Chapter 6
Intracellular Pathways Associated with Neuronal Survival and Death in Epilepsy

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Abstract Epilepsy has been characterized a disease whose social and occupational behavioural has had devastating economical consequences and is associated with great cumulative brain damage and neurological deficits. From different forms of epilepsy, the most frequent type is temporal lobe epilepsy (TLE), being the most common form of drug refractory epilepsy. Although there are a great amount of studies about the mechanisms involved in neuronal damage and death during critical phases of epileptogenesis, it is crucial to construct strategies for neuroprotection that may prevent the development of epilepsy. In this chapter, some molecular mechanisms involved in the neuronal death, which are induced by excitotoxicity phenomena following the signalling pathways activation and studied in animal models under seizure conditions or expressed in the epilepsy are discussed, mainly those as the mitogen-activated protein kinases, Jak/Stat, and Pi3k/Akt pathways those genes responsible to participate in the apoptosis and cell cycle regulation are also analysed.

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L. Rocha and E.A. Cavalheiro (eds.), *Pharmacoresistance in Epilepsy: From Genes and Molecules to Promising Therapies*, DOI 10.1007/978-1-4614-6464-8_6,
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In summary, the structural and molecular changes at cellular level are believed to play a key role in the generation of convulsive seizures and its possible identification should facilitate the develop of potential therapeutic targets heading towards of specific genes, proteins, and signalling pathways altered during the different stages of epileptogenesis process.

Keywords TLE • Epileptogenesis • Apoptosis • Excitotoxicity • Cell death • Cell damage • Intrinsic pathway • Extrinsic pathway

6.1 Introduction

Epilepsy has devastating behavioural, social, and occupational consequences and is associated with cumulative brain damage and neurological deficits. In addition, it is characterized by the occurrence of repeated and sudden transitory episodes of motor, sensory, autonomic, and physical origin known as seizures, which at the cellular level are characterized by synchronized discharges of large groups of neurons that interfere their functions. Temporal lobe epilepsy (TLE) is the most common form of partial epilepsy and affects 40% of the patients. Seizures arising from the mesial temporal lobe structures (i.e., amygdala and hippocampus) can be progressive and often becomes refractory to drug treatment. It is characterized by the presence of complex partial seizures and generalized tendency to produce multiple epileptic foci. One of the most common histologic abnormalities observed in approximately 66% of patients with TLE is hippocampal sclerosis or mesial temporal sclerosis, characterized by a remarkable loss of neurons in the hippocampus leading to excessive glial proliferation, particularly in the hilar region of the dentate gyrus and the CA1 and CA3 regions (Thom et al. 2005). The majority of the patients with TLE suffer from symptomatic focal epilepsies, which are frequently a consequence of brain trauma, complicated febrile convulsions, prolonged seizures (*status epilepticus*—SE), ischemic lesions and brain tumours, encephalitis or childhood febrile seizures (Cendes 2002; Engel 2001; French et al. 2004). It has been established that each of these initial events leads to the activation of molecular signalling cascades, which in turn induce selective cell death that is directly related to the epileptogenic process, although even now it is not well known if cell death is the cause or effect of the establishment of the phenomenon of epilepsy. Hippocampal sclerosis (HS), also known as Ammon's horn sclerosis, is characterized by the loss of pyramidal cells and gliosis in CA1 (Ammon's horn) and end folium, dispersion of the granule cell layer of the dentate gyrus (DG), neurogenesis of granule cells, axonal sprouting, and synaptic reorganization of the mossy fibres (Wieser 2004; Thom et al. 2005). Cell loss is typically asymmetric between the hippocampus; the most affected regions are the CA1 and CA3 subfields and hilar region of the DG, while the CA2 subfield and granule cells of the DG usually show much less cell loss (Mathern et al. 1997). In spite of damage to other limbic regions, the cerebellum and cerebral cortex are also commonly affected.

TLE represents the final stage of a long and complex process of cellular and molecular events that are determined by the initial stimulus that triggers the process. There is usually a latent period of several years between this injury and the emergence of the chronic TLE characterized by spontaneous recurrent seizures originating from the temporal lobe, as well as learning and memory impairments (Bartolomei et al. 2005; Detour et al. 2005; Devinski 2004).

The TLE can be reproduced in laboratory animals (typically rodents) by the systemic or intracerebral administration of powerful convulsant agents such as glutamatergic (kainic acid) or cholinergic (pilocarpine) agonists (Pitkänen et al. 2005; Covolan et al. 2000). Over the last few decades, there has been considerable progress in the pharmacotherapy of epilepsy, including the introduction of several new antiepileptic drugs (AEDs) (McCabe 2000). The mechanisms of action of most clinically used drugs in human epilepsies are based upon the synchronized neuronal activity and imbalance between inhibitory and excitatory neurotransmission, events commonly linked to the pathogenesis of epilepsy (Dalby and Mody 2001). However, approximately 30–40% of all patients with TLE are estimated to be drug resistant, therefore identification of specific biological processes and biochemical pathways that trigger cell death during critical phases of epileptogenesis is crucial to design strategies for neuroprotection that may prevent epileptogenesis process.

6.2 Epileptogenesis and Animal Models

The term epileptogenesis refers to the transformation through the normal process of the plastic neuronal network into a chronically hyperexcitable state. The epileptogenic processes emerge after precipitating insults (i.e., local infections, *SE*, ischemia, or trauma) in concert with genetic susceptibility factors, which they are possible to trigger such persistent pathophysiological changes (Coulter and DeLorenzo 1999). However, the study of human epileptic hippocampus does not allow revealing the sequence of events leading to neuronal loss and the regulation of plastic events. For this reason, different animal models have been designed using electrical or drug stimulation, among which include systemic administration of kainic acid, an analogue of glutamate, or cholinergic agonist-pilocarpine (Pitkänen et al. 2005). Both experimental models and postmortem human studies support the idea that cell death is a common pathological feature of insult to the brain, which triggers a chronic epileptic condition (Sutula 2004). Briefly, such injuries invariably set in signal various cell and molecular processes including gliosis, inflammation and vascular changes, neurogenesis and rewiring, axonal reorganization, dispersion of granule cells, and changes in expression of ion channels and signalling molecules including neuronal death. Collectively, this process is identified as epileptogenesis (Blumcke et al. 1999; Mathern et al. 1993; Sloviter 1999).

In the animal models of TLE, the damage within the hippocampus precedes the appearance of spontaneous seizures. Moreover *SE* induced by systemic injection of pilocarpine or kainic acid or by repeated electrical stimulation-caused structural

brain damage in rats (Sloviter 2008). Cell loss has been observed in these models in the hilus and CA3 regions, as well as amygdala and entorhinal cortex (Turski et al. 1989; Bartolomei et al. 2005). Moreover, prominent mossy fibre sprouting occurred. The primary cause of neuronal death following seizures is probably over-activation of ion channels gated by glutamate, the principal excitatory neurotransmitter in the brain (Meldrum 1991; Fujikawa 2006). In these conditions, biological processes, including activation of signalling pathways related to stress response, ion transport, signal transduction, and synaptic transmission are triggered (Aronica and Gorter 2007).

6.3 Mechanisms of Cell Death

6.3.1 Excitotoxicity

The *excitotoxicity* is a pathological process into the cells. The pioneering work of Meldrum (1993) provided evidence that seizure-induced cell death and other events that induce neurodegeneration result from over-activation of ionotropic glutamate receptors which leads to increased intracellular levels of Ca^{2+} and Na^{+} and causes swelling and cell lysis. There is also energy failure, production of free radicals, activation of enzymatic complex, and cell death (Smolders et al. 2009). The death of cells has been classified generally into two distinct types: apoptosis and necrosis. Both forms of cell death can be induced by excitotoxicity. It has been shown that the excitotoxic damage induced by seizures activates programmed cell death pathways through changes in the expression of specific genes (Engel and Henshall 2009).

6.3.2 Apoptotic Pathways

Apoptotic signals are given through a highly ordered molecular cascade that is energy dependent. Several different stimuli can initiate the apoptotic death of neurons. Complex intracellular and intercellular cell-death-regulatory pathways are increasingly recognized as important contributors to seizure-induced neuronal death; however, apoptotic pathways converge on a restricted number of common effector (Sastry and Rao 2000; Engel and Henshall 2009). Two principal pathways have been described as apoptotic death. These two signalling pathways and the final caspase executor activation pathway are also regulated by several proteins such as glycogen synthase kinase (GSK3), ataxia-telangiectasia-mutated protein (ATM)/p53, Bcl-2, cyclin-dependent kinases (CDKs), and mitogen-activated protein kinases (MAPKs), which act on both pathways (Wang et al. 2007; Kroemer et al. 2007).

There are two main cell-signalling pathways that have been identified in the control of apoptosis: the intrinsic pathway, or core pathway, and the extrinsic pathway.

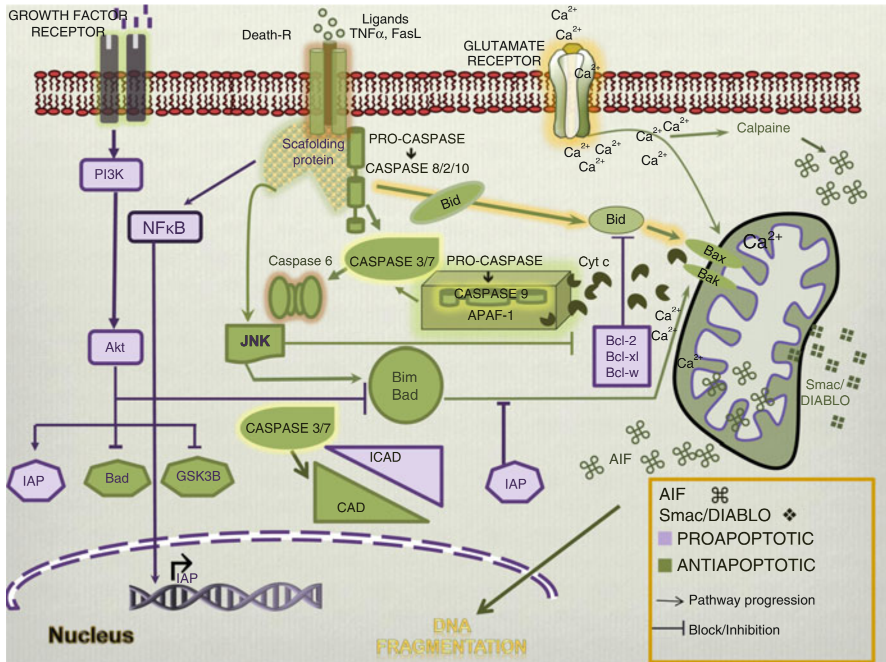

Fig. 6.1 Major apoptosis pathways activated by seizures. Both the excitotoxicity which may directly trigger mitochondrial dysfunction or activate enzymatic pathways (e.g., mitogen activated protein kinases-MAPK) and the pathway activated by death receptors (DR) converge in the activation of modulators (e.g., inhibitor of caspase-activated DNase-ICAD) and executors proteins of death process (e.g., caspases). Other pathways may compensate these processes (e.g., pathway mediated by Growth-factor receptor or cytokines) promoting activity or expression (e.g., inhibitors of apoptosis proteins-IAP) of anti-apoptotic proteins or decreasing their activity (e.g., Bad and glycogen synthase kinase-3β (GSK-3β))

The extrinsic pathway mediates cell death in response to extracellular stimuli and is initiated by cell-surface receptors called death receptors (DR) of the tumour necrosis factor (TNF) superfamily (Wajant 2002). Ligands for these receptors include TNFα, Fas ligand, and TNF receptor apoptosis-inducing ligand (TRAIL). Activation follows binding of the ligand to its receptor and oligomerization of the receptor. In the case of Fas, the cell death signal is propagated inside the cell by recruitment of Fas-Associated protein with Death Domain (FADD) and an initiator caspase (e.g., 8 or 10 caspases) to the intracellular side of the plasma membrane, resulting in formation of a death-inducing signalling complex (DISC). In contrast, activation of TNFR1 leads to direct association with TNF receptor-associated death domain (TRADD); the recruited to this complex can then modulate the nuclear factor-κB pathway (Fig. 6.1) (Wajant 2002; Strasser et al. 2000).

Extrinsic pathway activation after seizures is documented in various seizure models where the presence or activity for 2 and 8 caspases has been reported (Henshall et al. 2001a, b). While death receptors are constitutively expressed in the

brain, their participation has been linked to the activity of caspase 8 observed after seizures through scaffolding proteins and receptors for TNFα and FasL, both present in the adult rat hippocampus before and after seizures (Shinoda et al. 2003; Henshall et al. 2001a, b).

On the other hand, *an intrinsic pathway* that is associated to apoptosis is regulated by mitochondria, which integrates a lethal or pro-survival signal that eventually determines the cell density. It is initiated after cell stressors that perturb intracellular organelle function (Danial and Korsmeyer 2004; Xu et al. 2005) and include raised intracellular Ca^{2+} and reactive oxygen species (ROS) (Orrenius et al. 2003). Mitochondrial apoptosis in neurons can be triggered by a variety of structurally related agents (Sastry and Rao 2000). A key event in this pathway is the release of apoptogenic molecules from mitochondria, which is caused by a change in permeability of the outer mitochondrial membrane and the release of molecules from mitochondria, and in particular *cytochrome c* (*cyt c*), binds the apoptotic protease, activating factor 1 (Apaf1) and recruiting caspase 9. This forms the so-called apoptosome, which processes downstream effector caspases such as caspase 3, culminating in cleavage of various structural and other proteins (Fig. 6.1) (Bratton and Salvesen 2010).

The permeability of the mitochondrial outer membrane (MOMP) is regulated by the activity of several proteins that belong to the Bcl2 family. The Bcl-2 gene family comprises more than 20 different members that either positively or negatively regulate apoptosis primarily by affecting the mitochondria (Cory and Adams 2002; Liou et al. 2003; Kroemer et al 2007). The proteins of this family with anti-apoptotic function include Bcl-2, Bcl-X_L, and Mcl-1, which preserve the integrity of the outer mitochondrial membrane. The major pro-apoptotic proteins here include Bad, Bid, Bik, Bim, Noxa, p53-upregulated mediator of apoptosis (PUMA), Bax, and Bak. Interestingly, Bid protein constitutes one link between the extrinsic and intrinsic pathways through the cleavage of caspase-8, which further amplifies the apoptotic death signal. Bid interacts with Bax–Bak, which forms pores that allow the release of *cyt c* (Cory and Adams 2002). The anti-apoptotic Bcl-2 and Bcl-X_L proteins can prevent Bax translocation towards the mitochondria, but additionally Bcl-X_L may bind to Apaf-1 and in doing so suppresses caspase-9 activation (Fig. 6.1) (Kroemer et al. 2007; Stavrovskaya and Kristal 2005).

As previously mentioned, ROS production is another common stressor factor that is triggered by excitotoxicity. One target of ROS is DNA, which is extremely sensitive to oxidative stress. One of the sensors of DNA damage is the ATM, which belongs to the family of phosphatidylinositol-3 kinases (PI3K) (Roos and Kaina 2006; Chipuk and Green 2009). Once activated, ATM stimulates p53 (a nuclear transcription factor). Thus, DNA damage and the subsequent p53 activation; both contribute to other apoptotic signals that the mitochondria receive through the intrinsic pathway. In fact, if the neuronal DNA damage cannot be repaired, overactivation of p53 triggers the neuronal apoptotic process. In a second step, p53 mainly induces the expression of the pro-apoptotic Bcl-2 family members PUMA (Roos and Kaina 2006; Chipuk and Green 2009). Noxa is another p53-activated mediator that can contribute to apoptosis. Interestingly, PUMA activates the

intrinsic apoptotic pathway by binding to Bax, which acts directly on the mitochondria (Chipuk and Green 2009). In addition, PUMA can bind with and consequently inhibit anti-apoptotic Bcl-2 family members, including Bcl-2, Bcl-X_L, Bcl-w, and Mcl-1 (Fig. 6.1) (Roos and Kaina 2006). A tight balance exists between the activities of pro- and anti-apoptotic Bcl-2 family members in resting conditions. The cell's fate will progress to apoptosis only when this equilibrium is altered toward enhanced activity of pro-apoptotic proteins.

While both extrinsic and intrinsic pathways have different beginnings, they eventually converge in the massive activation of catabolic enzymes (including a class proteases known as caspases, no caspases proteases, lipases, and endonucleases); at present there are 14 known mammalian caspases (named from cysteinyl-aspartate-specific proteases) that are enzymes that cleave other proteins next to an aspartate residue. The apoptosis regulatory caspases are divided into initiators of apoptosis that include caspases 8, 9, and 10, and the apoptotic executioners are caspases 3, 6, and 7 (Schindler et al. 2006; Bozzi et al. 2011). Each caspase is initially synthesized as a zymogen and requires processing at specific cleavage sites to generate the active enzymes. The caspases that are the first to be activated trigger downstream other caspases giving rise to a proteolytic cascade that culminates in the execution of apoptosis. Different subsets of caspases are activated depending on the pro-apoptotic stimulus (Salvesen and Riedl 2007). For example, caspases 3, 6, and 8 are part of the Fas/TNFα-mediated death pathway, while caspases 3 and 9 together with apoptosis protease-activated factor 1 (Apaf1) and *cyt c* participate in mitochondria-associated cell death (see Fig. 6.1) (Bratton and Salvesen 2010).

Activation of intrinsic, mitochondria-dependent cell death pathways after seizures would be predicted based on the assumed significance of glutamate excitotoxicity and mitochondrial dysfunction due to both calcium (Ca^{2+}) and ROS loading (Orrenius et al. 2003). Several authors have observed that neuroprotection is also less pronounced when mitochondrial-activated caspase-9 is blocked after seizures, and other data suggests the extrinsic cell death pathway-associated caspase 8 is activated following seizures in vitro (Henshall et al. 2001b, c; Meller et al. 2006).

6.3.3 Apoptosis and Cell Cycle Regulation

The biochemical mechanisms of the different phases of the cell cycle are highly regulated by intracellular signalling elements such as protein kinases (e.g., MAPKs) as well as their target substrates in particular cell cycle regulators. A family of cyclins act as regulatory subunits for CDKs, and thus regulate passage through the four phases of the cell cycle. The activities of the various cyclin/CDK complexes regulate the progression through G1/S/G2/M phases of the cell cycle (Nigg 1995). MAPKs are involved in regulating the protein expression of cell cycle regulators; in particular those that regulate passage of cells of phase G_0 to G_1 (Yeste-Velasco et al. 2009).

In particular, the differentiated neurons are post-mitotic cells and completely lacking in replicative capability. These cells enter a phase of mitotic quiescence commonly referred to as the Go phase, and as such were believed to be unable to re-enter the cell cycle. Postmortem studies have revealed pathological evidence of aberrant cell cycle re-entry occurring in neurons of patients with Alzheimer's disease (Yang et al. 2001, 2003), epilepsy (Nagy and Esiri 1998), and Parkinson's disease (Jordan-Sciutto et al. 2003). Moreover, it has been observed experimentally that cell cycle regulators such as CDKs are produced and abnormally activated in different models of induction of cell damage (e.g., ischemia, epilepsy, excitotoxicity, and trauma) (Timsit and Menn 2007; Sutula 2004). The activation of these events leads to cell death. Various markers of this event have been detected before neuronal death occurs suggesting its participation as an initiator of the execution of the cell death program (Katchanov et al. 2001; Timsit and Menn 2007). The atypical expression of mitogenic genes may promote entry and progression of neurons into the cell cycle through an increase in the expression level of cyclin D and phosphorylation of the retinoblastome protein (Rb), regulating the E2F activity which induces modifications to the transcription of pro-apoptotic molecules as caspases 3, 8, and 9, as well as Apaf-1 or members of the Bcl-2 family (Greene et al. 2004).

Although few studies have evaluated the role of cell cycle regulators in epilepsy, there is enough evidence to link changes in the expression and activity of these molecules in epileptogenesis. A study following kainate-induced seizures showed that the cyclin D1 mRNA was induced in the vulnerable CA3 region, and to a lesser extent, in non-vulnerable regions, while that the expression of CDK4 and cyclin D1 was upregulated in neurons of the rat piriform cortex and amygdala 1–3 days after KA administration in vivo. CDK4 and cyclin D1 proteins were induced in the cytoplasm and nuclei of neurons, with a concomitant increase of CDK4- and cyclin D1-positive microglia in the affected areas; these results suggest that CDK4 and cyclin D1 are essential for KA-induced neuronal apoptosis *in vivo* (Timsit and Menn 2007; Ino and Chiba 2001).

6.4 Signal Pathways in Survival or Cell Damage

Epilepsy activates several signalling cascades that are essential to regulate the survival or cell damage, which are evoked by multiple stimuli, including excitotoxicity, oxidative stress, and inflammation processes (Henshall and Murphy 2008; Okamoto et al. 2010). Particularly the inflammatory processes, including activation microglia and astrocytes and production of proinflammatory cytokines and related molecules, have been described in human epilepsy patients as well as in experimental models of epilepsy (Vezzani et al. 2008). A number of proinflammatory mediators, thus initiating a cascade of processes in brain tissue, alter neuronal excitability and affect the physiological functions of glia by paracrine or autocrine actions, thus interfering with the neuronal communications and may compromise neuronal survival (Riazi et al. 2010; Vezzani et al. 2008). Chronic brain inflammation may also contribute to susceptibility to seizures and comorbidity in chronic epilepsy patients. Prototypical inflammatory cytokines such as interleukine-1β (IL-1β), TNF-alpha,

and interleukine-6 (IL-6) are over-expressed in experimental models of seizures in brain areas of seizure generation and propagation, and are prominent in glia, and to a lesser extent by neurons. Cytokines receptors are also upregulated, and the related intracellular signalling is activated in both cell populations highlighting autocrine and paracrine actions of cytokines in the brain (Riazi et al. 2010; Vezzani et al 2008). The recent demonstration of functional interactions between cytokines and classical neurotransmitters such as glutamate and gamma amino butyric acid (GABA), as well as intracellular signalling mechanisms, suggest the possibility that these interactions underlie the cytokine-mediated changes in neuronal excitability, thus promoting seizure phenomena and the associated neuropathology (Balosso et al. 2008, 2009; Stellwagen et al. 2005; Pickering et al. 2005).

6.4.1 Protein Kinases Activated by Mitogen

Extracellular stimuli evoked by neurotransmitters, neurotrophins, and growth factors in the brain regulate critical cellular events, including synaptic transmission, neuronal plasticity, morphological differentiation, and survival. A pathway known to influence seizure-induced neuronal damage and epileptogenesis includes the MAPK cascades (Liou et al. 2003; Shinoda et al. 2003). The MAPK pathways are used by eukaryotic cells for the transduction of extracellular signals to the nucleus and other intracellular targets (Chang and Karin 2001).

There are two known major pathways of MAPK including the extracellular signal-regulated kinases (ERK) and stress-activated protein kinases (SAPK). The latter is divided into the kinases c-Jun NH_2-terminal (JNK/SAPK) and p38 kinase pathway (p38/SAPK) (Pearson et al. 2001; Okuno et al. 2004). The MAPKs are also involved in apoptosis and may, therefore, play a role in neurodegeneration (Borsello and Forloni 2007; Guan et al. 2006; Kyosseva 2004). These kinases are activated by phosphorylation on threonine and tyrosine residues. Subsequently, phosphorylated and other intracellular enzymes or transcription factors regulate the expression of genes involved in cellular response (Kyosseva 2004). The substrates that are identified are phosphorylated; for MAPKs in the nucleus they include some hormone receptors, as well as transcription factors such as the activator protein-1 (AP-1), the family of Jun factors (c-Jun, Jun-B, and Jun-D), Elk-1, p53, transcription factor-2 (ATF-2), JDP2, c-Myc, the NAFT family, the STAT family, and the PAX family (Chen et al. 2001; Kyosseva 2004).

Commonly the activation of signalling pathways JNK/SAPK and p38/SAPK has been associated with the promotion of cell damage (Borsello and Forloni 2007; Guan et al. 2006; Kyosseva 2004). Nevertheless, extracellular signal-regulated kinase1/2 (ERK1/2) has been implicated in several cellular functions including regulation of cell proliferation, differentiation, survival, and apoptosis in response to a wide variety of external stimuli (Cheung and Slack 2004; Miller and Gauthier 2007; Yoon and Seger 2006).

ERK pathway exhibits dynamic changes following several types of seizure activity and may function in the regulation of neuronal excitability (Dudek and Fields 2001; Houser et al. 2008). This signalling pathway is strongly activated in neurons

following severe, chemically induced seizures. In an initial *SE* episode-increased ERK activation may be neuroprotective and limit the damage of some neurons, such as dentate granule cells (Choi et al. 2008). Conversely, a lack of ERK activation in other neurons may contribute to their vulnerability to excitotoxic damage (Choi et al. 2007). At later stages, ERK phosphorylation may decrease as a compensatory mechanism to control increased network excitability (Dudek and Fields 2001).

Certain evidence has shown that neuronal activity-dependent modulation of the ERK signalling pathway plays an important role in synaptic plasticity (Yoon and Seger 2006).

Moreover, *in vivo* studies have implicated that the SAPKs play an important role in mediating glutamate receptor (GluR) responses, possibly involving the normal physiology of glutamate and associated pathophysiology. For example, the activation of the *N*-methyl-D-aspartate (NMDA) receptor stimulates JNK and p38 MAPK in cultured CGCs (Kawasaki et al. 1997); and in hippocampal neurons α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainite (KA) receptors stimulate ERKs, JNK, and p38 kinase (Mukherjee et al. 1999).

The JNK pathway has a central position in cellular damage particularly in apoptosis and participates in the death cell program through regulation of the function of pro-apoptotic activators members of bcl-2 family (BH3-only) or phosphorylates Bim- and Bcl2-associated agonist of cell death (Bad) at distinct serine residues (Donovan et al. 2002; Putcha et al. 2003). Moreover, JNK enhances *bim*-gene expression through activation of the transcription factor c-Jun. Therefore, the deletion or inhibition of JNKs components substantially limits the cellular potential to undergo death in neuronal and non-neuronal cells, principally the caspases dependent. The most convincing evidence to suggest that JNK is implicated in excitotoxic neuronal death has come from studies using JNK3 knockout mice, where KA-mediated seizures in vivo failed to cause apoptosis in hippocampal neurons, coincident with the reduction of *c-Jun* phosphorylation (Yang et al. 1997). The principal substrate for JNK is c-Jun; however, it is not known which isoform is responsible for its phosphorylation. High expression of both the gene and protein of c-Jun precedes or coincides with periods of cell death, such as that occurring during embryonic development (Sun et al. 2005), after trauma (Raivich et al. 2004), cerebral ischemia (Wessel et al. 1991), and seizures (Morgan and Curran 1991).

This dual role of MAPKs may make it possible to design alternative and/or synergistic approaches to the management of degenerative diseases, either by using specific inhibitors of the MAPKs involved in apoptosis or by increasing the activation of the MAPKs involved in neuronal survival and differentiation.

6.4.2 JAK/STAT and PI3K/AKT Pathways

The Janus kinases (JAKs) are a family of non-receptor protein tyrosine kinases. They are activated in a variety of different ways. In the canonical pathway, two JAK molecules bind to two receptors that dimerized in response to ligand binding and the

juxtaposed JAKs trans and/or autophosphorylate resulting in their activation (Yamaoka et al. 2004). This mode of activation applies, for example, to cytokine receptors, growth hormone-like receptors, and the leptin receptor. Alternatively, JAKs may be activated following stimulation of G protein-coupled receptors and/or via intracellular Ca^{2+} changes. Once activated, JAKs phosphorylate and activate downstream targets. For instance, the recruitment of JAK2 mediates the activation of several signalling pathways, including STAT5, ERK/MAPK, and PI3K/Akt (Silva et al. 1999; Kretz et al. 2005). The best-established downstream effector of JAK is the signal transducer and activator of transcription (STAT) family. Seven STAT isoforms, named STAT1 to STAT4, STAT5A, STAT5B, and STAT6, have been identified (Battle and Frank 2002). Once phosphorylated by JAK, STATs dimerize and are translocated to the nucleus where they regulate the expression of numerous genes (Aaronson and Horvath 2002). The JAK/STAT pathway is involved in many physiological processes including those governing cell survival, proliferation, differentiation, development, and inflammation. There is increasing evidence that this pathway also has neuronal specific functions in the central nervous system (Yadav et al. 2005). The cellular and molecular mechanism by which the JAK/STAT pathway is involved in neuronal function is unknown. However, it has been shown that STAT can regulate the expression or function of several neurotransmitter receptors, including GABA (Lund et al. 2008), muscarinic acetylcholine (Chiba et al. 2009), and NMDA and AMPA receptors. Particularly, STAT5 is a predominantly pro-survival signal (Debierre-Grockiego 2004).

A role for Akt in mediating neuronal survival was first demonstrated by Datta and colleagues (Datta et al. 1997) in a primary postnatal cerebellar granule cell culture model, in which apoptosis is induced by either low potassium or growth factor withdrawal (D'Mello et al. 1993). Moreover, Akt is a serine/threonine kinase with diverse roles related to the regulation of cell growth, proliferation, migration, glucose metabolism, transcription, protein synthesis, and angiogenesis (Bazil et al. 2002). Activation of Akt occurs following the binding of a protein growth factor to its receptor on the cell surface. Ligand binding induces autophosphorylation of tyrosine residues in the cytoplasmic portion of the receptor, resulting in the recruitment and activation of phosphatidylinositol 3-kinase (PI3K).

One best-characterized signalling survival mediated by Akt is activated by NMDA receptors (Datta et al. 1997); the inhibition of this kinase activity contributes to NMDA receptor-mediated apoptosis. Moreover the protein kinase serine/threonine (Akt), also known as protein kinase B (PKB) has two sites of phosphorylation that determine the regulation of Akt activity: threonine 308 (Thr308), located in the kinase domain, and serine 473 (Ser473), which is in the regulatory domain (Coffer and Woodgett 1991; Song et al. 2005).

The activation of Akt by trophic factors depends on PI3-K (Burgering and Coffer 1995). When the trophic factor specifically binds to its receptor, PI3-K is recruited by activating Akt modulating, and an anti-apoptotic effect may be through:

1. Direct regulation of the apoptotic pathway
2. Transcriptional control of molecules that promote cell survival, and
3. Regulation of cellular metabolism (Song et al. 2005)

With respect to direct regulation, Akt can phosphorylate different members of the pro-apoptotic Bcl-2 family such as Bad (Datta et al. 1997) and Bim. Once phosphorylated bind to proteins called chaperones 14-3-3 in the cytoplasm; they are thereby inactive in a pro-apoptotic function. Other direct effects involve the inactivation of caspase-9 by phosphorylation or the negative regulation of JNK/SAPK. Regarding the transcriptional control, Akt can phosphorylate different transcription factors indirectly by modulating its activity. Phosphorylation of the family of FoxO transcription factors, whose function includes the induction of apoptosis through the redistribution of these factors from the nucleus to the cytoplasm, prevents its activity (Huang and Tindal 2007).

6.5 Mechanisms of Neuronal Death in Both Experimental Models and Patients with Intractable TLE

6.5.1 Experimental Models of TLE and Cell Signalling

Molecular analyses of epilepsy-induced hippocampal plasticity have largely focused on individual candidate genes, with particular emphasis on genes with known functions for specific pathogenetic aspects (Aronica and Gorter 2007; Mefford et al. 2010). Various studies have reported changes in gene expression in the *SE* induced by kainic acid (Hunsberger et al. 2005) and pilocarpine (Becker et al. 2003), as well as by electric stimulation (Gorter et al. 2006; Engel and Henshall 2009). Moreover, the analyses of these studies have shown an overlap in gene expression profiling in epileptogenesis revealing that the biological process emerges as the most frequently encountered in this context and is related to glial activation, immune response (e.g., inflammation), signal transduction, synaptic transmission (e.g., dopaminergic, glutamatergic, and GABAergic), and the induction of immediate early genes (IEGs) (De Lanerolle and Lee 2005; Aronica and Gorter 2007; Okamoto et al. 2010).

In the work of Okamoto et al. (2010), all possible changes in the rat transcriptome were monitored at distinct time points corresponding from the latent to chronic phase of the pilocarpine model of epilepsy, one the most extensively studied models of TLE. Genes identified as being differentially expressed were classified based on their respective biological functions to envisage processes and pathways likely implicated in epileptogenesis. The hyper-expression of 128 genes was described in this model, indicating stable modulation of the p38/MAPK, JAK/STAT, and PI3K signalling pathways (Okamoto et al. 2010), some of which displaying a parallel expression pattern in humans with epilepsy.

The involvement of caspases in *SE*-induced neurodegeneration has also been studied after systemic injection of kainic acid or lithium-pilocarpine, both of which produce vast and severe neuronal damage (Fujikawa et al. 1999, 2000). Henshall et al (2001b) and Li et al (2006) reported that caspases-8 and -9 are activated in the hippocampus after focal *SE* was induced by kainic acid. The expression of activated

caspase-3 in hippocampal neurons and astrocytes have been also detected after pilocarpine-induced *SE* (Narkilahti et al. 2003; Weise et al. 2005). The different location of caspase after *SE* suggests different functions in the brain.

Additionally, López–Meraz et al. (2010) using the lithium–pilocarpine model of *SE* in 2-week-old rat pups showed that dying neurons in the DG and CA1-subiculum area do not share the same mechanism of death. In CA1-subiculum, caspase-8 upregulation preceded caspase-3 activation in morphologically necrotic neurons, while in the DG dying neurons were caspases-9 and -3 immunoreactive and morphologically apoptotic. *SE*-induced neuronal necrosis can be an active mechanism involving the activation of a caspase cascade (Niquet et al. 2007; Lopez-Meraz et al. 2010).

Weak evidence for apoptotic mitochondrial pathways has been described after lithium–pilocarpine-induced *SE* in degenerating neuronal populations (Fujikawa et al. 2002). Some works have shown that *SE* triggered by intra-amygdala kainic acid in mice causes rapid p53 accumulation and subsequent hippocampal damage. Expression of PUMA, a pro-apoptotic protein under p53 control, was increased within a few hours of *SE*. Induction of PUMA was blocked by pharmacologic inhibition of p53, and hippocampal damage was also reduced. Compared to PUMA-expressing mice, PUMA-deficient mice had significantly smaller hippocampal lesions after *SE*. Moreover, PUMA-deficient mice were found to develop fewer epileptic seizures than wild-type animals after *SE* (Engel et al. 2010). Nevertheless, functional-proteomics studies are needed to determine which molecules are active during the process of epileptogenesis or after *SE* (Engel and Henshall 2009).

On the other hand, the neuronal stem cells in the hippocampus appear to be sensitive to a prolonged seizure resulting in an increase in stem or progenitor cell numbers (Walker et al. 2008). In agreement, a quantitative real-time PCR analysis of cell cycle genes confirmed hyper-expression of Cdk1, a gene regulating the G1 to S and G2 to M transition of the cell cycle, and Nestin, a marker of neural stem cells and neural progenitor cells. However, expression of the cell cycle inhibitor *p18*(*INK4c*) was paradoxically enhanced after *SE* induced by pilocarpine and coincided with the peak of Cdk1 and Nestin expression at day 3 post-*SE* (Okamoto et al. 2010). These findings suggest that the proliferative stage may be inhibited by such activation *p18* in pilocarpine model.

Cells born after seizure have altered synaptic inputs and neurotransmitter expression (Jessberger et al. 2007; Parent et al. 2006; Jakubs et al. 2006). These alterations have also been shown in neurogenesis in pilocarpine-induced *SE* (Radley and Jacobs 2003). Experimental TLE is associated with an increase in neurogenesis following amygdala kindling (Parent et al. 1998, 2006; Scott et al. 1998); since many of the newborn neurons eventually integrate into hippocampal circuitry and they may either contribute to the hippocampal network plasticity associated with epilepsy or, possibly, limit seizure activity (Jakubs et al. 2008; Overstreet-Wadiche et al. 2006).

Moreover, phosphorylated ERK (pERK) is increased in many hippocampal neurons following recurrent spontaneous seizures in pilocarpine-treated mice (Houser et al. 2008). Its activity appears to be involved in regulation of seizure-induced neurogenesis during the first few days after *SE*, since ERK activation returns to control levels within 1 week (Choi et al. 2008).

Experimental evidence suggests that seizures evoked by microinjection of kainic acid into the amygdala of the rat activate multiple cell death pathways involving Bcl-2 and caspase family proteins in brain regions destined to die, whereas survival promoting responses predominate in cortical populations that survive (Henshall 2001a). Pro-apoptotic BAD and the counteractive effects of Akt-pathway may underlie in part, the cell death outcome after seizures, providing a more complete understanding of the mechanisms by which seizures damage brain and highlighting novel targets for treatment of brain injury associated with seizure disorders (Henshall 2001a, b). Moreover, the end effector of the signalling pathway regulated by STAT5 proteins includes Bcl-xL and XIAP. Both have shown anti-apoptotic effects in diverse damage animal models (Okamoto et al. 2010).

In summary, although there are several models for the study of epileptogenesis, *SE*, and convulsive seizures, it is important to continue with additional studies for search potential molecular elements that can participate in the process of neuroprotection and/or as therapeutic targets for the treatment of epilepsy.

6.5.2 Studies of Signalling in Patients with Pharmacoresistant TLE

Studies have shown that both pro- and anti-apoptotic proteins, as well as death receptors are found in surgically removed brain samples from patients with pharmacoresistant TLE (Nagy and Esiri 1998; Henshall et al. 2000a, b; Dorr et al 2002).

Analysis of hippocampus from patients with intractable TLE from several groups has confirmed altered expression of Bcl-2 and caspase family genes. In particular, it has been observed that the Bcl-2 and bax immunoreactivity increases predominantly in cells with the morphologic appearance of neurons, whereas bcl-x_L immunoreactivity augments in cells with the appearance of glia (for review Engel and Henshall 2009). In another studies, the expression of anti-apoptotic proteins Bcl-2, Bcl-x, and Bcl-w has been reported to be higher in brain tissue obtained from patients with intractable seizures; however, some pro-apoptotic changes are also seen in this gene family. It may suggest the possible predominance of apoptotic protective pathway-Bcl2 in human epileptic brain (Henshall et al. 2000a, b; Shinoda et al. 2004). DNA fragmentation was also detected in some but not all brain sections from patients undergoing temporal lobectomy for intractable seizures (Henshall et al. 2000a, b).

Nagy and Esiri (1998) described cell cycle disturbances and a possible apoptotic mechanism of hippocampal neuronal cell death in hippocampus obtained from patients with pharmacoresistant epilepsy, suggesting that neurons have re-entered the cell division cycle and reached the G_2 phase. Another interaction was described between the cell cycle machinery and the intrinsic processes in apoptotic neurons, with evidence that Cdk1 activates pro-apoptotic bad protein. Moreover, that interaction has also been associated with different pathological conditions such as stress, depression, and epilepsy (Becker and Bonni 2004).

Other studies trying to prove a causal relationship between changes in neurogenesis and the disease state (i.e., depression and epilepsy) have been controversial (Jung et al. 2006; Malberg et al. 2000; Scharfman et al. 2000).

6.6 Conclusions and Perspectives

In TLE, structural changes in the hippocampus are believed to play a key role in the generation of epileptic seizures. However, given the complexity of hippocampal circuitry and cell damage in case of hippocampal sclerosis, structural repair of epileptic hippocampal networks will require complex strategies in which proper integration and rewiring of the implanted neurons will be of crucial importance. The activation of cell-signalling pathways in response to acute seizures has dramatic consequences such as neuronal loss and irreversible loss of function. In the past 10 years, researchers have found molecular "signatures" of gene-directed cell death signalling strategy linked to apoptosis in brain samples from a subpopulation of patients with pharmacoresistant epilepsy who experience frequent seizures. Using animal models, researchers have shown that evoked seizures or epilepsy often activates the same signalling pathways, and drugs or genetic modulation of these cascades can reduce brain injury.

The analysis of molecular responses to seizure is not simple because biological processes are not uniform and experimental protocols differ. The identification of potential therapeutic targets should be facilitated by the knowledge of genes, proteins, and altered signalling pathways during the different stages of epilepsy development.

It is important to consider that most of the functional studies reviewed here support targeting apoptosis signalling pathways to prevent seizure-induced neuronal death. However, not all groups detect an apoptotic signature (Henshal and Murphy 2008). Also, protection of some cell populations may be more critical than others. Translating short-term to long-term neuroprotection will also be challenging. Apoptosis-regulatory genes with neuromodulatory properties may be particularly promising but, of course, raises concerns of its effects on brain function that targeting apoptosis pathways was originally expected to avoid.

A deep understanding of signalling pathways involved in both acute and long-term responses to seizures continues to be crucial to unravel the origins of epileptic behaviours. Therefore, additional studies would be necessary to identify those genes related to neuroprotection and/or those involved in neuronal activities related to epileptogenesis and could potentially represent target genes in design new preventive drugs for epilepsy.

Acknowledgements This work was partially supported by grants: CYTED (http://www.cyted.org/) through Group study of neuroscience Iberoamerican Network and CONACyT 00177594 to CBZ.

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