

Molecular Factors of Seizures – Induced Neuronal Cell Death in Epilepsy and Potential Possibility for Neuroprotective Therapy

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Summary

Epilepsy is a chronic brain disorder of various etiology, characterized by recurrent seizures of short duration, associated with a variety of clinical and laboratory manifestations. Apoptosis is a type of specialized physiological cell death, which occurs during development, normal tissue homeostasis or as a result of different cellular insults. Epilepsy is a common brain disorder. Apoptosis is a major barrier to oncogenesis. Status epilepticus and/or repetitive epileptic seizures lead to neuronal loss and neuronal cell death. The neuronal cell death indicates features consisted with both apoptosis and necrosis. The interaction between epilepsy and apoptosis is important to be study.

Introduction

Epilepsy is a chronic brain disorder of various etiology, characterized by recurrent seizures, associated with a variety of clinical and laboratory manifestations. Epilepsy is a common brain disease. At present about 50 millions of people in the world suffer from epilepsy.

After a severe episode of epilepsy, such as status epilepticus* (Status epilepticus consists of recurrent seizures without recovery of consciousness between the seizures or of unusually prolonged epileptic seizure) or repetitive epileptic seizures, neuronal loss has been found within structures such as hippocampus (2, 19, 23, 33, 36) and others (3, 32, 33). Studies have determined that status epilepticus and repetitive epileptic seizures induce a mixed patterns of neuronal cell death that indicate features consisted with both apoptosis and necrosis (3, 10, 11, 16, 28, 34) which are completely different processes of cell death with completely different morphological cell changes (31).

Apoptosis is a type of physiological cell death that occurs during development, normal tissue homeostasis or as a result of different cellular insults (12).

Publications concerning the interaction between apoptosis and epilepsy are few in contrast to study concerning apoptosis and some other medical problems such as neoplasia, Alzheimer disease etc.

It was found that apoptosis may be responsible for a significant component of seizure - induced neuronal death (2, 22, 33). High – power microscopic examination of TUNEL - positive cells (16) confirmed that apoptotic cell death were frequently present (23). Such neuronal loss may cause cognitive dysfunctions, a problem common to patients with poorly managed epileptic seizures and worsen seizures severity in epilepsy (18, 20, 33, 40). It has been unclear whether neuronal loss occurs following infrequent seizures or affects the progression of epilepsy (1, 9, 27, 33, 35).

The mechanisms by which seizures induce neuronal cell death remains incompletely understood (23) Cumulated knowledge of stress- activating pathways and some genes regulating apoptotic death pathways contribute to better understanding of mechanisms of neuronal cell death in the brain structures in result to epileptic seizures. The role of cell death regulatory genes in the neuronal cell death of epilepsy has been explored only recently (16).

It was established that seizures activate multiple apoptotic death pathways (16, 26) involving the members of a family of proteins BIM, BAX, BAD of the Infringing apoptotic pathway, “Death receptors” apoptotic pathway, AIF caspase – independent apoptotic pathway and “Programmed necrosis” pathway (26).

BIM – BAX apoptotic pathway

BIM and BAX are members of a family of proteins called Bcl-2 (B- cell lymphoma 2) family which comprises antiapoptotic and proapoptotic members. Antiapoptotic members include Bcl-2, Bcl-XL, Bcl-w, Mcl-1 and A1 (26). Proapoptotic members are subdivided to BAX subfamily (which includes BIM, BAD, BID, BIK, BMF, PUMA, NOXA and HRK) (26). The balance between proapoptotic and antiapoptotic members of Bcl -2 family determines their effects in seizure-induced brain injury (1, 16). BIM, also known as BOD (17) has been received increasing attention in the setting of epilepsy since its over expression is particularly common during neuronal cell death (33, 38).

BIM expression is controlled by transcription factors of the forehead in rbdomyosarcoma (FKHR) family, including FKHR and FKHR – like – 1 (FKHRL – 1) (26, 33). It was established that seizures induce dephosphorylation of FKHR and FKHRL – 1 factors (33). This lead to its activation and to the upregulation of proapoptotic BIM which interacts with the antiapoptotic Bcl – w neutralizing in this way its effect (29, 33) and triggering BAX activation (26). BAX (Bcl – 2 associated X protein) translocates during apoptosis from the cytosol to the mitochondrion. It is responsible of triggering and release of cytochrome – C, a critical factor in the initiation of cell death pathway originating from the mitochondria (37) and caspase – dependent apoptotic neuronal death (4, 17, 38).

Hippocampal injury following prolonged seizures is associated with FKHR / FKHRL – 1 activation and increased BIM levels, while BIM is down regulated in patients with temporal lobe epilepsy (33). BIM regulation may be one of keys determinant neuronal survival, which suggests that its targeting might prevent neuronal loss after prolonged seizures and maintain epileptic brain in a state less susceptible to further cell loss (33). It was established that activation of protein kinase B, AKT result in cortical neuroprotection and is associated with FKHR inhibition and reduced BIM expression in human temporal lobe epilepsy (16). Although AKT activation contributed to neuronal survival in the cortex following seizures (16) the mechanism of this protection is yet unknown (33). The resistance of the cortex to seizures – induced neuronal death may reside with differences in neuroanatomy, neurotransmitter receptor expression and cell death modulatory pathways in addition to AKT (16).

BAD – BAX apoptotic pathway

Proapoptotic BAD normally resides in an inactive state complexed with the chaperone proteins of the 14-3-3 family (23). Another ptoapoptotic Bcl-2 family member BAX resides in an inactive complex with antiapoptotic Bcl-xl (16). Following epileptic seizures and calcium (Ca 2+) entry via NMDA receptors in the cell the phosphatase calcineurin is activated (23). Calcineurin than dephosphorylates BAD which is released from 14-3-3 and interact with the BAX / Bcl-xl complex (23). BAD displaces BAX resulting in BAD/Bcl-xl dimmer (16). Released BAX than translocates to mitochondria where it triggers cytochrome –C release and caspase cascade activation leading to apoptosis (23).

BAD – BAX apoptotic pathway is most extensive within the hippocampus whereas cortex is primarily spared, because of prosurvival response, such as Akt phosphorylation (16). Phosphorylation Akt may inhibit BAD and in this way may protect neurons from cell death (8). It was found that calcineurin inhibitor FK 506 may interrupt the BAD – BAX pathway resulting in neuroprotection (5, 24). It is evident that BAX is integral of both BID – BAX death pathway and BAD – BAX death pathway to trigger the release of cytochrome –C from the mitochondria.

When cytochrome–C is released into the cytoplasm it binds to an adaptor protein Apaf – 1 (apoptotic protease – activating factor 1) and to the presence of procaspase – 9 to form a complex “apoptosome” (1). In the presence of ATP caspase - 9 is activated which then activates caspase – 3 (activated also from caspase – 8). This leads to widespread activation of other caspases, which activate each other through multiple feedback loops forming in this way caspase cascade and provoking cell death (26).

“Dead receptors” apoptotic pathway

This apoptotic pathway which does not require mitochondrial participation involves engagement of particular “dead receptors” that belong to the tumor necrosis factor receptors (TNF-R) family (14). The cell – surface receptor Fas, a member of the TNF –R family of receptors, is a key component of the extrinsic pathway (25).

Expression of Fas and FADD, components of death receptor signaling is increased following seizures (15). Activated TNFR -1 and Fas receptors then induce activation of catalytic enzymes caspase -8 and caspase -3, which in turn induce apoptotic neuronal death (14). Caspase -8 and caspase -3 are members of the great caspase family of the cell death effector proteases (6).

Apoptosis has been conceptually tied to the activation of catabolic hydrolases in two waves (6). During the 80’s it was generally assumed that DN-ases were responsible for execution of the cell (6). When this notion turns out to be wrong, during the 90’s most investigators become convinced that caspases were responsible for cell death (6). Of the 14 identified members of caspase family caspase – 8 is a key initiator of apoptosis and caspase -3 (Apopain or Yama) has been shown to be main executioner (15, 16). It was also established that caspase -8 activates directly caspase -3 (6, 14). There is a cross – talk between the extrinsic and intrinsic pathways (26). The proapoptotic BID is a link between these pathways (14). Caspase -8 cleaves and activates BID which in turn activates BAX and BAK to initiate mitochondrial events leading to “apoptosome” formation and activation of caspase -9 and caspase -3 (14). It was also found that cytochrome –C release may activate caspase -8 (21). These data support the role for caspase -8 and caspase -3 in mediating seizure – induced neuronal death and suggest that therapeutic treatments targeted at the caspases may prove useful as an adjunct to anticonvulsant therapy in human epileptics (15).

AIF Caspases – independent pathway

AIF- caspases - independent pathway does not require caspase activation (6). AIF (apoptosis inducing factor) is a major factor determining caspase-independent neuronal death (6). Evidence that AIF induces caspase – independent cell death is based on experiments in which caspase activation is suppressed by the addition of caspase inhibitors (6). AIF is a phylogenetically old flavoprotein which in healthy cells is confined to the mitochondrial intermembrane space. Upon epileptic seizures AIF translocates via the cytosol to the nucleus where it binds to DNA and provokes caspase - independent chromatin condensation (6). It was found that AIF may also produce a necrotic appearance (40). There are also evidences of cross – talk between AIF and caspase cascade at several levels (6). AIF and caspase may thus cooperate in the cell death (6).

The Bcl-2 family members regulate the release of AIF (13). Proapoptotic Bcl-2 family proteins BAX, BID and BAK, which participate in the apoptotic permeabilization of the mitochondrial membranes, directly trigger the release of cytochrome *c* and AIF (7). BIM, BAD and BAX were found to be a deadly combination in epileptic seizures (26). The role of BAX is particularly important (13).

All data do suggest incorporation of neuroprotective strategies targeting both the Bcl-2 family members and AIF which may have potential clinical applications.

Programmed necrosis pathway

It is well known that seizures activity increases intracellular calcium (Ca^{2+}) entry to neurons (23). The functional significance of this pathway is based on the fact that epileptic seizures deplete energy reserves, leading to mitochondrial calcium overloading and opening of the mitochondrial transition pores and / or mitochondrial swelling and rupture of the outer membrane (26). Subsequent cytochrome *c* release activates the caspase cascade and leads to neuronal death, which is characterized by a predominantly necrotic morphology (10, 11, 26).

Neuronal death pathways list may be extended by other death pathways with the participation of endonuclease G, cathepsins, Smac/Diablo and others, but at present our understanding of these pathways is at its infancy stage and merits further investigations to substantiate links between findings in experiment seizure models and brain injury.

Conclusion

Understanding of neuronal death pathways is a critical precondition for new therapeutic strategies. Appropriate neuroprotective medication concerning neuronal death pathways may be able to modify epileptogenesis and reduce seizures – induced brain injury in epileptic patients.

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