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Probing the molecular mechanisms of neuronal degeneration: importance of mitochondrial dysfunction and calcineurin activation

Hiroyuki Uchino[1], Yasuhiro Kuroda[2], Saori Morota[1,3,4], Go Hirabayashi[1], Nagao Ishii[1], Futoshi Shibasaki[5], Yukiho Ikeda[1], Magnus J. Hansson[3], and Eskil Elmér[3]

[1] Department of Anesthesiology, Hachioji Medical Center, Tokyo Medical University, 1163 Tate-machi, Hachioji, Tokyo 193-0998, Japan
[2] Emergency Medical Center, Kagawa University Hospital, Kagawa, Japan
[3] Laboratory for Experimental Brain Research, Department of Clinical Sciences, Lund University, Lund, Sweden
[4] Laboratory of Cellular Neurobiology, School of Life Science, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan
[5] Department of Molecular Physiology, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

Abstract
Cerebral injury is a critical aspect of the management of patients in intensive care. Pathological conditions induced by cerebral ischemia, hypoxia, head trauma, and seizure activity can result in marked residual impairment of cerebral function. We have investigated the potential mechanisms leading to neuronal cell death in pathological conditions, with the aim of discovering therapeutic targets and methods to minimize neuronal damage resulting from insults directed at the central nervous system (CNS). Over the years, deeper understanding of the mechanisms of neuronal cell death has indeed evolved, enabling clinical critical care management to salvage neurons that are at the brink of degeneration and to support recovery of brain function. However, no substantial breakthrough has been achieved in the quest to develop effective pharmacological neuroprotective therapy directed at tissues of the CNS. The current situation is unacceptable, and preservation of function and protection of the brain from terminal impairment will be a vital medical issue in the twenty-first century. To achieve this goal, it is critical to clarify the key mechanisms leading to neuronal cell death. Here, we discuss the importance of the calcineurin/immunophilin signal transduction pathway and mitochondrial involvement in the detrimental chain of events leading to neuronal degeneration.

Key words Mitochondria · Neuroprotection · Permeability transition · Calcineurin · Cyclophilin · Brain

Introduction

In clinical anesthesiology, pathological conditions induced by cerebral ischemia, hypoxia, head trauma, and seizure activity can result in marked residual impairment of cerebral function. However, no effective pharmacological treatment directed at tissues of the central nervous system (CNS) has been established to prevent these pathological conditions from evolving. Therefore, all aspects of the basic mechanisms responsible for brain damage should be urgently elucidated. Recently, our research has been directed at the involvement and importance of calcium and the calcineurin/immunophilin signal transduction pathway, and we have demonstrated that immunosuppressants display potent neuroprotective effects in several animal models of ischemic brain damage, effects that are believed to be separate from the action of these agents on immune competent cells [1–7]. In this article, we outline the role of increased intracellular calcium in ischemic neuronal cell death, with special emphasis on the calcineurin/immunophilin signal transduction pathway and the so-called mitochondrial permeability transition (MPT), a pathological state of the inner mitochondrial membrane, leading to bioenergetic failure [8–13].

Pathological conditions involving neurodegeneration in the clinical setting

Pathological conditions that involve neuronal degeneration can be broadly divided into several categories: e.g., (i) global ischemia due to an extended period of cardiac arrest [14–16]; (ii) cerebral infarction (focal ischemia) that occurs after the occlusion of cerebral arteries [17]; (iii) direct injuries due to head trauma, cerebral compression associated with hematoma, or cerebral edema [18,19]; (iv) increased intracranial pres-

Address correspondence to: H. Uchino
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sure (ICP), and secondary hypoxic brain damage due to cerebrovascular spasm [20–23]; (v) encephalitis or meningitis caused by viruses, bacteria, parasites, fungi, and spirochetes [24–28]; and (vi) seizures caused by head trauma, cerebral tumor, cerebrovascular disorders, intracranial infections, and abnormal metabolism [29–31]. It is likely that these conditions share many aspects of the pathological mechanisms that result in brain damage and neurological impairment. However, the most crucial mechanisms responsible for brain damage are not yet clear, and the elucidation of the basic mechanisms for each of these conditions is of great importance to be able to develop effective neuroprotective pharmaceutical agents.

Susceptibility to ischemia: selective vulnerability and the penumbra

A prominent feature of neuronal cell death in animal models of transient ischemia (and also other insults such as status epilepticus) is the so-called "selective vulnerability" [32–36]. For a given ischemic insult, with comparable reduction in blood flow, some regions and cells are more vulnerable than others. Another aspect of transient ischemia is the temporal development of injury, a striking phenomenon where the cell death of, e.g., hippocampal CA1 pyramidal cells, striatal cells, or layer III and V cells of the neocortex occurs a few days after an ischemic episode [37]. The nature and extent of cells displaying selective vulnerability and delayed degeneration is entirely dependent on the intensity (the magnitude of blood flow reduction) and duration of the ischemic insult. In addition, in models mimicking embolic stroke, in contrast to models of cardiac arrest, there will be a gradient in the reduction of blood flow—severe close to the occluded vessel, and less severe in regions more distal to the embolus—corresponding to regions supplied with collateral blood flow. The region outside the immediate core of the infarct, the so-called penumbra [38–44], will display features of selective vulnerability and delayed neuronal death. Here, functional recovery can be expected after reperfusion, and this is an important target region for pharmaceutical agents. The penumbra can, due to its blood flow (although restricted), be reached with potential neuroprotective agents that can support the tissue in the precarious balance between life and death.

Induction of ischemic neuronal cell death: the glutamate-Ca^{2+} theory

Discontinuation of aerobic metabolism due to cerebral ischemia provokes immediate loss of energy substrates and promotes anaerobic glycolysis with the accumulation of intracellular lactic acid and H^+, leading to intracerebral acidosis. Further, there will be a loss of energy-dependent ion homeostasis, primarily caused by inhibition of the plasma membrane ATP-dependent Na^+/K^+ exchanger, resulting in an increase in extracellular K^+, as well as intracellular Na^+, leading to cellular depolarization. The ion gradients normally established across the plasma membrane are used, for example, to extrude intracellular Ca^{2+} and for the reuptake of extracellular glutamate. These functions are abolished during ischemia. Moreover, Ca^{2+} influx via voltage-dependent Ca^{2+} channels can contribute to glutamate release from presynaptic terminals to the extracellular space. The excessive release of glutamate further provokes an increase in intracellular Ca^{2+} and Na^+ levels by binding to its postsynaptic receptors (i.e., *N*-methyl D-aspartate [NMDA], and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid [AMPA]), which activates signal transduction pathways mediated by the activation of Ca^{2+}-dependent enzymes including, for example, nitric oxide synthase (NOS), phospholipase A2 (PLA2), and calmodulin kinase, triggering the degradation of lipid membrane components, increased levels of free fatty acids, altered gene expression, phosphorylation and de-phosphorylation of proteins, degradation of proteins and the cytoskeleton, and enzymatic and mitochondrial production of free radicals such as reactive oxygen species (ROS; e.g., superoxide, hydroxyl radical, and H_2O_2) or reactive nitrogen species (RNS; Fig. 1). In addition, the increased intracellular Ca^{2+} levels will trigger mitochondrial dysfunction (described separately below and in Figs. 2 and 3). As a result, the neuronal cellular membranes and organelles will deteriorate, and a downstream cascade of increased Ca^{2+} cycling and Ca^{2+} overload, activation of suicide programs, disturbance of axonal transport, and activation of macrophages by expressed adhesion factors and platelet aggregation, associated with microvascular dysfunction, will lead to unavoidable cell death (Fig. 1).

This glutamate-Ca^{2+} theory of so-called excitotoxic neuronal cell death is widespread [45–47]. According to this theory, the most important aspect of the pathogenesis of cerebral ischemia is substrate and oxygen restriction to the mitochondrial respiratory system and the induction of a cellular ATP crisis. It is the loss of cellular energy and its repercussions (as described above) that trigger acute or delayed neuronal cell death. However, recent analyses of the role played by heart and liver mitochondria in reperfusion injury [48,49] have strongly indicated that, in neuronal cell death, there is a possibility of direct calcium-triggered mitochondrial dysfunction and neuronal cell death associated with the induction of the so-called mitochondrial permeability transition (MPT) in situations of disturbed cellular

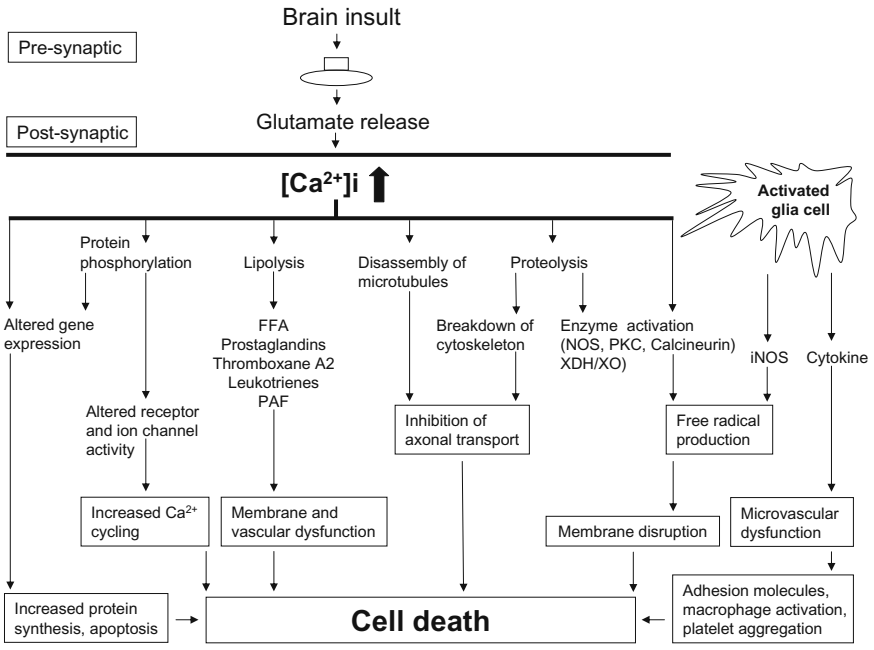

Fig. 1. Excitotoxicity following brain insults. An increase in the intracellular Ca^{2+} (*[Ca²⁺]i*) level (Ca^{2+} overload) is considered to be a key element in the process of neuronal cell death caused by such diverse conditions as cerebral ischemia, hypoxia, head trauma, and seizure activity. Intracellular calcium overload activates signal transduction pathways mediated by activation of Ca^{2+}-dependent enzymes including, for example, nitric oxide synthase (*NOS*), and calmodulin kinase (CaM), triggering degradation of lipid membrane components, increased levels of free fatty acids (*FFA*), altered gene expression, phosphorylation and dephosphorylation of proteins, degradation of proteins and the cytoskeleton and enzymatic systems (e.g., the xanthine system [*XDH/XO*]), and the mitochondrial production of free radicals. In addition, the increased intracellular Ca^{2+} levels will trigger mitochondrial dysfunction (described separately in Figs. 2 and 3). However, the most crucial mechanisms responsible for brain damage are not yet clear, and elucidation of the basic mechanisms is of great importance to be able to develop effective neuroprotective pharmaceutical agents. *PAF*, platelet-activating factor; *iNOS*, inducible isoform of nitric oxide synthase; *PKC*, protein kinase C

energy (lowered levels of adenonucleotides) and increased oxidative stress (Figs. 2 and 3). During the past 10 years we have investigated and characterized the MPT in mitochondria isolated from the CNS, as well as examined the role of inhibitors of this mechanism in in vivo models of brain disease. The MPT is an exciting new putative therapeutic target for intervention in ischemia reperfusion injury [1,3,9,10,12,50–59].

The mitochondrial response and sensitivity to increased intracellular Ca^{2+} ($[Ca^{2+}]i$) levels associated with ischemia-reperfusion

As mentioned above, an increase in the $[Ca^{2+}]i$ level (Ca^{2+} overload) is considered to be significantly involved in the process of neuronal cell death caused by ischemia-reperfusion injury. In addition to loss of energy-dependent Ca^{2+} transport and resulting increases in the $[Ca^{2+}]i$ levels, the activation of PLA2 and the generation of free radicals disturb the function and organization of phospholipids and structural proteins of receptors as well as ion channels, and induce a change in membrane permeability, leading to a persistent $[Ca^{2+}]i$ increase [60–68] (Fig. 1). As previously mentioned, a persistent increase in the $[Ca^{2+}]i$ level will, under a situation of cellular stress, affect the mitochondrial membrane potential ($\Delta\Psi m$) and oxidative phosphorylation, provoking mitochondrial dysfunction and bioenergetic failure—but how does this occur?

Mitochondria electrophoretically and very rapidly take up Ca^{2+} through the so-called calcium uniporter located in the inner membrane, using $\Delta\Psi m$ as the driving force [69] and Ca^{2+} is, in comparison, released slowly through Na^+/Ca^{2+} antiporters linked to an Na^+/H^+ exchange system [70,71]. It should be noted that the accumulation of Ca^{2+} by mitochondria takes precedence over oxidative phosphorylation [72], pointing to its importance in cellular function [73], and the uptake occurs as soon as the intracellular levels reach a certain, very defined, extramitochondrial concentration threshold [74]. In the presence of physiological levels of phosphate, mitochondria accumulating calcium extrude 1 H^+ per Ca^{2+}, leading to enhanced matrix alkalinization. The

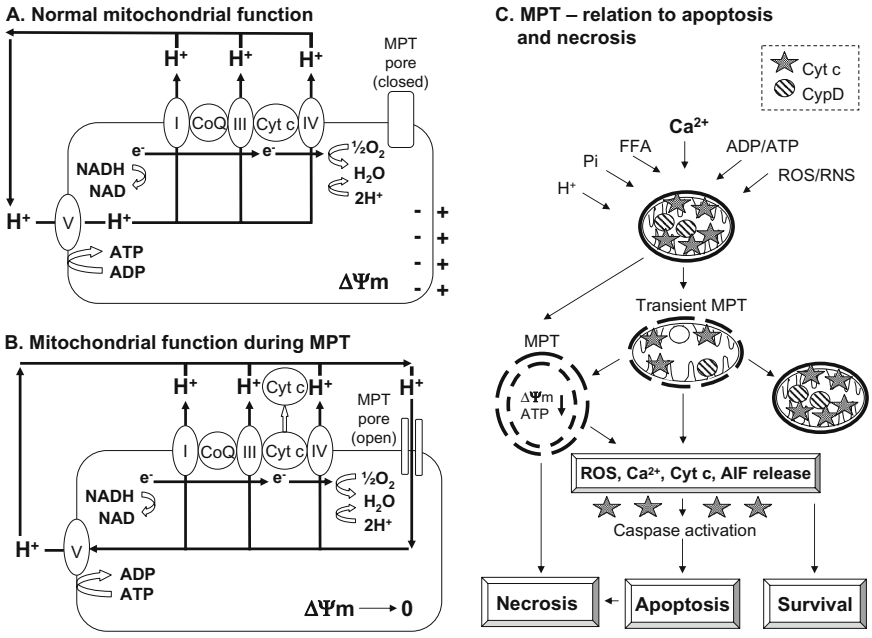

Fig. 2A–C. Bioenergetic consequences of the mitochondrial permeability transition (*MPT*). The consequences of MPT are dramatic when the inner membrane rapidly changes its permeability to solutes of up to 1500 Da. **A** Normal electron transport and the coupled generation of ATP. **B** Following MPT, the proton gradient and mitochondrial membrane potential ($\Delta\Psi m$) are rapidly lost as the extruded hydrogen ions in the electron transport chain rapidly fall back through the MPT pores, uncoupling the oxidation of metabolic substrates and respiration from the phosphorylation of adenonucleotides. **C** The changes in the intracellular environment during, for example, ischemia-reperfusion injury, prolonged seizure activity, traumatic brain injury, and hypoglycemic coma, will increase the risk of MPT activation. The loss of adenonucleotides (*ADP/ATP*), the increase in Ca^{2+}, phosphate levels (*Pi*) and free fatty acids (*FFA*), and the generation of free radicals (reactive oxygen species [*ROS*]/reactive nitrogen species [*RNS*]) are factors that are key elements in sensitizing the MPT to induction. The mitochondrial matrix is dense in proteins, and the induction of MPT will result in mitochondrial swelling and the release of pro-apoptotic proteins, such as cytochrome c (*Cyt c*) and apoptosis-inducing factor (*AIF*). The extent and duration of the brain insult, the resulting Ca^{2+} overload, and the availability of ATP will determine the fate of the cell. *NADH*, Nicotinamide adenine dinucleotide, reduced; *NAD*, nicotinamide adenine dinucleotide; *CypD*, cyclophilin D

alkaline matrix milieu promotes the transport of $H_2PO_4^{2-}$ and two proton dissociations to PO_4^{3-}, which allows for the stable formation of osmotically inactive calcium-phosphate complexes in the form of $Ca_3(PO_4)_2$ [74,75]. The concentration of PO_4^{3-} will vary with the cube of the proton concentration, and free Ca^{2+} will be kept low despite the accumulation of large amounts of total calcium. An alkaline matrix pH is therefore essential in the strict regulation of intramitochondrial free Ca^{2+}, and the pH may determine the total amount of calcium that can be sequestered before the MPT is activated [74]. Supporting the notion of respiration-dependent calcium transport, it has been shown that, in isolated liver mitochondria, the uniporter activity increases exponentially as a function of external calcium, until respiration becomes rate-limiting [76]. In general, three distinct and sometimes sequential types of mitochondrial uptake of calcium can be defined: (i) uptake regulating the activity of tricarboxylic acid enzymes, normally as a response to an increased cellular energetic demand (ii) extensive accumulation and storage of calcium without large influence on mitochondrial function, and (iii) accumulation of calcium with activation of the MPT, causing severe energetic and functional consequences (Fig. 2).

It has been demonstrated that neuronal mitochondria, in the presence of inorganic phosphate (Pi) and physiological levels of adenonucleotides, can buffer very large amounts of calcium [74,77,78] and are highly resistant to calcium-induced MPT induction [77,79], a finding that is reasonable considering the bioenergetic consequences of MPT (Fig. 2). Which are the available (free) cytoplasmic concentrations of adenonucleotides available to interact with and inhibit the MPT (via binding to the adenine nucleotide translocase, see below)? In brain mitochondria, the effect of ADP on the MPT was seen to center around 10 μM, with the maximal effect around 100 μM, indicating that small changes in ADP concentration result in large effects on calcium uptake and MPT sensitivity [79]. It has been argued that 10–100 μM is the physiological range of free ADP in the neuronal cytoplasm [80,81] and that the free ADP level is around 20-fold lower that the measured

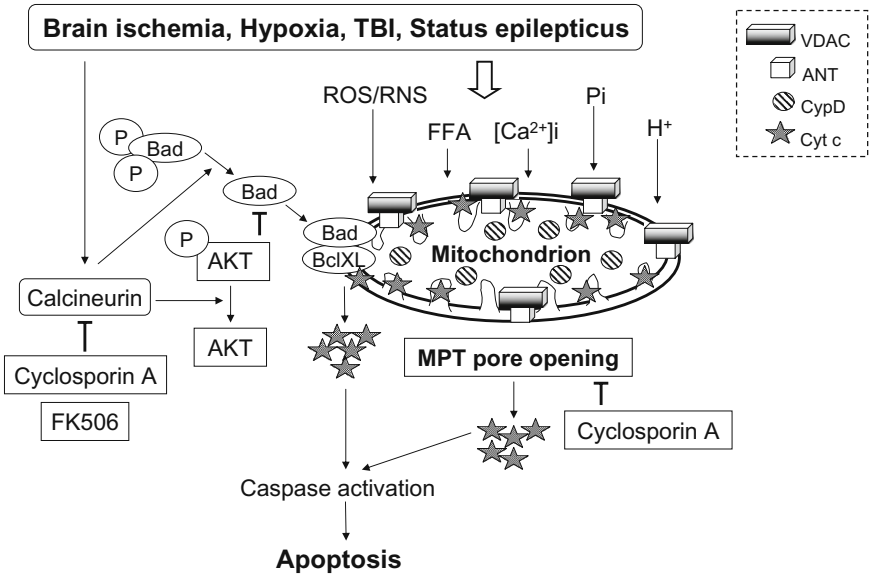

Fig. 3. The role of calcineurin activity in cell death—the mitochondrial connection. Shibasaki et al. [115] have demonstrated interaction between the antiapoptotic Bcl-2-family and calcineurin activity, indicating an important role for calcineurin in the regulation of cell death during apoptosis. Calcineurin specifically participates in a Ca^{2+}-inducible mechanism for apoptosis induction by regulating Bad (a Bcl-2 proapoptotic family member) phosphorylation. Dephosphorylated Bad binds to BclXL (an antiapoptotic Bcl-2 molecule) and thereby facilitates the release of cytochrome c (*Cyt c*) from the mitochondrial intermembrane space. The induction of MPT (the proposed complex of an inner membrane protein (adenine nucleotide translocase [ANT]), an outer membrane component (voltage-dependent anion channel [VDAC]/porin), and a matrix modulator (cyclophilin D [*CypD*] are depicted) will also result in the release of pro-apoptotic proteins, but this occurs by osmotic expansion of the inner mitochondrial membrane and disruption of the outer membrane. The calcineurin and CypD pathways converge on the immunosuppressant cyclosporin-A (which inhibits both pathways), and cyclosporins and other pharmacological agents modulating the activity of either (or both) calcineurin and CypD may finally provide the first class of effective neuroprotective agents for clinical use. *TBI*, traumatic brain injury; *Pi*, inorganic phosphate; *AKT*, protein kinase B or Akt (PKB/Akt), a serine/threonine kinase

ADP content in mitochondria-containing tissues, supporting a role for the MPT in situations of hypoxia or ischemia, where the energetic state is rapidly altered. Cerebral tissue ATP levels have been demonstrated to decline to approximately 10% (from 3 mM to 300 µM) within only 2 min following cardiac arrest in a model of global ischemia. Furthermore, ischemia is associated with a net degradation and further loss of adenine nucleotides as the resultant ADP is rapidly degraded to nucleosides and bases. Supporting a protective role for adenine nucleotides in the MPT, it has been shown that the sensitivity to calcium of mitochondria from different regions of the brain correlated with the susceptibility of these regions to ischemic damage and their content of adenine nucleotides [82,83]. The differences in sensitivity disappeared when the adenonucleotides were depleted with pyrophosphate [82]. Thus, during ischemia and early reperfusion, the physiological restraints on the MPT provided by the high free concentrations of ATP and ADP would be relieved, increasing the probability of pore opening. This dramatic alteration of adenonucleotides will act in concert with other intracellular changes affecting the sensitivity of the MPT to calcium. In other brain insults, such as status epilepticus and traumatic brain injury, increased levels of, for example, free fatty acids and/or ROS may be more important than altered adenonucleotide levels for the facilitated induction of the MPT (Fig. 2C).

The molecular basis of MPT

Mitochondrial permeability transition (MPT) is traditionally considered to be mediated by the formation of an MPT pore, a dynamic complex of several proteins. The protein complex has been proposed to be located at contact sites between the inner and outer mitochondrial membranes, sites important for metabolic regulation and interaction between the cytosol, intermembrane space, and the matrix compartments [84,85]. Parallel to the calcium uniporter, despite rigorous analyses, the accurate molecular constituents are yet to be resolved for the proteins or molecular entities mediating the MPT. A general hypothesis has been that the MPT is formed by the proteins—voltage-dependent anion channel (VDAC or porin)—of the outer membrane, the adenine nucleotide translocase (ANT) of the inner membrane, and cyclophilin D (CypD) located in

the matrix compartment [85]. The core proteins of the MPT pore have also been proposed to attract several other proteins which can regulate the complex, such as the peripheral benzodiazepine receptor (PBR) and hexokinase. To date, in vitro and in vivo pharmacological analyses targeting VDAC, PBR, ANT, and CypD have been conducted, and these studies support the hypothesis that these molecules can participate in or facilitate MPT pore formation [86–88].

Further, analysis of proteins associated with the contact sites, using cerebral mitochondria, has detected VDAC, ANT, hexokinase, and CypD [89], and when these proteins were inserted into liposome systems they formed pores with characteristics similar to those of the MPT [89,90]. On the other hand, it was demonstrated that the formation of a structure similar to the MTP pore was possible even without VDAC or ANT being present [89]. This notion is supported by recent results where gene deletion of ANT or CypD in mice modulated the sensitivity of the MPT but did not prevent its occurrence [54,91–94]. Further, a recent genedeletion study questions the role of VDAC as an essential component and regulator of the MPT [95]. It is likely that the increased permeability of the inner membrane can be accomplished/facilitated by the concerted action of other proteins such as the uncoupling proteins, the Tom/Tim transport system, or the aggregation of misfolded membrane proteins. However, the proposed core components of the MPT pore, in particular ANT and CypD, are the proteins likely to be involved in the MPT phenomenon during calcium overload under pathophysiological conditions.

In summary, the obligate molecular components of the MPT have not been resolved. Initially, there was a hypothesis that the MPT required the complex of an inner membrane protein (ANT), an outer membrane component (VDAC/porin), and a matrix modulator (CypD). However, data are emerging which emphasize that some of these initial components are not obligate in the formation of the calcium-induced increased permeability of the inner mitochondrial membrane, and they can be replaced with other known proteins present in mitochondria, or other proteins yet to be identified [96,97].

The role of MPT in neurodegeneration

The detailed regulation of the MPT has been extensively investigated, predominantly in liver and heart mitochondria [8,12,98,99], and more recently also in mitochondria from the CNS [56,58,74,100–105]. It is clear that many of the changes in the intracellular environment during, for example, ischemia-reperfusion injury, prolonged seizure activity, traumatic brain injury, and hypoglycemic coma, will increase the risk of MPT activation. The loss of adenonucleotides, the increases in calcium, phosphate levels and free fatty acids, and the generation of free radicals are factors that are key elements in sensitizing the MPT to induction (Figs. 2 and 3).

The consequences of MPT are obviously dramatic when the inner membrane rapidly changes its permeability to solutes of up to 1500 Da (Fig. 2). The proton gradient and $\Delta\Psi m$ are rapidly lost as the extruded hydrogen ions in the electron transport chain rapidly fall back through the MPT pores, uncoupling oxidation of metabolic substrates and respiration from the phosphorylation of adenonucleotides. Importantly, the transition, if prolonged, may have consequences in respiration that depend on the substrate being oxidized. MPT induced in mitochondria energized with complex-I linked substrates may be followed by complete respiratory inhibition, due to the loss of pyridine nucleotides [106,107]. Complex II-linked respiration is, rather, followed by uncoupling [108] when MPT is induced. If prolonged and extensive in the cellular population of mitochondria MPT will lead to abolished ATP production and necrotic cell death if the energy balance cannot be compensated by anaerobic metabolism.

The mitochondrial matrix is dense in proteins, and the induction of MPT pores will result in an osmotic influx of water into the matrix, causing the inner membrane to unfold and expand, with mitochondrial swelling, outer membrane rupture, and induction of the release of pro-apoptotic proteins, such as cytochrome c (Cyt c), apoptosis-inducing factor (AIF), omi, and smac (Fig. 2). However, caspase-dependent apoptosis will only be executed to its completion if there is continuous and sufficient ATP produced by remaining mitochondria or anaerobic processes. If not, the cell death pathway will be redirected to the necrotic type. The intracellular energy levels following brain insults are not only hampered by decreased ATP production but also by an increased ATP consumption rate. As described above, the disturbed ion gradients will increase energy-dependent transport across the plasma membrane and, further, the increase in oxidative stress, generated from mitochondria and enzymatic processes in the cytoplasm, will activate, for example, Poly (ADP-ribose) polymerase (PARP). Normally PARP participates in genome repair, DNA replication, and the regulation of transcription [109], and overactivation following brain insults can deplete the cell of nicotinamide adenine dinucleotide $(NAD)^+$ and adenonucleotides, ultimately leading to energy failure, sensitization of MPT to Ca^{2+}, and cell death. Importantly, the loss of $\Delta\Psi m$ caused by MPT will trigger the reversal of complex V (ATP synthase) and convert it to an ATPase, consuming ATP in a futile attempt to uphold the $\Delta\Psi m$ (Fig. 2).

Calcineurin and cell death

Calcineurin was first discovered by Wang et al. [110] in 1976 as an inhibitor of calmodulin (CaM)-dependent cyclic phosphodiesterase. Calcineurin is a serine/threonine phosphatase regulated by Ca^{2+}/CaM and is highly enriched in neural tissue; in fact, it comprises more than 1% of the total protein content in brain tissue [111], pointing to its importance as a regulator of protein phosphorylation, and thereby cellular function, in the CNS. Calcineurin is abundantly distributed in the hippocampus, striatum, and cerebral cortex. Subcellularly, it can primarily be found bound to membrane or cytoskeleton elements and is enriched in postsynaptic densities. The best known aspect of calcineurin function is as a target for the widely used immunosuppressive molecules cyclosporin-A (CsA) and FK506 [112]. In this process, the binding proteins of CsA and FK506, the so-called immunophilins (cyclophilins and FK-binding proteins, respectively) play an important role in the inhibition of calcineurin and in the immunosuppressive effect. CsA or FK506 form complexes with specific immunophilins, causing steric hindrance of the calcineurin catalytic site, inhibiting its activity. Under physiological conditions, the effects of calcineurin are enormously multifaceted, and it can, for example dephosphorylate NMDA receptors, IP3 receptors, and ryanodine receptors, which are all relevant to the regulation of intracellular Ca^{2+} levels. Further, Morioka et al. [113] have indicated that calcineurin can play a role as a Ca^{2+}-buffering protein, and another report suggests that calcineurin exercises neuroprotective effects by promoting the expression of the antioxidant superoxide dismutase (SOD), via nuclear factor (NF)κB after cerebral ischemia [114]. Shibasaki et al. [115] have demonstrated an interaction between the antiapoptotic Bcl-2-family and calcineurin activity, indicating an important role for calcineurin in the regulation of cell death during apoptosis. Further pinpointing the role of calcineurin in apoptosis, they have demonstrated that calcineurin specifically participates in a Ca^{2+}-inducible mechanism for apoptosis induction by regulating Bad (a Bcl-2 proapoptotic family member) phosphorylation [116] (see Fig. 3).

Inhibition of calcineurin activity and the MPT modulator cyclophilin D are distinct and separate key pharmacological targets in the development of neuroprotective agents

The pharmacological inhibition or genetic downregulation of calcineurin activity is clearly neuroprotective [116] and the anti-ischemic effect of calcineurin-inhibiting immunosuppressive agents started to attract attention when Sharkey et al. [117] first reported the effect of FK506 in a rat model of focal ischemia in 1994. The following year, Uchino et al. [5] demonstrated an effect of CsA in forebrain ischemia. Since these initial findings, a bulk of experimental evidence supports a protective role for calcineurin inhibition in the pathogenesis of brain damage in a wide range of disease models (reviewed in [1,13,118]). However, the effect of CsA and FK506 is not restricted to the inhibition of calcineurin, and for CsA this is evident when examining the effects of non-calcineurin inhibiting (non-immunosuppressive) cyclosporins, such as DEBIO-025 (MeAla^{3}EtVal^4 CsA) or NIM811 (MeIle^4 CsA), which retain potent effects on mitochondrial function in vitro [57,119] and are neuroprotective in vivo [120–123]. These amino acid variants of the CsA molecule bind, just like CsA, to intracellular cyclophilins, but the NIM811- and DEBIO-025-cyclophilin complexes do not inhibit calcineurin. The inhibition of mitochondrial CypD, the proposed MPT pore component, as described above, results in decreased sensitivity to calcium-induced MPT (similar to findings in mutant mice with genetic deletion of CypD [54,91–94]). FK506 does not exert any effect on mitochondrial CypD or the MPT phenomenon. The calcineurin and the CypD pathways converge on the immunosuppressant CsA (which inhibits both pathways), and cyclosporins and other pharmacological agents modulating the activity of either (or both) calcineurin and CypD may finally provide the first class of effective neuroprotective agents for clinical use.

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