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Mitochondrial Regulators of Synaptic Plasticity in the Ischemic Brain

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Abstract

Synaptic plasticity is a process by which neurons adapt or alter the strength of information transfer, and it is known to play a role in memory formation, learning, and recovery after injury. In this chapter, we describe how ischemic insults alter neuronal intracellular mechanisms and signaling pathways, and we discuss how, after neuronal injury, synaptic plasticity is regulated prior to and during death or rehabilitation and recovery. In addition, recently described regulators of synaptic plasticity will be introduced.

Keywords: ischemia, mitochondrial metabolism, neuroprotection, Bcl-xL, ATP synthase

1. Cellular mechanisms after cerebral ischemia

Cerebral ischemia occurs as a result of a lack of, or insufficiency of, blood supply to the brain, which results in the failure to meet neuronal metabolic demands. Thrombotic or embolic stroke (focal), and cardiac arrest or cardiac surgery (global) are common causes of cerebral ischemia. The loss of oxygen and glucose flow to the brain eventually leads to neuronal energy deficits. These energy deficits result in the failure of adenosine triphosphate (ATP)-dependent ion pumps expressed on the neuronal plasma membrane, permitting an unregulated surge of ion influx into the neuronal cytoplasm [1–3]. Calcium influx induces the release of neurotransmitters from presynaptic neurons and activation of postsynaptic glutamate receptors such as N-methyl-D-aspartate (NMDA) receptors and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [4–6]. Uncontrolled opening of postsynaptic receptors provokes failure of intracellular ion homeostasis, resulting in excessive postsynaptic entrance of calcium or sodium through NMDA- or AMPA-regulated channels; this initiates signaling pathways.

Calcium is a trigger for a number of important cell-signaling pathways. Increased levels of intracellular calcium activate phospholipase C, which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP₂) and forms diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP₃) [1]. The hydrophobic DAG molecule is expressed on the cell membrane, and recruits protein kinase C (PKC) from the cytosol. PKC regulates synaptic function by phosphorylating ion channels [7–9] and glutamate receptors [10, 11], and enhancing neuronal outgrowth [12, 13]. On the other hand, hydrophilic IP₃ travels into the cytosol and binds with the IP₃ receptor expressed on the endoplasmic reticulum (ER). The ER membrane-embedded IP₃ receptor releases calcium from the ER to mitochondria, depolarizing mitochondrial inner membranes and further compromising ATP production.

Ischemic conditions also activate death receptors (e.g., Fas, tumor necrosis factor (TNF) α R, DR) that cause caspase activation and ultimately lead to neuronal apoptosis [14, 15]. Death receptor-ligand binding releases caspase 8, which directly cleaves either caspase 3 (which can activate downstream death-inducing enzyme pathways) or BH3 interacting-domain death agonist (Bid) to form truncated tBid [16, 17]. tBid translocates to the mitochondria and initiates activation of the pro-apoptotic proteins Bax and Bak. Oligomerization of Bax on the mitochondrial membrane causes cytochrome c release. Cytochrome c forms an apoptosome complex made up of cytochrome c, apoptotic protease-activating factor 1 (APAF 1), and caspase 9. Caspase 9 cleaves and activates effectors such as caspase 3 and caspase 6 which results in neuronal apoptosis. Numerous proteins are subjected to caspase-mediated cleavage [18] including regulators for synaptic function such as glutamate receptors [19, 20], synaptic adhesion molecules [21], ion channels [2], neuronal growth/pruning regulators [22, 23], and inflammatory cytokines [24, 25].

In addition, ischemic stimulation increases the permeability of the blood-brain barrier, and activates neuroinflammatory responses in the brain. Inflammatory infiltration such as the entrance of leukocytes (e.g. neutrophils, macrophages, and lymphocytes) activates microglia and astrocytes which then release inflammatory regulators, including cytokines (e.g. interleukin-1 β (IL-1 β), IL-6), tumor necrosis factor α , chemokines (e.g. chemokine C-C motif ligand 2 (CCL2), CXC-chemokine ligand 1 (CXCL1)), nitric oxide, reactive oxygen species (ROS), and growth factors [26–29]. Neuroinflammation is a dual-purpose response that can hasten neuronal death or facilitate repair depending on the circumstances. For example, TNF- α is one of the most well-studied pro-inflammatory cytokines increased by ischemic events; it is clearly responsible in large part for ischemia-induced brain injury [30]. However, TNF- α also enhances synaptic strength by increasing the expression of AMPA receptors [31] and through the regulation of the transcription factor, NF κ B [32], increasing the expression of anti-apoptotic proteins (Bcl2, and Bcl-xL) and facilitating the production of neurotrophic factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) [33, 34] which play a role in neuroprotection [35]. Taken together, ischemia triggers a multifunctional and complex process in the brain that can lead to neuronal death or facilitate the defense system to rescue the brain against neurotoxic stimulation.

2. Synaptic plasticity in the ischemic brain

The process of early neuronal growth and neurite elongation is critical for synapse formation and neuronal network development. Projection of filopodia, where actin and microtubules

become polymerized and attach to substrates, anchors a growth cone and guides a peripheral domain within the thin outer edge of the growth cone known as the lamellipodium. After the lamellipodium contacts the substrate, the central domain of the growth cone, where actin is reorganized and microtubules predominate, moves toward its target [36–38]. These steps occur repeatedly during neurite sprouting and branching. When the tip of the axon reaches its target (either dendrite, soma, or another axon), it differentiates to become suitable for neurotransmission. Presynaptic terminals contain a high density of mitochondria, presynaptic vesicles, and endosomes to enhance communication within the synapse. The target of presynaptic contact is the postsynapse, which matures by the expression of neurotransmitter receptors. Neurexin-neurologin, N-cadherin, ephrin, and synaptic cell-adhesion molecules play a role in the interaction between presynaptic and postsynaptic neurons [37, 39–42].

Neuronal development is far from static in the mature brain. Recent studies have found that neurons are capable of plasticity during the entire human lifespan [43–46]. Mature neurons have the ability to repair their synaptic network after neurotoxic insults. Stroke induces changes in numerous genes including the ones involved in axonal sprouting in both young and aged animals [47]. Alteration of neuronal connectivity and degradation of neurites are well described after ischemic stroke [47–49], and strategies to strengthen the synaptic network to regain neuronal function and enhance brain repair after episodes of brain injury have been reported [47, 50]. Our study demonstrates examples of the dynamic and adaptive changes that occur in neurites after glutamate neurotoxic challenge. Neurite branches initially become fragmented and damaged (**Figure 1A and D**), but then neurites regain structure (**Figure 1B and C**) over time, or undergo degradation if severely damaged; in some case collateral sprouting occurs to compensate for lost neurites and to regain synaptic connectivity with healthy neighboring cells (**Figure 1D–F**).

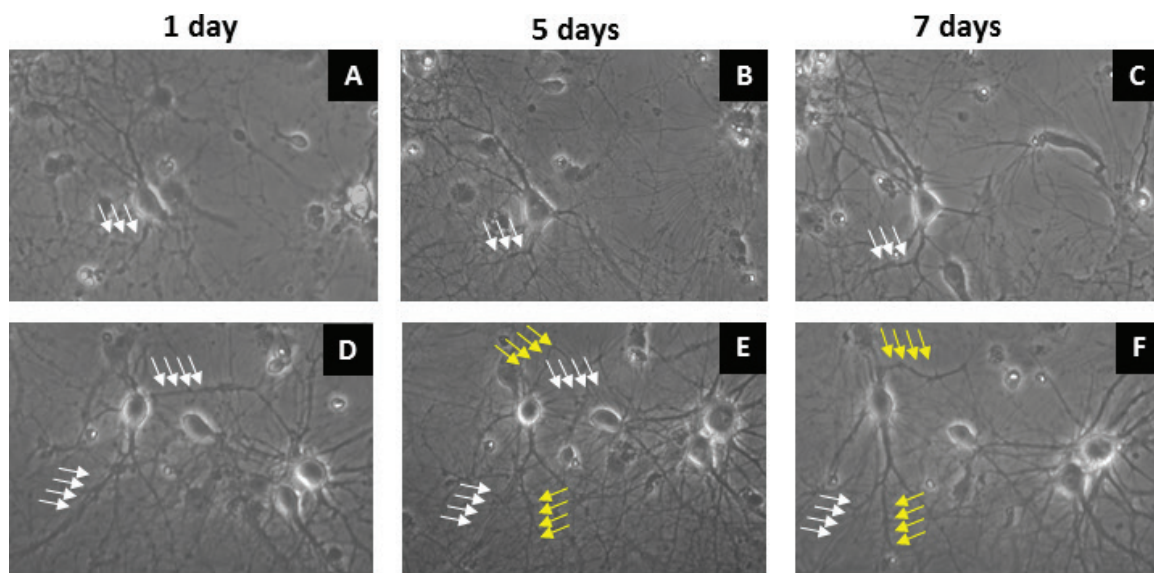


Figure 1. Adaptation of neurite sprouting and pruning after glutamate-induced neurotoxicity. Primary hippocampal neurons were treated with 20 μ M glutamate and then imaged at days 1, 5 and 7 after introduction of the insult. Bar, 20 μ m. (A) Damaged and fragmented neurite at 1 day after insult (white arrows). (B) Early recovery of injured neurite at day 5 (white arrows). (C) Thickening of recovering neurite at day 7 (white arrows). (D) Fragmented and damaged neurites at day 1 (white arrows) are cleared at day 5 (E, white arrows), but strengthening of collateral branches was found at day 7 (F, yellow arrows) to supplement synaptic networking.

Numerous studies have reported the modification of synaptic plasticity during or after ischemic events. Some studies describe synaptic modification as a part of death signaling; on the other hand, other studies suggest changes in synaptic function as a mechanism of protection or rehabilitation. Synaptic plasticity plays a role in the decision for the neuron to live or die. Synaptic transmission demands high levels of energy production [51]. Failure to control neurotransmitter release [6, 52], and abnormalities of synaptic transmitter release due to lack of energy supply after ischemic insult are well described previously [53]. Regulation of the postsynaptic receptor is critical to control synaptic plasticity [54, 55], and studies have reported that NMDAR or AMPAR are subject to alterations after ischemic events. Earlier studies have shown that brains of animals undergoing four vessel occlusion (4VO)-induced global ischemia show impaired voltage-dependent NMDAR responses, display NMDA-mediated hyperexcitability and loss of long-term potentiation (LTP), and manifest morphological changes of pyramidal neurons prior to the onset of delayed death in the CA1 region of hippocampus [56, 57]. Recent studies expand on these mechanisms and distinguish roles for individual ion channel subunits [58–61]. Studies have reported that ischemia is responsible for the alteration of AMPA receptor expression, especially the GluR2 subunit, transforming a non-calcium permeable into a calcium-permeable AMPA receptor, thereby further mediating calcium entry into CA1 neurons after global ischemia. Although this role contributes to delayed neuronal death in the globally ischemic rodent brain [62–65], these changes may also occur during normal events in synaptic plasticity [54, 66]. Despite the close relationship between ischemia-induced neuronal death and NMDA receptor activation, the application of NMDAR antagonists fails to prevent stroke-related brain injury in clinical trials [67] perhaps indicating involvement of NMDAR in neuronal survival. Indeed, functioning NMDARs are required for synaptogenesis, and the NMDAR plays a neuroprotective role against apoptotic stimulation and oxidative stress [61, 68–70]. In summary, synaptic changes that occur during ischemia may be protective or detrimental depending on the severity and temporal sequence of ischemic events.

2.1. Synaptic failure may lead to ischemic death

Structural damage including degradation of axons or dendrites, and loss of synaptic connectivity associated with synaptic dysfunction are described in various cerebral ischemic models [53, 71]. Animals undergoing ischemic surgery exhibit a reduction of the total neuronal population and an increased appearance of degenerating neurons and apoptotic cells [72–76]. Shy of frank cell demise, axonal morphological changes after brain ischemia have been reported within a variety of brain regions including cortex, striatum, and hippocampus. Degenerating axons exhibit swelling and the appearance of varicosities in both an acute and chronic manner; changes are observed over a period between 6 h and 4 weeks after ischemia reperfusion [49]. Cerebral ischemic insults also lead to cytoskeletal disruption in neurites such as a reduction in the amount of microtubule-associated protein (MAP2), an enhancement in neurofilament proteolysis and an alteration of tau in some neurons [77–79]. Ischemia causes an increase in intracellular calcium levels and ROS production [15] and triggers neuronal death by mechanisms such as enhancing mitochondrial permeability which can facilitate both apoptotic and necrotic death and neuritic degeneration [80]. Therefore,

ischemia is one of the major causes of structural and functional failure of both somata and neuronal processes during stroke.

2.2. Synaptic repair after ischemic events

Although ischemic stroke induces neuronal death that leads to functional disability, studies have reported evidence for synaptic plasticity contributing to recovery after stroke [81]. Rats undergoing neocortical ischemia had induction of the growth-associated protein 43 (GAP-43) which is enriched in the growth cone, promoting synaptogenesis and behavioral recovery [82, 83]. Axonal sprouting and plasticity in the intracortical circuitry was observed in post-ischemic brain regions [84, 85]. Plasticity was not limited to neurons but also occurred in other cells including reorganization of vascular structures. Enhancement of the dendritic network such as increased dendritic density has been reported in post-ischemic brain [86, 87]. Moreover, the brain is capable of generating new neurons. The subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus are reported to be the site of neurogenesis, exhibiting therapeutic potential for the treatment of brain diseases [88]. In particular, studies have shown that stroke increases neuronal progenitor cell populations in the brain [89, 90], and enhances cell proliferation in the SVZ [91] and in the ischemic penumbra of stroke patients [92]. These studies indicate that ischemia-induced signaling, even denervation itself, may act as a stimulus for the functional and structural recovery of synapses.

2.3. Preconditioning in synaptic potentiation

Despite the possibility of severe ischemia to trigger adaptive responses on its own, studies have also reported that non-noxious, lower levels of injury (preconditioning) may augment ischemic tolerance. Moderate levels of neurotoxic stimuli such as glutamate, ROS, or inflammation initiate survival mechanisms without impairing brain function. Thus, preconditioning builds a latent neuroprotective environment in the brain and provides for a reprogrammed defense response when the truly injurious stimulation occurs [93, 94].

Zukin's group has reported that ischemic preconditioning downregulates AMPA receptors, and blocks mitochondrial release of death molecules such as Smac and DIABLO without altering the pro-survival inhibitor of apoptosis (IAP) family, therefore attenuating ischemia-induced damage in rodent hippocampal neurons [95, 96]. Neuroprotective mechanisms of preconditioning are further evidenced as a change in dynamics of mitochondrial proteins. Preconditioning prevents translocation of the pro-apoptotic protein Bad, enhances the availability of the pro-survival protein Bcl-xL, blocks activation of caspase 3, decreases release of Smac, DIABLO, and cytochrome *c*, and prevents large conductance mitochondrial channel activity, eventually rescuing hippocampal neurons after ischemic insult [72]. In addition, mitochondrial Bcl2 protein family [97–99], redox regulators [100, 101], and transcription factors such as hypoxia-inducible factor (HIF) [102, 103], NFκB [104, 105], c-Fos [106], CREB [97, 105], Nrf2 [101, 107], and AP1 [108] are involved in gene regulation after preconditioning, further modifying neuronal functions such as neurotransmitter release, channel activity, and synaptic networking by regulating the expression of new proteins.

3. Regulators of mitochondrial function and synaptic plasticity

Ischemic insults damage the mitochondrial electron transport chain, decrease mitochondrial ATP production, impair ATP-dependent transporters, and allow intracellular calcium entrance that triggers opening of the mitochondrial permeability transition pore (mPTP) [80, 109, 110]. Ischemia also impairs electron transfer and causes ROS generation from mitochondrial proteins such as complex I and III [111]; ROS greatly contributes to mPT-mediated responses in the mitochondrial membrane [112]. On the other hand, ischemia alters proteins that reside in the mitochondria, and changes levels of transcription factors that target the promoter regions of mitochondrial and nuclear genes. Thus, mitochondria are an essential organelle in ischemia-mediated neuronal responses. There are several regulators that modify mitochondrial function and plasticity to either enhance synaptic recovery after injury or signal for synaptic decline prior to neuronal death. We describe these individually and then show how they work together to support synaptic function during stress.

3.1. Mitochondrial reactive oxygen species

Oxidative phosphorylation is the metabolic process that produces ATP, but it is also the main source of production of superoxide in the mitochondria that can be converted into hydrogen peroxide. Due to the high metabolic rate of neurons, ROS is highly produced in the brain. However, the brain is also more vulnerable than other organs to ROS-induced damage, because it is rich in polyunsaturated fatty acids, and lacking in catalase activity [113, 114]. Indeed, ROS is one of the main causes of mPTP opening, release of mitochondrial death molecules and cell death in ischemic brain [15]. Although numerous studies have reported that an ischemia-induced surge of ROS causes functional and structural injury to neurons, ROS plays multifunctional roles. Physiological levels of ROS regulate synaptic signaling. Approaches that block production or enhance clearance of superoxide using depletion of NADPH oxidase, or overexpression of superoxide dismutase (SOD), respectively, failed to allow for LTP in hippocampal slice [115–117]. Moreover, SOD-overexpressing mice demonstrate defective hippocampal memory formation as measured by water maze learning and fear conditioning [117, 118].

Nitric oxide synthase (NOS) is an enzyme that generates nitric oxide (NO) gas from arginine. NO is considered to be a ROS, due to its ability to generate highly reactive peroxynitrite [119]. Indeed, 4VO-induced global ischemia causes induction of NO in various regions of the brain including hippocampus, cortex, striatum, and cerebellum [120]. Excessive NO production is reported to be neurotoxic as it damages mitochondria, and exacerbates excitotoxicity [121–123]. However, NO is also required for synaptic transmission and synaptic plasticity [124, 125]. NADPH oxidase (NOX) is a cell membrane-bound enzymatic complex that generates superoxide from NADPH, and it also expresses in the mitochondria [126]. NOX contributes to ROS-induced neuroinflammation and apoptosis [127, 128]. Studies reported an upregulation of NOX mRNA and protein level in response to experimental stroke [129, 130]. Inhibition of NOX using the pharmacological inhibitor apocynin improved ischemia-induced brain damage and mortality [131, 132]. However, mice lacking NOX subunits had impaired long-term

potentiation and manifested hippocampus-mediated memory deficits, indicating that a physiological level of NOX and ROS may be required for synaptic plasticity and memory formation [114, 115].

3.2. Mitochondrial permeability transition pore (mPTP) and ATP synthase

Ischemia-induced death signaling opens a calcium-sensitive inner mitochondrial membrane pore called mPTP, causing permeabilization of the mitochondrial inner membrane [80, 109, 110]. Loss of mitochondrial inner membrane integrity leads to leakage of intermembrane molecules such as cytochrome *c*, Smac, and DIABLO into the cytoplasmic space, facilitates death-signaling cascades, and results in the impairment of mitochondrial outer membrane structure and cell death. Thus, mPTP is recognized as an important target for neuroprotection; inhibition of mPTP opening may delay or prevent mitochondrial-mediated cell death. Since the discovery of the calcium-induced mitochondrial membrane permeability transition (PT) [133, 134], several molecular participants in mPTP structure or formation have been reported. However, the identification of mPTP is still under investigation.

Cyclophilin D (Cyp D) is a peptidyl-prolyl *cis/trans* isomerase that is localized to the mitochondrial matrix. Cyp D has been considered as a main element of mPTP. However, recent studies have reported that depletion of Cyp D did not eliminate mPTP [135–137] indicating it may not be the critical component of pore opening. One target of Cyp D is a complex of proteins that includes the voltage-dependent anion channel (VDAC), localized to the mitochondrial outer membrane, and the adenine nucleotide translocase (ANT), an ADP/ATP translocator localized to the mitochondrial inner membrane. Therefore, this protein complex was widely studied for its possible role in forming mPTP [138]. Studies showed that mitochondrial swelling after stimulation facilitates the formation of VDAC and ANT complexes along with Cyp D binding, leading to opening of pores in the mitochondrial inner and outer membranes [139–143]. Studies have continuously reported new participants of Cyp D-ANT-VDAC complex: the mitochondrial phosphate carrier binds to Cyp D and ANT [144], spastic paraplegia 7 forms a heterooligomeric complex with VDAC [145], and hexokinase binds to VDAC [146, 147]. However, a recent study revealed that animals lacking VDAC genes did not show improvement of mPTP-mediated mitochondrial stress. They exhibited equivalent levels of cytochrome *c* release, caspase activation, and cell death compared to control animals [135]. In addition, mitochondria from mice lacking ANT also displayed mPT and cytochrome *c* release at similar rates compared to wild-type controls [148]. These studies indicate that further investigations are required to clarify the roles of VDAC and ANT in mPTP activation.

In contrast to the studies of VDAC and ANT, knockdown of the membrane-embedded portion (c-subunit) of the F_1F_0 ATP synthase does regulate the ability of mitochondria to undergo PT. Several recent studies have reported that F_1F_0 ATP synthase is an important candidate to form mPTP [149–157]. F_1F_0 ATP synthase is localized in the mitochondrial inner membrane (F_0 unit) and matrix (F_1 unit), and produces ATP by using the potential energy of the hydrogen ion gradient. F_1F_0 ATP synthase gained attention as a putative candidate for mPTP when it was found that it binds CypD [152] and when it was reported to regulate the efficiency of mitochondrial energy metabolism via controlling an inner mitochondrial membrane ion leak

[158]; the c-subunit of the ATP synthase was found to be required for mPTP [149]; the c-subunit of ATP synthase was revealed to form a voltage-sensitive channel, the opening of which is correlated with PT and neuronal death [155]. Bonora and Alavian also showed that shRNA-mediated c-subunit depletion protects neurons from PT-induced cell death, such as excitotoxic and oxidative stress. Together, the findings suggest that the c-subunit forms the pore component of mPTP [155]. Moreover, the depletion of c-subunit of F_1F_0 ATP synthase causes resistance to calcium-induced mPTP opening, while overexpression of c-subunit accelerates calcium-mediated responses, decreases mitochondrial potential, and promotes mitochondrial fragmentation [149, 155]. In addition to the role of the ATP synthase monomer, dimerization of F_1F_0 ATP synthase in mPTP has been correlated with the onset of mPT [151, 159–161].

3.3. B-cell lymphoma-extra large (Bcl-xL) and other Bcl2 family proteins regulate the synapse

Bcl-xL is a member of the Bcl-2 family of proteins. It is traditionally known for anti-apoptotic properties through its role to inhibit the activation/oligomerization of pro-apoptotic Bax and Bak on mitochondrial membranes [162, 163], and its ability to block mitochondria-mediated cytochrome c release and cell death [164]. However, recent studies have reported multifunctional roles of Bcl-xL in the brain. Bcl-xL facilitates reorganization and biogenesis of mitochondria by regulation of fission and fusion [165–167]. Inhibition of Bcl-xL decreases neuronal ATP levels but increases oxygen flux, indicating that Bcl-xL enhances the efficiency of mitochondrial metabolism by preventing the wasteful leak of H^+ ions through the inner membrane [158, 168]. Inefficient leakage of H^+ through a mitochondrial inner membrane pore prevents ATP production. These latter studies provided the first evidence for a functional role for Bcl-xL at the mitochondrial inner membrane and F_1F_0 ATP synthase. Bcl-xL interacts with α - and β -subunits [158, 169] of F_1F_0 ATP synthase in the mitochondrial matrix. These protein-protein interactions may cause conformational changes of the Bcl-xL- F_1F_0 ATP synthase complex, favoring closure of the c-subunit channel (putative mPTP), enhanced mitochondrial energy metabolism, and increased ATP production with minimal oxygen use (decreased inner membrane uncoupling).

It is therefore not surprising that Bcl-xL is an important player in energy-demanding processes such as neuronal outgrowth [74, 170] and synapse formation [167, 171]. Depletion of Bcl-xL in primary hippocampal neurons impairs neuronal branching and elongation [74]. Abnormalities of neurite sprouting caused by Bcl-xL depletion do not induce immediate cytotoxicity, but cause delayed neuronal death, presumably as more synapses fail. Despite their low propensity toward death in the absence of stress, neurons depleted of Bcl-xL are significantly vulnerable to hypoxic insult compared with control neurons, presumably due to failure of synaptic connections and impaired metabolism. In contrast to depletion, Bcl-xL overexpression increases levels of pre- and postsynaptic markers on axons and on the opposing dendrites and enhances the number of mitochondria and synaptic vesicles in the presynaptic bouton [167]. Bcl-xL also enhances synaptic vesicle recycling during presynaptic plasticity, by forming a complex of clathrin, Bcl-XL, and Drp1 which is necessary for normal or enhanced endocytosis [171]. In addition, Bcl-xL is reported to have multiple binding partners besides those of traditional Bcl2 family proteins that regulate apoptotic pathways (**Table 1**); thus, additional functions of Bcl-xL in synaptic plasticity need to be further investigated.

| Protein | Roles | References |
|---|--|-----------------|
| <i>Bcl2 family protein</i> | | |
| Bak | Regulates apoptosis | [163, 208] |
| Bax | Regulates apoptosis | [209–211] |
| Beclin 1 | Prevents autophagy | [212] |
| Bad | Regulates apoptosis and cell cycle | [213–215] |
| Bim | Regulates apoptosis | [216, 217] |
| Bid | Regulates apoptosis | [217] |
| PUMA | Regulates apoptosis | [209, 218] |
| <i>Non-Bcl2 family proteins</i> | | |
| Apoptotic protease-activating factor 1 (Apaf-1) | Regulates caspase 9-mediated apoptosis | [219, 220] |
| Apoptosis regulatory protein Siva (Siva-1) | Sequesters Bcl-xL and induces apoptosis | [221] |
| F ₁ F ₀ ATP synthase | Regulates mitochondrial energy metabolism | [158, 169] |
| Aven | Stabilizes Bcl-xL and regulates caspase-dependent apoptosis | [222, 223] |
| Dynamin-related protein 1 (Drp1) | Regulates synaptic vesicle endocytosis | [165, 167, 171] |
| IP3 receptor | Regulates calcium signaling and apoptosis | [224, 225] |
| Phosphoglycerate mutase family 5 (PGAM5) | Enhances Keap1-dependent degradation | [226, 227] |
| PTEN-induced putative kinase 1 (PINK1) | Regulates phosphorylation of Bcl-xL to prevent its cleavage | [228] |
| Voltage-dependent anion channel (VDAC) | Regulates mitochondrial calcium, cytochrome c release, ATP release | [164, 229, 230] |
| Tumor protein p53 | Regulates cytochrome c release and apoptosis | [231, 232] |

Table 1. List of proteins that bind to Bcl-xL.

In contrast to its neuroprotective properties, Bcl-xL is also capable of decreasing synaptic strength [172, 173] and inducing neurotoxicity [73, 174]. Bcl-xL is subject to caspase-mediated fragmentation [174–176], and forms N-terminus truncated Δ N-Bcl-xL. The N-terminally localized BH4 region has been reported as the functional domain that carries out the anti-apoptotic role of Bcl-xL [177, 178], and cleavage of Bcl-xL to remove this domain gives pro-apoptotic characteristics to this molecule. Δ N-Bcl-xL is reported to induce large channel activity in the synaptic mitochondria [172], to cause decline of the amplitude of post synaptic potentials [173], and to increase cytochrome c release [179]. Studies show that transient global ischemia induces Δ N-Bcl-xL formation prior to delayed neuronal death in the CA1 region of hippocampus [72, 73]. The strategies to block Δ N-Bcl-xL formation such as administration of pharmacological inhibitors, or mutation of the caspase cleavage site protect rodent brains against ischemic injury [73, 172].

Bcl-2-associated x protein (Bax) is a pro-apoptotic member of the Bcl2 family containing BH1, BH2, and BH3 domains but lacking the BH4 domain found in many anti-apoptotic

family members. Bax forms channel activity in lipid bilayers [180], induces cytochrome c release [162, 181], and cooperates with mPTP candidates such as ANT and VDAC [138, 164]. Interestingly, although it is mostly known as a pro-apoptotic protein, it also has important functions in healthy synapses undergoing plasticity. Injection of Bax protein into the presynaptic terminal induces enhanced neurotransmitter release, similarly to effects of pro-survival Bcl-xL, indicating that Bax is capable of supporting normal synaptic plasticity in unstressed neurons [182]. In healthy hippocampal neurons, Bax is necessary for the formation of synaptic plasticity known as NMDA receptor-dependent LTD. Despite comparable expression of NMDA receptors in Bax knockout animals, these animals fail to demonstrate hippocampal LTD induction [183].

Bid is a pro-apoptotic BH3 only protein. Bid is normally expressed in the cytoplasm, but during cytotoxic stimulation, caspase cleaves Bid into truncated Bid (tBid) which activates other members of the pro-apoptotic Bcl2 family [184] or antagonizes anti-apoptotic Bcl2 proteins [17]. tBid contributes to the mobilization of cytochrome c by Bax and alters mitochondrial cristae independent of its function to activate Bax, and it opens mitochondrial intermembrane spaces [185]. Bid enhances mitochondrial membrane permeabilization, cooperates with mPT or Bax, and mediates large-channel conductances [186]. Studies show that Bid is an important activator in ischemia-induced brain injury [187, 188]. Cleavage of Bid was also found after middle cerebral artery occlusion (MCAO)-induced stroke in mouse. Bid knockout animals show decreased levels of cytochrome c release and infarct volume [187, 188].

3.4. Hypoxia-inducible factor 1 (HIF1- α)

HIF1- α is a transcriptional factor activated in response to hypoxia. In normoxic conditions, HIF1- α is generally degraded by prolyl hydroxylases (PHD)-mediated ubiquitination, but hypoxia inhibits PHD activity and leads to stabilization of HIF1- α which then is translocated to the nucleus and regulates gene expression. Although HIF1- α is not generally considered as a mitochondrial protein, HIF1- α is reported as an important player in mitochondrial function [189–191]. HIF1- α directly binds with the promoter region of Bcl-xL [192] and targets the expression of BNIP3, a BH3-only protein member of the Bcl2 family that mediates hypoxia-induced mitochondrial autophagy [193–195]. HIF1- α regulates a subunit of cytochrome c oxidase [196] which is an essential member in the mitochondrial electron transport chain. In addition, a recent study showed localization of HIF1- α to both the nucleus and mitochondria after hypoxia [197].

Since ischemia is closely related to hypoxic stimulation, there are studies reporting functions of HIF1- α in models of cerebral ischemia. HIF1- α is enhanced by MCAO-induced stroke in rodent brains, and co-regulated with death-signaling molecules such as caspases, inflammatory cytokines, and apoptotic molecules [198–202]. On the other hand, the neuroprotective role of HIF1- α has been studied by several groups during the past decade [203–205]. Impairment of dopaminergic differentiation and reduction of vascular endothelial growth factor are reported in a HIF-1 α knockout model [206]. Tomita et al. reported that neuron-specific-HIF1- α knockout mice have reduced numbers of neurons and impaired spatial memory [207]. Therefore, understanding both physiological and pathological roles of HIF-1 α and its targets is important to understand synaptic plasticity in cerebral ischemia.

4. Conclusion

Mitochondria are the center of intracellular energy production, and the executor of cellular fate. Ischemic injury triggers or is caused by mitochondria-mediated signaling pathways. We have discussed in this chapter the intracellular signals activated during and after episodes of ischemia, the alteration in the dynamics of structural components of the synapse, and how these elements play a role in attenuation of synaptic plasticity or recovery of synaptic responses. Since after ischemia, increased energy demands are inherent in the formation of new synapses and repair of the normal operation of existing synapses, we are particularly focused on mitochondrial components that regulate mPTP and neuronal energy metabolism. Although details of the structure of mPTP are still in question, it is clear that prevention of mPTP and conservation of energy are critical in management of ischemia-induced damage in the brain. We have also highlighted non-canonical roles of mitochondrial Bcl2 family proteins in the synapse besides their known functions in apoptosis; these roles should be further studied to elucidate the crucial functions of mitochondria in synaptic plasticity.

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