurotransmitter GABA, with sharp increases in their concentrations when CBF dropped below approximately 20 ml/100 g/min. Nonessential amino acids not considered transmitters in the cortex (Tau, alanine, asparagine, serine, and glutamine) showed less marked alterations in concentrations during ischemia (1.5–5-fold), and no threshold-type relation between CBF and concentration was found. The extracellular concentrations of other essential amino acids (phenylalanine, valine, isoleucine, and Leu) were not related to CBF reduction and did not change during ischemia.

The changes in ECoG power and AEP amplitude during ischemia resembled those of extracellular EAA neurotransmitter concentration. Electrical impairment started at the same CBF threshold as EAA neurotransmitter accumulation (Figure 5).

Discussion

To study the dependency of amino acid liberation on CBF, a model that permits various levels of graded ischemia ranging from slightly below normal to cessation of perfusion is required. We achieved this prerequisite by occluding the major brain vessels

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentration (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>0.30±0.03</td>
</tr>
<tr>
<td>Glutamate</td>
<td>0.96±0.12</td>
</tr>
<tr>
<td>Asparagine</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>Serine</td>
<td>0.86±0.11</td>
</tr>
<tr>
<td>Glutamine</td>
<td>9.92±0.81</td>
</tr>
<tr>
<td>Taurine</td>
<td>1.29±0.17</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.04±0.18</td>
</tr>
<tr>
<td>a-Aminobutyric acid</td>
<td>0.02±0.003</td>
</tr>
<tr>
<td>Valine</td>
<td>0.97±0.10</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.39±0.04</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.40±0.04</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.69±0.09</td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=14.
The onset of increases in the extracellular concentrations of amino acids depends on the severity of cerebral ischemia; complete global ischemia induces 4–8-fold increases in the concentrations of Glu and aspartate after 10 minutes and >100-fold increases after 30 minutes, but the increases differ for the various amino acids. This can be seen in Figure 3, where significant changes are obvious in the cats with the most severe ischemia for Glu after 10, for GABA after 20, and for Tau after 30 minutes. As described previously, the extracellular accumulation of amino acids increases with time, reaching maximal values after 60 minutes. To obtain maximal effects of mild and moderate ischemia but not lose the effects on nontransmitter amino acids, we used the extracellular concentrations after 50–60 minutes of ischemia to establish the relation with severity of ischemia. Using this approach, different behaviors of the various amino acids could be described, with a steep increase in the concentrations of neurotransmitters below a threshold of approximately 20 ml/100 g/min, a slight increase in the concentrations of nonessential amino acids (some with possible modulator action), and no increase in concentrations of amino acids essential for protein synthesis. This selective increase in the concentrations of EAA neurotransmitters near the CBF threshold for electrophysiologic function indicates the liberation of these amino acids from nerve terminals due to depolarization of nerve cells and forms the basis of excitotoxicity in the brain cortex. EAAs can trigger early cell death by activating the kainate receptors that gate sodium channels and promote Cl− and water uptake, leading to osmolyis; these kainate receptors are abundant on pyramidal cells. Membrane depolarization caused by influx of Na+ might open voltage-dependent calcium channels, leading to an influx of Ca2+. The NMDA receptor also gates a calcium channel. Therefore, EAAs open two pathways by which Ca2+ influx mediates delayed neuronal injury. The selective vulnerability of nerve cells depends on the distribution of NMDA receptors, which might be related to the occurrence of seizure-like activity during ischemia-induced Ca2+ accumulation. Seizure-like activity is a common finding in the hippocampus of ischemic gerbils, but it has also been demonstrated in the cortex of cats during ischemia.
and early reperfusion,26,27 and epileptic seizures are repeatedly documented in patients with acute ischemic cerebrovascular disease.28-30

While the importance of EAAs for ischemic neuronal damage is well established and supported by our finding increases in their concentrations at CBF levels below the threshold for neuronal function and synaptic transmission, the extracellular accumulation of the inhibitory neurotransmitter GABA, which should protect against excitability-induced cell injury, needs further discussion. That the increased concentration of GABA, which is liberated from depolarized inhibitory interneurons, does not protect against or at least ameliorate neuronal damage might be due to the fact that, contrary to the Glu receptors (which are resistant to ischemia31,32), the functioning of GABAergic receptors is impaired in ischemia.33 This might also explain why GABA agonists are not very effective in preventing ischemic cell damage.34 Whether graded CBF-related increases in concentrations of Tau, aspartate, serine, and glutamine (of which at least Tau has a modulatory property in regulating osmolality of the brain35) affect ischemic cell damage has not been investigated.

We conclude that EAAs are released at CBF values close to the thresholds for neuronal function
and synaptic transmission, consistently found to be 12–18 ml/100 g/min (see Reference 36). With more severe and longer-lasting ischemia, the extracellular concentrations of EAAs increase further since release continues and neuronal and glial reuptake is impaired. This explains why extremely high concentrations are reached after 50–60 minutes of severe cerebral ischemia. This gradual increase in the concentrations of EAAs starting at CBF values above the cerebral ischemia. This gradual increase in the concentrations of EAAs starting at CBF values above the cerebral ischemia: A study utilizing cerebral microdialysis. J Neurochem 1985;50:1714–1718


References


38. Choi DW: Calcium-mediated neurotoxicity: Relationship to specific channel types and role in ischemic damage. *Trends Neurosci* 1988;11:465-469

**KEY WORDS** • cerebral blood flow • neurotransmitters • amino acids • cats
Differences in ischemia-induced accumulation of amino acids in the cat cortex.
N Shimada, R Graf, G Rosner and W D Heiss

Stroke. 1990;21:1445-1451
doi: 10.1161/01.STR.21.10.1445

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/21/10/1445