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The Mitochondrial Permeability Transition as a Target for Neuroprotection

Bruce S. Kristal,[1,2,3,5] Irina G. Stavrovskaya,[1] Malini V. Narayanan,[4] Boris F. Krasnikov,[1,2] Abraham M. Brown,[1,3] M. Flint Beal,[2] and Robert M. Friedlander[4]

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Mitochondria serve as checkpoints and amplifiers on cell death pathways. In the central nervous system, mitochondrial involvement seems essential for normal expression of cell death phenotypes, and interference with these pathways thus seems a reasonable approach to neuroprotection. We have been involved in examining the potential involvement of the mitochondrial permeability transition (mPT) as one of several possible mechanisms by which mitochondria may be drawn into these death cascades. This possibility, though still controversial, is supported by evidence that factors that may stimulate mPT induction are associated with some forms of cell death (e.g., in stroke) and are modulated by diseases of the central nervous system (e.g., Huntington's). Evidence of neuroprotection seen with compounds such as *N*-Met-Val cyclosporine also support this possibility.

KEY WORDS: Mitochondria; permeability transition; neuroprotection; apoptosis; oxidants; zinc; aldehydes; Huntington's disease.

INTRODUCTION

Recognition that mitochondria could release triggers of cell death, and that at least one of these triggers, cytochrome c, was sufficient for the latter stages of cell death, highlighted the potential gatekeeper role of mitochondria in cell death cascades. We focus our work in this area on the mitochondrial permeability transition (mPT), which has been defined primarily based on studies in purified liver and heart mitochondria. The term mPT refers to the opening of pores in the inner mitochondrial membrane that allows free diffusion of all solutes <1.5 kD. Therefore, mPT induction leads to loss of the proton gradient, to inability to conduct oxidative phosphorylation, and to a potentially lethal efflux of mitochondrially sequestered calcium into the cytosol. mPT-like events have been observed in mitochondria isolated from CNS tissues (Kristal and Dubinsky, 1997), and mPT induction also has been experimentally associated with release of cytochrome c, AIF, and SMAC/DIABLO, which are the direct activators of the downstream cascades in both caspase-dependent and -independent cell death. Induction of an mPT has been linked to cytotoxicity following pathological insults, including stroke and excitotoxicity (Friberg and Wieloch, 2002). This said, the involvement of mPT in mitochondria from the CNS remains controversial, and available data suggest both PT-dependent and PT-independent events may be initiated by different pathogenic insults. Consistent with this, data from studies at the level of isolated mitochondria, cells, and intact animal models support the existence of "PT-like" events in the nervous system, but equally compelling data, suggest that "PT-like" events in the nervous system must be significantly different than those in the liver or heart, and that cell death in the CNS may often be "PT-independent" (Andreyev *et al*., 1998; Andreyev and Fiskum, 1999; Berman *et al*., 2000; Berman and Hastings, 1999; Kristal and Dubinsky, 1997; Martinou

[1] Dementia Research Service, Burke Medical Research Institute, 785 Mamaroneck Ave., White Plains, New York 10605.
[2] Department of Neurology and Neuroscience, Weill Medical College of Cornell University, New York.
[3] Department of Biochemistry, Weill Medical College of Cornell University, New York.
[4] Neuroapoptosis Laboratory, Department of Neurosurgery, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts.
[5] To whom correspondence should be addressed; e-mail: bkristal@burke.org.

and Green, 2001). Here, we will focus on some of the pieces of evidence from our work that supports a role for PT in acute neurological injury and in chronic neurodegeneration.

BEYOND CALCIUM AND NON-SPECIFIC ROS—WHAT MODIFIES mPT SUSCEPTIBILITY?

One question about the relevance of mPT for cell death in vivo has followed from acknowledgement that the conditions used to study mPT in vitro, such as the lack of adenine nucleotides, may not translate well to in vivo circumstances. A second question concerns whether there may be disease-specific modifiers of mPT induction. We have been trying to address these issues by examining small molecules that may contribute to mPT induction in specific circumstances. Potential small molecule modulators of the mPT include, among others, Zn^{2+}, ganglioside GD3 (Kristal and Brown, 1999), the reactive aldehydes 4-hydroxyhexenal (HHE, a lipid peroxidation byproduct; Kristal *et al*., 1996), 3,4-dihydroxyphenylacetaldehyde (DOPAL; Kristal *et al*., 2001), peroxynitrite (Packer and Murphy, 1995; Brookes and Darley-Usmar, 2004), and arachidonic acid (Scorrano *et al*., 2001). We have also examined whether changes in physiological status (e.g., Huntington's disease, impaired respiration) may be associated with changes in susceptibility to mPT induction.

Elevation of free intracellular Zn^{2+} has been observed in both heart and brain after ischemia/reperfusion or excitotoxic insults. Koh *et al.* (1996) found a one-to-one correspondence between neurons with elevated Zn^{2+} and markers of cell death following ischemia-reperfusion. Strikingly, in this study, neurons were protected from ischemia-reperfusion injury by chelators of Zn^{2+} but not Ca^{2+}, implicating Zn^{2+} in cell death. These and other studies implicate Zn^{2+} at the cellular and organism level, studies by ourselves and others implicate Zn^{2+} at the mitochondrial level as an mPT inducer and as an inhibitor of α-ketoglutarate dehydrogenase (Brown *et al*., 2000). Induction of an mPT in the presence of Zn^{2+} shows both quantitative and qualitative similarities and differences relative to that involving Ca^{2+}. As two examples, effects of Zn^{2+} rapidly plateau and reverse, whereas those of Ca^{2+} do not. Zn^{2+} is also associated with a rapid, irreversible depolarization.

Monoamine-derived aldehydes have been suggested as possible toxicants in Parkinson's and Alzheimer's diseases. Consistent with a possible involvement of mPT, we had previously shown that hydroxyhexenal (HHE), a cytotoxic lipid peroxidation byproduct, accelerates mPT induction at femtomolar concentrations (Kristal *et al*., 1996). We have demonstrated that 3,4-dihydroxyphenylacetaldehyde (DOPAL), the direct MAO metabolite of dopamine, is more cytotoxic in neuronally differentiated PC12 cells than dopamine and several of its metabolites. DOPAL is also a potent mPT inducer (Kristal *et al*., 2001). These data and others are consistent with a model in which DOPAL-induced mitochondrial damage, including induction of the mPT, contributes to disease progression in PD. Furthermore, DOPEGAL [3,4-dihydroxyphenylglycolaldehyde], the monoamine metabolite of epinephrine and norepinephrine, and a molecule suggested to contribute to Alzheimer's disease, is also a costimulators of the mPT.

Huntington's disease is a chronic progressive neurological disease that may also include systemic manifestations, including wasting. These aspects of the disease are modeled in several available transgenic mouse lines, including the R6/2 mouse. We have shown that the advanced stages of illness in this animal model are associated with an increased susceptibility to induction of the mPT in isolated liver mitochondria. This data has both similarities and differences from that previously presented by others (Panov *et al*., 2002). Increased susceptibility was robustly observed under several different experimental conditions. Comparison with previous work on the mPT in diabetic rodents suggests that the effects observed are not a consequence of the diabetes that occurs in the R6/2 model. Increased susceptibility to mPT induction were independent of alterations in mitochondrial Ca^{2+} transport, endogenous Ca^{2+} load, respiration, or initial mitochondrial membrane potential ($\Delta\Psi$). Additional data obtained are consistent with the existence of a subpopulation of mitochondria that readily or constitutively exhibit the open conformation of the mPT pore in vivo. These data implicate further a systemic role for mutant huntingtin, and provide further evidence for a mitochondrial defect as a consequence of the gene mutation.

Activity of the tricarboxylic acid cycle component *α-ketoglutarate dehydrogenase complex* (KGDHC) is notably decreased in Alzheimer's and in several other neurodegenerative conditions. If this change is causally-linked to disease processes, then it is reasonable to expect that this linkage would be mediated by effects on mitochondrial physiology. In isolated rat forebrain nonsynaptosomal mitochondria, inhibition of KGDHC exerts coincident effects on $\Delta\Psi$, Ca^{2+} transport, and Ca^{2+} retention as well as ruthenium red insensitive, Ca^{2+}-mediated loss of mitochondrial membrane potential. The latter phenomenon is conceptually similar to the changes associated with mPT. In isolated liver mitochondria, a system more amenable to mechanistic evaluation, inhibition of KGDHC facilitated

mPT induction. This facilitation was independent of $\Delta\Psi$ during state 4 respiration, Ca^{2+} transport, and overall oxygen consumption. In contrast, progressive inhibition of respiration mediated by other substrates minimally affected or delayed mPT induction. These data suggest the potential for direct linkages between impaired KGDHC activity and neurodegenerative, in addition to cognitive, changes.

INTERVENTIONS

While this evidence is consistent with induction of an mPT during neurodegeneration and neurological changes, it remains unclear whether mPT is on the causative pathway of cell death, or whether it is simply a downstream effect related to overall cellular collapse, which includes, for example, oxidative damage to overall cellular collapse, which includes, for example, oxidative damage to components of the oxidative phosphorylation system. Although neuroprotection mediated by CsA was initially cited as evidence for causal involvement of mPT in ischemic injury, this is now appreciated to be problematic as CsA also affects calcineurin, the blockade of which itself has been shown to be neuroprotective. Similar "lack of specificity" arguments hold for other compounds, such as minocycline.

Minocycline is a second generation tetracycline antibiotic known to be protective in models of stroke, spinal cord injury, and neonatal hypoxia-reperfusion injury. While our recent work links minocycline to prevention of mPT-mediated release of mitochondrially-sequestered protein factors that facilitate both caspase-dependent and -independent cell death path ways, other actions of minocycline have been identified, and the use of minocycline to build a case for mPT involvement awaits a more mechanistic study of the actions of minocycline. In addition, while minocycline appears to prevent mPT-mediated release of cytochrome c, protection is highly atypical and displays unexpected properties, including an associated loss of mitochondrial membrane potential, an apparently stoichiometric response, and a sharp, biphasic dose response.

Arguably, the best direct test of the hypothesis that mPT lies on the causative pathway of clinically relevant cell death, at least in stroke, comes from the studies using *N*-Met-Val-CysA—a nonimmunosuppressive analog of CsA reputed not to interact with calcineurin. This compound reduces infarct size in a rat model of transient focal ischemia (Friberg and Wieloch, 2002). The universal acceptance of mPT involvement in stroke remains limited however, at least in part, because of the reliance on data from a single drug, and the limited availability and characterization of its analog. Furthermore, even in stoke it appears that availability of CsA is limited by the blood–brain barrier. Thus, there is a need to show that other characterized agents can modulate mPT induction and protect again cerebral infarction, both to answer this central mechanistic question in the pathogenesis of stroke-related neuropathology and to help reduce its clinical effects.

Over the past 2 years, we were 1 of 30 projects re-examining FDA-approved drugs. The purpose of these screens was to identify previously unknown activities of FDA-approved compounds so that these drugs might be moved rapidly into clinical trials to treat previously unexpected conditions. Our assay examined the ability of such compounds to inhibit the mPT. Screening identified a subset of neuroactive medicines, including tricyclics and phenothiazines, as being protective against mPT induction (Stavrovskaya *et al*., 2004). Indeed, some of these medications have been in clinical use since the 1950s, are known to cross the blood–brain barrier, and have been well-tolerated for long-term use, despite their side effects. Initial screens have identified drugs that appear protective at doses approaching those in clinical use. Literature searches reveal data that some of these compounds (e.g., desipramine, trifluoperazine) exert cytoprotective effects in vitro and protect against ischemia-reperfusion in some animal models, supporting the potential for these drugs to be protective against excitotoxic injury. Because the side effects of different tricyclics vary, yet the protection appears mediated via similar mechanisms, it may be possible to use combinations of multiple tricyclics to reduce side effects while strengthening protection.

In summary, the mPT remains a plausible candidate for therapeutic intervention in stroke and other problems of both acute neurotoxicity and chronic neurologic neurodegeneration. Disease specific conditions that facilitate mPT induction may exist.

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