Review

Molecular mediators of hypoxic–ischemic injury and implications for epilepsy in the developing brain

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Abstract

Perinatal hypoxia–ischemia (HI) is the most common cause of cerebral palsy, and an important consequence of perinatal HI is epilepsy. Epilepsy is a disorder in which the balance between cerebral excitability and inhibition is tipped toward uncontrolled excitability. Selected neuronal circuits as well as certain populations of glial cells die from the excitotoxicity triggered by HI. Excitotoxicity, a term referring to cell death caused by overstimulation of the excitatory glutamate neurotransmitter receptors, plays a critical role in brain injury caused by perinatal HI. Ample evidence suggests distinct differences between the immature and mature brain with respect to the pathology and consequences of hypoxic–ischemic brain injury. Thus, the intrinsic vulnerability of specific cell types and systems in the developing brain is particularly important in determining the final pattern of damage and functional disability caused by perinatal HI. These patterns of neuronal vulnerability are associated with clinical syndromes of neurologic disorders such as cerebral palsy, epilepsy, and seizures. Recent studies have uncovered important molecular and cellular aspects of hypoxic–ischemic brain injury. The cascade of biochemical and histopathological events initiated by HI can extend for days to weeks after the insult is triggered, which may provide a ''therapeutic window'' for intervening in the pathogenesis in the developing brain. Activation of apoptotic programs accounts for the majority of HI-induced pathophysiology in neonatal brain disorders. New experimental approaches to protecting brain tissue from the effects of neonatal HI include administration of neuronal growth factors and effective inhibition of the death effector pathways, such as caspase cascade, and their downstream targets, which execute apoptosis and/or induction of their regulatory cellular proteins. Our recent findings that a novel neuronal protein, neuronal pentraxin 1 (NP1), is induced following HI in neonatal brain and that NP1 gene silencing is neuroprotective suggest that NP1 could be a new molecular target in the central neurons for preventing HI injury in developing brain. Most importantly, the specific interactions between NP1 and the excitatory glutamate receptors and their colocalization further implicate a role for this novel neuronal protein in the excitotoxic cascade. Recent experimental work suggests that these approaches may be effective during a longer therapeutic window after the insult, as they are acting on events that are relatively delayed, creating the potential for therapeutic interventions for these lifelong neurological disabilities.
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Keywords: Perinatal hypoxia–ischemia; Excitotoxicity; Glutamate receptors; Brain injury; Epilepsy; Apoptosis; Neuroprotection; Neuronal pentraxin 1; Epileptogenesis

1. Introduction

Epilepsy is one of the most common neurological disorders [1], and ∼40% of epilepsies are acquired, meaning that the epileptic condition is acquired through an injury to the central nervous system [2,3]. Neuronal injury such as status epilepticus (SE), stroke, and traumatic brain injury produces long-term plasticity changes in the neurons, resulting in spontaneous recurrent seizures commonly known as acquired epilepsy [4]. Epileptic seizures in children are one of the most common and frightening neurological conditions. The incidence of seizures is significantly higher in children than in adults,

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with the highest incidence occurring during the first year of life [5]. Although there are many common features of the disorder across the age groups, it has become clear that there are also significant differences between young children and adults with respect to both the pathogenesis and consequences of epilepsy and seizures. The molecular basis for developing acquired epilepsy is still not completely understood. Perinatal hypoxic–ischemic brain injury is one of the most important causes of epilepsy, and often manifests as complex partial seizures, which occur in the majority (15–60%) of children with cerebral palsy [6–8]. Cerebral palsy is a known risk factor for complex partial epilepsy and also for generalized tonic–clonic, myoclonic, and focal seizures [9]. The frequent association of epilepsy with cerebral palsy is of special interest. Various studies reveal that the age at onset of epilepsy is closely correlated with neurological abnormalities. Children with cerebral palsy have significantly earlier seizure onset than those with normal neurodevelopment [10]. Further, larger studies are required to delineate other prognostic factors. The development of a novel animal model that mimics more closely human epilepsy, especially that associated with perinatal hypoxia–ischemia (HI), may facilitate efforts to characterize relevant epileptogenic mechanisms and to identify effective antiepileptogenic treatments. The neurobiological basis of hypoxic–ischemic injury in the developing brain and its implications in various neuropathological entities that may lead to clinical forms of epilepsy are reviewed here.

2. Pathophysiology of hypoxic–ischemic brain damage

Hypoxia–ischemia is a common cause of damage to the fetal and neonatal brain. Survivors of such injury can experience substantial and lifelong cognitive, sensory, and motor disabilities, including paralysis, learning disabilities, cerebral palsy, and epilepsy, for which currently there is no promising therapy, and even death [11–14]. Clinical studies conducted with magnetic resonance imaging (MRI) have supplemented postmortem studies that demonstrated patterns of selective vulnerability in the developing brain at different ages. Hypoxic–ischemic insults during critical cellular and tissue differentiation processes have a serious impact on brain maturation. Thus, the gestational/perinatal age of the infant is one of the main variables in determining the neuropathological picture of hypoxic–ischemic brain injury. For this reason alone, the developmental stage at which a hypoxic–ischemic insult occurs is of great importance. However, the mechanism(s) underlying the neuronal damage and death triggered by HI in the developing brain leading to various forms of neurological disabilities remains inadequately understood. Hypoxia–ischemia during early phases of gestation has

toxic effects on the white matter, in contrast to the immature basal ganglia and motor cortex, which are vulnerable to injury at a later phase. For instance, injury to the developing white matter during the early weeks of gestation results in the spastic form of cerebral palsy [15]. This differential susceptibility to injury may be related to the development of interneuronal connections and excitatory glutamate receptors that create the possibility of excessive neurotransmitter release and receptor overstimulation. Moreover, most forms of HI in neonates cause injury to selected areas of the brain rather than the entire brain [16,17]. One of the central features of human temporal lobe epilepsy with complex partial seizures is hippocampal lesions resulting from neuronal death in the hilar, CA1, and CA3 areas [18]. A similar pattern of neuronal death has been observed in the neonatal rat model of HI, in which the CA3 region is the most susceptible population followed by the granule cell layer, CA1, and the hilus [19]. These findings further suggest that the pattern of neuronal loss appears to depend on the stage of development during which the HI insult occurred [19]. Williams and co-workers recently reported induction of epilepsy and synaptic reorganization in the hippocampus in a perinatal rat model of HI [8], similar to that used in our study [20]. Over the last few years MRI has also revealed a special pattern of symmetric injury to the putamen, thalamus, and cerebral cortex after severe or near-total asphyxia [16,21]. These regions of the brain are the targets of excitatory glutamatergic input [22]. There is good evidence from the laboratory as well as from human experimental studies that this special pattern of damage reflects the dysfunction of a selected set of excitatory neuronal circuits triggering selective neuronal death [23]. Selective vulnerability of neuronal structures in the perinatal brain to HI appears to be related to the developmental characteristics of excitatory synapses in the developing neuronal network. Thus, sustained excitatory activity in the corticothalamic connections initiated by HI could be responsible for selective neuronal injury in these brain areas and for seizures and proximal EEG activity. Although systemic and cerebrovascular factors play an important role in the initial phases of hypoxic–ischemic injuries, recent studies have uncovered important cellular and molecular aspects of injury and brain damage.

3. Neurotoxic events triggered following hypoxia–ischemia

Hypoxic–ischemic damage at the prenatal/perinatal stage has a severe impact on brain maturation and is a leading cause of serious human neurological disorders. A great deal of laboratory work on cerebral blood flow and perfusion suggests that most hypoxic injuries in fetuses and infants reflect a combination of hypoxia

and ischemia [24]. In general, the syndrome of hypoxic–ischemic encephalopathy is an integral component of the evolving injury, reflecting a cascade of biochemical and molecular events that evolves over hours to several days [19]. The mechanisms leading to neuronal damage and death triggered by HI and the pathological consequences leading to various neurological disorders remain inadequately understood. Therapeutic intervention in the neuropathological disorders caused by HI will likely require a greater understanding of the discrete neurotoxic molecular mechanism(s) of the neuronal cell death program triggered by HI in the developing brain. Our laboratory and collaborators have been interested in the problem of neuronal vulnerability and injury in developing brain initiated by hypoxic–ischemic insult. Our major focus is to understand the underlying molecular and cellular events contributing to the mechanism of injury using a well-established perinatal animal model of hypoxic–ischemic brain injury. Study of this in vivo model of HI, as well as in vitro experiments using primary neuronal cultures, indicates that HI triggers a cascade of biochemical and molecular events—energy failure, membrane depolarization, brain edema, increased neurotransmitter release and inhibition of neurotransmitter uptake, increased intracellular calcium, production of oxygen free radicals and lipid peroxidation—that induce neuronal injury, neurodegeneration, and cell death [16,25–28]. Understanding the mechanisms behind these complex molecular cascades of injury in neonates will provide much of the insight required to meet the challenges posed by HI-induced brain damage and to develop novel strategies to prevent or avert the long-term consequences of various neurological disorders such as epilepsy and cerebral palsy.

4. Excitotoxicity and hypoxic–ischemic brain injury

The fundamental process responsible for hypoxic–ischemic damage of neurons is called *excitotoxicity*, a term popularized in 1970s by John Olney that refers to cell death caused by excessive stimulation of extracellular excitatory amino acid receptors [25]. The amino acid glutamate mediates almost all excitatory synaptic transmission in the brain [29]. Several studies indicate that glutamate receptor-mediated excitotoxicity is a key player in neuronal cell death and that it is more critically involved in the developing brain than in the adult brain [16,25–28]. The neuronal injury occurring with cerebral HI has been attributed to overstimulation of the *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtypes of excitatory amino acid glutamate receptors [25,27,30]. Acute energy deprivation leads to the excessive release of extracellular glutamate and uncontrolled activation of ionotrophic glutamate receptors NMDA,

AMPA, and kainate [25,31]; impedes energy-dependent reuptake of glutamate [32]; and causes a rise in intracellular Ca^{2+} concentration [33]. The massively increased levels of intracellular second messenger Ca^{2+} trigger activation of toxic intracellular pathways involving kinases, phosphatases, proteases, free radicals, mitochondrial dysfunction, inflammation, DNA damage, and, ultimately, irreversible neuronal injury and death [31,34,35]. Previously we found that quinolinic acid, an endogenous metabolite of tryptophan, which mediates neuronal injury through NMDA receptor activation [36], produces substantial neuronal injury following intracerebroventricular injection into rat brain [37]. This quinolinate-induced model of brain injury is considered a model of the relatively late events of hypoxic–ischemic injury [36].

Cell death due to excitotoxicity occurs in many types of cells in the newborn brain, and the initial trigger may be impairment of glutamate uptake by glia, resulting in overactivation of glutamate receptors [23]. Developmental differences in the function and expression of glutamate receptors dictate the response of the newborn to injury. For example, oligodendrocyte progenitor cells express glutamate receptors including NMDA, AMPA, and kainate subtypes. Experimental data indicate that blockade of these receptors protects against hypoxic–ischemic injury to the white matter in the immature rodent [38]. Several studies suggest that mitochondria also play a critical role in determining whether neurons live or die following hypoxic–ischemic insult [39]. Injured mitochondria switch cells to anaerobic metabolism, raising the level of lactic acid from enhanced glycolysis. Experimental evidence suggests that brain mitochondria become dysfunctional in tissue that is destined to die in the course of HI [40]. Studies in rodents following HI have found that important functional deficits of mitochondria can be blocked by NMDA receptor blockade [40]. Furthermore, calcium entering from the NMDA receptor channel may also be concentrated in the mitochondria, which, in turn, disrupts their function [40,41]. NMDA channel overactivity, calcium entry into neurons, production of nitric oxide, and mitochondrial dysfunction are intimately linked in a potentially lethal cycle [42]. There is abundant evidence that excitotoxic injury to neuronal mitochondria is linked to activation of neuronal death, and mitochondria appear to play a central role in determining expression of injury as necrosis and/or apoptosis [40,41,43–45].

Another important component of glutamate synaptic dysfunction caused by HI is postsynaptic neuronal membrane depolarization with secondary opening of voltage-sensitive channels [46]. Membrane depolarization in the face of high levels of synaptic glutamate produces maximum channel opening and flooding of calcium and sodium into neurons [25]. Although high levels of glutamate can produce some degree of mem-

brane depolarization in the face of normal mitochondrial function, maximal excitotoxicity probably occurs when there is synergism between high synaptic glutamate levels due to disruption of glucose delivery and oxidative stress. The prominent role of the NMDA receptor–channel complex in perinatal HI is related in part to its special transient role in brain development. The NMDA receptor subunits in the developing brain create populations of receptors that flux more calcium, open more easily, and block less frequently than mature forms, allowing them to serve these special developmental roles [23]. However, this makes the immature brain more susceptible to excitotoxic injury if critical levels of energy failure are exceeded. For instance, neurotoxicity mediated by NMDA is more enhanced in the neonatal rat brain than the adult brain [26]. Furthermore, the important role that NMDA receptors play in activity-dependent neuronal plasticity during development makes them particular targets for neonatal HI [23]. Therefore, development-dependent changes in the expression of NMDA receptor subunits and their composition are, at least, partially responsible for the fact that immature brains are far more excitable and epileptogenic than the adult brain [5].

5. Apoptosis as a basic mechanism of hypoxic–ischemic cell death

Excitotoxic cell death occurs through necrosis and/or apoptosis. Early HI-induced neuronal death occurs through necrosis (primary damage) [47]. Delayed neuronal death (secondary damage) occurs hours or days later through a series of complex and highly regulated biochemical and molecular events leading to apoptosis [12,48,49]. This apoptosis–necrosis morphological continuum of neuronal death after HI [17,50] is similar to that observed in neonatal rats after excitotoxic activation of NMDA and non-NMDA glutamate receptors, suggesting that hypoxic–ischemic neuronal injury is triggered by the excitotoxic cascade [51–53]. Apoptosis is the mechanism for refining cell connections and pathways during development [54] by removing many neurons that will not be needed in adulthood [17,55]. Accumulating data suggest that apoptosis plays a prominent role in the evolution of hypoxic–ischemic injury in the neonatal brain and may be more important than necrosis after injury [56]. During neonatal brain injury, excitotoxicity, oxidative stress, and inflammation all contribute to accelerated cell death by means of either apoptosis or necrosis, depending on the region of the brain affected and the severity of the insult [52]. It is noteworthy that activation of apoptosis-executing caspases is much greater in the immature brain than in the adult brain [56,57]. Activation of caspase-3, a ''terminator protein'' that triggers the execution phase of apopto-

sis, has been implicated in neuronal death in various neonatal HI models [12,48,50,56,58,59]. Activated caspases, in turn, cleave a large number of cellular substrates, including the DNA repair enzyme poly(-ADP-ribose) polymerase (PARP) and deoxyribonuclease, which play a role in apoptosis. Various studies have revealed that activation of caspase-3 is required for glutamate-mediated apoptosis in cultured cerebellar granule neurons. Using a perinatal rat model of HI, we observed prolonged activation of caspase-3 in the injured cerebral cortex and the hippocampal CA1 and CA3 regions of neonatal rat brain following HI (unpublished data). These results are consistent with other studies reporting that caspase-3 is strongly activated in animal models of HI, and that pancaspase inhibitors are strongly neuroprotective when given several hours after the insult [56,58].

Apoptosis is characterized by a series of distinct morphological and biochemical changes such as cell shrinkage, membrane blebbing, and DNA fragmentation [49,60,61]. Two predominant pathways mediate apoptosis in mammalian cells [62]. The death receptor pathway is activated by the binding of specific ligands such as FasL (CD95 ligand) and tumor necrosis factor (TNF) (or TNF-related apoptosis-inducing ligand, TRAIL) to cell surface receptors such as Fas (CD95) and TNFR [63], which, in turn, activate caspase-8. Caspase-8 is then able to activate the downstream executioner caspase-3, which cleaves a variety of proteins, resulting in the classic cytoplasmic and nuclear signs of apoptosis. The c-Jun N-terminal protein kinase (JNK) signaling pathway is also a potential cascade mediating neuronal cell death through apoptosis triggered following focal and global ischemia [64]. There is strong evidence that JNK may be a key mediator for transmission of apoptotic signals to mitochondrial apoptosis-related proteins [65,66]. The other mitochondrial pathway can be triggered in response to diverse environmental and intracellular stresses, such as growth factor withdrawal and DNA damage [67], which also activate caspase-3, but release of cytochrome c from mitochondria is reported to play a key role in this process [62]. These molecules and other signals influence the balance between pro-apoptotic and anti-apoptotic members of the family of BH1–3 multidomain Bcl-2 family of proteins (e.g., Bcl-2, Bax, Bid, Bak) located on the surface of the mitochondria [68]. JNK has been shown to have significant association with Bax and Bak in the mediation of apoptosis [65,66]. In addition, Bcl-2 proteins are also present in nuclear membrane and regulate intracellular calcium, which regulates the gene expression necessary for apoptotic cell death during hypoxia [69]. Thus, the death receptor and mitochondrial apoptosis pathways converge at the caspases that cleave multiple cellular substrates, ending in DNA fragmentation and cell death [70]. It has also been reported that caspase activation

is a constant feature of reperfusion injury in ischemic neurons leading to late cell death [49,61]. Recently, inhibitors of apoptosis proteins (IAPs) and the mito-chondrial protein SMAC/DIABLO (an IAP inhibitor) have been found to play important roles in regulating caspase activation and the commitment to apoptosis [71,72]. Most importantly, these death receptor proteins have been documented in the brain and the cerebrospinal fluid of newborns after brain injury [73,74], suggesting that this pathway may be a potential therapeutic target.

6. Excitatory synaptic function

Most excitatory neurotransmission in the brain is mediated by glutamate acting on AMPA and NMDA receptors [29]. AMPA receptors mediate rapid excitatory synaptic transmission, whereas NMDA receptors have a more modulatory role during synaptic plasticity and development due to their high Ca^{2+} permeability. The predominant charge carrier during routine fast excitatory synaptic transmission is the AMPA-type receptor, whereas NMDA receptors contribute a significant calcium current that is thought to modulate second messenger systems and kinases [75]. One of the earliest events in excitatory synapse formation is the clustering of postsynaptic glutamate receptors, and nearly all excitatory synapses are associated with postsynaptic receptor clusters [76]. The targeting and clustering of AMPA- and NMDA-type glutamate receptors to the synapses in the central nervous system (CNS) are essential for efficient excitatory synaptic transmission [77]. Recent studies have shown that members of the "long pentraxins" family of proteins, such as neuronal pentraxin 1 (NP1) and neuronal activity-regulated pentraxin (Narp, also called NP2), are linked to glutamate receptors at synaptic sites [77,78], and regulate AMPA receptor clustering [79,80]. Thus, it has been proposed that changes in the expression or function of long pentraxins may contribute to changes in excitatory synaptic transmission induced by neuronal stimulation [81]. Further studies reveal that long pentraxins including NP1 and Narp have several structural and functional characteristics that might play a role in promoting excitatory synapse formation and remodeling [82,83]. Exclusive expression of NP1 in the CNS, its characteristic feature as a secretory protein, and its large molecular size relative to the classic pentraxins (>50 kDa vs 30 kDa) suggest that NP1 may have novel unknown functions. Most recently, we showed that the neuronal pentraxin NP1 is induced in hypoxic–ischemic injury in neonatal brain, and that antisense oligonucleotides directed against NP1 mRNA prevent hypoxia-induced neuronal death [20]. Our results establish the neuronal protein NP1 as a novel molecular mediator of hypoxic–ischemic brain injury.

This is the first evidence that a novel neuronal protein (NP1) is part of the neuronal death program initiated by HI in the developing brain. We also found that NP1 is induced in response to HI primarily in the cerebral cortex and hippocampal pyramidal layers of the CA3 and CA1 regions. This differential expression suggests that NP1 serves distinct functions in different neuronal populations/subpopulations. It is possible that selective induction of NP1 in certain subpopulations of neurons reflects their particular vulnerability to injury. Particularly interesting and important are the findings that NP1 is associated with the fast excitatory glutamate receptor AMPA and that hypoxia induces a time-dependent increase in NP1–GluR1 interactions. Thus, hypoxia recruits NP1 protein to GluR1 subunits concurrent with the hypoxic excitotoxic cascade. These results suggest a novel mechanism by which NP1 induction during HI accentuates excitotoxicity and thereby contributes to HI-induced neuronal death. Our study identifies a new molecular target in neurons for preventing hypoxic–ischemic injury, which could lead to more effective neuroprotective strategies against hypoxic–ischemic brain injury in the developing brain.

7. Neurotrophic factors and neuroprotection against injury and death

Since discovery of the potent survival-promoting effects of neurotrophic factors, there has existed the possibility that they could be used in the treatment of diseases of the CNS. The protection of neurons, and perhaps most cells, from excitotoxicity and hypoxic–ischemic injury as well may require extracellular ligand–receptor interactions and the activation of specific intracellular signaling cascades. In the CNS, these signals are provided, in part, by neurotrophic growth factors such as nerve growth factor (NGF), fibroblast growth factors (FGFs), neurotrophin (NT)-3, insulin-like growth factor (IGF), ciliary neurotrophic factor (CNTF), and brain-derived neurotrophic factor (BDNF) [84–86]. These neurotrophic factors support growth, differentiation, maturation, maintenance, and neuronal survival [87], and exhibit neuroprotective activity in multiple neuronal populations after injury [84,85]. To test the potential therapeutic use of growth factors, it is important to elucidate which growth factors can protect against death and dysfunction of particular neuronal populations in specific disease states. The neurotrophic factor BDNF and its protein receptor tyrosine kinase B (Trk B) are expressed in a broad range of neuronal types [88], and are reported to have marked protective effects against tissue loss due to neonatal HI in vivo [89]. The neurotrophin NGF, which acts at receptor tyrosine kinase A (Trk A), has also been reported to be neuroprotective in the Postnatal Day 7 rat HI model [90]. NGF may exert its effect by

inhibiting apoptosis, as NGF withdrawal causes apoptosis in the sympathetic ganglionic neurons that can be blocked by caspase inhibitors. IGF also appears to be a potent neuronal rescue agent after hypoxic–ischemic injury in fetal lamb [91].

We have examined the neuroprotective actions of acidic fibroblast growth factor (FGF-1) and the underlying mechanism(s) of neuroprotection against excitotoxicity [37,92]. FGFs are essential molecules for mammalian growth and development. The nine known FGF ligands and the four signaling FGF receptors are expressed in specific spatial and temporal patterns in the immature and adult CNS [93–95]. Basic FGF (FGF-2) and the structurally related acidic FGF (FGF-1) are induced and released from cells in response to injury [96]. FGF-2, the most extensively studied member of the FGF family, induces neurite outgrowth, and protects against glutamate-induced neurotoxicity of cultured striatal and hippocampal neurons in vitro [97,98] and of striatal and cortical neurons in adult animal models [99]. On the other hand, of the nine known FGF factors, FGF-1 is the only one that is capable of activating all known FGF receptor variants and may therefore have unique neuroprotective properties within the brain [95]. FGF-1 has been found to protect against experimental facial nerve injury and hippocampal injury induced by experimental seizures, and may enhance neuronal regeneration following spinal cord transection [100,101]. We have shown that endothelial cell-based delivery of a human acidic FGF (FGF-1) transgene to the CNS substantially protects the developing rat brain from quinolinic acid-induced excitotoxic injury in vivo [37]. Further investigations using primary neuronal cultures confirmed our in vivo findings of FGF-1-mediated neuroprotection against excitotoxicity [92]. Therefore, FGFs are neuroprotective in neonatal brain, but it is unclear how protective they are when given after injury.

8. Second messenger signaling pathways and neuronal survival

The intracellular signaling pathways by which growth factors promote cell survival and, in particular, survival of CNS neurons are only partially characterized. Survival-promoting effects of neurotrophins are elicited via activation of different intracellular pathways depending on such factors as cell type, culture conditions, and the injurious stimulus. In addition, the ability of different neurotrophic factors to act on neurons is often developmentally regulated [102], and the impact of developmental maturation on effects of intracellular signaling is not completely understood. The mechanism and time course of neuronal death in response to different injuries can be age-dependent [103] and potentially influence the response to intracellular signaling. Recent

studies have revealed that growth and neurotrophic factors could activate several intracellular signal transduction systems in various types of neuronal and nonneuronal cells. These include the mitogen-activated protein kinase (MAPK) kinase (MEK)/extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3-K)/Akt kinase pathways [104–108]. In several neuronal cell types in vitro, the PI3-K/Akt cascade appears to mediate the survival-promoting and neuroprotective effects of growth factors [107]. NGF-mediated activation of the PI3-K pathway appears to be required for the survival of pheochromocytoma 12 (PC12) cells [109] and superior cervical ganglion neurons [110], whereas activation of PI3-K by BDNF in motoneurons [111] and IGF in cerebellar granule neurons, oligodendrocytes, and PC12 cells also appears to be important for survival [112–114]. Studies from our laboratory have demonstrated that FGF-1 protects cerebellar granule neurons against excitotoxicity in a PI3-K/Akt-dependent and MAPK/CREB-independent manner [92].

Although PI3-K is clearly important for growth factor-mediated neuronal survival in certain cells and conditions, in other neuronal cell types and under different conditions, growth factor-mediated activation of the MEK/ERK pathway appears to mediate survival effects. For example, activation of MEK, an activator of MAPK, promotes survival of PC12 cells in response to NGF [104] and IGF-1 [114], as well as survival of primary cortical neurons by estrogen [108]. However, other studies have revealed MAPK-independent survival of sympathetic neurons, PC12 cells, and cerebellar granule neurons mediated by NGF, insulin, and BDNF [105,115]. One study demonstrated that BDNF neuroprotection of cortical neurons can be mediated via the ERK or PI3-K pathway depending on the injurious stimulus [116]. Thus, it appears that the growth and neurotrophic factor-mediated protection of neurons may occur via different intracellular second messenger signaling pathways depending on factors such as cell type, conditions, and injurious stimulus. These findings may have important implications in terms of understanding the cellular signaling responsible for the actions of different neurotrophic factors in CNS neurons in vivo, as well as for developing therapeutic strategies to protect the neonatal brain from hypoxic–ischemic damage.

9. Clinical relevance

These studies indicate that therapy for hypoxic–ischemic injury in developing brain and associated neurological disorders in infants and children will be feasible in the future. It is difficult to predict during the neonatal period which neonate will suffer the most profound damage after the insult to the CNS, as more than 30% of neonates presenting with moderate encephalopathy

have normal outcomes [117]. Studies in laboratory animals have shown that the immature brain responds differently to treatment than does the mature brain. Furthermore, therapy designed to ameliorate brain injury in adults may worsen outcomes in neonates, possibly by accentuating the apoptotic cell death cascade. Control of apoptosis involves a balance between expression of numerous apoptotic and anti-apoptotic proteins after injury, providing many potential approaches to modifying outcome [58,102,118]. Several neurotrophic factors that have been reported to protect against excitotoxicity and hypoxic–ischemic injury in immature animal models may act by inhibiting apoptosis [37,99]. We recently observed that transgenic expression of FGF-1 in neonatal brain protects against hypoxic–ischemic injury by blocking activation of the components of the caspase cascade (unpublished data). The delayed activation of caspase-dependent cell death components after neonatal HI [12,50] suggests that there is a prolonged therapeutic window during which the effects of the activation of survival-promoting signaling pathways by both transcription-dependent and -independent mechanisms could influence survival. It is also plausible that hypothermia, which is currently receiving considerable clinical attention as a neuroprotective strategy, may slow or reduce the excitotoxic cascade by altering processes favoring apoptosis [42,119,120]. Furthermore, our finding that the neuronal protein NP1 is induced in neonatal brain following HI could provide a new molecular target in the neurons for preventing neuronal injury, which could lead to more effective neuroprotective strategies against hypoxic–ischemic brain injury in the developing brain [20]. However, developing novel strategies to treat and avert the consequences of hypoxic–ischemic brain injury in children will require further understanding of the unique mechanisms of injury initiation and propagation in the immature brain.

10. Summary

The neonatal brain is far more electrically excitable and prone to excitotoxicity than the adult brain, probably because many excitatory circuits are enhanced during development to promote activity-dependent neuronal plasticity. Hypoxia–ischemia rapidly and profoundly disrupts excitatory circuits, causing a buildup of glutamate and other excitatory amino acids and depolarization of neuronal membranes. Furthermore, neurons connected to established neuronal circuits appear to be particularly vulnerable to excitotoxic damage. Brain injury from HI is not immediate but evolves over a period after the initial insult. Work on experimental HI in immature animal models demonstrates that delayed neuronal damage is triggered initially by overstimulation of excitatory glutamate receptors and executed

by a cascade of events that includes calcium entry, nitric oxide production, mitochondrial disruption, and delayed death by either necrosis or apoptotic cell death programs. Early glutamate-mediated events mediated by NMDA and AMPA receptors constitute the important primary triggering phase of the hypoxic–ischemic insult in perinatal brain, but the excitatory cascade rapidly becomes self-perpetuating, ultimately causing neuronal injury and degeneration. Activation of apoptotic programs accounts for the majority of HI-induced pathophysiology in neonatal brain disorders. Thus, the concept of an extended excitotoxic cascade provides a rational framework for designing effective neuroprotective strategies for neonates. Recent work with growth factors and caspase inhibitors offers the prospect of neuronal rescue. It is noteworthy that identification of novel neuronal target molecules acting upstream in regulating the onset of the cascade of delayed events in the cell death pathway has the potential to prevent the serious neuropathological effects of perinatal brain injury. An understanding of the basic molecular mechanisms of neonatal brain injury combined with advances in neuroimaging and developmental biology may result in the prevention of or reduction in the incidence of such lifelong disabilities as cerebral palsy, epilepsy, and behavioral and learning disorders.

Acknowledgments

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