

Astrocyte Mitochondrial Mechanisms of Ischemic Brain Injury and Neuroprotection*

Linda Bambrick,^{1,2,3} Tibor Kristian,¹ and Gary Fiskum^{1,2,4}

(Accepted August 25, 2003)

Research on ischemic brain injury has established a central role of mitochondria in neuron death (1–3). Astrocytes are also damaged by ischemia (4), although the participation of mitochondria in their injury is ill defined. As astrocytes are responsible for neuronal metabolic and trophic support, astrocyte dysfunction (5) will compromise postischemic neuronal survival. Ischemic alterations to astrocyte energy metabolism and the uptake and metabolism of the excitatory amino acid transmitter glutamate may be particularly important. Despite the significance of ischemic astrocyte injury, little is known of the mechanisms responsible for astrocyte death and dysfunction. This review focuses on differences between astrocyte and neuronal metabolism and mitochondrial function, and on neuronal–glial interactions. The potential for astrocyte mitochondria to serve as targets of neuroprotective interventions is also discussed.

KEY WORDS: Apoptosis; calcium; glutamate; lactate; metabolism.

INTRODUCTION

Ischemic brain injury comprises: (i) the initial ischemic insult; (ii) a maturation period, during which cells may resume various functions if adequate reperfusion follows the ischemia; and (iii) a delayed series of events leading to cell death. Ischemia causes mitochondrial dysfunction through oxygen and glucose deprivation. The loss of oxygen blocks the oxidation of pyruvate (and other substrates) by the mitochondria, drastically reducing ATP production. Reduced ATP stimulates gly-

colytic metabolism of residual glucose and glycogen, thereby increasing the production of lactate plus H^+ . Loss of ATP also inhibits various membrane ion pumps, leading to a loss of cellular and mitochondrial ion gradients with cellular influx of Ca^{2+} and Na^+ and efflux of K^+ (1,6,7).

DISCUSSION

Ischemia/Reperfusion: Pathology

The influx of Ca^{2+} into cells is the most significant event in the pathogenesis of ischemic brain damage. Ca^{2+} influx triggers mitochondrial dysfunction, leading to immediate or delayed cell death (8–11). In neurons, the principle route of Ca^{2+} influx is through NMDA glutamate receptors (12). Astrocytes lack NMDA receptors, and recent studies suggest that astrocyte Ca^{2+} influx during ischemia/reperfusion occurs through reverse action of the Na^+/Ca^{2+} exchanger in response to the lowered extracellular Na^+ (13).

*Special issue dedicated to Professor John B. Clark.

¹ Department of Anesthesiology.

² Program in Neuroscience.

³ Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland.

⁴ Address reprint requests to: Gary Fiskum, Department of Anesthesiology, University of Maryland School of Medicine, 685 West Baltimore Street, MSTF 5.34, Baltimore, Maryland 21201. Tel: 410-706-4711; Fax: 410-706-2550; E-mail: gfish001@umaryland.edu

Mitochondria in Ischemia

Mitochondrial dysfunction plays a central role in cerebral ischemia/reperfusion cell injury and death (14). After transient ischemia, the reperfusion period is characterized by a gradual rise in cytoplasmic free Ca^{2+} (15), Ca^{2+} sequestration in mitochondria (16,17), and mitochondrial respiratory dysfunction (18). Data showing a translocation of cytochrome *c* from the mitochondria to the cytosol during reperfusion suggest that mitochondrial membranes are damaged or that their permeability properties are altered (19–21). Mitochondrial respiratory dysfunction can be induced by several ischemia/reperfusion-associated events, including membrane lipid peroxidation (22,23), direct oxidation of respiratory enzymes, for example, the pyruvate dehydrogenase (PDH) complex (24), and Ca^{2+} -induced loss of critical respiratory components (e.g., NAD(H) and cytochrome *c*) (25,26).

Comparison of Neuron and Astrocyte Injury in Ischemia

Forebrain ischemia of brief to intermediate duration gives rise to delayed cell death over several hours or days (for review see [1]). Vulnerability to ischemia varies among neuronal subtypes. For example, within the hippocampus, the pyramidal cells of the CA1 sector are the most vulnerable whereas neurons in the dentate gyrus are the most resistant (27–29). It was once assumed that ischemia causes a purely neuronal cell death (for review see [30]). However, recent morphological studies suggest that glia are also damaged by ischemia (4). Mitochondria expressing pathological morphology were observed in non-neuronal cells in a permanent middle cerebral artery occlusion (MCAO) model in monkeys (31). Swollen mitochondria within astrocytes were found in a feline model of transient MCAO (32), and astrocyte death was reported within 1 day after rat focal ischemia (5). As is the case for neurons, vulnerability to insult varies across astrocyte subtypes. Protoplasmic cortical astrocytes are reported to be more sensitive than fibrous astrocytes to MCAO (33) and astrocytes cultured from cortex, striatum, and hippocampus differ in their sensitivity to oxygen/glucose deprivation or H_2O_2 exposure (34). Pathological changes in mitochondria present in oligodendrocytes and astrocytes were observed 30 min after the arterial occlusion (35), suggesting that at least the glia of the cerebral white matter are highly vulnerable to the effects of ischemia.

In the 1970s a technique was developed to isolate energetically functional synaptic and nonsynaptic (“free”) mitochondrial from brain (36–38). Synaptic mitochondria

are derived from neurons; nonsynaptic mitochondria are a mix of neuron- and glia-derived organelles. There are differences between the two populations of mitochondria. Metabolic enzyme activities and rates of respiration vary between synaptic and nonsynaptic mitochondria isolated from normal brains (37,39). Further, when isolated following ischemia, both synaptic and nonsynaptic mitochondria exhibit a loss of respiratory activity, but the degree of inhibition of respiratory chain complex I necessary for inhibition of oxidative phosphorylation is 25% for CA1 synaptic mitochondria, compared to 60% for nonsynaptic mitochondria (40). Such differences in mitochondrial function and response to inhibition may underlie the higher susceptibility of neurons than glia to ischemic injury and death.

The presence of both neuronal and glial mitochondria in nonsynaptic preparations limits the conclusions that can be drawn regarding the relative contributions of neuronal and non-neuronal cells to ischemia-induced changes in mitochondrial function. *In vitro* models of ischemia using primary cultures of astrocytes and neurons have been developed to more directly address the question of how ischemia affects cell-selective mitochondrial injury and cell survival. One hour of oxygen-glucose deprivation did not lead to astrocyte death but did produce a long-lasting decrease in mitochondrial membrane potential and a loss of mitochondrial cytochrome *c* (41). Longer periods of oxygen-glucose deprivation produced astrocyte death in some but not all studies (42,43), while combinations of hypoxia and acidosis or hypoxia/acidosis and ischemia-relevant changes in ion concentrations were very effective at killing astrocytes *in vitro* (44,45). When astrocyte death was observed, it was associated with a prelethal reduction in glutathione, oxidative damage, and impaired active uptake of glutamate (46–48). Astrocyte glutamate uptake and glutathione production are important for neuron survival, thus ischemic impairment of these activities may promote neuron death even in the absence of astrocyte cell death.

Neuroglial Interactions: Glutamate Excitotoxicity

Glutamate is the principal excitatory neurotransmitter in the mammalian brain. However, high levels of glutamate lead to excitotoxic neuronal death mediated by Ca^{2+} influx, principally through NMDA-gated channels (see e.g., [49–51]). Massive release of glutamate occurs during ischemia and the consequent excitotoxicity plays a major role in neuronal death, as shown by the protective effects of the NMDA antagonist MK-801 (52).

Astrocyte glutamate uptake via the GLAST and GLT-1 transporters is essential for maintaining extracel-

lular glutamate below neurotoxic levels (53,54). These transporters operate by exchanging 3 Na⁺ in and 1 K⁺ out for every glutamate-H⁺ transported. Glutamate uptake therefore causes membrane depolarization and is compensated by (or driven by) the ATP-dependent Na⁺/K⁺-ATPase. During cerebral ischemia/reperfusion, loss of ATP and increased intracellular Na⁺ and extracellular K⁺ would seriously compromise glutamate uptake (54). In addition, oxidative stress can inhibit astrocyte glutamate uptake (55,56). Impaired glutamate transport has indeed been suggested to play a role in neuronal injury following ischemia and hypoxia (57–60). Antisense knock-down of the glial transporter GLT-1, but not a neuronal glutamate transporter, increased neuron death following focal cerebral ischemia in the rat (56,61). This result suggests that astrocytic, not neuronal, uptake of glutamate is the important modulator of excitotoxic ischemic damage.

Astrocytes can also release glutamate. Astrocyte release of glutamate is known to be important for neuron-astrocyte signaling (62,63). Two principal mechanisms of astrocyte glutamate release have been demonstrated. First, the glutamate transporters can function in reverse. This abnormal activity may occur during ischemia, in which cell swelling resulting from excess Na⁺ influx appears to cause the reversal of a transporter and lead to increased extracellular glutamate after transient forebrain ischemia (64,65). Second, astrocytes also exhibit a Ca²⁺-mediated release of glutamate via a mechanism involving exocytosis (66,67). Ca²⁺ influx into astrocytes could therefore trigger glutamate release directly, contributing to neuronal excitotoxicity.

Neuroglial Interactions: Glutathione

The reduced form of glutathione (GSH) is an important antioxidant in the brain (68,69). This metabolite formed from cysteine acts as a free radical scavenger and is important in maintaining other antioxidants in their reduced, active form. A major change observed following cerebral ischemia/reperfusion is a loss of GSH (70). Astrocytes support neuronal GSH levels by supplying glutathione or cysteine-containing precursors (71,72). Astrocyte levels of GSH are reduced following prolonged glucose deprivation *in vitro* (46). GSH in astrocytes may be critical for limiting nitric oxide toxicity in neurons (73). Depletion of astrocyte glutathione has been correlated with increased neuron death in an oxidative stress/co-culture system (73). Ischemia-induced loss of astrocyte glutathione could therefore directly compromise astrocyte function and cell viability and indirectly affect neuronal survival.

Neuron/Astrocyte Differences: Energy Metabolism

Differences in metabolism between astrocytes and neurons will affect their responses to ischemia. Astrocytes are the principal repositories of glycogen in the brain (74), whereas neurons have very little glycogen; thus, neurons and astrocytes may respond differently to ischemic glucose deprivation. In a study of *in vitro* hypoxia, an increase in glycolysis and glycogenolysis was critical for astrocyte survival (75). Glucose is metabolized to pyruvate and then either by LDH to lactate, or via PDH to acetylCoA (Fig. 1). PDH activity is inhibited after ischemia/reperfusion; possibly leading to decreased ATP production and increased lactate generation and tissue acidosis. One therapeutic intervention based on overcoming the postischemic PDH metabolic roadblock is intravenous acetyl-L-carnitine (ALCAR). ALCAR reduced neurological morbidity and mortality in a clinically relevant canine cardiac arrest and resuscitation model (76,77) and in rat permanent focal cerebral ischemia (78). Improvement in neurological outcome was associated with a reduction in postischemic brain lactate levels and markers of oxidative stress (76,77,79). The most likely mechanisms of action of ALCAR involve the intramitochondrial transfer of the acetyl moiety to coenzyme A forming acetylCoA. (80), a primary source of fuel for oxidative metabolism, thus bypassing the metabolic roadblock caused by ischemia-induced decreases in PDH immunoreactivity and enzyme activity (79). By stimulating aerobic metabolism, ALCAR limits the need for continuously high levels of glycolysis

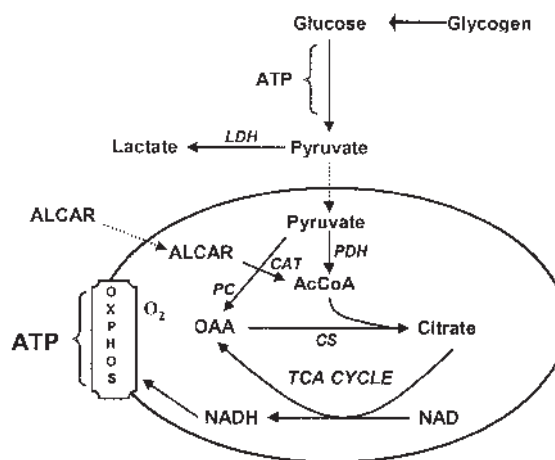


Fig. 1. Possible metabolic mechanisms of neuroprotection by acetyl-L-carnitine (ALCAR). LDH, Lactate dehydrogenase; PDH, pyruvate dehydrogenase; CAT, carnitine acetyltransferase; PC, pyruvate carboxylase; CS, citrate synthase; OAA, oxaloacetate; AcCoA, acetyl coenzyme A.

and thereby reduces excessive tissue lactate production and acidosis. Astrocytes carry out most of the glycolysis in the brain; therefore this effect of ALCAR would be larger in astrocytes than in neurons. Effective utilization of acetylCoA generated from ALCAR requires the presence of oxaloacetate as cosubstrate for the citrate synthase reaction. Compared to neurons, the postischemic levels of oxaloacetate in astrocyte mitochondria should be high because of the relatively greater activity of mitochondrial pyruvate carboxylase in astrocytes (81). Thus the neuroprotective effects of ALCAR *in vivo* may be at least partially mediated by astrocyte mitochondrial metabolism.

Neuron/Astrocyte Differences: Mitochondrial Permeability Transition

Studies performed with isolated mitochondria, particularly from liver and heart, revealed that when mitochondria accumulate excessive Ca^{2+} or when they are exposed to oxidative stress, both of which occur in ischemia, a large conductance pore in the inner mitochondrial membrane is opened. This mitochondrial permeability transition (MPT) pore leads to collapse of the mitochondrial membrane potential and dissipation of proton and ion gradients. One consequence is osmotic swelling of the matrix, which leads to rupture of the outer membrane and release of intermembrane contents, such as cytochrome *c* (82–84), with uncoupling of oxidative phosphorylation and cessation of mitochondrial ATP synthesis. The MPT pore is a high-conductance mitochondrial inner membrane channel. Multiple effectors regulate pore opening and closing (84–87). Pore opening is favored by high intramitochondrial Ca^{2+} concentrations, by oxidizing agents and by high levels of inorganic phosphate (P_i). The MPT is inhibited by cyclosporin A (CsA), most likely via binding to cyclophilin D, an endogenous MPT modulator, and by magnesium ions, ADP, and ATP.

MPT pore regulation is complex, and one cannot readily predict when and under what conditions the MPT is induced in intact tissue. Recent studies with mitochondria isolated from brain suggest that, unlike mitochondria in heart and liver, brain mitochondria exhibit clear heterogeneity in their response to calcium-induced MPT, with CsA unable to completely inhibit the permeability transition (88–90). This result may reflect the presence in the preparation of mitochondria from neurons and astrocytes, because our data from studies of mitochondria in permeabilized cells indicate that CsA can protect mitochondria in astrocytes, but not neurons, from Ca^{2+} insult (91). Encephalopathic levels of ammo-

nia are also capable of inducing a CsA-sensitive MPT in intact, cultured astrocytes (92). CsA administered *in vivo* can protect against both mitochondrial dysfunction and cell death following cerebral ischemia (93–96); thus it is possible that astrocyte mitochondria may be the primary target of CsA.

Activation of a CsA-sensitive MPT in astrocytes during cerebral ischemia/reperfusion would affect astrocytes and astrocyte–neuronal interactions in several ways (Fig. 2). Depending on the extent of MPT activation, its impairment of aerobic energy production could either limit specific energy-requiring activities (e.g., glutamate uptake) or lead to total metabolic failure, ionic dyshomeostasis, and neurotic cell death. Moreover, MPT uncoupling of respiration from ATP synthesis can result in elevated, albeit inefficient, metabolism that could shift the flux of glycolytic carbon to the TCA cycle. Because neurons utilize astrocyte-generated lactate as a significant source of energy, particularly following an ischemic episode (97), MPT-mediated uncoupling could reduce this metabolic trafficking between astrocytes and neurons. Alternatively, when the MPT causes mitochondrial osmotic swelling sufficient to disrupt the outer membrane, intermembrane proteins, including cytochrome *c*, are released into the cytosol. Loss of cytochrome *c* can dramatically inhibit even uncoupled respiration (26), and this could cause a shift in metabolism from aerobic to anaerobic, with an increase in lactate production and possibly a pathological

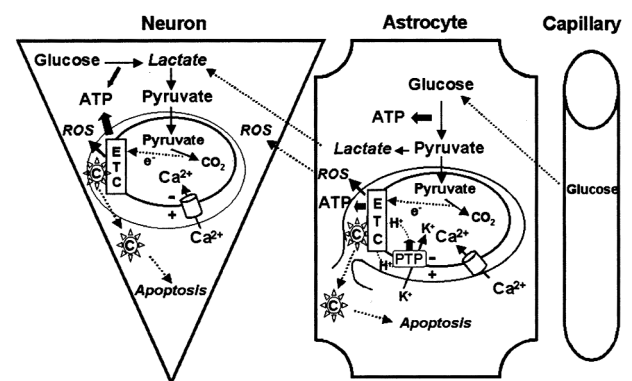


Fig. 2. Potential effects of astrocyte mitochondrial permeability transition on astrocyte and neuronal metabolism. Activation of the permeability transition pore (PTP) by Ca^{2+} and/or oxidative stress in astrocyte mitochondria may cause respiratory uncoupling, osmotic swelling, loss of cytochrome *c*, and stimulation of mitochondrial production of reactive oxygen species (ROS). Uncoupling can increase the oxidative metabolism of pyruvate, lowering astrocyte lactate production, which is an important source of energy for neighboring neurons, particularly following cerebral ischemia. Astrocyte ROS production can also affect nearby neurons by many mechanisms, including those that activate the mitochondrial pathway toward apoptosis.

decrease in brain pH. Such inhibition of respiration can also promote ROS generation at early steps in the electron transport chain (ETC) (e.g., complex I [98]). Astrocyte-generated diffusible ROS can adversely affect nearby neurons and deplete astrocytes of their glutathione, and therefore of antioxidant defenses they share with their neuronal partners. Finally, mitochondrial release of cytochrome *c* can activate the caspase cascade of events that lead to apoptosis, known to contribute to glial cell death following cerebral ischemia.

The MPT is not the only, or even necessarily the most active, mechanism responsible for release of cytochrome *c* in cell death paradigms, including ischemia/reperfusion. An alternative mechanism involves the translocation of the proapoptotic Bcl-2 family member Bax to the mitochondria and the disruption of the outer mitochondrial membrane protein permeability barrier as a result of Bax oligomerization and pore formation (99). Several intra- and extracellular signals can trigger either an increase in Bax gene expression or its translocation from the cytosol to mitochondria. As oxidative stress is one of the more effective of these triggers (100), astrocyte ROS production is likely involved in Bax-mediated apoptosis of both astrocytes and neurons. Mitochondrial Bax translocation is associated with cytochrome *c* release in rat models of transient focal ischemia (19,101). The relative Bax and cytochrome *c* redistribution in neurons and astrocytes has not, however, been investigated. In addition, the contribution of the Bax and MPT mechanisms of cytochrome *c* release and other forms of mitochondrial dysfunction are unknown. Such information will prove useful in neuroprotective drug development targeting potentially cell-selective mitochondrial mechanisms of brain injury.

CONCLUSION

The study of cell death following cerebral ischemia has focused primarily on neurons. However, recent work, both *in vivo* and *in vitro*, indicates that ischemia also causes damage to astrocytes. Mitochondria are central mediators of both necrosis and apoptosis and are the primary targets of neuronal excitotoxicity. In contrast, relatively little is known regarding the involvement of mitochondrial dysfunction in ischemic or hypoxic astrocyte injury. Studies of isolated brain mitochondria following cerebral ischemia typically use preparations containing mitochondria from both neurons and glia and have not provided information on mitochondrial injury within specific cell types. Differences in energy metabolism and mitochondrial function exist between astro-

cytes and neurons that affect their responses to ischemia/reperfusion and possibly also to therapeutic interventions that target metabolic failure (e.g., ALCAR, or apoptosis, CsA).

REFERENCES

- Kristian, T. and Siesjo, B. K. 1998. Calcium in ischemic cell death. *Stroke* 29:705–718.
- Fiskum, G., Murphy, A. N., and Beal, M. F. 1999. Mitochondria in neurodegeneration: Acute ischemia and chronic neurodegenerative diseases. *J. Cereb. Blood Flow Metab.* 19:351–369.
- Lipton, P. 1999. Ischemic cell death in brain neurons. *Physiol. Rev.* 79:1431–1568.
- Petito, C. K., Olarte, J. P., Roberts, B., Nowak, T. S. J., and Pulsinelli, W. A. 1998. Selective glial vulnerability following transient global ischemia in rat brain. *J. Neuropathol. Exp. Neurol.* 57:231–238.
- Liu, D., Smith, C. L., Barone, F. C., Ellison, J. A., Lysko, P. G., Li, K., and Simpson, I. A. 1999. Astrocytic demise precedes delayed neuronal death in focal ischemic rat brain. *Brain Res. Mol. Brain Res.* 68:29–41.
- Erecinska, M. and Silver, I. A. 1994. Ions and energy in mammalian brain. *Prog. Neurobiol.* 43:37–71.
- Hansen, A. J. 1985. Effect of anoxia on ion distribution in the brain. *Physiol. Rev.* 65:101–148.
- Choi, D. W. 1998. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1:623–634.
- Morley, P., Hogan, M. J., and Hakim, A. M. 1994. Calcium-mediated mechanisms of ischemic injury and protection. *Brain Pathol.* 4:37–47.
- Siesjo, B. K., Katsura, K., Zhao, Q., Folbergrova, J., Pahlmark, K., Siesjo, P., and Smith, M. L. 1995. Mechanisms of secondary brain damage in global and focal ischemia: A speculative synthesis. *J. Neurotrauma* 12:943–956.
- Tymianski, M. and Tator, C. H. 1996. Normal and abnormal calcium homeostasis in neurons: A basis for the pathophysiology of traumatic and ischemic central nervous system injury. *Neurosurgery* 38:1176–1195.
- Budd, S. L. and Nicholls, D. G. 1996. Mitochondria, calcium regulation, and acute glutamate excitotoxicity in cultured cerebellar granule cells. *J. Neurochem.* 67:2282–2291.
- Bondarenko, A. and Chesler, M. 2001. Calcium dependence of rapid astrocyte death induced by transient hypoxia, acidosis, and extracellular ion shifts. *Glia* 34:143–149.
- Fiskum, G. 2000. Mitochondrial participation in ischemic and traumatic neural cell death. *J. Neurotrauma* 17:843–855.
- Silver, I. A. and Erecinska, M. 1992. Ion homeostasis in rat brain *in vivo*: Intra- and extracellular $[Ca^{2+}]$ and $[H^+]$ in the hippocampus during recovery from short-term, transient ischemia. *J. Cereb. Blood Flow Metab.* 12:759–772.
- Dux, E., Mies, G., Hossmann, K. A., and Siklos, L. 1987. Calcium in the mitochondria following brief ischemia of gerbil brain. *Neurosci. Lett.* 78:295–300.
- Zaidan, E. and Sims, N. R. 1994. The calcium content of mitochondria from brain subregions following short-term forebrain ischemia and recirculation in the rat. *J. Neurochem.* 63:1812–1819.
- Sims, N. R. and Pulsinelli, W. A. 1987. Altered mitochondrial respiration in selectively vulnerable brain subregions following transient forebrain ischemia in the rat. *J. Neurochem.* 49:1367–1374.
- Fujimura, M., Morita-Fujimura, Y., Murakami, K., Kawase, M., and Chan, P. H. 1998. Cytosolic redistribution of cytochrome *c* after transient focal cerebral ischemia in rats. *J. Cereb. Blood Flow Metab.* 18:1239–1247.

20. Perez-Pinzon, M. A., Xu, G. P., Born, J., Lorenzo, J., Busto, R., Rosenthal, M., and Sick, T. J. 1999. Cytochrome c is released from mitochondria into the cytosol after cerebral anoxia or ischemia. *J. Cereb. Blood Flow Metab.* 19:39-43.
21. Sugawara, T., Fujimura, M., Morita-Fujimura, Y., Kawase, M., and Chan, P. H. 1999. Mitochondrial release of cytochrome c corresponds to the selective vulnerability of hippocampal CA1 neurons in rats after transient global cerebral ischemia. *J. Neurosci.* 19:RC39.
22. Nakahara, I., Kikuchi, H., Taki, W., Nishi, S., Kito, M., Yonekawa, Y., Goto, Y., and Ogata, N. 1992. Changes in major phospholipids of mitochondria during postischemic reperfusion in rat brain. *J. Neurosurg.* 76:244-250.
23. Gilboe, D. D., Kintner, D., Fitzpatrick, J. H., Emoto, S. E., Esanu, A., Braquet, P. G., and Bazan, N. G. 1991. Recovery of postischemic brain metabolism and function following treatment with a free radical scavenger and platelet-activating factor antagonists. *J. Neurochem.* 56:311-319.
24. Wagner, K. R., Kleinholz, M., and Myers, R. E. 1990. Delayed decreases in specific brain mitochondrial electron transfer complex activities and cytochrome concentrations following anoxia/ischemia. *J. Neurol. Sci.* 100:142-151.
25. Schild, L., Huppelsberg, J., Kahlert, S., Keilhoff, G., and Reiser, G. 2003. Brain mitochondria are primed by moderate Ca^{2+} rise upon hypoxia/reoxygenation for functional breakdown and morphological desintegration. *J. Biol. Chem.* (2003).
26. Polster, B. M., Kinnally, K. W., and Fiskum, G. 2001. Bcl-2 death domain peptide induces cell type-selective mitochondrial outer membrane permeability. *J. Biol. Chem.* 276:37887-37894.
27. Smith, M. L., Auer, R. N., and Siesjo, B. K. 1984. The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia. *Acta Neuropathol. (Berl.)* 64:319-332.
28. Pulsinelli, W. A., Brierley, J. B., and Plum, F. 1982. Temporal profile of neuronal damage in a model of transient forebrain ischemia. *Ann. Neurol.* 11:491-498.
29. Ito, U., Spatz, M., Walker, J. T. Jr., and Klatzo, I. 1975. Experimental cerebral ischemia in mongolian gerbils: I. Light microscopic observations. *Acta Neuropathol. (Berl.)* 32:209-223.
30. Siesjo, B. K., Katsura, K., and Kristian, T. 1996. Acidosis-related damage. *Adv. Neurol.* 71:209-233.
31. Garcia, J. H., Cox, J. V., and Hudgins, W. R. 1971. Ultrastructure of the microvasculature in experimental cerebral infarction. *Acta Neuropathol. (Berl.)* 18:273-285.
32. Garcia, J. H., Kalimo, H., Kamijyo, Y., and Trump, B. F. 1977. Cellular events during partial cerebral ischemia: I. Electron microscopy of feline cerebral cortex after middle-cerebral-artery occlusion. *Virchows Arch. B Cell. Pathol.* 25:191-206.
33. Lukaszevicz, A. C., Sampaio, N., Guegan, C., Benchoua, A., Couriaud, C., Chevalier, E., Sola, B., Lacombe, P., and Onteniente, B. 2002. High sensitivity of protoplasmic cortical astroglia to focal ischemia. *J. Cereb. Blood Flow Metab.* 22:289-298.
34. Xu, L., Sapolsky, R. M., and Giffard, R. G. 2001. Differential sensitivity of murine astrocytes and neurons from different brain regions to injury. *Exp. Neurol.* 169:416-424.
35. Pantoni, L., Garcia, J. H., and Gutierrez, J. A. 1996. Cerebral white matter is highly vulnerable to ischemia. *Stroke* 27:1641-1646.
36. Clark, J. B. and Nicklas, W. J. 1970. The metabolism of rat brain mitochondria: Preparation and characterization. *J. Biol. Chem.* 245:4724-4731.
37. Lai, J. C. and Clark, J. B. 1976. Preparation and properties of mitochondria derived from synaptosomes. *Biochem. J.* 154:423-432.
38. Booth, R. F. and Clark, J. B. 1979. A method for the rapid separation of soluble and particulate components of rat brain synaptosomes. *FEBS Lett.* 107:387-392.
39. Leong, S. F., Lai, J. C., Lim, L., and Clark, J. B. 1984. The activities of some energy-metabolizing enzymes in nonsynaptic (free) and synaptic mitochondria derived from selected brain regions. *J. Neurochem.* 42:1306-1312.
40. Davey, G. P., Canevari, L., and Clark, J. B. 1997. Threshold effects in synaptosomal and nonsynaptic mitochondria from hippocampal CA1 and paramedian neocortex brain regions. *J. Neurochem.* 69:2564-2570.
41. Reichert, S. A., Kim-Han, J. S., and Dugan, L. L. 2001. The mitochondrial permeability transition pore and nitric oxide synthase mediate early mitochondrial depolarization in astrocytes during oxygen-glucose deprivation. *J. Neurosci.* 21:6608-6616.
42. Zhao, G. and Flavin, M. P. 2000. Differential sensitivity of rat hippocampal and cortical astrocytes to oxygen-glucose deprivation injury. *Neurosci. Lett.* 285:177-180.
43. Almeida, A., Delgado-Esteban, M., Bolanos, J. P., and Medina, J. M. 2002. Oxygen and glucose deprivation induces mitochondrial dysfunction and oxidative stress in neurons but not in astrocytes in primary culture. *J. Neurochem.* 81:207-217.
44. Swanson, R. A., Farrell, K., and Stein, B. A. 1997. Astrocyte energetics, function, and death under conditions of incomplete ischemia: A mechanism of glial death in the penumbra. *Glia* 21:142-153.
45. Bondarenko, A. and Chesler, M. 2001. Rapid astrocyte death induced by transient hypoxia, acidosis, and extracellular ion shifts. *Glia* 34:134-142.
46. Papadopoulos, M. C., Koumenis, I. L., Dugan, L. L., and Giffard, R. G. 1997. Vulnerability to glucose deprivation injury correlates with glutathione levels in astrocytes. *Brain Res.* 748:151-156.
47. Robb, S. J. and Connor, J. R. 1998. An *in vitro* model for analysis of oxidative death in primary mouse astrocytes. *Brain Res.* 788:125-132.
48. Chen, D., Lan, J., Pei, W., and Chen, J. 2000. Detection of DNA base-excision repair activity for oxidative lesions in adult rat brain mitochondria. *J. Neurosci. Res.* 61:225-236.
49. Castilho, R. F., Hansson, O., Ward, M. W., Budd, S. L., and Nicholls, D. G. 1998. Mitochondrial control of acute glutamate excitotoxicity in cultured cerebellar granule cells. *J. Neurosci.* 18:10277-10286.
50. Ward, M. W., Rego, A. C., Frenguelli, B. G., and Nicholls, D. G. 2000. Mitochondrial membrane potential and glutamate excitotoxicity in cultured cerebellar granule cells. *J. Neurosci.* 20:7208-7219.
51. Rego, A. C., Ward, M. W., and Nicholls, D. G. 2001. Mitochondria control ampa/kainate receptor-induced cytoplasmic calcium deregulation in rat cerebellar granule cells. *J. Neurosci.* 21:1893-1901.
52. Bullock, R. and Fujisawa, H. 1992. The role of glutamate antagonists for the treatment of CNS injury. *J. Neurotrauma* 9(Suppl 2):S443-S462.
53. Rothstein, J. D., Dykes-Hoberg, M., Pardo, C. A., Bristol, L. A., Jin, L., Kuncl, R. W., Kanai, Y., Hediger, M. A., Wang, Y., Schielke, J. P., and Welty, D. F. 1996. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 16:675-686.
54. Anderson, C. M. and Swanson, R. A. 2000. Astrocyte glutamate transport: Review of properties, regulation, and physiological functions. *Glia* 32:1-14.
55. Miralles, V. J., Martinez-Lopez, I., Zaragoza, R., Borrás, E., Garcia, C., Pallardo, F. V., and Vina, J. R. 2001. Na⁺ dependent glutamate transporters (EAAT1, EAAT2, and EAAT3) in primary astrocyte cultures: Effect of oxidative stress. *Brain Res.* 922:21-29.
56. Fukumachi, S., Furuta, A., Ikeda, T., Ikenoue, T., Kaneoka, T., Rothstein, J. D., and Iwaki, T. 2001. Altered expression of glutamate transporter subtypes in rat model of neonatal cerebral hypoxia-ischemia. *Brain Res. Dev. Brain Res.* 132:131-139.
57. Torp, R., Lekieffre, D., Levy, L. M., Haug, F. M., Danbolt, N. C., Meldrum, B. S., and Ottersen, O. P. 1995. Reduced postischemic expression of a glial glutamate transporter, GLT1, in the rat hippocampus. *Exp. Brain Res.* 103:51-58.
58. Rao, V. L. R., Rao, A. M., Dogan, A., Bowen, K. K., Hatcher, J., Rothstein, J. D., and Dempsey, R. J. 2000. Glial glutamate transporter GLT-1 down-regulation precedes delayed neuronal death in gerbil hippocampus following transient global cerebral ischemia. *Neurochem. Int.* 36:531-537.
59. Martin, L. J., Brambrink, A. M., Lehmann, C., Portera-Cailliau, C., Koehler, R., Rothstein, J., and Traystman, R. J. 1997. Hypoxia-

- ischemia causes abnormalities in glutamate transporters and death of astroglia and neurons in newborn striatum. *Ann. Neurol.* 42:335–348.
60. Inage, Y. W., Itoh, M., Wada, K., and Takashima, S. 1998. Expression of two glutamate transporters, GLAST and EAAT4, in the human cerebellum: Their correlation in development and neonatal hypoxic-ischemic damage. *J. Neuropathol. Exp. Neurol.* 57:554–562.
 61. Rao, V. L., Dogan, A., Todd, K. G., Bowen, K. K., Kim, B. T., Rothstein, J. D., and Dempsey, R. J. 2001. Antisense knockdown of the glial glutamate transporter GLT-1, but not the neuronal glutamate transporter EAAC1, exacerbates transient focal cerebral ischemia-induced neuronal damage in rat brain. *J. Neurosci.* 21:1876–1883.
 62. Innocenti, B., Parpura, V., and Haydon, P. G. 2000. Imaging extracellular waves of glutamate during calcium signaling in cultured astrocytes. *J. Neurosci.* 20:1800–1808.
 63. Pasti, L., Volterra, A., Pozzan, T., and Carmignoto, G. 1997. Intracellular calcium oscillations in astrocytes: A highly plastic, bidirectional form of communication between neurons and astrocytes *in situ*. *J. Neurosci.* 17:7817–7830.
 64. Phillis, J. W. and O'Regan, M. H. 1996. Mechanisms of glutamate and aspartate release in the ischemic rat cerebral cortex. *Brain Res.* 730:150–164.
 65. Seki, T. and Arai, Y. 1999. Different polysialic acid-neural cell adhesion molecule expression patterns in distinct types of mossy fiber boutons in the adult hippocampus. *J. Comp. Neurol.* 410:115–125.
 66. Pasti, L., Zonta, M., Pozzan, T., Vicini, S., and Carmignoto, G. 2001. Cytosolic calcium oscillations in astrocytes may regulate exocytotic release of glutamate. *J. Neurosci.* 21:477–484.
 67. Araque, A., Li, N., Doyle, R. T., and Haydon, P. G. 2000. SNARE protein-dependent glutamate release from astrocytes. *J. Neurosci.* 20:666–673.
 68. Liu, S. Y. 1990. [Protective effects of vitamin E and selenium on myocardial mitochondria in rats: A study on the pathogenic factors and pathogenesis of Keshan disease]. *Chung. Hua. Yu. Fang. I. Hsueh. Tsa. Chih.* 24:214–216.
 69. McConkey, D. J., Nicotera, P., and Orrenius, S. 1994. Signalling and chromatin fragmentation in thymocyte apoptosis. *Immunol. Rev.* 142:343–363.
 70. Anderson, M. F. and Sims, N. R. 2002. The effects of focal ischemia and reperfusion on the glutathione content of mitochondria from rat brain subregions. *J. Neurochem.* 81:541–549.
 71. Dringen, R., Gutterer, J. M., and Hirrlinger, J. 2000. Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. *Eur. J. Biochem.* 267:4912–4916.
 72. Bona, E., Hagberg, H., Loberg, E. M., Bagenholm, R., and Thoresen, M. 1998. Protective effects of moderate hypothermia after neonatal hypoxia-ischemia: Short- and long-term outcome. *Pediatr. Res.* 43:738–745.
 73. Chen, Y., Vartiainen, N. E., Ying, W., Chan, P. H., Koistinaho, J., and Swanson, R. A. 2001. Astrocytes protect neurons from nitric oxide toxicity by a glutathione-dependent mechanism. *J. Neurochem.* 77:1601–1610.
 74. Dringen, R., Gebhardt, R., and Hamprecht, B. 1993. Glycogen in astrocytes: Possible function as lactate supply for neighboring cells. *Brain Res.* 623:208–214.
 75. Niitsu, Y., Hori, O., Yamaguchi, A., Bando, Y., Ozawa, K., Tamatani, M., Ogawa, S., and Tohyama, M. 1999. Exposure of cultured primary rat astrocytes to hypoxia results in intracellular glucose depletion and induction of glycolytic enzymes. *Brain Res. Mol. Brain Res.* 74:26–34.
 76. Liu, Y., Rosenthal, R. E., Starke-Reed, P., and Fiskum, G. 1993. Inhibition of postcardiac arrest brain protein oxidation by acetyl-L-carnitine. *Free Radic. Biol. Med.* 15:667–670.
 77. Rosenthal, R. E., Williams, R., Bogaert, Y. E., Getson, P. R., and Fiskum, G. 1992. Prevention of postischemic canine neurological injury through potentiation of brain energy metabolism by acetyl-L-carnitine. *Stroke* 23:1312–1317.
 78. Lolic, M. M., Fiskum, G., and Rosenthal, R. E. 1997. Neuroprotective effects of acetyl-L-carnitine after stroke in rats. *Ann. Emerg. Med.* 29:758–765.
 79. Bogaert, Y. E., Rosenthal, R. E., and Fiskum, G. 1994. Postischemic inhibition of cerebral cortex pyruvate dehydrogenase. *Free Rad. Biol. Med.* 16:811–820.
 80. Calvani, M. and Arrigoni-Martelli, E. 1999. Attenuation by acetyl-L-carnitine of neurological damage and biochemical derangement following brain ischemia and reperfusion. *Int. J. Tissue React.* 21:1–6.
 81. Bouzier, A. K., Thiaudiere, E., Biran, M., Rouland, R., Canioni, P., and Merle, M. 2000. The metabolism of [3-(13)C]lactate in the rat brain is specific of a pyruvate carboxylase-deprived compartment. *J. Neurochem.* 75:480–486.
 82. Bernardi, P., Broekemeier, K. M., and Pfeiffer, D. R. 1994. Recent progress on regulation of the mitochondrial permeability transition pore: A cyclosporin-sensitive pore in the inner mitochondrial membrane. *J. Bioenerg. Biomembr.* 26:509–517.
 83. Halestrap, A. P., Connern, C. P., Griffiths, E. J., and Kerr, P. M. 1997. Cyclosporin A binding to mitochondrial cyclophilin inhibits the permeability transition pore and protects hearts from ischaemia/reperfusion injury. *Mol. Cell Biochem.* 174:167–172.
 84. Zoratti, M. and Szabo, I. 1995. The mitochondrial permeability transition. *Biochim. Biophys. Acta* 1241:139–176.
 85. Gunter, T. E. and Pfeiffer, D. R. 1990. Mechanisms by which mitochondria transport calcium. *Am. J. Physiol.* 258:C755–C786.
 86. Bernardi, P. and Petronilli, V. 1996. The permeability transition pore as a mitochondrial calcium release channel: A critical appraisal. *J. Bioenerg. Biomembr.* 28:131–138.
 87. Bernardi, P. 1999. Mitochondrial transport of cations: Channels, exchangers, and permeability transition. *Physiol. Rev.* 79:127–1155.
 88. Kristal, B. S. and Dubinsky, J. M. 1997. Mitochondrial permeability transition in the central nervous system: Induction by calcium cycling-dependent and -independent pathways. *J. Neurochem.* 69:524–538.
 89. Andreyev, A., Fahy, B., and Fiskum, G. 1998. Cytochrome c release from brain mitochondria is independent of the mitochondrial permeability transition. *FEBS Lett.* 439:373–376.
 90. Kristian, T., Gertsch, J., Bates, T. E., and Siesjo, B. K. 2000. Characteristics of the calcium-triggered mitochondrial permeability transition in nonsynaptic brain mitochondria: Effect of cyclosporin A and ubiquinone O. *J. Neurochem.* 74:1999–2009.
 91. Fiskum, G., Bambrick, L., Kristian, T., Chandrasekaran, K., and Chinopoulos, C. 2003. Calcium-induced damage to neuron, astrocyte, and brain mitochondria [Abstract]. *J. Neurochem.* 85(Suppl. 1):56.
 92. Bai, G., Rama Rao, K. V., Murthy, C. R., Panickar, K. S., Jayakumar, A. R., and Norenberg, M. D. 2001. Ammonia induces the mitochondrial permeability transition in primary cultures of rat astrocytes. *J. Neurosci. Res.* 66:981–991.
 93. Uchino, H., Elmer, E., Uchino, K., Lindvall, O., and Siesjo, B. K. 1995. Cyclosporin A dramatically ameliorates CA1 hippocampal damage following transient forebrain ischaemia in the rat. *Acta Physiol. Scand.* 155:469–471.
 94. Uchino, H., Elmer, E., Uchino, K., Li, P. A., He, Q. P., Smith, M. L., and Siesjo, B. K. 1998. Amelioration by cyclosporin A of brain damage in transient forebrain ischemia in the rat. *Brain Res.* 812:216–226.
 95. Friberg, H., Ferrand-Drake, M., Bengtsson, F., Halestrap, A. P., and Wieloch, T. 1998. Cyclosporin A, but not FK 506, protects mitochondria and neurons against hypoglycemic damage and implicates the mitochondrial permeability transition in cell death. *J. Neurosci.* 18:5151–5159.
 96. Yoshimoto, T. and Siesjo, B. K. 1998. Posttreatment with the immunosuppressant cyclosporin A in transient focal ischemia. *Brain Res.* 839:283–291.
 97. Schurr, A. 2002. Lactate, glucose and energy metabolism in the ischemic brain [Review]. *Int. J. Mol. Med.* 10:131–136.

98. Starkov, A. A., Polster, B. M., and Fiskum, G. 2002. Regulation of hydrogen peroxide production by brain mitochondria by calcium and Bax. *J. Neurochem.* 83:220–228.
99. Kroemer, G. and Reed, J. C. 2000. Mitochondrial control of cell death. *Nat. Med.* 6:513–519.
100. Naderi, J., Hung, M., and Pandey, S. 2003. Oxidative stress-induced apoptosis in dividing fibroblasts involves activation of p38 MAP kinase and over-expression of Bax: Resistance of quiescent cells to oxidative stress. *Apoptosis* 8:91–100.
101. Cao, G., Minami, M., Pei, W., Yan, C., Chen, D., O'Horo, C., Graham, S. H., and Chen, J. 2001. Intracellular Bax translocation after transient cerebral ischemia: Implications for a role of the mitochondrial apoptotic signaling pathway in ischemic neuronal death. *J. Cereb. Blood Flow Metab.* 21:321–333.