Calcium in Ischemic Cell Death

Tibor Kristián, PhD; Bo K. Siesjö, MD, PhD

**Background**—This review article deals with the role of calcium in ischemic cell death. A calcium-related mechanism was proposed more than two decades ago to explain cell necrosis incurred in cardiac ischemia and muscular dystrophy. In fact, an excitotoxic hypothesis was advanced to explain the acetylcholine-related death of muscle end plates. A similar hypothesis was proposed to explain selective neuronal damage in the brain in ischemia, hypoglycemic coma, and status epilepticus.

**Summary of Review**—The original concepts encompass the hypothesis that cell damage in ischemia-reperfusion is due to enhanced activity of phospholipases and proteases, leading to release of free fatty acids and their breakdown products and to degradation of cytoskeletal proteins. It is equally clear that a coupling exists between influx of calcium into cells and their production of reactive oxygen species, such as \( \cdot \mathrm{O}_2^- \), \( \mathrm{H}_2\mathrm{O}_2 \), and \( \cdot \mathrm{OH} \). Recent results have underscored the role of calcium in ischemic cell death. A coupling has been demonstrated among glutamate release, calcium influx, and enhanced production of reactive metabolites such as \( \cdot \mathrm{O}_2^- \), \( \cdot \mathrm{OH} \), and nitric oxide. It has become equally clear that the combination of \( \cdot \mathrm{O}_2^- \) and nitric oxide can yield peroxynitrate, a metabolite with potentially devastating effects. The mitochondria have again come into the focus of interest. This is because certain conditions, notably mitochondrial calcium accumulation and oxidative stress, can trigger the assembly (opening) of a high-conductance pore in the inner mitochondrial membrane. The mitochondrial permeability transition (MPT) pore leads to a collapse of the electrochemical potential for \( \mathrm{H}^+ \), thereby arresting ATP production and triggering production of reactive oxygen species. The occurrence of an MPT in vivo is suggested by the dramatic anti-ischemic effect of cyclosporin A, a virtually specific blocker of the MPT in vitro in transient forebrain ischemia. However, cyclosporin A has limited effect on the cell damage incurred as a result of 2 hours of focal cerebral ischemia, suggesting that factors other than MPT play a role. It is discussed whether this could reflect the operation of phospholipase A\(_2\) activity and degradation of the lipid skeleton of the inner mitochondrial membrane.

**Conclusions**—Calcium is one of the triggers involved in ischemic cell death, whatever the mechanism. *(Stroke. 1998;29:705-718.)*

**Key Words:** calcium ■ cerebral ischemia ■ free radicals ■ mitochondria

---

The calcium hypothesis of ischemic cell death was originally launched to explain the relationship between excessive calcium influx and the cell damage that is incurred in myocardial ischemia\(^1\) as well as in muscle dystrophy.\(^2\) In fact, studies conducted at that time led to the formulation of an excitotoxic hypothesis, predicting that excessive release of acetylcholine at the motor end plate was what caused damage to skeletal muscle.\(^3,4\) The work performed at that time on ischemic muscle damage was almost visionary since it forestalled the pivotal role of release of transmitters in enhancing the influx of \( \mathrm{Ca}^{2+} \) at postsynaptic sites and also predicted that a nonphysiological rise in \([\mathrm{Ca}^{2+}]\) could exert its adverse effects by overactivating cellular proteases and lipases.\(^5,6\) A link to mitochondrial dysfunction was also suggested since free fatty acids, liberated as a result of PLA\(_2\) activity,\(^5,6\) were supposed to increase the permeability of mitochondrial membranes and to uncouple respiration and oxidative phosphorylation in isolated mitochondria.\(^7\)

In 1977, Nicholson et al\(^8\) showed that anoxia triggers rapid translocation of \( \mathrm{Ca}^{2+} \) from extracellular to intracellular spaces of cerebellar tissues. This, as well as other findings, led to the hypothesis of calcium-mediated neuronal death in ischemia/hypoxia, hypoglycemia, and status epilepticus.\(^9\) As applied to brain tissues, the hypothesis of calcium-mediated cell death has some special features. The most important of these is that since the BBB has a very low permeability to \( \mathrm{Ca}^{2+} \), the calcium translocated into cells is, at least in the short perspective, that contained in the cerebral extracellular fluids. It was tempting to speculate, therefore, that certain neurons in the brain are selectively vulnerable to ischemic, hypoglycemic, and epileptic insults because they possess a high density of calcium channels in their plasma membranes.\(^9\) At that time, many neurons were known to have an innate proclivity to fire synchronously in an epileptic fashion, the epileptiform activity being driven by calcium spikes, ie, by calcium influx through VSCC, localized to the dendritic fields of these neurons.\(^10\) Accordingly, it could...
Selected Abbreviations and Acronyms

AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
BBB = blood-brain barrier
CaA = cyclosporin A
EAA = excitatory amino acid
ER = endoplasmic reticulum
IP₃, IP₄ = inositol 1,4,5-triphosphate, inositol 1,3,4,5-tetrakisphosphate
MCA = middle cerebral artery
MPT = mitochondrial permeability transition
NMDA = N-methyl-d-aspartate
NO = nitric oxide
NOS = nitric oxide synthase
PBN = α-phenyl-N-tet-butylnitrone
PLA₂ = phospholipase A₂
ROS = reactive oxygen species
VSCC = voltage-sensitive calcium channel

Cell Calcium Homeostasis

There is normally a very large electrochemical driving force tending to translocate calcium into cells. This force has two components: the difference in calcium concentration (extracellular fluids having a 10 000-fold higher concentration than intracellular ones) and the electrical potential across plasma membranes (the inside being 60 to 90 mV negative to the outside). The electrochemical potential is upheld because calcium fluxes associated with signal transduction are usually small and tightly regulated and because a rise in [Ca²⁺], triggers the extrusion of calcium by 3Na⁺–Ca²⁺ exchange and by an ATP-driven Ca²⁺–2H⁺ exchanger.⁴²,²⁵ The pump-leak relationships thus upheld normally maintain [Ca²⁺], at values of 100 to 200 nmol/L, but the concentration rises transiently during cell activation.

Presynaptic and postsynaptic calcium channels (“conductances”) at an excitatory synapse in which signal transduction is mediated by glutamate encompass N, P, L, and T types of VSCCs. The first two may be mainly localized to presynaptic endings, while the L and T types abound at postsynaptic membranes (see, for example, References 26 through 29). Two ionotropic glutamate receptors exist, one being selectively activated by AMPA and the other by NMDA.

Under normal circumstances, signal transduction begins with release of glutamate from the presynaptic ending and with activation of AMPA and NMDA receptors. Since the AMPA receptor–gated channel is permeable to monovalent cations, Na⁺ will enter the cell along its electrochemical gradient, depolarizing the postsynaptic membrane. The depolarization has two effects: it relieves the Mg²⁺ block of the Ca²⁺-permeable channel gated by the NMDA receptor, and it opens VSCCs. Calcium thus enters the cell by multiple pathways. At least in some cells additional VSCCs exist, and it is debated at present whether changes in the subunit composition of the AMPA receptor can render the channel it gates permeable to calcium.³⁰–³² Additional channels may be opened under adverse conditions, eg, those comprising unspecific cation channels and those activated by ROS, notably H₂O₂ (F. Mendez and R. Penner, personal communication, 1997). It is of interest that the latter inactivate very slowly, if at all. Under some circumstances, such as depolarization and intracellular Na⁺ accumulation, the 3Na⁺–Ca²⁺ exchanger can operate in the reversed mode, causing calcium to accumulate in the cell (see below).

Since physiological signals usually have a short duration, mechanisms must exist for terminating the glutamate (and calcium) transients. This occurs by several mechanisms. The major mechanism involves reuptake of glutamate through an electrogenic Na⁺/glutamate symporter, which derives its energy from the electrochemical gradient for Na⁺.²⁵ Although some glutamate may be taken up by presynaptic and postsynaptic neuronal elements, glial cells represent major sinks for glutamate. The glial cells convert glutamate to glutamine or lactate, which are then exported to neurons for resynthesis of glutamate or energy production.³⁴,³⁵ Other mechanisms encompass activation of Ca²⁺ or ATP-dependent K⁺ channels or of γ-aminobutyric acid–activated Cl⁻ channels.³⁶,³⁷ Activation of such channels would tend to repolarize or hyperpolarize membranes. Yet another mechanism, of theoretical and practical interest, can be deduced from the known dependence of NMDA-activated ion currents on pH. Since Ca²⁺ ion conductance decreases steeply when extracellular pH is reduced below 7.0,⁴⁶–⁴⁸ one can envisage that strong excitatory stimuli (which reduce intracellular and extracellular pH) are subjected to feedback inhibition by this mechanism. However, since the pK₅, for the effect of pH on NMDA channel currents is approximately 6.7, one can also deduce that alkalis (ie, an increase in pH₁ above normal) increases the tendency to calcium-mediated cell firing, perhaps acting as a trigger for epileptogenic discharges.⁴¹
Fig 1 summarizes cell calcium metabolism in a wider perspective, taking into account not only pathways of Ca\(^{2+}\) entry but also mechanisms for extrusion, binding, and sequestration. The upper part of the figure illustrates what has already been discussed, ie, the pump-leak relationship for Ca\(^{2+}\) across the plasma membrane. Undoubtedly, this relationship will set, or at least modulate, the value of [Ca\(^{2+}\)]\(_i\). Another determinant is the corresponding calcium traffic across the membranes of the ER, which is supposed to contain fluids having a free cytosolic calcium concentration close to that of the extracellular fluid.\(^4\) The calcium sequestered by the ER can be released by the operation of a sequence of events that starts with activation of surface receptors coupled to phospholipase C, continues with the formation of IP\(_3\) and its activation of IP\(_3\) receptors on ER membranes, and ends with the release of Ca\(^{2+}\) from the ER. This is undoubtedly an important source of calcium, which could contribute to a rise in [Ca\(^{2+}\)]\(_i\).\(^\text{42-45}\) Furthermore, since resquestration of Ca\(^{2+}\) into the ER requires ATP, the traffic of Ca\(^{2+}\) across the ER membranes represents another aspect of the pump-leak relationship for Ca\(^{2+}\).

A third determinant of intracellular calcium movements, and thereby of [Ca\(^{2+}\)]\(_i\), is the mitochondrion. It is now widely accepted that the balance between influx and efflux of Ca\(^{2+}\) across the inner membrane regulates mitochondrial dehydrogenases, which are rate-limiting for citric acid cycle metabolism.\(^4\) In summary, when cell activity leads to a substantial or excessive rise in [Ca\(^{2+}\)]\(_i\), the mitochondria may accumulate large amounts of calcium.\(^3\) This is because the uniporter, carrying calcium into the mitochondrion along the electrochemical gradient, has a much higher total activity than the export pathways, which encompass 2Na\(^+\)-Ca\(^{2+}\) exchange. In other words, if the net influx of Ca\(^{2+}\) exceeds the capacity of the extrusion pathway through 2Na\(^+\)-Ca\(^{2+}\) exchange, intramitochondrial Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_m\)) increases, and Ca\(^{2+}\) will be sequestered within the mitochondria. Under special circumstances, which will be discussed below, a large conductance pore will be opened. This, which is called the MPT pore, allows Ca\(^{2+}\) to leave the mitochondria.

Given this background, we will discuss calcium fluxes during and after ischemia. Before we do that, though, we wish to bring up two issues of conceptual importance. These relate to the total tissue and total cell calcium content and to the type of ischemia encountered.

As remarked, intracellular and extracellular (“free”) calcium concentrations are normally approximately 0.1 and 1000 \(\mu\)mol/L, respectively. However, since the total cell calcium content (excluding the extracellular spaces) is approximately 1000 \(\mu\)mol/L (ie, 1 \(\mu\)mol/L \(\cdot\) kg\(^{-1}\)), it follows that more than 99% of the total cell calcium content is bound to proteins or phospholipids, or sequestered in so-called calciosomes and mitochondria. Clearly, although the source of a rise in [Ca\(^{2+}\)]\(_i\) is often the extracellular calcium content, the cell contains enough (bound or sequestered) calcium to markedly increase [Ca\(^{2+}\)]\(_i\). This could occur if the bound calcium is displaced from its binding sites or if the calcium sequestered in ER, or in mitochondria is released to the cytosol. An uncontrolled release of calcium from the ER (or related intracellular stores) is now believed to predispose to Ca\(^{2+}\)-related cell damage.\(^3\) Furthermore, calcium metabolism of the mitochondria has become the focus of interest (see below).

As discussed elsewhere,\(^2\)\(^,\)\(^3\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^6\) it appears justified that a distinction is made between ischemia of the “cardiac arrest” type and that of the “stroke type.” The former, which is usually of short duration (5 to 15 minutes), can be studied as such, ie, in models of cardiac arrest (eg, Reference 52); however, most workers in the field prefer to use forebrain ischemia in gerbils and rats.\(^5\) This is because these models allow some remaining flow to brain stem centers during ischemia, obviating the use of intensive care measures during reperfusion. The second type of ischemia (“stroke”) is a focal one, which is usually of much longer duration, if not permanent. It is commonly induced by permanent or transient occlusion of one MCA.

The most important differences between the two types of ischemia relate to the duration of the ischemia (and its density). In global/forebrain ischemia of brief duration, the tissue damage is usually confined to the neuronal population, and cell death is characteristically delayed by hours or days.\(^5\)\(^,\)\(^6\) Cells outside the selectively vulnerable areas are usually not affected,
nor are glial cells or vascular endothelium. Furthermore, any inflammatory component is of moderate degree. In contrast, focal ischemia of long duration leads to pan necrosis, ie, to the death of all types of cells (infarction). This implies that vascular damage is a prominent feature of the lesions and that a strong inflammatory response is elicited.

Forebrain or Global Ischemia of Brief to Intermediate Duration

Calcium Fluxes and Changes in Extracellular and Intracellular Calcium Concentration

Under this heading, we will discuss events occurring after brief periods of forebrain ischemia, under optimal reflow conditions. However, we will also consider results obtained with sudden, complete ischemia since they give unequivocal information on rates of calcium influx (for reviews, see References 22, 37, and 62).

Figure 2 illustrates calcium influx into cells of neocortical tissue after complete ischemia, as this influx is reflected in [Ca\(^{2+}\)]. In normoglycemic animals the time to ischemic depolarization is approximately 50 seconds, and calcium influx is rapid. The raised plasma (and tissue) glucose concentrations in hyperglycemic subjects lead to delayed depolarization and to a two-phase influx of Ca\(^{2+}\), the second one being very slow. Clearly, hyperglycemia delays depolarization, probably by providing additional substrate (anaerobic) ATP production, and it reduces the rate of calcium influx. The latter effect is obviously due to the exaggerated tissue acidosis since excessive hypercapnia, induced in normoglycemic animals, duplicates the effects of preischemic hypercapnia. Finally, since the NMDA antagonist MK-801 has effects similar to those of hyperglycemia and of hypercapnia, the results must largely reflect the blocking effect of acidosis on NMDA receptor-gated Ca\(^{2+}\) influx.

During ischemia, [Ca\(^{2+}\)], decreases to values less than 10% of control, ie, to approximately 0.1 mmol/L. In addition, the extracellular fluid space decreases to approximately 50% of control. This means that almost all extracellular calcium is translocated into cells. With an extracellular fluid space of 20% of tissue volume and a [Ca\(^{2+}\)] of approximately 1.3 mmol/L, the calcium load to which cells are exposed is approximately 250 μmol·kg\(^{-1}\) of tissue. Since it is not likely that much calcium uptake occurs into glial cells, the calcium load of neurons could well be twice that figure, meaning that the total neuronal cell calcium content may increase to 150% of control or more. Clearly, if some neurons have very high calcium conductances, they could be exposed to an even higher load.

In the hippocampus, CA1 cells have a very high density of NMDA receptors. It is of interest, therefore, that Silver and Erecinska demonstrated that ischemia causes [Ca\(^{2+}\)], to increase from approximately 0.1 μm to values of 30 to 60 μm. Clearly, these are nonphysiological increases in [Ca\(^{2+}\)].

Additional information on ischemic calcium transients has been collected in experiments in which [Ca\(^{2+}\)] and DC potential shifts were measured in transient ischemia of the forebrain type. This type of ischemia has the drawback of giving a less distinct “onset” of ischemia but the advantage of readily allowing reperfusion events to be studied. Figure 3 illustrates the [Ca\(^{2+}\)], transients accompanying ischemia of 15 minutes’ duration in hypoglycemic, normoglycemic, and hyperglycemic animals. The results illustrate that hyperglycemic animals show a delay before depolarization occurs (see above) compared with normoglycemic animals but also that they repolarize earlier than their normoglycemic controls (see also Fig 4 for 5- and 2.5-minute ischemia). This means that the duration of the ischemic calcium transient is shorter in hyperglycemic than in normoglycemic animals (for review, see Reference 67); however, the ischemic damage is exaggerated in hyperglycemic animals.

These experiments challenge the postulate that any damage incurred by hypoxic cells is proportional to the duration of the calcium transient and to the rise in Ca\(^{2+}\), ie, to the time integral under the Ca\(^{2+}\) curve. Although it is noteworthy that hyperglycemic animals have a worse outcome after a 10-
minute period of ischemia, whether one uses a neurological or histopathological end point, the difference in duration of calcium uptake and DC potential shift between normoglycemic and hyperglycemic animals is relatively trivial. However, it becomes more obvious if the nominal ischemic period is only 5 minutes (Fig 4, data from Reference 69). With this period of ischemia, the duration of the Ca\textsuperscript{2+} \textit{i}/DC potential transient in hyperglycemic subjects is only 50% of that observed in normoglycemic animals. Despite that, the former incurred at least as much neuronal damage and, in contrast to normoglycemic animals, the hyperglycemic subjects is only 50% of that observed in normoglycemic animals. Despite that, the former incurred at least as much neuronal damage and, in contrast to normoglycemic animals, they showed a tendency to develop postischemic seizures. Clearly, although it is likely that Ca\textsuperscript{2+} \textit{i} must have been small, and other factors must have contributed to the delayed neuronal death. Such factors could encompass acidosis caused by the anaerobic glycolysis and/or the redox change that accompanies ischemia/reperfusion.

**Calcium and Delayed Neuronal Death**

In this type of ischemia, the neuronal injury is truly delayed since cells repolarize and resume physiological and metabolic functions before they suffer secondary damage and die. Signs of ongoing adverse processes are present, however. These encompass a sustained depression of metabolic rate and of overall protein synthesis. Other signs of metabolic perturbation are the expression of mRNAs for immediate early genes and for neurotrophins and the synthesis of “stress” or “heat shock” proteins (for extensive discussions of these issues, see recent volumes edited by Moskowitz and Caplan and by Siesjö and Wieloch).

The question arises of how the perturbation of cell calcium metabolism during and immediately after a transient period of ischemia influences the cascade of events that leads to delayed cell injury. There are many possibilities since Ca\textsuperscript{2+} \textit{i} activates phospholipases, endonucleases, and proteases, since it affects protein phosphorylation by altering the activity of protein kinases and phosphatases, and since it activates enzymes that give rise to the production of ROS and NO. Further, a sustained suppression of protein synthesis could be equally harmful since it bereaves the cells of molecules required for survival, such as antioxidative enzymes and trophic factors.

The second hypothesis is one in which cell death is assumed to be due to a sustained perturbation of cell calcium metabolism, leading to a slow, gradual rise in [Ca\textsuperscript{2+}] \textit{i}, and to eventual mitochondrial calcium overload. We will describe the origin of that hypothesis, attempt to integrate it with data suggesting a failure of the signal transduction pathway, and discuss data that give a novel perspective on postischemic mitochondrial dysfunction.

In the beginning of the 1980s, it was known that ischemia is accompanied by translocation of Ca\textsuperscript{2+} \textit{i} from extracellular to intracellular fluids and also that reperfusion restored
by guest on September 12, 2017 http://stroke.ahajournals.org/ Downloaded from

The depolarization could be prevented by antibodies to IP3 or signs of cell death. The results showed that the total tissue calcium content increased incorporation of $^{45}\text{Ca}^{2+}$ after 48 and 72 hours of reperfusion. \textit{**P<0.01 ANOVA, post hoc Dunnett’s test. Data from Reference 81.}

$[\text{Ca}^{2+}]$, to normal within 15 to 20 minutes. However, it was not known whether any changes occurred in total tissue $\text{Ca}^{2+}$ content, nor was there information on calcium metabolism in the period of reperfusion preceding the delayed neuronal death. In 1984, Dienel reported results on transient forebrain ischemia of 20 minutes’ duration. His data revealed an increased incorporation of $^{45}\text{Ca}^{2+}$ into the subiculum of the CA1 sector and into the lateral caudoputamen after 24 hours of reperfusion; however, this increased incorporation seemed to occur without an increase in the total cell calcium content. On the basis of these results, our laboratory explored the time course of changes in the total tissue calcium concentration of the dorsal hippocampus, correlating it with light microscopic signs of cell death. The results showed that the total tissue calcium content during reperfusion did not increase until late (between 24 and 48 hours) and that an increase in $[\text{Ca}^{2+}]_i$, seemed to precede morphological signs of cell death. The results, which are illustrated in Fig 5, were subsequently confirmed by measurements with proton-intensified x-ray emission (PIXE), allowing analyses of the different layers of the CA1 and CA3 sectors. Based on these results and on the theory proposed by Alkon and Rasmussen, we advanced the hypothesis of delayed calcium-related cell death. This hypothesis predicts that the initial ischemic transient gives rise to a sustained perturbation of plasma membrane handling of $\text{Ca}^{2+}$, resulting in a gradual rise in $[\text{Ca}^{2+}]_i$. When the latter exceeds the “set point” for calcium sequestration in the mitochondria, these begin accumulating $\text{Ca}^{2+}$ until they are “overloaded” and become dysfunctional. It should be emphasized that the plasma membrane may not be the only type of membranes that are perturbed by the ischemic transient. Thus, evidence for an involvement of ER membranes in the delayed cell death was reported by Tsukokawa et al., who induced ischemia of 5 minutes in gerbils, allowed reperfusion for 2.5 to 3.5 days, and prepared hippocampal slices for patch clamping of CA1 cells. Tetanic stimulation of the input to these cells caused irreversible depolarization, as did injection of IP$_3$ via the patch pipette. The depolarization could be prevented by antibodies to IP$_3$ or to the kinase that converts IP$_3$ to IP$_4$. This suggests that release of calcium from the ER could contribute to the rise in $[\text{Ca}^{2+}]_i$, and that a perturbed signal transduction affecting the IP$_3$-IP$_4$ system could be part of the pathogenetic defect leading to dysregulation of calcium metabolism (see also Reference 45).

Figure 5. Changes in total tissue calcium content in dorsal hippocampus in control animals (C) and at 6, 24, 48, and 72 hours of reperfusion after 15 minutes of forebrain ischemia. There was a significant increase in tissue $\text{Ca}^{2+}$ after 48 and 72 hours of reperfusion. **P<0.01 ANOVA, post hoc Dunnett’s test. Data from Reference 81.

This hypothesis requires (1) that mitochondria accumulate $\text{Ca}^{2+}$ before cell death becomes manifest and (2) that $[\text{Ca}^{2+}]_i$ rises gradually during reperfusion. Both of these requirements seem to be fulfilled. Thus, Dux et al. showed that a second wave of $\text{Ca}^{2+}$ precipitates in the mitochondria of CA1 neurons after 24 hours of reperfusion following 5 minutes of transient ischemia in the gerbil. Furthermore, Zaidan and Sims, studying forebrain ischemia of 20 minutes’ duration in rats and employing fractionation of tissue by centrifugation, directly showed a two-phase accumulation of calcium by the mitochondria, one occurring immediately after ischemia and the other many hours later. Finally, the results reported by Silver and Erecinska demonstrated that although reperfusion in rats subjected to forebrain ischemia normalized $[\text{Ca}^{2+}]_i$ within 10 to 20 minutes, continued reperfusion seemed to give rise to a secondary, gradual rise in $[\text{Ca}^{2+}]_i$.

The third hypothesis is that published by Abe et al. Like the second of the other two hypotheses discussed, it predicts that the ultimate cell damage is due to mitochondrial failure. However, the mechanisms proposed are different. The background is that the respiratory complexes, i.e., the enzymes that shuttle electrons along the respiratory chain and extrude $\text{H}^+$, are encoded for by both mitochondrial and nuclear DNA. For mitochondria to gain genetic material for synthesis of all relevant proteins, the mitochondria must be transported to the nucleus by being propelled along cytoskeletal elements by transport proteins such as dynorphin and kinesin. Abe et al. submit that this process is halted when the cytoskeleton is broken down by calcium-activated proteases and by calcium-dependent disassembly of microtubuli (see Reference 93). The long-term result of this would be reduced activities of respiratory enzymes, such as complex I or complex IV, with devastating effects on mitochondrial generation of ATP.

**Calcium Accumulation and Mitochondrial Dysfunction**

The postulate that mitochondrial dysfunction contributes to delayed neuronal death has recently received support from results obtained with the immunosuppressant drug CsA. The background is as follows. It has been known for decades that massive calcium accumulation triggers mitochondrial damage. This was originally thought to reflect activation of mitochondrial PLA$_2$ which, by breaking down the lipid backbone of the inner mitochondrial membrane, gives rise to a nonspecific increase in mitochondrial membrane permeability. However, a different mechanism giving rise to such an increase in permeability involves the formation (“assembly”) of a proteinaceous pore, the MPT pore, which has such a high conductance that it allows the passage of ions and molecules with a molecular mass less than 1500 D. According to the chemiosmotic theory of Mitchell, electron transport in the respiratory chain of mitochondria causes the extrusion of $\text{H}^+$, creating a large electrochemical potential difference across the inner mitochondrial membrane. This potential ($\Delta \Psi_\text{H}^+$) consists of an $\text{H}^+$ concentration gradient and an electrical potential difference ($\Delta \Psi_e$). The membrane is normally impermeable to $\text{H}^+$ and other ions, and it only allows passage of ions (or substrates) for which specific transport systems exist. This means that $\text{H}^+$ can normally only reenter...
Mitochondrial ATP production thus depends on the regulated entry of H⁺ across the inner mitochondrial membrane. However, the literature on mitochondrial function in vitro contains many reports demonstrating that exposure of mitochondria to calcium causes them to swell and to release intramitochondrial components into the medium. It is now realized that this sequence of events reflects the assembly of an MPT pore in the inner mitochondrial membrane. This pore allows the release of Ca²⁺ and Mg²⁺ as well as of various low- and high-molecular-weight compounds. In this process the mitochondria show osmotic swelling. Furthermore, the assembly of the pore leads to the collapse of ΔµH⁺ and thereby to cessation of ATP production. As will be discussed below, an additional consequence is a burst of production of ROS.

The seminal work of Crompton and collaborators identified the major factors triggering the assembly of an MPT pore in isolated mitochondria. These were a decrease in the ATP and increase in the P, concentration, oxidative stress, and calcium accumulation. The last two factors have emerged as major determinants in other experimental paradigms. Furthermore, the coupling among a decrease in mitochondrial membrane potential, the assembly of an MPT pore, and enhanced mitochondrial production of ROS has been established in thymocytes that have been committed to die by an apoptotic mechanism, after exposure to dexamethasone. In these and other rapidly proliferating cells, the first event in the sequence leading to (apoptotic) cell death is a decrease in ΔΨᵣ. What then follows is a burst of free radical production, cell shrinkage, and cell death.

The conclusions that can be drawn from these experiments are that oxidative stress and mitochondrial calcium accumulation predispose to an MPT and to the consequences of such an event, e.g., the depolarization-coupled production of ROS. This is where the action of CsA comes in. In all experimental paradigms studied in vitro, whether on cells of nonneuronal or neuronal origin, CsA proved to be an almost specific inhibitor of the MPT. This is presumably because CsA, which combines with a series of cyclophilin proteins, blocks the MPT pore by competing with the effect of Ca²⁺-cyclophilin for occupancy on the transition pore proteins.

Two years ago, our laboratory obtained results showing that CsA dramatically ameliorates the CA1 damage, provided that it can pass the BBB. The results were obtained in experiments in which growth factor–producing cells were injected into the CA1 sector of one hemisphere, CsA being given to suppress the immune response (Uchino et al). Analyses of the primary experiments and additional experiments revealed that the combination of systematically injected CsA and a unilateral needle lesion almost eliminated the CA1 damage after 7 or 10 minutes of forebrain ischemia (Fig 6). We interpreted the results to show that the needle lesion enhanced the BBB permeability of CsA and that CsA acted by preventing the assembly of an MPT pore in calcium-loaded mitochondria. At present, this is a tentative interpretation since CsA has effects other than that of blocking the MPT pore. These may be related to its immunosuppressant effects and its ability to combine with cyclophilin, a modulator of the phosphatase calcineurin. This enzyme affects several metabolic events, including NO production by NOS. However, FK 506, which is a stronger immunosuppressant than CsA and which, like CsA, inhibits calcineurin, is less efficacious than CsA in forebrain ischemia. Since FK 506 does not act as blocker of the MPT, the results suggest that CsA works by preventing a Ca²⁺-triggered MPT during reperfusion.

Calcium-Mediated Mechanisms of Delayed Neuronal Death: A Speculative Synthesis

The hypotheses discussed encompass events that are possibly linked. For example, a perturbation of signal transduction may alter the pump-leak relationship for calcium across plasma and intracellular membranes so that the result is a gradual rise in Ca²⁺, and eventual mitochondrial calcium overload. Since the hypothesis of Abe et al requires further support, we will discuss the hypothesis of delayed calcium-mediated cell death. In its simplest form, this hypothesis predicts that the initial ischemic transient gives rise to a sustained perturbation of membrane handling of calcium. This then sets the stage for a gradual, secondary rise in [Ca²⁺], which eventually causes mitochondrial calcium overload. If this hypothesis is viewed against the background of our present knowledge of the MPT, affecting mitochondria that accumulate calcium, we can envisage a coupling among plasma membrane perturbation, gradual cell calcium accumulation, mitochondrial dysfunction, and delayed ischemic cell death.

Release of excitatory transmitters, depolarization, and an increase in [Ca²⁺], trigger enhanced production of the traditional ROS (·O₂⁻, H₂O₂, and ·OH) as well as of NO. General activation of PLA₂ leads to the production of ROS because arachidonic acid, generated by PLA₂ activity, is metabolized by cyclooxygenase (and lipooxygenase) to yield a variety of degradation products and, in that process, ·O₂⁻ is formed. Another source of ROS is activation of the Ca²⁺-calmodulin–dependent NOS pathway, which produces NO from arginine. As proposed by Beckman et al, NO can then react with ·O₂⁻ to yield peroxynitrite (ONOO⁻), the latter decomposing with the production of ·OH, a highly toxic free radical. The reaction sequences yield NO, peroxy nitrate, and...
with reperfusion translocates protein kinase C to membranes, downregulates protein kinase A, alters the activity of Ca\(^{2+}\)–calmodulin–protein kinase II, reduces or arrests protein synthesis, and alters gene expression.\(^{80,124,125}\) Any of these events could modulate, over long periods, membrane handling of Ca\(^{2+}\). We recognize that a rise in [Ca\(^{2+}\)]
per se could trigger many of these effects. As discussed above, this does not necessarily imply that it is the plasma membrane function that is altered since a correspondingly altered pump-leak relationship for calcium could exist at the level of ER membranes. The important feature of the hypothesis is that ischemia and reperfusion perturb membrane handling of calcium in such a way that [Ca\(^{2+}\)]
is gradually increased, triggering sequestration of calcium in mitochondria. Cell death could then be the direct result of a calcium–triggers of a pore opening in mitochondria. It has been shown beyond doubt that transient forebrain ischemia is accompanied by an enhanced production of ROS, which is maximal during the first 10- to 60-minute period of reperfusion.\(^{118-120}\) However, it has been more difficult to demonstrate an anti-ischemic effect of free radical scavengers. For example, although the spin trap PBN ameliorates neurological deficit and ischemic cell death in the gerbil,\(^{118,121}\) it lacks an effect in rats subjected to forebrain ischemia.\(^{122}\) However, a fairly robust effect was obtained with a very high dose of the •OH scavenger dimethylthiourea in rats subjected to 15 minutes of forebrain ischemia.\(^{123}\) It seems likely, therefore, that the postischemic production of ROS contributes to delayed neuronal death in both gerbils and rats.

Fig 7 illustrates how these findings can be incorporated into the general hypothesis discussed. Realizing that ischemia leads to the influx of Ca\(^{2+}\), causing [Ca\(^{2+}\)]
to rise, and that reperfusion triggers the extrusion of the calcium accumulated with normalization of [Ca\(^{2+}\)], we must probe into the mechanisms that are triggered during and immediately after the ischemic transient, when ROS are formed. As proposed in a recent article by Siesjö et al.,\(^{82}\) ROS could directly modulate Ca\(^{2+}\) entry pathways or Ca\(^{2+}\) extrusion mechanisms by oxidation of proteins or lipids, thereby resetting the pump-leak relationship for calcium. An alternative possibility is that the effect of ROS is indirect, reflecting activation of protein kinases and phosphatases or of endonucleases, which indirectly affect membrane handling of calcium. For example, ischemia

Global or Focal Ischemia of Long Duration

When global or forebrain ischemia is prolonged, the free interval between the insult and the final damage is shortened and, if the ischemia is of very long duration, recirculation may fail to restore mitochondrial function and cellular bioenergetic state. Experiments with extended periods of ischemia also reveal a rapidly developing, massive calcium accumulation in the tissue. We will consider in turn recovery of mitochondrial function and changes in calcium metabolism.

Recovery of Mitochondrial Function

Under adverse conditions, recirculation may fail to be accompanied by resumption of oxidative phosphorylation of isolated mitochondria or lead to partial recovery for a limited period only. Such conditions encompass ischemia of long duration and ischemia with superimposed hyperglycemia. For example, incomplete forebrain ischemia of 30 minutes’ duration in fed rats (which became hyperglycemic) was followed by additional deterioration of mitochondrial respiratory rates during recirculation.\(^{132,133}\) Furthermore, rapid maturation of mitochondrial damage has been found in hyperglycemic dogs subjected to anoxia-ischemia,\(^{129}\) in rats subjected to long ischemic peri-
The reason why mitochondrial respiratory functions are not resumed after long ischemic periods, particularly in hyperglycemic subjects, is not known. It has been generally held that mitochondria are either damaged by PLA₂-mediated breakdown of the lipid backbone of the inner mitochondrial membrane or by oxidation of protein components mediating electron transport, H⁺ extrusion, or ATP production. However, no agreement has been reached on the targets. Thus, some studies implicate the pyruvate dehydrogenase complex, others one or more respiratory complexes (I through V), and still others the adenylate translocase. In fact, the pyruvate dehydrogenase complex has also been incriminated in ischemia of brief duration in starved animals. Since PLA₂ is a calcium-dependent enzyme and since calcium activates several enzymes producing ROS, the mitochondrial failure, whether acute or delayed, can be traced back to a perturbed calcium metabolism. However, the molecular defect remains unclarified.

In this context, recent results obtained in experiments on focal ischemia of 2 hours’ duration are intriguing. In core and penumbral tissues, this period of MCA occlusion was accompanied by a decrease in tissue ATP content to approximately 10% and 25%, respectively, and by increases in lactate content to approximately 15 mmol/L × kg⁻¹. After 1 hour of reperfusion, ATP increased to 50% to 70% of control. Since the adenine nucleotide pool (Σ-ATP + ADP + AMP) had decreased accordingly, this degree of recovery must reflect a virtually complete rephosphorylation of the ADP available to ATP. However, recirculation for 2 hours did not further increase ATP concentration or the size of the adenine nucleotide pool and, after 4 hours, signs of a secondary decrease in ATP concentration were apparent. Since tissue lactate concentrations showed little tendency to decrease at 1 and 2 hours and increased further at 4 hours, the results suggest partial recovery of cell energy metabolism and mitochondrial functions at 1 and 2 hours and secondary failure at 4 hours.

Subsequent results showed that the time course of changes in cellular bioenergetic state was paralleled by corresponding changes in ADP- and uncoupler-stimulated respiration of tissue homogenates in focal and penumbral tissues. These results suggest that the partial recovery of ATP concentrations and the failure of recovery of normal lactate concentrations reflect sustained mitochondrial dysfunction. It could further be shown that the free radical spin trap PBN and the immunosuppressant FK-506, both of which ameliorate tissue damage shown that the free radical spin trap PBN and the immunosuppressant FK-506, both of which ameliorate tissue damage, would promote mitochondrial production of free radicals and accelerate breakdown of mitochondrial phospholipids. Very likely, conditions during reperfusion favor the opening of an MPT pore, particularly if NO and peroxynitrite are formed.

Calcium Metabolism

After brief to intermediate periods of ischemia, massive calcium accumulation, reflecting net transfer of Ca²⁺ from blood to tissue, is observed many hours (or days) after the initial insult (see above). After long periods of ischemia, the “free” interval is shortened, and substantial amounts of calcium may accumulate during the first 2 to 3 hours of reperfusion. A similarly accelerated influx of calcium into the brain occurs in permanent or transient focal ischemia. In some published studies the calcium content was measured by atomic absorption spectrophotometry, giving quantitative values for calcium content and the rate of calcium flux from blood to tissue. In others, Ca autoradiography was used. This technique gives good spatial resolution, but the data cannot be used for quantitative estimates since the tissue activity must depend on both calcium content and Ca transfer rates across the BBB. Despite this reservation, however, published data are consistent in showing a rapidly developing true increase in tissue calcium content, whether the ischemia is permanent or sustained for 1 to 3 hours.

Although the results are clear-cut, they raise a series of important questions. First, is there an influx of calcium from blood to tissue because Ca²⁺ is reduced, creating a suitable transport gradient? Second, is there a rise in total tissue calcium content because cells fail to extrude Ca²⁺ against whatever gradient may be existing? Third, if cells continuously accumulate Ca²⁺, is this “extra” calcium sequestered by the mitochondria?

Recent results obtained in an experimental setting of 2 hours of MCA occlusion, with reperfusion for 1 to 8 hours, provide some answers to these questions. First, in the focus of the lesion, reperfusion leads to rephosphorylation of ADP to ATP (see above) and to normalization of K⁺, but Ca²⁺ is only restored to approximately 50% of control, leaving a transport gradient for translocation of Ca²⁺ from plasma to tissue (T.K. and B.K.S, unpublished data, 1997). Second, the 2-hour period of ischemia leads to a 20% increase in the total tissue calcium content, reflecting the influx of plasma calcium during the ischemic period. During the first 3 to 4 hours of recirculation, the calcium content remains the same or increases slowly, but after 6 hours (or 24 hours) there is a substantial increase in tissue calcium content (T.K. and B.K.S, unpublished data, 1997). Changes in a neocortical penumbral area were qualitatively similar, and massive calcium “loading” occurred after 6 (and 24) hours of recovery (T.K. and B.K.S, unpublished data, 1997).

These results lead to a number of questions. A major question can be posed as follows: Why is Ca²⁺ in focal areas of forebrain ischemia. In vivo, and perhaps also when homogenates are used to study mitochondrial respiratory functions in vitro, increased FFA concentrations may uncouple mitochondria or inhibit their respiration. An additional possibility is that the mitochondria are partially calcium-loaded and prone to assemble a permeability transition pore. As remarked, this would promote mitochondrial production of free radicals and accelerate breakdown of mitochondrial phospholipids. Very likely, conditions during reperfusion favor the opening of an MPT pore, particularly if NO and peroxynitrite are formed.
not normalized during recirculation? Provided that one can assume that calcium influx occurs along a transport gradient created by the low Ca\(^{2+}\), where does the calcium accumulate? Does it accumulate in mitochondria? However, if mitochondria accumulate calcium, how can such accumulation be reconciled with the notion of an MPT, which should be accompanied by release of calcium to the cytosol? This leads to a general question: Where is the accumulated calcium localized? Furthermore, how is this localization/sequstration related to cell death? Does it cause cell death, or does it occur because dying cells accumulate calcium? To provide answers to some of these questions, we will examine in vitro experiments.

**Excitotoxicity, Calcium Influx, and Cell Death In Vitro**

There is now extensive literature on pathological calcium transients in cultured neurons. A major part of this work was inspired by the discovery that glutamate and related EAAs are neurotoxic and that the toxicity is related to Ca\(^{2+}\) influx into cells\(^{14,17}\) (for early reviews, see References 16 and 77). During the last 8 to 10 years, a large body of evidence has been accumulated that expands the central postulate, i.e., that damage is prone to develop when cells are exposed to sufficiently high concentrations of EAAs for a sufficiently long period of time and that the damage depends heavily on the Ca\(^{2+}\) influx that occurs in response to the EAA exposure.\(^{157,158}\)

Exposure of cultured cells to EAAs in vitro has been claimed to be a useful paradigm for ischemic disease in vivo. Two facts suggest that the extrapolation from in vitro conditions is fraught with difficulties. First, while in vitro results demonstrate that 2 to 3 minutes of glutamate exposure leads to extensive neuronal necrosis in vitro,\(^{159}\) this duration of ischemia does not lead to cell damage in any region other than the CA1 sector of the hippocampus.\(^{46,69}\) In other words, cells are more vulnerable in vitro to anoxic/excitotoxic transients. Second, it has been demonstrated that acidosis in vitro protects cells against anoxic and excitotoxic insults\(^{160–162}\); however, it has been demonstrated beyond doubt that acidosis in vivo exaggerates ischemic brain damage (for reviews, see References 67 and 163).

It seems likely that these differences can be explained by the fact that, in vitro, cells are exposed to an unlimited source of calcium, allowing translocation of large amounts of Ca\(^{2+}\) into cells.\(^{164}\) This conclusion is supported by results obtained in vitro, demonstrating that the total calcium content during a 5- to 10-minute exposure to glutamate can increase severalfold.\(^{165}\) Such a massive influx does not occur in vivo, since the amount acutely accumulated is restricted by the calcium content of the extracellular fluid (see above). By analogy, and recalling that acidosis reduces the rate of calcium influx through NMDA- and voltage-operated channels,\(^{166}\) we can deduce that, in vitro, acidosis protects cells by limiting ischemia-induced calcium influx into cells. In vivo, acidosis can retard the rate of calcium influx in ischemia, but since the calcium source is restricted, factors other than the total calcium load determine the final outcome.\(^{25}\)

Despite these reservations, it must be concluded that experiments on neurons in vitro represent a useful paradigm for studies of ischemia in vivo. The following results, obtained on cells in vitro, are particularly relevant.

1. A transient insult leads to a delayed increase in Ca\(^{2+}\), after the initial increase. It has been documented in numerous publications that a glutamate transient leads to a marked rise in Ca\(^{2+}\). A few studies have documented that if the excitotoxic insult is sufficiently severe or sufficiently prolonged, recovery of Ca\(^{2+}\), occurs after the initial insult but is followed by a secondary rise in Ca\(^{2+}\), obviously preceding deregulation of Ca\(^{2+}\) homeostasis and cell death.\(^{158,167,168}\) In fact, if the excitotoxic insult is sufficiently severe, Ca\(^{2+}\), will not recover when the stimulus is removed.\(^{158,169}\) We recognize that these results mimic those obtained in vivo after brief and sustained ischemic periods.

2. The cell damage incurred in EAA transients is not related to changes in Ca\(^{2+}\), but to changes in total calcium influx. Results obtained from several laboratories\(^{158,170,171}\) suggest that the (initial) rise in Ca\(^{2+}\), is a poor indicator of the outcome. A variable that correlates better with the outcome of an excitotoxic insult is the total amount of calcium translocated into the cell,\(^{156,172,173}\) (for review, see Reference 165). There are several possible implications of the findings, supporting this notion. One is that activation of NMDA receptors may be detrimental because the receptor-gated channel translocates more Ca\(^{2+}\) into the cell than other agonist-operated channels or voltage-dependent channels. Another implication is that the vulnerability of neurons in vitro is exaggerated because much more calcium can be translocated into intracellular fluids in vitro because of the unlimited source of Ca\(^{2+}\) available (see above).

3. The initial calcium load, at least in part, is sequestered by the mitochondria. Data obtained more than one decade ago suggested that the mitochondria served as a “sink” for calcium loads associated with intense physiological activity or pathological transients.\(^{47}\) Subsequent work performed on neuronal cultures in vitro gave substance to this assumption and provided information on pathological calcium transients (Thayer and Miller\(^{174}\) [1990], Werth and Thayer\(^{175}\) [1994], White and Reynolds\(^{176}\) [1995], Wang et al\(^{177}\) [1994], White and Reynolds\(^{178}\) [1997]). The concept emerging from these studies is that when substantial amounts of Ca\(^{2+}\) enter the cell, a large part is sequestered in the mitochondria. This process occurs by uptake of Ca\(^{2+}\) through the electrogenic uniporter, while egress of Ca\(^{2+}\) probably occurs by Ca\(^{2+}\)-2Na’ exchange. The absolute amounts of Ca\(^{2+}\) taken up are not known, however, ie, we do not know how much of a given tissue calcium “overload” can be attributed to calcium sequestration.

4. Mitochondrial calcium accumulation is associated with enhanced production of ROS. As mentioned previously, ischemia with reperfusion is associated with enhanced production of ROS.\(^{184–186}\) Evidence now exists that a substantial part of this production emanates from mitochondria.\(^{179,180}\) This notion is supported by results obtained in vitro on primary neuronal cultures. The concept is based on the hypothesis that excitotoxic cell death is, at least in part, mediated by a coupling between glutamate-induced production of ROS and EAA-induced cell damage.\(^{30}\) Subsequent results showed that exposure of cells to glutamate gave rise to production of ROS and that a substantial part of this production occurred in the mitochondrial fraction.\(^{180,181}\)
(5) Cell death is preceded by mitochondrial membrane depolarization and is inhibited by CsA. Recently the EAA stimulation of surface receptors, increase in Ca\(^{2+}\), sequestration of Ca\(^{2+}\) by mitochondria, and mitochondrial generation of ROS have been further investigated. Thus, results have been published which show that the effect of EAA on cells encompasses a decrease in the mitochondrial AVi; furthermore, CsA was found to not only reduce the incidence (and degree) of depolarization but also to reduce the incidence of cell death. There are thus reasons to believe that EAA not only increase cell death at brief periods of ischemia, the rapidly maturing cell death after long periods of ischemia, or cell death compromised by preischemic hyperglycemia.

Acknowledgments

This study was supported by the Swedish Medical Research Council (14X-263), the US Public Health Service through the National Institutes of Health (5 R01-NS-07838), and the Queen’s Medical Center, Honolulu, Hawaii.

References

12. Murphy VA, Rapoport SI. Increased transfer of Ca into brain and cerebrospinal fluid from plasma during chronic hypocalcemia in rats. Brain Res. 1988;454:315–320.
49. Paschen W. Disturbances of calcium homeostasis within the endoplasmic reticulum may contribute to the development of ischemic-cell damage. Med Hypotheses. 1996;47:283–288.


99. Bernardi P, Petronilli V. The permeability transition pore as a mito-

100. Mitchell P. Chemiosmotic coupling in oxidative and photosynthetic 

101. Crompton M, Ellinger H, Costi A. Inhibition by cyclosporin A of a Ca²⁺
dependent pore in heart mitochondria activated by inorganic phosphate 

102. Richter C. Pro-oxidants and mitochondrial Ca²⁺: their relationship 

M, Walter P, Yaffe M. Oxidants in mitochondria: from physiology to 

104. Zamzami N, Marchetti P, Castedo M, Decaudin D, Macho A, Hirsch T, 
Sousa S, Petit P, Mignotte B, Kroemer G. Sequential reduction of mito-
chondrial transmembrane potential and generation of reactive oxygen 

Kroemer G. Reduction in mitochondrial potential constitutes an early 

106. Uchino H, Elmér E, Uchino K, Lindvall O, Siejsjo BK. Cyclosporin A 
dramatically ameliorates CA1 hippocampal damage following transient 

107. Dawson TM, Snyder SH. Gases as biological messengers: nitric oxide 

108. Tokime T, Nozaki K, Kikuchi H. Neuroprotective effect of FK506, an 
immunosuppressant, on transient global ischemia in gerbil. Neurol Sci. 

109. Drake M, Frinberg H, Bors-Moller F, Sakata K, Wieloch T. The immu-

110. Floyd RA, Carney JM. Free radical damage to protein and DNA: mech-
a

111. Halliwell B. Reactive oxygen species and the central nervous system. 

defects in brain mitochondria at low oxygen tension. 

113. Pahlmark K, Folbergrova J, Smith M-L, Siejsjo BK. Effects of dimethyl-
thiourea on selective neuronal vulnerability in forebrain ischemia in rats. 

114. Pahlmark K, Hu B, Shamoo A. Aberrant cell signaling in the postischemic 
brain: an integrated view. In: Welch KMA, Caplan LR, Reis DJ, Siejsjo BK, 
Weir B, eds. Primer on Cerebrovascular Diseases. New York, NY: 

115. Matson M. Trophic factors and brain cell survival. In: Welch KMA, 
Caplan LR, Reis DJ, Siejsjo BK, Weir B, eds. Primer on Cerebrovascular 

116. Ljunggren B, Norberg K, Siejsjo BK. Influence of tissue acidosis upon 
restoration of brain energy metabolism following total ischemia. Brain Res. 

117. Hillered L, Siejsjo BK, Arfors K-E. Mitochondrial response to transient 
1984;4:438–446.

118. Sims N, Pulinelli W. Altered mitochondrial respiration in selectively 
vulnerable brain subregions following transient forebrain ischemia in the 

119. Wagner K, Kleinholz M, Myers R. Delayed onset of neurologic deteri-
oration following anoxia/ischemia coincides with appearance of impaired 
brain mitochondrial respiration and decreased cytochrome oxidase activity. 

120. Kocher M. Metabolic and hemodynamic activation of postischemic rat 
brain by cortical spreading depression. J Cereb Blood Flow Metab. 1990;10: 
564–571.

121. Phillis J, Clough-Helfman C. Protection from cerebral ischemic injury in 
primates with oxygen radicals and scavengers in cerebral ischemic injury. 

122. Ljunggren B, Norberg K, Siejsjo BK. Influence of tissue acidosis upon 
restoration of brain energy metabolism following total ischemia. Brain Res. 

123. Kuroda S, Katsura K, Hillered L, Bates TE, Siejsjo BK. Delayed treatment 
of FK506 ameliorates secondary mitochondrial dysfunction following 


125. Almeida A, Allen KL, Bates TE, Clark JB. Effect of reperfusion following 
cerebral ischemia on the activity of the mitochondrial respiratory-chain in 

126. Rehncrona S, Mela L, Siejsjo BK. Recovery of brain mitochondrial 
function in the rat after complete and incomplete cerebral ischemia. Stroke. 

127. Kuroda S, Smith M-L, Siejsjo BK. Lactic acidosis and recovery of mito-
chondrial function following forebrain ischemia in the rat. J Cereb Blood 
Flow Metab. 1985;5:259–266.

128. Sun D, Gilboe DD. Ischemia-induced changes in cerebral mitochondrial 
free fatty acids, phospholipids, and respiration in the rat. J Neurochem. 

129. Kocher M. Metabolic and hemodynamic activation of postischemic rat 
brain by cortical spreading depression. J Cereb Blood Flow Metab. 1990;10: 
564–571.

130. Katsura K, Folbergrova J, Gido G, Siejsjo BK. Functional, metabolic, and 
circulatory changes associated with seizure activity in the postischemic 

131. Almeida A, Allen KL, Bates TE, Clark JB. Effect of reperfusion following 
cerebral ischemia on the activity of the mitochondrial respiratory-chain in 

132. Rehncrona S, Mela L, Siejsjo BK. Recovery of brain mitochondrial 
function in the rat after complete and incomplete cerebral ischemia. Stroke. 

133. Kuroda S, Katsura K, Hillered L, Bates TE, Siejsjo BK. Delayed treatment 
of FK506 ameliorates secondary mitochondrial dysfunction following 

134. Sims NB. Mitochondrial function and calcium sequestration during 
reperfusion. In: Welch KMA, Caplan LR, Reis DJ, Siejsjo BK, Weir B, 
eds. Primer on Cerebrovascular Diseases. New York, NY: Academic Press, 
Inc; 1997:184–186.

135. Folbergrova J, Zhao Q, Katsura K, Siejsjo BK. N-Tert-butyl-α-
phenylnitrone improves recovery of brain energy state in the rats follow-
ing transient focal ischemia. Proc Natl Acad Sci U S A. 1995;92: 
5047–5061.

failure after transient focal ischemia is due to mitochondrial injury. Acta 

137. Zhao Q, Pahlmark K, Siejsjo M-L, Siejsjo BK. Delayed treatment with the 
spin trap α-phenyl-N-tert-butyl nitrone (PBN) reduces infarct size follow-

138. Shanker J, Butcher S. Immunophilins mediate the neuroprotective effects 

139. Kuroda S, Siejsjo BK. Postischemic administration of FK 506 reduces 
infarct volume following transient focal ischemia. Neurosci Res Commun. 

140. Kuroda S, Katsura K, Hillered L, Bates TE, Siejsjo BK. Delayed treatment 
with α-phenyl-N-tert-butyl nitrone (PBN) attenuates secondary mito-
chondrial dysfunction after transient focal cerebral ischemia in the 

141. Nakai A, Kuroda S, Kristian T, Siejsjo BK. The immunosuppressant drug 
FK 506 ameliorates secondary mitochondrial dysfunction following 
Ca\textsuperscript{2+} and Brain Ischemia


177. Schinder AF, Olson EC, Spitzer NC, Montal M. Mitochondrial dysfunction is a primary event in glutamate neurotoxicity. J Neurosci. 1996;16:6125–6133.

Calcium in Ischemic Cell Death
Tibor Kristián and Bo K. Siesjö

Stroke. 1998;29:705-718
doi: 10.1161/01.STR.29.3.705

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/29/3/705

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/