Apoptosis signalling pathways in seizure-induced neuronal death and epilepsy

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Abstract
Delineating the molecular pathways underlying seizure-induced neuronal death may yield novel strategies for brain protection against prolonged or repetitive seizures. Glutamate-mediated excitotoxicity and necrosis is a primary contributing mechanism but seizures also activate programmed (apoptotic) cell death pathways. Apoptosis signalling pathways are typically initiated following perturbation of intracellular organelle function (intrinsic pathway) or by activated cell-surface-expressed death receptors (extrinsic pathway), with signalling cascades orchestrated in part by the Bcl-2 and caspase gene families. In this review, evidence for these pathways from experimental seizure modelling and clinical material from patients with intractable temporal lobe epilepsy is examined. Seizures cause mitochondrial dysfunction and activate intrinsic pathway components including pro-apoptotic Bcl-2 family proteins and caspases, processes that may be partly calcium-induced. The ER (endoplasmic reticulum) has emerged as a major intrinsic pathway trigger for apoptosis and its function may also be compromised following seizures and in epilepsy. The extrinsic, death-receptor-dependent pathway is also rapidly engaged following experimental seizures and in patient brain, supporting a previously unexpected apical role for a calcium-independent pathway. When considered alongside emerging functions of apoptosis-regulatory proteins in non-cell-death processes, including regulating intracellular calcium release and neuronal (re)structuring, apoptosis signalling pathways can be viewed as an important developing focus of research into how to obviate the deleterious impact of seizures on the brain.

Epilepsy and the impact of seizures on the brain
Epilepsy is a common chronic neurological disorder characterized by recurrent seizures. Meldrum and co-workers proved that seizures could kill neurons in the 1970s and work by Olney’s group revealed the central mechanism with the demonstration of the neurotoxic properties of glutamate and its analogues (reviewed in [1]). The damaging consequences of prolonged seizures (status epilepticus) are now recognized both experimentally and in patients, supporting a need for adjunctive neuroprotective treatment after certain seizures [1,2]. Neuroimaging studies also suggest that refractory (drug-resistant) TLE (temporal lobe epilepsy) patients may be at risk of progressive structural and associated cognitive impairments [3]. Whether neuroprotection against seizure damage has additional benefits against epileptogenesis is not clear because epilepsy may develop after a precipitating insult even when the brain is largely neuroprotected [4]. However, both seizure-induced cognitive impairments and epileptic seizure severity are influenced by the extent of damage caused by seizures [5,6].

Mechanisms of cell death after seizures: glutamate and necrosis
Seizure-induced neuronal death results in large part from excitotoxic glutamatergic neurotransmission gating excessive Na+ and Ca2+ entry with resultant osmolytic stress and cell swelling/rupture, free radical production which damages DNA and protease activation leading to proteolysis of cell and organelle membranes, culminating in necrosis [1]. Glutamate receptor antagonists protect against seizure damage but their therapeutic value is unlikely to be realized because of neurotoxic side effects, particularly in the young, and the profound disturbance these drugs impart on normal brain function [2]. Accordingly, alternative approaches to targeting seizure-induced neuronal death are required.

Apoptosis signalling pathways
Experiments in the mid-1990s identified DNA fragmentation patterns in seizure-damaged brain that were known to be a biochemical hallmark of apoptosis. Apoptosis is a morphologically distinct form of cell death characterized by cytoplasmic condensation, preservation and packaging of intracellular organelles, DNA fragmentation, dispersal and phagocytosis of the cell as apoptotic bodies. Apoptosis may be triggered by two main pathways. In the extrinsic pathway, activation of cell-surface-expressed death receptors of the TNF (tumour necrosis factor) superfamily leads to formation of an intracellular complex known as the DISC (death-inducing signalling complex) containing
intracellular molecular adaptors such as FADD (Fas-associated death domain) and caspase 8 or 10 [7]. Caspases are aspartate-specific cysteine proteases present in cells aszymogens that form the active enzyme following proteolytic cleavage. A caspase cascade is initiated that culminates in activation of ‘executioner’ caspases, such as caspase 3, which cleave key intracellular structural and survival proteins and activate the enzyme responsible for the characteristic DNA fragmentation pattern [7].

The intrinsic pathway is triggered following disruption to intracellular organelle homeostasis or DNA damage. Several triggers for this have been identified, including raised intracellular calcium, free radicals and dimerization interactions of Bcl-2 family proteins, pro- and anti-apoptotic regulators of apoptosis that regulate mitochondrial and other intracellular organelles (dys)function [7]. Mitochondrial dysfunction culminates in the release of apoptogenic factors such as cytochrome c, which activates Apaf-1 (apoptotic protease-activating factor 1) and caspase 9, followed by downstream executioner caspases. Caspase-independent mechanisms have also been identified including AIF (apoptosis-inducing factor), a mitochondrial protein that translocates to the nucleus to induce large-scale DNA fragmentation and apoptosis [7].

### Evidence of intrinsic pathway activation by seizures

Our research began with the reasoning that the intrinsic apoptosis pathway might be activated by seizures because in vitro experiments, including those by Jun Chen’s laboratory [8], revealed that brain mitochondria release cytochrome c when exposed to high calcium. Using a rat model of focally evoked short-duration status epileptics we demonstrated cytochrome c release in the damaged hippocampus within 2 h following seizures, whereupon it associated with Apaf-1, commensurate with the appearance of activated caspases 9 and 3 and subsequently DNA fragmentation [9,10]. Corroborating reports of caspase activation after seizures have since emerged from many but not all laboratories [1,7].

While raised intracellular calcium alone may be sufficient to activate the intrinsic pathway, we pursued evidence of Bcl-2 family involvement. Upstream pro-apoptotic BH3 domain (Bcl-2 homology domain 3)-only members BAD (Bcl-2-associated death protein), Bid and Bim (Bcl-2-interacting mediator of cell death), can be activated via calcium-dependent mechanisms and each was found to be activated by seizures in vivo [11–13].

Proving the intrinsic apoptosis pathway was functionally significant initially relied on pharmacological interventions, the specificity of which remains limited. Targeting caspases or pro-apoptotic Bcl-2 proteins reduced seizure-induced neuronal death, albeit not completely [9,10,14]. Genetic approaches in mice offer a more selective means to resolve these issues and ongoing studies in our laboratory with recently developed mouse models are confirming that knockout of certain pro- or anti-apoptotic Bcl-2 family genes significantly alters hippocampal damage after seizures.

### Clinical findings

In parallel with our experimental modelling, we began studies on temporal lobe material surgically obtained from patients with intractable epilepsy. Temporal neocortex from TLE patients expressed raised levels of Bcl-2, Bcl-Xi, and activated caspase 3 [15]. More recent work with hippocampus has corroborated caspase 3 activation and demonstrated altered regulation of the pro-apoptotic Bim gene [13,16]. The nuclear accumulation of caspase-activated DNase (but not AIF) in such tissue implies downstream substrates of caspases are also reached [16]. However, the extent to which these pathways culminate in cell death in patient brain is still at issue because few cells exhibit end-stage DNA fragmentation in such specimens, suggesting concurrent anti-apoptotic processes may also be engaged [13,15,17].

### Intrinsic apoptosis pathways: ER (endoplasmic reticulum)

The ER has recently emerged as a key trigger of the intrinsic pathway, although some of the molecular machinery of this process remains incompletely understood [18]. ER stress is a feature of seizure-damaged brain and we recently reported overexpression of ER chaperone proteins and raised microsomal levels of cleaved caspases 9 and 7 in human TLE hippocampus [17].

### Evidence of extrinsic pathway activation

A role for death receptors and the extrinsic pathway contributing to seizure-induced neuronal death was less anticipated because there is no apparent requirement for calcium in the activation mechanism. Nevertheless, caspase 8 cleavage is an early event after seizures [12]. Studies by our group also determined TNFR1 (TNF receptor 1) is activated and a DISC is present in seizure-damaged rat hippocampus [19,20]. We have since detected similar events in the hippocampus of TLE patients [21]. Caspase 8 inhibition is neuroprotective and also reduces some of the downstream events common to the mitochondrial pathway such as caspase 9 cleavage and cytochrome c release [12].

TNFR1 does not ordinarily trigger apoptosis when activated because of a concomitant induction of survival proteins [22]. However, under conditions of protein synthesis blockade, TNFR1 can trigger apoptosis and so the ER stress in patient hippocampus means TNFR1 activation could be damaging during seizures [17,20]. These provocative data suggest glutamate excitotoxicity is not the only trigger for neuronal death after seizures. Additional effort in our laboratory is underway to tease out the temporal ordering of intrinsic and extrinsic pathways and evaluate redundancy.

### Apoptosis-regulatory protein functions beyond cell death relevant to epilepsy

While we approached the role of these pathways from the perspective of learning their contribution to seizure-induced neuronal death, their significance for epilepsy may be broader...
still. Several apoptosis-regulatory proteins have homeostatic functions in cells that could be important in the setting of epilepsy and seizure susceptibility, including Bax and Bcl-2 which regulate intracellular calcium handling, particular at the ER membrane [23]. Accordingly, altered levels of Bcl-2 family proteins in TLE patient brain may influence a spectrum of intracellular processes including nerve excitability. Caspases may have non-lethal functions in cells, and Asla Pitkanen’s group [24] recently demonstrated active caspase 6 within dendrites during hippocampal remodelling and epileptogenesis in rats. Finally, TNFα has been shown to regulate the surface expression of glutamatergic and GABA (γ-aminobutyric acid) receptors in neurons [25] and the Fas receptor regulates neurite extension and dendritic arborization [26,27].

In summary, apoptosis signalling pathways appear to contribute to the neuronal loss that follows damaging seizures and may also be engaged in involved temporal lobe structures in TLE patients. The adoption of mouse models to target genes of interest in long-term studies of epileptogenesis should further clarify the relevance of these signalling pathways. Finally, novel functions for certain genes in apoptosis signalling pathways mean altered levels or activity may influence seizure susceptibility and neuronal restructuring during epileptogenesis.

References

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