

Mitochondrial calcium and oxidative stress as mediators of ischemic brain injury

Anatoly A. Starkov^a, Christos Chinopoulos^b, Gary Fiskum^{b,*}

^a Department of Neurology, Weil Medical College, Cornell University, New York, NY 10021, USA

^b Department of Anesthesiology, University of Maryland School of Medicine, 685 W. Baltimore St., Baltimore, MD 21201, USA

Received 14 February 2004; accepted 18 February 2004

Abstract

Acute ischemic and brain injury is triggered by excitotoxic elevation of intraneuronal Ca^{2+} followed by reoxygenation-dependent oxidative stress, metabolic failure, and cell death. Studies performed *in vitro* with neurons exposed to excitotoxic concentrations of glutamate demonstrate an initial rise in cytosolic $[\text{Ca}^{2+}]$, followed by a reduction to a normal, albeit slightly elevated concentration. This reduction in cytosolic $[\text{Ca}^{2+}]$ is due partially to active, respiration-dependent mitochondrial Ca^{2+} sequestration. Within minutes to an hour following the initial Ca^{2+} transient, most neurons undergo delayed Ca^{2+} deregulation characterized by a dramatic rise in cytosolic Ca^{2+} . This prelethal secondary rise in Ca^{2+} is due to influx across the plasma membrane but is dependent on the initial mitochondrial Ca^{2+} uptake and associated oxidative stress. Mitochondrial Ca^{2+} uptake can stimulate the net production of reactive oxygen species (ROS) through activation of the membrane permeability transition, release of cytochrome *c*, respiratory inhibition, release of pyridine nucleotides, and loss of intramitochondrial glutathione necessary for detoxification of peroxides. Targets of mitochondrially derived ROS may include plasma membrane Ca^{2+} channels that mediate excitotoxic delayed Ca^{2+} deregulation.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Calcium; Mitochondrial dysfunction; Brain injury

1. Calcium induced mitochondrial dysfunction in acute brain injury

Mitochondrial dysfunction contributes to the etiology of delayed death of neurons, oligodendrocytes, and astrocytes following cerebral ischemia, hypoxia, and trauma [1–3,4]. One of the key events that causes mitochondrial injury is an abnormal increase in intracellular Ca^{2+} [5–7]. Thus, transient ischemia is accompanied by a gradual rise in $[\text{Ca}^{2+}]$; [8], by calcium sequestration in mitochondria [9,10], and by mitochondrial bioenergetic dysfunction [11]. Pharmacologic agents that inhibit cellular Ca^{2+} influx protect mitochondria against ischemic injury [12–14]. The mechanisms of Ca^{2+} -mediated mitochondrial damage fall into two classes. The first involves activation of degradative enzymes, e.g., calpain proteases and phospholipases, or enzymes that generate reactive oxygen species (ROS) capable of oxidatively modifying mitochondrial proteins and lipids [15–19]. The second class involves the activation of the mitochondrial per-

meability transition (MPT) where a non-selective, large conductance pore within the inner membrane opens, resulting in uncoupling of oxidative phosphorylation, osmotic swelling, release of matrix metabolites, and even physical rupture of the mitochondrial outer membrane [4,20,21].

These direct and indirect effects of Ca^{2+} on mitochondrial structure and function can lead to either necrotic or apoptotic cell death (Fig. 1). While it is clear that both forms of death contribute to hypoxic, ischemic, and traumatic brain injury, the relative contribution of each form varies substantially with the experimental model, brain cell type, and age of the experimental animal [22–26]. The most important information obtained from studies on the molecular biology of apoptosis is the identity of numerous gene products that have a profound impact on the death or survival of brain cells *in vivo*, irrespective of how cell death is classified. This knowledge led to the development of neuroprotective interventions targeting specific enzymes, e.g., caspases [27,28], or processes, e.g., the MPT [29–33]. In addition, investigators are attempting to use anti-death gene products, e.g., Bcl-2 and Bcl-X_l, as neuroprotective drugs through generation of protein constructs containing “protein transduction domains” that allow these proteins to cross cell membranes

* Corresponding author. Tel.: +1-410-706-4711; fax: +1-410-706-2550.
E-mail address: gfishk001@umaryland.edu (G. Fiskum).

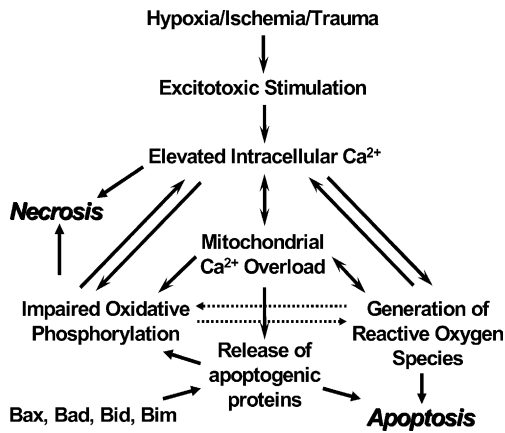


Fig. 1. Mitochondrial mechanisms of neural cell death following cerebral hypoxia, ischemia, and head trauma. Excitotoxic levels of intracellular Ca^{2+} accumulate within mitochondria causing metabolic failure, oxidative stress, and ultimately, both necrotic and apoptotic cell death. Proteinaceous signals, e.g., Bax, in addition to Ca^{2+} , trigger release of proteins, e.g., cytochrome *c*, from mitochondria. Once present in the cytosol, mitochondrial pro-apoptotic proteins activate the caspase cascade or proceed directly to the nucleus, leading to apoptosis. Moreover, loss of mitochondrial cytochrome *c* also contributes to impaired aerobic energy metabolism and stimulates mitochondrial ROS generation, contributing to oxidative stress. Mitochondrial Ca^{2+} overload together with oxidative stress result in a failure of neurons to maintain normal cytosolic Ca^{2+} following excitotoxic stimulation.

[34–37]. Bcl-2 “therapy” could be particularly effective as it exhibits at least two modes of action at the mitochondrion. Bcl-2 binds to pro-apoptotic proteins, e.g., Bax, preventing their oligomerization and pore formation in the outer membrane that allow cytochrome *c* and other pro-apoptotic proteins from escaping to the cytosol where they activate the caspase protease cascade [38]. Bcl-2 also inhibits the MPT via increasing mitochondrial redox capacity and resistance to MPT pore opening caused by Ca^{2+} and oxidative stress [39]. This effect can potentially protect against either necrotic or apoptotic cell death.

The deleterious effects of Ca^{2+} on mitochondrial bioenergetic activities are well established, at least in vitro. Recently, attention of investigators is also focused on more subtle effects of mitochondrial Ca^{2+} overload on the ability of neurons to maintain Ca^{2+} homeostasis and on mitochondrial participation in oxidative stress, as covered in the following sections.

2. Excitotoxicity, mitochondrial Ca^{2+} , and delayed neuronal Ca^{2+} deregulation

Excitotoxicity is a process whereby excessive synaptic release of glutamate activates postsynaptic glutamate receptors [40] leading to severe neuronal Ca^{2+} and Na^+ loading [41], culminating in cell death [42]. An important consequence of excitotoxic stimulation is delayed Ca^{2+} deregulation (DCD), as originally described by Manev et al.

[43] and further characterized by the groups of Thayer and coworker [44] and Tymianski et al. [45]. DCD refers to the latent loss of Ca^{2+} homeostasis of cultured neurons upon exposure to glutamate, and precedes neuronal cell death [45–48]. DCD is closely associated with mitochondrial dysfunction [49,50]. While glutamate-mediated Ca^{2+} influx results in mitochondrial Ca^{2+} uptake and neuronal cell death, Ca^{2+} influx by other means, e.g., voltage-dependent Ca^{2+} channel activation, is not nearly as toxic [45]. The linkage of *N*-methyl-D-aspartate (NMDA) receptor activation to cell death is associated with the spatial proximity of mitochondria to the plasma membrane and to NMDA receptors [51,52]. This proximity allows for preferential exposure of mitochondria to excitatory and excitotoxic Ca^{2+} . Thus, cytosolic Ca^{2+} microdomains are particularly important in glutamate excitotoxicity [53]. In addition to ionotropic receptor-mediated Ca^{2+} signals, neurons also release Ca^{2+} from intracellular stores, and this source of Ca^{2+} may also be involved in glutamate excitotoxicity [54,55] (Fig. 2).

The correlation between mitochondrial Ca^{2+} uptake and glutamate-induced excitotoxicity is strong. DCD is inhibited when cerebellar granule neurons are treated with mitochondrial poisons that block energy-dependent mitochondrial Ca^{2+} uptake [56]. DCD is associated with MPT in striatal neurons and is inhibited by the mitochondria-specific cyclophilin ligand *N*-methyl-valine-CsA [57]. This study also demonstrated that DCD is preceded by the CsA-sensitive loss of mitochondrial Ca^{2+} . When extracellular Ca^{2+} is replaced with Ba^{2+} , which proceeds through the NMDA receptor but is not sequestered by mitochondria [58,59], evidence of MPT and DCD in hippocampal neurons is lost

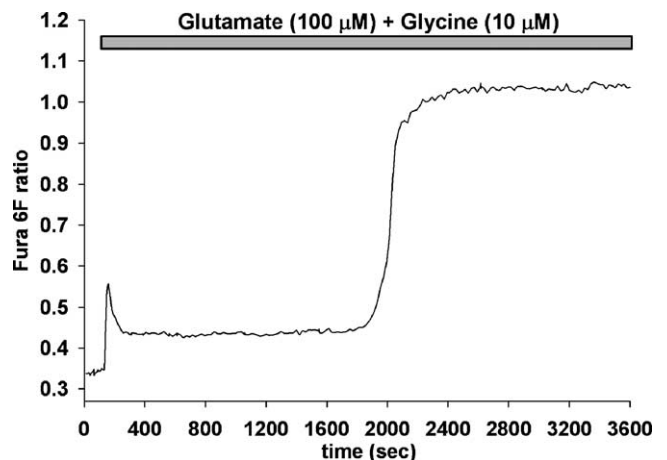


Fig. 2. Fluorescent measurements of cytosolic Ca^{2+} in a cortical neuron during exposure to excitotoxic glutamate. Primary cultures of rat cortical neurons were loaded with Fura 6F, a relatively low sensitivity fluorescent Ca^{2+} indicator. Cultures were exposed to 100 μM glutamate in the presence of 10 μM glycine. Addition of glutamate was followed by a very rapid transient rise and fall in $[\text{Ca}^{2+}]_i$. Approximately 1600 s later, the $[\text{Ca}^{2+}]_i$ spontaneously rose to a supranormal level ($>10 \mu\text{M}$) and did not return toward a normal level. This secondary irreversible rise in $[\text{Ca}^{2+}]_i$ is referred to as delayed Ca^{2+} deregulation (DCD).

[60]. Other measures of mitochondrial dysfunction, e.g., loss of mitochondrial membrane potential ($\Delta\Psi$), cytochrome *c* release into the cytosol, and enhanced ROS production appear consistently, but to varying degrees in different in vitro excitotoxicity models [61]. Ca^{2+} -induced cytochrome *c* release may indeed be responsible for enhanced mitochondrial ROS production during excitotoxicity [61,62], as explained in the next section. While release of cytochrome *c* into the cytosol is a potent trigger of apoptotic cell death [63], excitotoxic cell death is primarily necrotic. Respiratory impairment caused by the loss of cytochrome *c*, a member of the electron transport chain, could easily result in metabolic failure and subsequent loss of ionic homeostasis. There is, however, a lack of correlation between glutamate-induced neuronal cell death and depletion of ATP [64]. The role of cytochrome *c* release in glutamate-induced neuronal death is therefore more likely mediated by downstream effects of oxidative stress or by other as yet unidentified mechanisms.

Mitochondrial Ca^{2+} accumulation and ROS generation each contribute to DCD [56]. However, mitochondrial ROS generation per se is not sufficient to trigger acute neuronal degeneration [65], with NMDA activation also being necessary. Neuronal ROS production is stimulated by Ca^{2+} influx via NMDA receptor activation but not via voltage-dependent calcium channels [66]. However, oxidative stress also occurs in response to both AMPA and kainate receptor activation [67,68], that also elicit DCD. While Ca^{2+} -triggered mitochondrial ROS production plays an important role in glutamate-induced DCD and cell death, the molecular links between these events are at this juncture unknown.

Supporting evidence for the participation of mitochondrial Ca^{2+} uptake in glutamate excitotoxicity comes from the observation that exposure of cerebellar granule neurons to high levels of glutamate causes major ultrastructural alterations of mitochondria, suggesting the activation of some form of permeability transition pore [69]. While the PTP is apparently involved in DCD, it is unclear whether it is the cause or result of DCD. One possible mechanism by which activation could trigger DCD is through stimulation of mitochondrial ROS production [50]. Recently, Ca^{2+} -permeable plasma membrane cation channels were identified that are activated by oxidative stress [70–72]. These channels are all members of the transient receptor potential (TRP) family [73–75], which is prominently expressed in nervous tissue [74,76,77]. We hypothesize that these one or more subsets of these channels are activated as a consequence of Ca^{2+} -stimulated mitochondrial ROS production and that they are largely responsible for the delayed Ca^{2+} and Na^+ influx characteristic of DCD that irreversibly commits neurons to die.

3. Influence of Ca^{2+} on mitochondrial ROS production and the potential relationship to hypoxic brain injury

A compelling body of evidence indicates that oxidative stress caused by reactive oxygen species (ROS) is intimately

involved in pathways leading to tissue damage induced by ischemia and reperfusion [49,78–83]. ROS are generated in large amounts during reperfusion; [49,81–83] mitochondria are thought to produce most of ROS, however, the mechanisms and regulation of mitochondrial ROS are not well understood. Numerous reports imply that massive mitochondrial Ca^{2+} accumulation promotes ROS generation during ischemia/reperfusion and during excitotoxicity [49,83–88]. The mechanism by which such Ca^{2+} sequestration can promote mitochondrial ROS production is, however, obscure. In this section we summarize what is known about mitochondrial ROS production in the context and in relation to the known consequences of massive Ca^{2+} accumulation in mitochondria.

4. Effects of mitochondrial Ca^{2+} accumulation on ROS production at Complex III of the electron transport chain

The primary ROS produced by mitochondria is superoxide [89]. This highly reactive free radical is extremely short-lived [90,91–94] and dismutates either spontaneously or with the help of the mitochondrial superoxide dismutase forming the more ROS, H_2O_2 [95]. It is not known what mitochondrial redox site or sites are responsible for superoxide production in vivo. Experiments in vitro demonstrate that superoxide can be produced in mitochondria at multiple sites that vary in their V_{max} for production and in their mechanisms of regulation.

The most studied site of mitochondrial superoxide generation is located in the quinol-oxidizing center of Complex III of mitochondrial respiratory chain. Superoxide is apparently produced in a reaction of O_2 with an unstable semiquinone, which is elevated in the presence of a specific electron transport inhibitor, antimycin A. In the presence of antimycin A, Complex III generates substantial superoxide. However, no superoxide production at this site has ever been directly demonstrated in the absence of antimycin A or a similar electron transport chain inhibitor.

While the contribution of Complex III to mitochondrial ROS production in the absence of respiratory inhibitors remains unresolved, two known effects of mitochondrial Ca^{2+} could conceivably promote ROS production at this site. When mitochondrial Ca^{2+} accumulation triggers cytochrome *c* release by MPT-dependent or independent pathways, respiration is inhibited and the redox state of redox sites proximal to cytochrome *c* shift to a maximally reduced level. Under some circumstances, this release is accompanied by increased mitochondrial ROS production [96]. However, it is unlikely that Complex III is responsible for this increase in ROS generation as the partially reduced semiquinone would not be formed under these (reviewed in [97–99]). Another possible mechanism by which Ca^{2+} could promote ROS production at Complex III is via stimulation of respiration. A physiologically relevant increase in ex-

tramitochondrial Ca^{2+} stimulates respiration through activation of both intramitochondrial pyruvate-, isocitrate-, and α -ketoglutarate dehydrogenases through increased transfer of electrons to Complex I [100]. Ca^{2+} may also stimulate electron flow from Complex III to IV independent of its effects on matrix dehydrogenases [101]. The mechanism of Ca^{2+} -induced stimulation of electron flow through Complex III is not known; therefore, the possibility of an effect of elevated Ca^{2+} on ubiquinone in Complex III favoring superoxide production cannot be excluded. Nevertheless, the available information on the mechanism of electron transfer in Complex III [102–104] and the mechanism of superoxide formation at this site [105–107] does not support the hypothesis that Ca^{2+} fluctuations in or around mitochondria promote superoxide production at Complex III.

5. Effects of mitochondrial Ca^{2+} accumulation on ROS production at Complex I of the electron transport chain

One or more sites of superoxide production are located in Complex I of the respiratory chain (reviewed in [97–99]). The mechanism of ROS generation is not known, primarily because the mechanism of electron transfer in Complex I is not yet clear. The ROS production associated with the physiological electron flow from NADH of the mitochondrial matrix to coenzyme Q in the inner mitochondrial membrane requires the presence of NAD-linked respiratory substrates, e.g., pyruvate, glutamate, α -ketoglutarate, and malate. Recently, it was demonstrated that ROS production supported by oxidation of NAD-reducing substrates is stimulated by both high membrane potential ($\Delta\psi$) [108] and the reduced redox state of intramitochondrial NAD(P)H [108,109]. Because of these relationships, ROS production is stimulated three to four folds by the transition from phosphorylating respiration (State 3) to a resting state (State 4), this transition results in an increase in both $\Delta\psi$ and the level of reduced NAD(P)H. An even greater, 10-fold stimulation of ROS production is observed when the Complex I inhibitor rotenone is added to mitochondria during State 3 respiration. Remarkably, the stimulation of ROS production by rotenone is species and tissue-dependent. For example, rotenone stimulates ROS generation in guinea pig submitochondrial particles (SMP), has no effect on horse heart SMP [110], and even inhibits ROS production by mouse kidney mitochondria [111]. Importantly, ROS production in the presence of NAD-linked substrates is also stimulated by inhibiting the respiratory chain at any level downstream of Complex I, as this increases the level of reduced NAD(P)H. As the interruption in electron transport by release of cytochrome *c* also causes reduction in NAD(P)H, this is a plausible explanation for the increase in ROS production we and others observed after removal of the outer membrane or outer membrane pore formation by the addition of Bax [96,108]. While supra-physiological mitochondrial Ca^{2+} uptake can also in-

duce the release of cytochrome *c*, Ca^{2+} uptake exerts other effects on mitochondrial bioenergetics that oppose ROS formation. Massive Ca^{2+} uptake typically results in mitochondrial membrane depolarization and oxidation of NAD(P)H. These effects may indeed explain the inhibition of ROS production that we recently reported for brain mitochondria in the presence of NAD-linked oxidizable substrates and in the absence of respiratory chain inhibitors [96].

Another set of conditions that promote ROS production at Complex I are those that promote reverse electron transport from ubiquinone to Complex I. Energetically, this reversal of the normal flow of electrons requires a highly reduced redox state of ubiquinone and a high $\Delta\psi$. The source of reducing power is from FADH_2 -dependent substrates, e.g., succinate or α -glycerophosphate. Although the physiological concentration of succinate in tissues is very low (0.2–0.4 mM), it rises substantially during ischemia or hypoxia (up to 4–7 mM) [112,113]. It is therefore possible that during early reoxygenation, the oxidation of accumulated succinate generates the high $\Delta\psi$ and reducing power necessary for reversal of electron transfer and ROS production at Complex I. Stimulation of ROS generation via reversed electron transport by mitochondrial uptake of Ca^{2+} is, however, unlikely. The electrophoretic transport of Ca^{2+} into the mitochondrial matrix lowers $\Delta\psi$, and once accumulated, Ca^{2+} decreases $\Delta\psi$ through activation of the PTP or other mechanisms. A decrease in $\Delta\psi$ by merely $\sim 10\%$ inhibits succinate-supported ROS production in rat heart mitochondria by at least $\sim 90\%$ [114]. Almost complete inhibition of succinate-dependent ROS production by Ca^{2+} uptake in brain mitochondria was also demonstrated [96]. Thus, while reverse electron transport through Complex I is a viable mechanism for a burst of ROS production during reoxygenation and reperfusion, this mechanism is unlikely to be stimulated by high intracellular Ca^{2+} .

6. Stimulation of ROS production by Ca^{2+} -activated mitochondrial permeability transition

Activation of the mitochondrial permeability transition pore (PTP) is the most frequently observed consequences of extensive Ca^{2+} accumulation by mitochondria from various tissues. The PTP is thought to be a large channel in the inner mitochondrial membrane which is normally closed and can be opened by Ca^{2+} overloading and other factors including oxidative stress. The following are characteristics of the PTP observed in mammalian mitochondria:

- (1) Permeability to solutes with molecular weight < 1500 [115].
- (2) Reversibility, at least in vitro under some conditions [116,117].
- (3) Large-amplitude mitochondrial swelling associated with a decrease in membrane potential and loss of matrix solutes [118–120].

- (4) Sensitivity to inhibition by cyclosporine A [116,121] and bongkreic acid [116], however [122] sensitivity to cyclosporine A depends on levels of Ca^{2+} and physiological factors that suppress the PTP, e.g., Mg^{2+} and adenine nucleotides [123–125].
- (5) PTP [126–129] opening is substantially enhanced by the presence of ROS, products of ROS reactions, and other pro-oxidants [39,118,130].
- (6) The extent to which mitochondria swell in response to accumulation of Ca^{2+} varies considerably with experimental conditions and with tissue type [29,131,132].
- (7) Release of cytochrome *c* typically accompanies the osmotic swelling and rupture of the outer membrane evoked by the PTP [116,118,133].
- (8) Loss of mitochondrial matrix pyridine nucleotides is another measure of the PTP [119], distinguishing it from a mechanism of depolarization mediated by activation of a “low conductance” pore [134].

Several reports demonstrate that opening of PTP correlates with an increase in ROS production by isolated mitochondria [88,135], and in cells [136]. However, the mechanism by which PTP opening causes mitochondrial ROS production is unknown. PTP opening actually uncouples mitochondria, thereby causing a drop in $\Delta\Psi$ and a net oxidation in the pyridine nucleotide redox state. However, inhibition of electron transport at any point distal to the sites of ROS production will overcome the effects of pore opening, resulting in a net reduced shift in NAD(P)H redox state as well as the redox state of sites of ROS production, e.g., that present in Complex I. One way that PTP opening can cause such respiratory inhibition and reduced shift in redox state is through osmotic swelling, rupture of the outer membrane, and loss of cytochrome *c*. PTP opening may also cause respiratory inhibition by release of matrix NAD(P)H as these molecules mediate the transfer of electrons from several steps in the TCA cycle to Complex I of the electron transport chain. While it loss of pyridine nucleotides should inhibit the production of ROS at Complex I or other distal sites, it would cause a net reduction in redox sites present in TCA cycle dehydrogenases. The significance of this effect relates to our recent observations that both α -ketoglutarate and pyruvate dehydrogenases are significant sites of ROS production, particularly in the presence of high levels of reduced substrates, e.g., α -ketoglutarate, and low levels of electron acceptors, i.e., NAD^+ [137]. Thus, while loss of mitochondrial NAD(P)H inhibits ROS production by components of the electron transport chain, it may promote ROS production by mitochondrial matrix dehydrogenases. The “steady-state” production of ROS will also be enhanced in permeabilized mitochondria because of the loss of glutathione from the matrix space, as it was demonstrated in mitochondria from reperfused brain [138]. Reduced glutathione is a substrate for mitochondrial matrix glutathione peroxidase, an antioxidant enzyme that detoxifies H_2O_2 and other peroxides.

References

- [1] G. Fiskum, B. Polster, A. Starkov, et al., Role of mitochondria in neural cell fate, in: Restorative Neurology and Neuroscience, Special Section Abstracts of the International Conference on “Cellular Signaling in Neuroprotection Plasticity”, vol. 20 (issue 1/2), 2002, p. 62.
- [2] K. Blomgren, C. Zhu, U. Hallin, H. Hagberg, Mitochondria and ischemic reperfusion damage in the adult and in the developing brain, *Biochem. Biophys. Res. Commun.* 304 (2003) 551–559.
- [3] N.R. Sims, M.F. Anderson, Mitochondrial contributions to tissue damage in stroke, *Neurochem. Int.* 40 (2002) 511–526.
- [4] H. Friberg, T. Wieloch, Mitochondrial permeability transition in acute neurodegeneration, *Biochimie* 84 (2002) 241–250.
- [5] L. Schild, J. Huppelsberg, S. Kahler, G. Keilhoff, G. Reiser, Brain mitochondria are primed by moderate Ca^{2+} rise upon hypoxia/reoxygenation for functional breakdown and morphological desintegration, *J. Biol. Chem.* 278 (2003) 25454–25460.
- [6] M. Puka-Sundvall, B. Gajkowska, M. Cholewinski, K. Blomgren, J.W. Lazarewicz, H. Hagberg, Subcellular distribution of calcium and ultrastructural changes after cerebral hypoxia–ischemia in immature rats, *Brain. Res. Dev. Brain Res.* 125 (2000) 31–41.
- [7] K.A. Abo-Hashema, M.H. Cake, I.C. Potter, Liver mitochondria, confirmed as intact by complete suppression of succinate uptake and oxidation, possess a carnitine palmitoyltransferase I that is totally inhibited by malonyl CoA, *Biochem. Biophys. Res. Commun.* 258 (1999) 778–783.
- [8] I.A. Silver, M. Erecinska, Ion homeostasis in rat brain in vivo: intra- and extracellular $[\text{Ca}^{2+}]$ and $[\text{H}^+]$ in the hippocampus during recovery from short-term, transient ischemia, *J. Cereb. Blood Flow Metab.* 12 (1992) 759–772.
- [9] E. Dux, G. Mies, K.A. Hossmann, L. Siklos, Calcium in the mitochondria following brief ischemia of gerbil brain, *Neurosci. Lett.* 78 (1987) 295–300.
- [10] E. Zaidan, N.R. Sims, The calcium content of mitochondria from brain subregions following short-term forebrain ischemia and recirculation in the rat, *J. Neurochem.* 63 (1994) 1812–1819.
- [11] N.R. Sims, W.A. Pulsinelli, Altered mitochondrial respiration in selectively vulnerable brain subregions following transient forebrain ischemia in the rat, *J. Neurochem.* 49 (1987) 1367–1374.
- [12] B.H. Verweij, J.P. Muizelaar, F.C. Vinas, P.L. Peterson, Y. Xiong, C.P. Lee, Improvement in mitochondrial dysfunction as a new surrogate efficiency measure for preclinical trials: dose–response and time-window profiles for administration of the calcium channel blocker Ziconotide in experimental brain injury, *J. Neurosurg.* 93 (2000) 829–834.
- [13] M. Kudo, M. Aono, Y. Lee, G. Massey, R.D. Pearlstein, D.S. Warner, Effects of volatile anesthetics on *N*-methyl-D-aspartate excitotoxicity in primary rat neuronal–glial cultures, *Anesthesiology* 95 (2001) 756–765.
- [14] R.E. Rosenthal, F. Hamud, G. Fiskum, P.J. Varghese, S. Sharpe, Cerebral ischemia and reperfusion: prevention of brain mitochondrial injury by lidoflazine, *J. Cereb. Blood Flow Metab.* 7 (1987) 752–758.
- [15] J.M. Wingrave, K.E. Schaefer, E.A. Sribnick, et al., Early induction of secondary injury factors causing activation of calpain and mitochondria-mediated neuronal apoptosis following spinal cord injury in rats, *J. Neurosci. Res.* 73 (2003) 95–104.
- [16] I. Nakahara, H. Kikuchi, W. Taki, Changes in major phospholipids of mitochondria during postischemic reperfusion in rat brain, *J. Neurosurg.* 76 (1992) 244–250.
- [17] D.D. Gilboe, D. Kintner, J.H. Fitzpatrick, Recovery of postischemic brain metabolism and function following treatment with a free radical scavenger and platelet-activating factor antagonists, *J. Neurochem.* 56 (1991) 311–319.

- [18] K.R. Wagner, M. Kleinholz, R.E. Myers, Delayed decreases in specific brain mitochondrial electron transfer complex activities and cytochrome concentrations following anoxia/ischemia, *J. Neurol. Sci.* 100 (1990) 142–151.
- [19] A. Almeida, K.L. Allen, T.E. Bates, J.B. Clark, Effect of reperfusion following cerebral ischaemia on the activity of the mitochondrial respiratory chain in the gerbil brain, *J. Neurochem.* 65 (1995) 1698–1703.
- [20] P. Bernardi, Mitochondrial transport of cations: channels exchangers and permeability transition, *Physiol. Rev.* 79 (1999) 1127–1155.
- [21] B.K. Siesjo, E. Elmer, S. Janelidze, et al., Role and mechanisms of secondary mitochondrial failure, *Acta Neurochir. Suppl. (Wien)* 73 (1999) 7–13.
- [22] L.J. Martin, N.A. Al Abdulla, A.M. Brambrink, J.R. Kirsch, F.E. Sieber, C. Portera-Cailliau, Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: a perspective on the contributions of apoptosis and necrosis, *Brain Res. Bull.* 46 (1998) 281–309.
- [23] L.J. Martin, F.E. Sieber, R.J. Traystman, Apoptosis and necrosis occur in separate neuronal populations in hippocampus and cerebellum after ischemia and are associated with differential alterations in metabotropic glutamate receptor signaling pathways, *J. Cereb. Blood Flow Metab.* 20 (2000) 153–167.
- [24] F.J. Northington, D.M. Ferriero, E.M. Graham, R.J. Traystman, L.J. Martin, Early neurodegeneration after hypoxia–ischemia in neonatal rat is necrosis while delayed neuronal death is apoptosis, *Neurobiol. Dis.* 8 (2001) 207–219.
- [25] S.T. Hou, J.P. MacManus, Molecular mechanisms of cerebral ischemia-induced neuronal death, *Int. Rev. Cytol.* 221 (2002) 93–148.
- [26] D. Dewar, S.M. Underhill, M.P. Goldberg, Oligodendrocytes and ischemic brain injury, *J. Cereb. Blood Flow Metab.* 23 (2003) 263–274.
- [27] M. Rabuffetti, C. Sciorati, G. Tarozzo, E. Clementi, A.A. Manfredi, M. Beltramo, Inhibition of caspase-1-like activity by Ac-Tyr-Val-Ala-Asp-chloromethyl ketone induces long-lasting neuroprotection in cerebral ischemia through apoptosis reduction and decrease of proinflammatory cytokines, *J. Neurosci.* 20 (2000) 4398–4404.
- [28] K. Fink, J. Zhu, S. Namura, Prolonged therapeutic window for ischemic brain damage caused by delayed caspase activation, *J. Cereb. Blood Flow Metab.* 18 (1998) 1071–1076.
- [29] T. Kobayashi, S. Kuroda, M. Tada, K. Houkin, Y. Iwasaki, H. Abe, Calcium-induced mitochondrial swelling and cytochrome *c* release in the brain: its biochemical characteristics and implication in ischemic neuronal injury, *Brain Res.* 960 (2003) 62–70.
- [30] P.A. Li, T. Kristian, Q.P. He, B.K. Siesjo, Cyclosporin A enhances survival, ameliorates brain damage, and prevents secondary mitochondrial dysfunction after a 30-minute period of transient cerebral ischemia, *Exp. Neurol.* 165 (2000) 153–163.
- [31] S. Matsumoto, H. Friberg, M. Ferrand-Drake, T. Wieloch, Blockade of the mitochondrial permeability transition pore diminishes infarct size in the rat after transient middle cerebral artery occlusion, *J. Cereb. Blood Flow Metab.* 19 (1999) 736–741.
- [32] B. Alessandri, A.C. Rice, J. Levasseur, M. DeFord, R.J. Hamm, M.R. Bullock, Cyclosporin A improves brain tissue oxygen consumption and learning/memory performance after lateral fluid percussion injury in rats, *J. Neurotrauma* 19 (2002) 829–841.
- [33] P.G. Sullivan, M. Thompson, S.W. Scheff, Continuous infusion of cyclosporin A postinjury significantly ameliorates cortical damage following traumatic brain injury, *Exp. Neurol.* 161 (2000) 631–637.
- [34] G. Cao, W. Pei, H. Ge, In vivo delivery of a Bcl-xL fusion protein containing the TAT protein transduction domain protects against ischemic brain injury and neuronal apoptosis, *J. Neurosci.* 22 (2002) 5423–5431.
- [35] G.P. Dietz, E. Kilic, M. Bahr, Inhibition of neuronal apoptosis in vitro and in vivo using TAT-mediated protein transduction, *Mol. Cell Neurosci.* 21 (2002) 29–37.
- [36] E. Kilic, G.P. Dietz, D.M. Hermann, M. Bahr, Intravenous TAT-Bcl-XL is protective after middle cerebral artery occlusion in mice, *Ann. Neurol.* 52 (2002) 617–622.
- [37] S. Asoh, I. Ohsawa, T. Mori, et al., Protection against ischemic brain injury by protein therapeutics, *Proc. Natl. Acad. Sci. U.S.A.* 99 (2002) 17107–17112.
- [38] B.M. Polster, K.W. Kinnally, G. Fiskum, Bcl-2 death domain peptide induces cell type-selective mitochondrial outer membrane permeability, *J. Biol. Chem.* 276 (2001) 37887–37894.
- [39] A.J. Kowaltowski, A.E. Vercesi, G. Fiskum, Bcl-2 prevents mitochondrial permeability transition and cytochrome *c* release via maintenance of reduced pyridine nucleotides, *Cell Death Differ.* 7 (2000) 903–910.
- [40] R. Sattler, M. Tymianski, Molecular mechanisms of glutamate receptor-mediated excitotoxic neuronal cell death, *Mol. Neurobiol.* 24 (2001) 107–129.
- [41] D.W. Choi, Ionic dependence of glutamate neurotoxicity, *J. Neurosci.* 7 (1987) 369–379.
- [42] M. Arundine, M. Tymianski, Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity, *Cell Calcium* 34 (2003) 325–337.
- [43] H. Manev, M. Favaron, A. Guidotti, E. Costa, Delayed increase of Ca²⁺ influx elicited by glutamate: role in neuronal death, *Mol. Pharmacol.* 36 (1989) 106–112.
- [44] R.D. Randall, S.A. Thayer, Glutamate-induced calcium transient triggers delayed calcium overload and neurotoxicity in rat hippocampal neurons, *J. Neurosci.* 12 (1992) 1882–1895.
- [45] M. Tymianski, M.P. Charlton, P.L. Carlen, C.H. Tator, Source specificity of early calcium neurotoxicity in cultured embryonic spinal neurons, *J. Neurosci.* 13 (1993) 2085–2104.
- [46] M. Tymianski, M.P. Charlton, P.L. Carlen, C.H. Tator, Secondary Ca²⁺ overload indicates early neuronal injury which precedes staining with viability indicators, *Brain Res.* 607 (1993) 319–323.
- [47] M.R. Witt, K. Dekermendjian, A. Frandsen, A. Schousboe, M. Nielsen, Complex correlation between excitatory amino acid-induced increase in the intracellular Ca²⁺ concentration and subsequent loss of neuronal function in individual neocortical neurons in culture, *Proc. Natl. Acad. Sci. U.S.A.* 91 (1994) 12303–12307.
- [48] D.D. Limbrick, S.B. Churn, S. Sombati, R.J. DeLorenzo, Inability to restore resting intracellular calcium levels as an early indicator of delayed neuronal cell death, *Brain Res.* 690 (1995) 145–156.
- [49] D.G. Nicholls, S.L. Budd, Mitochondria and neuronal survival, *Physiol. Rev.* 80 (2000) 315–360.
- [50] D.G. Nicholls, S. Vesce, L. Kirk, S. Chalmers, Interactions between mitochondrial bioenergetics and cytoplasmic calcium in cultured cerebellar granule cells, *Cell Calcium* 34 (2003) 407–424.
- [51] T.I. Peng, J.T. Greenamyre, Privileged access to mitochondria of calcium influx through *N*-methyl-D-aspartate receptors, *Mol. Pharmacol.* 53 (1998) 974–980.
- [52] N.B. Pivovarova, J. Hongpaisan, S.B. Andrews, D.D. Friel, Depolarization-induced mitochondrial Ca accumulation in sympathetic neurons: spatial and temporal characteristics, *J. Neurosci.* 19 (1999) 6372–6384.
- [53] M.R. Duchen, Mitochondria and calcium: from cell signalling to cell death, *J. Physiol.* 529 (Pt 1) (2000) 57–68.
- [54] P.B. Simpson, R.A. Challiss, S.R. Nahorski, Neuronal Ca²⁺ stores: activation and function, *Trends Neurosci.* 18 (1995) 299–306.
- [55] N. Emptage, T.V. Bliss, A. Fine, Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines, *Neuron* 22 (1999) 115–124.
- [56] R.F. Castilho, M.W. Ward, D.G. Nicholls, Oxidative stress mitochondrial function and acute glutamate excitotoxicity in cultured cerebellar granule cells, *J. Neurochem.* 72 (1999) 1394–1401.
- [57] C.C. Alano, G. Beutner, R.T. Dirksen, R.A. Gross, S.S. Sheu, Mitochondrial permeability transition and calcium dynamics in striatal neurons upon intense NMDA receptor activation, *J. Neurochem.* 80 (2002) 531–538.

- [58] J.G. McCormack, N.J. Osbaldeston, The use of the Ca₂(+)-sensitive intramitochondrial dehydrogenases and entrapped fura-2 to study Sr²⁺ and Ba²⁺ transport across the inner membrane of mammalian mitochondria, *Eur. J. Biochem.* 192 (1990) 239–244.
- [59] T.E. Gunter, K.K. Gunter, S.S. Sheu, C.E. Gavin, Mitochondrial calcium transport: physiological and pathological relevance, *Am. J. Physiol.* 267 (1994) C313–C339.
- [60] J.M. Dubinsky, Y. Levi, Calcium-induced activation of the mitochondrial permeability transition in hippocampal neurons, *J. Neurosci. Res.* 53 (1998) 728–741.
- [61] C.M. Luetjens, N.T. Bui, B. Sengpiel, et al., Delayed mitochondrial dysfunction in excitotoxic neuron death: cytochrome *c* release and a secondary increase in superoxide production, *J. Neurosci.* 20 (2000) 5715–5723.
- [62] J. Cai, D.P. Jones, Superoxide in apoptosis. Mitochondrial generation triggered by cytochrome *c* loss, *J. Biol. Chem.* 273 (1998) 11401–11404.
- [63] X. Liu, C.N. Kim, J. Yang, R. Jemmerson, X. Wang, Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*, *Cell* 86 (1996) 147–157.
- [64] G. Marcaida, M.D. Minana, S. Grisolia, V. Felipo, Lack of correlation between glutamate-induced depletion of ATP and neuronal death in primary cultures of cerebellum, *Brain Res.* 695 (1995) 146–150.
- [65] B. Sengpiel, E. Preis, J. Kriegelstein, J.H. Prehn, NMDA-induced superoxide production and neurotoxicity in cultured rat hippocampal neurons: role of mitochondria, *Eur. J. Neurosci.* 10 (1998) 1903–1910.
- [66] M. Lafon-Cazal, S. Pietri, M. Culcasi, J. Bockaert, NMDA-dependent superoxide production and neurotoxicity, *Nature* 364 (1993) 535–537.
- [67] S.G. Carriedo, H.Z. Yin, S.L. Sensi, J.H. Weiss, Rapid Ca²⁺ entry through Ca²⁺-permeable AMPA/Kainate channels triggers marked intracellular Ca²⁺ rises and consequent oxygen radical production, *J. Neurosci.* 18 (1998) 7727–7738.
- [68] S.G. Carriedo, S.L. Sensi, H.Z. Yin, J.H. Weiss, AMPA exposures induce mitochondrial Ca(2+) overload and ROS generation in spinal motor neurons in vitro, *J. Neurosci.* 20 (2000) 240–250.
- [69] N.K. Isaev, D.B. Zorov, E.V. Stelmashook, R.E. Uzbekov, M.B. Kozhemyakin, I.V. Victorov, Neurotoxic glutamate treatment of cultured cerebellar granule cells induces Ca²⁺-dependent collapse of mitochondrial membrane potential and ultrastructural alterations of mitochondria, *FEBS Lett.* 392 (1996) 143–147.
- [70] M. Balzer, B. Lintschinger, K. Groschner, Evidence for a role of Trp proteins in the oxidative stress-induced membrane conductances of porcine aortic endothelial cells, *Cardiovasc. Res.* 42 (1999) 543–549.
- [71] E. Wehage, J. Eisfeld, I. Heiner, E. Jungling, C. Zitt, A. Luckhoff, Activation of the cation channel long transient receptor potential channel 2 (LTRPC2) by hydrogen peroxide. A splice variant reveals a mode of activation independent of ADP-ribose, *J. Biol. Chem.* 277 (2002) 23150–23156.
- [72] R. Kraft, C. Grimm, K. Grosse, Hydrogen peroxide and ADP-ribose induce TRPM2-mediated calcium influx and cation currents in microglia, *Am. J. Physiol. Cell Physiol.* 286 (2003) C129–C137.
- [73] C. Zitt, C.R. Halaszovich, A. Luckhoff, The TRP family of cation channels: probing and advancing the concepts on receptor-activated calcium entry, *Prog. Neurobiol.* 66 (2002) 243–264.
- [74] C. Montell, Physiology, phylogeny, *Sci. STKE* 2001 (2001) RE1.
- [75] D.E. Clapham, L.W. Runnels, C. Strubing, The TRP ion channel family, *Nat. Rev. Neurosci.* 2 (2001) 387–396.
- [76] C. Montell, L. Birnbaumer, V. Flockerzi, The TRP channels, a remarkably functional family, *Cell* 108 (2002) 595–598.
- [77] C. Strubing, G. Krapivinsky, L. Krapivinsky, D.E. Clapham, Formation of novel TRPC channels by complex subunit interactions in embryonic brain, *J. Biol. Chem.* 278 (2003) 39014–39019.
- [78] G. Ambrosio, I. Tritto, Reperfusion injury: experimental evidence and clinical implications, *Am. Heart J.* 138 (1999) S69–S75.
- [79] R. Bolli, Causative role of oxyradicals in myocardial stunning: a proven hypothesis. A brief review of the evidence demonstrating a major role of reactive oxygen species in several forms of postischemic dysfunction, *Basic Res. Cardiol.* 93 (1998) 156–162.
- [80] G. Benzi, O. Pastoris, M. Dossena, Relationships between gamma-aminobutyrate and succinate cycles during and after cerebral ischemia, *J. Neurosci. Res.* 7 (1982) 193–201.
- [81] G. Fiskum, A.N. Murphy, M.F. Beal, Mitochondria in neurodegeneration: acute ischemia and chronic neurodegenerative diseases, *J. Cereb. Blood Flow Metab.* 19 (1999) 351–369.
- [82] M.A. Sciamanna, J. Zinkel, A.Y. Fabi, C.P. Lee, Ischemic injury to rat forebrain mitochondria and cellular calcium homeostasis, *Biochim. Biophys. Acta* 1134 (1992) 223–232.
- [83] G. Fiskum, Mitochondrial participation in ischemic and traumatic neural cell death, *J. Neurotrauma* 17 (2000) 843–855.
- [84] J.A. Dykens, Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated Ca²⁺ and Na⁺: implications for neurodegeneration, *J. Neurochem.* 63 (1994) 584–591.
- [85] A.J. Kowaltowski, L.E. Netto, A.E. Vercesi, The thiol-specific antioxidant enzyme prevents mitochondrial permeability transition. Evidence for the participation of reactive oxygen species in this mechanism, *J. Biol. Chem.* 273 (1998) 12766–12769.
- [86] A.J. Kowaltowski, R.F. Castilho, A.E. Vercesi, Ca(2+)-induced mitochondrial membrane permeabilization: role of coenzyme Q redox state, *Am. J. Physiol.* 269 (1995) C141–C147.
- [87] A.J. Kowaltowski, R.F. Castilho, A.E. Vercesi, Opening of the mitochondrial permeability transition pore by uncoupling or inorganic phosphate in the presence of Ca²⁺ is dependent on mitochondrial-generated reactive oxygen species, *FEBS Lett.* 378 (1996) 150–152.
- [88] A.J. Kowaltowski, E.S. Naia-da-Silva, R.F. Castilho, A.E. Vercesi, Ca²⁺-stimulated mitochondrial reactive oxygen species generation and permeability transition are inhibited by dibucaine or Mg²⁺, *Arch. Biochem. Biophys.* 359 (1998) 77–81.
- [89] G. Loschen, A. Azzi, C. Richter, L. Flohe, Superoxide radicals as precursors of mitochondrial hydrogen peroxide, *FEBS Lett.* 42 (1974) 68–72.
- [90] O. Dionisi, T. Galeotti, T. Terranova, A. Azzi, Superoxide radicals and hydrogen peroxide formation in mitochondria from normal and neoplastic tissues, *Biochim. Biophys. Acta* 403 (1975) 292–300.
- [91] A. Boveris, E. Cadenas, A.O. Stoppani, Role of ubiquinone in the mitochondrial generation of hydrogen peroxide, *Biochem. J.* 156 (1976) 435–444.
- [92] J.F. Turrens, A. Boveris, Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria, *Biochem. J.* 191 (1980) 421–427.
- [93] G. Krishnamoorthy, P.C. Hinkle, Studies on the electron transfer pathway, topography of iron–sulfur centers, and site of coupling in NADH-Q oxidoreductase, *J. Biol. Chem.* 263 (1988) 17566–17575.
- [94] L. Zhang, L. Yu, C.A. Yu, Generation of superoxide anion by succinate–cytochrome *c* reductase from bovine heart mitochondria, *J. Biol. Chem.* 273 (1998) 33972–33976.
- [95] D.T. Sawyer, J.S. Valentine, How super is superoxide? *Acc. Chem. Res.* 14 (1981) 393–400.
- [96] A.A. Starkov, B.M. Polster, G. Fiskum, Regulation of hydrogen peroxide production by brain mitochondria by calcium and Bax, *J. Neurochem.* 83 (2002) 220–228.
- [97] J.F. Turrens, Superoxide production by the mitochondrial respiratory chain, *Biosci. Rep.* 17 (1997) 3–8.
- [98] J.F. Turrens, Mitochondrial formation of reactive oxygen species, *J. Physiol.* 552 (2003) 335–344.
- [99] G. Lenaz, The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology, *IUBMB Life* 52 (2001) 159–164.

- [100] J.G. McCormack, R.M. Denton, The role of mitochondrial Ca^{2+} transport and matrix Ca^{2+} in signal transduction in mammalian tissues, *Biochim. Biophys. Acta* 1018 (1990) 287–291.
- [101] A.N. Murphy, J.K. Kelleher, G. Fiskum, Submicromolar Ca^{2+} regulates phosphorylating respiration by normal rat liver and AS-30D hepatoma mitochondria by different mechanisms, *J. Biol. Chem.* 265 (1990) 10527–10534.
- [102] Z. Zhang, L. Huang, V.M. Shulmeister, Electron transfer by domain movement in cytochrome bc1, *Nature* 392 (1998) 677–684.
- [103] A.R. Crofts, B. Barquera, R.B. Gennis, R. Kuras, M. Guergova-Kuras, E.A. Berry, Mechanism of ubiquinol oxidation by the bc(1) complex: different domains of the quinol binding pocket and their role in the mechanism and binding of inhibitors, *Biochemistry* 38 (1999) 15807–15826.
- [104] C.H. Snyder, E.B. Gutierrez-Cirlos, B.L. Trumpower, Evidence for a concerted mechanism of ubiquinol oxidation by the cytochrome bc1 complex, *J. Biol. Chem.* 275 (2000) 13535–13541.
- [105] J.F. Turens, A. Alexandre, A.L. Lehninger, Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria, *Arch. Biochem. Biophys.* 237 (1985) 408–414.
- [106] M. Ksenzenko, A.A. Konstantinov, G.B. Khomutov, A.N. Tikhonov, E.K. Ruuge, Effect of electron transfer inhibitors on superoxide generation in the cytochrome bc1 site of the mitochondrial respiratory chain, *FEBS Lett.* 155 (1983) 19–24.
- [107] A.A. Starkov, G. Fiskum, Myxothiazol induces H_2O_2 production from mitochondrial respiratory chain, *Biochem. Biophys. Res. Commun.* 281 (2001) 645–650.
- [108] A.A. Starkov, G. Fiskum, Regulation of brain mitochondrial H_2O_2 production by membrane potential and NAD(P)H redox state, *J. Neurochem.* 86 (2003) 1101–1107.
- [109] Y.E. Kushnareva, A.N. Murphy, A.Y. Andreyev, Complex I mediated reactive oxygen species generation: modulation by cytochrome *c* and NAD(P)⁺ oxidation–reduction state, *Biochem. J.* 368 (2002) 545–553.
- [110] A. Herrero, G. Barja, Localization of the site of oxygen radical generation inside the Complex I of heart and nonsynaptic brain mammalian mitochondria, *J. Bioenerg. Biomembr.* 32 (2000) 609–615.
- [111] L.K. Kwong, R.S. Sohal, Substrate and site specificity of hydrogen peroxide generation in mouse mitochondria, *Arch. Biochem. Biophys.* 350 (1998) 118–126.
- [112] Y. Kakinuma, T. Matsubara, T. Hashimoto, N. Sakamoto, Myocardial metabolic markers of total ischemia in vitro, *Nagoya J. Med. Sci.* 57 (1994) 35–42.
- [113] R.J. Wiesner, P. Rosen, M.K. Grieshaber, Pathways of succinate formation and their contribution to improvement of cardiac function in the hypoxic rat heart, *Biochem. Med. Metab. Biol.* 40 (1988) 19–34.
- [114] S.S. Korshunov, V.P. Skulachev, A.A. Starkov, High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria, *FEBS Lett.* 416 (1997) 15–18.
- [115] R.A. Haworth, D.R. Hunter, The Ca^{2+} -induced membrane transition in mitochondria. II. Nature of the Ca^{2+} trigger site, *Arch. Biochem. Biophys.* 195 (1979) 460–467.
- [116] M. Zoratti, I. Szabo, The mitochondrial permeability transition, *Biochim. Biophys. Acta* 1241 (1995) 139–176.
- [117] T. Minamikawa, D.A. Williams, D.N. Bowser, P. Nagley, Mitochondrial permeability transition and swelling can occur reversibly without inducing cell death in intact human cells, *Exp. Cell Res.* 246 (1999) 26–37.
- [118] J.J. Lemasters, A.L. Nieminen, T. Qian, The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy, *Biochim. Biophys. Acta* 1366 (1998) 177–196.
- [119] F. Di Lisa, R. Menabo, M. Canton, M. Barile, P. Bernardi, Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD^+ and is a causative event in the death of myocytes in postischemic reperfusion of the heart, *J. Biol. Chem.* 276 (2001) 2571–2575.
- [120] J. Huser, C.E. Rechenmacher, L.A. Blatter, Imaging the permeability pore transition in single mitochondria, *Biophys. J.* 74 (1998) 2129–2137.
- [121] N. Fournier, G. Ducet, A. Crevat, Action of cyclosporine on mitochondrial calcium fluxes, *J. Bioenerg. Biomembr.* 19 (1987) 297–303.
- [122] E.J. Harris, M. Al-Shaikhly, H. Baum, Stimulation of mitochondrial calcium ion efflux by thiol-specific reagents and by thyroxine. The relationship to adenosine diphosphate retention and to mitochondrial permeability, *Biochem. J.* 182 (1979) 455–464.
- [123] L. He, J.J. Lemasters, Regulated and unregulated mitochondrial permeability transition pores: a new paradigm of pore structure and function? *FEBS Lett.* 512 (2002) 1–7.
- [124] B.S. Kristal, J.M. Dubinsky, Mitochondrial permeability transition in the central nervous system: induction by calcium cycling-dependent and -independent pathways, *J. Neurochem.* 69 (1997) 524–538.
- [125] N. Brustovetsky, J.M. Dubinsky, Limitations of cyclosporin A inhibition of the permeability transition in CNS mitochondria, *J. Neurosci.* 20 (2000) 8229–8237.
- [126] N. Brustovetsky, J.M. Dubinsky, Dual responses of CNS mitochondria to elevated calcium, *J. Neurosci.* 20 (2000) 103–113.
- [127] N.V. Malkevitch, V.I. Dedukhova, R.A. Simonian, V.P. Skulachev, A.A. Starkov, Thyroxine induces cyclosporin A-insensitive, Ca^{2+} -dependent reversible permeability transition pore in rat liver mitochondria, *FEBS Lett.* 412 (1997) 173–178.
- [128] S.A. Novgorodov, T.I. Gudzh, Y.M. Milgrom, G.P. Brierley, The permeability transition in heart mitochondria is regulated synergistically by ADP and cyclosporin A, *J. Biol. Chem.* 267 (1992) 16274–16282.
- [129] S.A. Novgorodov, T.I. Gudzh, G.P. Brierley, D.R. Pfeiffer, Magnesium ion modulates the sensitivity of the mitochondrial permeability transition pore to cyclosporin A and ADP, *Arch. Biochem. Biophys.* 311 (1994) 219–228.
- [130] S. Chalmers, D.G. Nicholls, The relationship between free and total calcium concentrations in the matrix of liver and brain mitochondria, *J. Biol. Chem.* 278 (2003) 19062–19070.
- [131] A. Andreyev, G. Fiskum, Calcium induced release of mitochondrial cytochrome *c* by different mechanisms selective for brain versus liver, *Cell Death Differ.* 6 (1999) 825–832.
- [132] T. Kristian, T.M. Weatherby, T.E. Bates, G. Fiskum, Heterogeneity of the calcium-induced permeability transition in isolated non-synaptic brain mitochondria, *J. Neurochem.* 83 (2002) 1297–1308.
- [133] C. Chinopoulos, A.A. Starkov, G. Fiskum, Cyclosporin A-insensitive permeability transition in brain mitochondria: inhibition by 2-aminoethoxydiphenyl borate, *J. Biol. Chem.* 278 (2003) 27382–27389.
- [134] Y.E. Kushnareva, P.M. Sokolove, Prooxidants open both the mitochondrial permeability transition pore and a low-conductance channel in the inner mitochondrial membrane, *Arch. Biochem. Biophys.* 376 (2000) 377–388.
- [135] E.N. Maciel, A.E. Vercesi, R.F. Castilho, Oxidative stress in Ca^{2+} -induced membrane permeability transition in brain mitochondria, *J. Neurochem.* 79 (2001) 1237–1245.
- [136] M.V. Frantseva, P.L. Carlen, J.L. Perez Velazquez, Dynamics of intracellular calcium and free radical production during ischemia in pyramidal neurons, *Free Radic. Biol. Med.* 31 (2001) 1216–1227.
- [137] A. Starkov, G. Fiskum, Generation of reactive oxygen species by brain mitochondria mediated by alpha-ketoglutarate dehydrogenase, Program No. 194. 17. Washington, DC: Society for Neuroscience, 2002.
- [138] M.F. Anderson, N.R. Sims, The effects of focal ischemia and reperfusion on the glutathione content of mitochondria from rat brain subregions, *J. Neurochem.* 81 (2002) 541–549.