Excitotoxicity in Perinatal Brain Injury

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Excitotoxicity is an important mechanism involved in perinatal brain injuries. Glutamate is the major excitatory neurotransmitter, and most neurons as well as many oligodendrocytes and astrocytes possess receptors for glutamate. Perinatal insults such as hypoxia-ischemia, stroke, hypoglycemia, kernicterus, and trauma can disrupt synaptic function leading to accumulation of extracellular glutamate and excessive stimulation of these receptors. The activities of certain glutamate receptor/channel complexes are enhanced in the immature brain to promote activity-dependent plasticity. Excessive stimulation of glutamate receptor/ion channel complexes triggers calcium flooding and a cascade of intracellular events that results in apoptosis and/or necrosis. Recent research suggests that some of these intracellular pathways are sexually dimorphic. Age dependent expression of different glutamate receptor subtypes with varying abilities to flux calcium has been associated with special patterns of selective vulnerability at different gestational ages. For example, selective injury to the putamen, thalamus and cerebral cortex from near total asphyxia in term infants may be related to excessive activation of neuronal NMDA and AMPA type glutamate receptors, while brainstem injury may be related primarily to stimulation of neuronal AMPA/kainate receptors. In contrast, periventricular leukomalacia in premature infants has been linked to expression of AMPA/kainate receptors on immature oligodendrocytes. Insight into the molecular pathways that mediate perinatal brain injuries could lead to therapeutic interventions.

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Excitotoxicity has emerged as an important mechanism of injury in the brain, and the concept is important for understanding perinatal brain pathology. Following the original observation by Lucas and Newhouse in 1957 that glutamate can damage the retina (31), Olney described the excitotoxicity concept in the 1970s as neuronal death mediated by excessive stimulation of excitatory amino acid receptors at synapses (54). The initial description of this concept in the central nervous system was based on the observation that the food additive monosodium glutamate caused selective hypothalamic lesions and obesity when fed to neonatal mice (54). Glutamate is the predominant excitatory amino acid neurotransmitter in the brain, and most neurons and many glia possess extracellular receptors for glutamate. Neuronal pathways that utilize glutamate as their neurotransmitter are ubiquitous in the brain, mediating vision, hearing, somatosensory function, learning and memory and other functions. Cell death in these disorders is mediated by excessive activation of the multiple receptor subtypes that recognize the various conformations of glutamate, leading in turn to calcium flooding and downstream toxic effects on cellular metabolism (7, 61).

SYNAPSE DEVELOPMENT AND EXCITOTOXICITY

Neuronal death from excitotoxicity is linked directly to dysfunction of excitatory synapses (28) (Figure 1). The development of excitatory neuronal circuits, as well as expression of specific glutamate receptor subtypes in excitatory synapses, are dynamic in the perinatal brain, and these changes can be related to changing patterns of pathology at different gestational ages (8, 64). Glutamate is a di-carboxylic acid molecule with considerable structural flexibility, and multiple receptor subtypes have evolved to mediate its actions. These receptors were named for chemicals that are rigid analogues of glutamate. There are 3 major groups of receptors within the post-synaptic membrane that operate ion channels, so called ionotropic receptors, and a group of G-protein linked metabotropic glutamate receptors. The 3 major types of ionotropic receptors are the N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-isoazole-4-propionic acid (AMPA) and kainic acid (KA). Normally, glutamate is contained within the pre-synaptic nerve terminal until release is stimulated by neuronal depolarization; when release into the synaptic cleft does occur, the neurotransmitter is quickly taken up by high-capacity glutamate transporters in astroglia that surround the synapses and nerve terminals (Figure 1). Glutamate taken up into the astroglia is converted to glutamine before being transported back into the nerve terminal to recycle glutamate neurotransmitter (32). Hypoxia-ischemia and hypoglycemia impair the function of the astroglial glutamate transporters, causing glutamate to accumulate in the synaptic cleft where it can activate glutamate receptors (65). The glutamate transporter is dependent on a sodium gradient created by Na^+/K^+ ATPase that is powered by anaerobic glucose metabolism, and impaired delivery of glucose to the brain by ischemia and/or hypoglycemia impairs glutamate removal from the synapse (23, 32). Severe hypoxia associated with hypoxia-ischemia or ischemia also depolarizes nerve terminals, reversing their glutamate transporters and leading to additional accumulation of synaptic glutamate (60). Elevations in extracellular glutamate have been measured in animal models of perinatal hypoxia-ischemia using intracerebral microdialysis (17, 66).

While the accumulation of glutamate within synapses and the brain's extracellular space is a generic phenomenon that occurs in most regions of the brain where glutamate-containing pathways are present, the toxic effect of this accumulation is determined by the local repertoire of postsynaptic glutamate receptors. The distribution and molecular characteristics of NMDA-type glutamate receptors appears to be an especially important determinant

of the pattern of neuronal injury in the perinatal brain (Figure 2). The NMDA receptor channel complex requires co-activation by both glutamate and glycine, and is also voltage dependent, requiring depolarization of the post-synaptic membrane for the channel to open. The NMDA channel is blocked by magnesium at rest, but the block is released with membrane depolarization, allowing calcium to flux inward. These special features allow the NMDA receptor to play a role in activity-dependent synaptic plasticity, including long term potentiation (LTP) and refinement of synaptic connections (40). However, disruption of membrane potentials by hypoxia-ischemia can also overcome the magnesium block and open the NMDA channels. Drugs that block NMDA receptors or channels, such as dizocilpine (MK-801), dextromethorphan, ketamine, or magnesium, are strongly protective against hypoxic-ischemic injury if given before or shortly after hypoxic-ischemia or other insults in neonatal rodent models (18, 43). At around 7 days of age the rodent brain is much more sensitive to direct intracerebral injections of NMDA, ibotenic acid, hypoxia-ischemia or trauma than the adult brain (4, 39, 44). Molecular studies suggest that functional activity in NMDA receptors is controlled by changes in their heteromeric subunit composition (48, 49). Hypersensitivity to NMDA receptor activation during the neonatal period can be correlated with molecular features of the immature NMDA receptor operated channels that allow them to open more easily and flux more calcium than their adult counterparts. Autoradiographic studies of the development of glutamate binding to the NMDA receptor channel complex in rat hippocampus demonstrated an overshoot in receptor density compared to the adult and selective changes in binding to glutamate binding sites and channels (41). Electrophysiologic studies show that LTP and NMDA-mediated synaptic currents are heightened during postnatal days 3 to 7 in rat thalamocortical synapses, during a critical period for somatosensory cortical plasticity (9). NMDA receptors probably mediate much of the injury to neurons in structures such as cerebral cortex, basal ganglia, hippocampus and thalamus associated with hypoxic-ischemic injury in animal models.

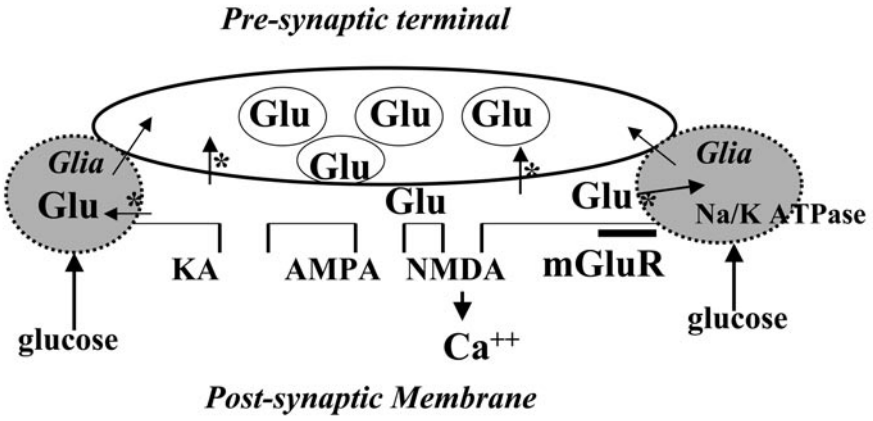

Figure 1. Excitotoxic injury is initiated by dysfunction at synapses, shown schematically. Four major types of glutamate receptors are present at excitatory synapses: NMDA, AMPA, kainate (KA), and metabotropic, G-protein linked (mGluR). Glutamate is normally released from the pre-synaptic nerve terminal into the synaptic cleft, then taken up by sodium dependent transporters in glia (shaded) and neuronal terminals. Glutamate taken up into glia is combined with ammonia to form glutamine, which diffuses into the nerve terminal and is converted to glutamate by glutaminase (not shown). The glutamate transporter is supported by a sodium gradient linked to Na+/K+ ATPase powered by anaerobic glycolysis. Glutamate accumulates in the synaptic cleft when the transporter is impaired by hypoxia and reduction in glucose delivery from ischemia. Hypoxia-ischemia leads to depolarization of the pre-synaptic nerve terminal, which reverses the neuronal glutamate transporter, and depolarization of the post-synaptic membrane leading to opening of NMDA channels.

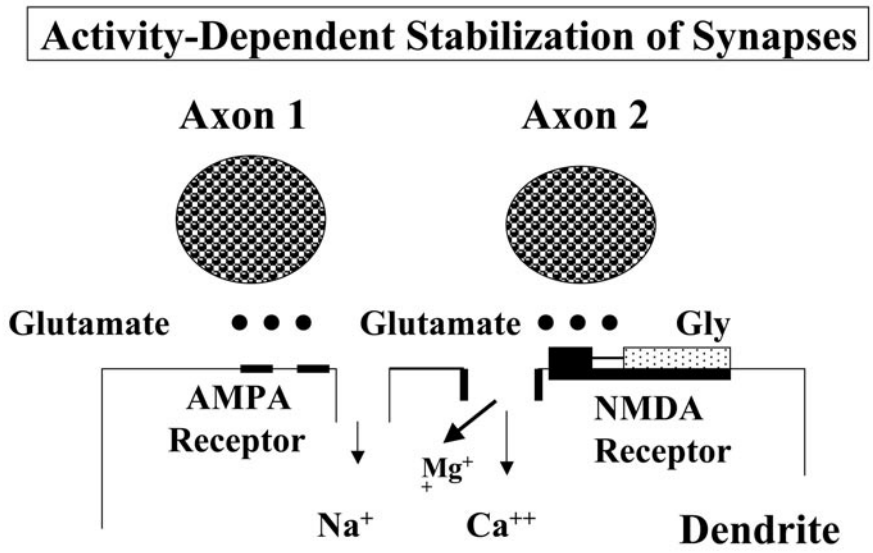

Figure 2. Normal function of glutamate ionotropic receptors in the developing brain, shown schematically. NMDA type glutamate receptors require receptor activation by glutamate and glycine, as well as membrane depolarization from activation of AMPA type glutamate receptors. Membrane depolarization is required to overcome the channel blockade from magnesium. Normally NMDA and AMPA receptor/channel complexes work hand in hand for normal tasks such as learning and memory and stabilization of synapses in the developing brain. During synaptic stabilization, coincident activation of two axons leads to opening of the NMDA receptor channel, which in turn leads to retrograde signaling by neuronal growth factors. These mechanisms are enhanced during development, and stresses such as hypoxia-ischemia can cause excititoxicity more easily than in the adult brain.

Activation of AMPA receptors, which primarily flux sodium and mediate most of the fast excitatory activity in the brain, also contribute to this injury. From a developmental standpoint, NMDA receptors are the first glutamate receptors to appear at new synapses, followed by AMPA receptors associated with increasing neuronal activity (8, 64). Immature AMPA receptor channels flux calcium like NMDA receptors, but increasing expression of GluR2 receptor subunits and RNA editing over the first 2 post-

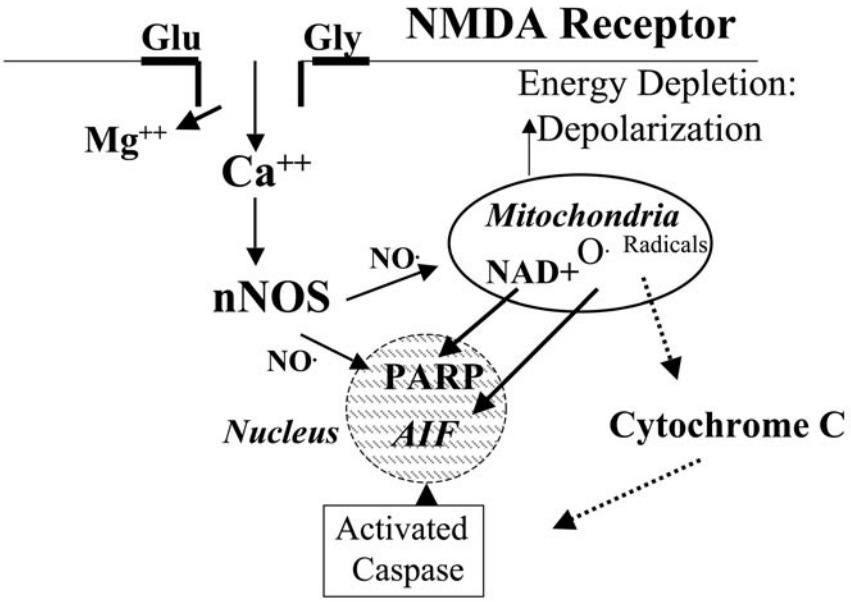

Figure 3. NMDA activated cascades involved in brain injury from hypoxia-ischemia, shown schematically. Calcium flooding through the NMDA channel activates neuronal nitric oxide synthase (nNOS) to form nitric oxide ($NO^{\cdot}$), which causes mitochondrial injury and DNA breaks that activate PARP-1. Direct mitochondrial injury from oxygen free radical stress can cause collapse of energy production leading to necrosis or release of cytochrome C, activation of caspases, and apoptosis. Activation of PARP-1 appears to trigger a parallel, non-caspase pathway to apoptosis by depleting nicotinamide adenine dinucleotide (NAD+) in mitochondria and stimulating transfer of apoptosis inducing factor (AIF) from mitochondria to the nucleus. The NMDA-$NO^{\cdot}$-PARP-AIF pathway appears to be sexually dimorphic: blocking this pathway protects males from brain injury, but blocking the same pathway in females either fails to protect or makes injury worse.

natal weeks in rodents creates a majority of calcium impermeable AMPA receptors in the mature brain (48, 63). Direct injections of AMPA agonists at various postnatal ages produce greater injury during the postnatal period than during adulthood, with a peak several days after that for NMDA (45). AMPA antagonist drugs are not as protective against hypoxic-ischemic neuronal injury as NMDA antagonists in the perinatal period, although the AMPA antagonist topiramate has been shown to be protective in combination with hypothermia in a model of hypoxic-ischemic injury in infant rats (30). The molecular features of both NMDA and AMPA receptors during the perinatal period are programmed to allow them to participate in activity-dependent neuronal plasticity and development. One indication of their importance for development is the observation that prolonged blockade of NMDA receptors causes apoptosis in cell culture and animal models (24). However, their vital role and enhanced function in the perinatal period also makes neurons more vulnerable to excitotoxicity. This creates a paradox: the immature brain can withstand longer periods of energy deprivation than the adult brain because of its low energy requirement, yet when a critical threshold of energy deprivation is reached, excitotoxic injury is enhanced because of developmentally activated excitatory pathways (25).

DOWNSTREAM EVENTS IN THE EXCITOTOXIC CASCADE

Calcium flooding through open NMDA and calcium permeable AMPA receptor channels, as well as through other types of calcium channels, combined with release of calcium from intracellular stores, triggers a cascade of intracellular events that mediate cell death (Figure 3). Recent studies indicate that calcium mediated activation of calpain can also contribute to this process by destroying the membrane NCX Na^{+}/Ca^{2+} exchanger that normally maintains low intracellular calcium levels (2). Calcium mediated activation of neuronal nitric oxide synthase (nNOS) and calcium overload together contribute to dysfunction of mitochondria and production of oxygen free radicals (1, 10, 14). Cytochrome C released from mitochondria, as well as activation of Fas extracellular death receptor pathways, in turn contribute to activation of caspases, leading to apoptosis (27). Activation of caspases is also enhanced in the perinatal brain compared to the adult (21, 51). Apoptosis is more prevalent as a mode of death in the perinatal brain than in the adult, and there is a continuum of apoptosis and necrosis that is dependent on brain region (34). Transcription in the nucleus also plays an important role in whether cells die or survive following a perinatal insult (29).

A parallel, non-caspase death pathway in the developing brain is also triggered by NMDA receptor-mediated activation of the DNA repair enzyme poly (ADP-ribose) polymerase 1 (PARP-1) (68). PARP-1 is activated by DNA breaks caused by nitric oxide, which is synthesized by nNOS in response to calcium fluxed preferentially through NMDA receptors. PARP-1 activation consumes NAD^{+} to form poly (ADP-ribose) polymer and reduction of the high-energy substrate, further degrading energy production by mitochondria already stressed by hypoxia and oxygen free radicals. This leads in turn to translocation of apoptosis inducing factor (AIF) from the mitochondria to the nucleus to produce apoptosis. Recent experiments in transgenic mice with PARP-1 knocked out showed that this step is sexually dimorphic in the perinatal brain: male knockout mice were protected from brain injury from hypoxia-ischemia, but females were not (20). Follow-up studies showed that this sex-based disparity is even greater in adult PARP-1 knockout mice, as males were protected by the gene knockout or by drugs that inhibit PARP-1, but injury in females was worse (37). These results from animal experiments suggest that there is a major sex-based difference in NMDA-mediated cell death pathways mediated through PARP-1 activation that has yet to be investigated in humans.

EXCITOTOXICITY AND HYPOXIC-ISCHEMIC ENCEPHALOPATHY IN TERM INFANTS

The excitotoxicity concept developed primarily using in vitro and animal models fits well with the clinical presentation of term infants with hypoxic-ischemic encephalopathy (HIE). The acute insult is generally followed by a latent period of 6 to 36 hours before signs of encephalopathy such as seizures, need for supported ventilation, severe hypotonia, and inability to feed

orally emerge. Evidence of neuronal excitation such as seizures and epileptic electroencephalographic (EEG) activity are nearly constant features of the encephalopathy that unfolds after a severe asphyxial insult, and there is generally a good correlation between the severity of these signs of excitability and the severity of injury. The seizures and abnormal EEG generally worsen and then improve over a period of a week (28). Positron emission tomography (PET) for glucose metabolism in term infants with HIE has shown hypermetabolism in the basal ganglia and cerebral cortex (5). A similar effect has been demonstrated in rodents (13). Glucose PET scans in the brain have been shown to be directly correlated with activity of the glutamate pumping from synapses through its connection with Na^+/K^+ ATPase. The severity of encephalopathy and outcome from HIE have also been shown to correlate with levels of the excitatory amino acids glutamate and aspartate in cerebrospinal fluid (19). Magnetic resonance spectroscopy has also shown elevations in the peak for glutamate/glutamine in the basal ganglia and thalami of infants with severe HIE (59). The characteristics of the NMDA, AMPA and kainate receptors appear to be similar to those of rodents when studied in postmortem tissue or in biopsy samples. Chahal and colleagues studied [3H]-MK-801 binding to NMDA channels in postmortem tissue from neonates, infants and adults, and found that infants have an excess of NMDA receptors that are hyper-responsive to glutamate, resembling the rodent studies (6).

NEAR TOTAL ASPHYXIA: BASAL GANGLIA AND BRAINSTEM DAMAGE

Magnetic resonance imaging (MRI) has markedly improved the understanding of the patterns of brain injury from perinatal asphyxia. The pattern produced by so-called "near total" asphyxia is easily recognized on early MRI scans or later scans and includes relatively selective injury to the putamen, thalamus and peri-Rolandic cerebral cortex and often includes injury to the brainstem as well (3, 58) (Figure 4). This pattern is similar to the pathological pattern of diencephalic and brainstem injury described by Myers in his model of acute total asphyxia in subhuman primates developed in the early 1970s (50). It can be distinguished from the injury produced by a partial prolonged insult that results in a primary, but more extensive cortical injury. In most infants, white matter is relatively spared, although soon after injury, a transient increase in signal is often seen in the posterior internal capsule (62). The insult that produces this pattern of injury is a relatively brief period of intense asphyxia from an insult such as complete umbilical cord compression that lowers cardiac output severely. Infants who sustain this type of insult generally must be vigorously resuscitated in order to survive, and have severe metabolic acidosis when cord blood gases are analyzed. The injury shows up within the first 2 weeks as increased signal on T1-weighted images in the posterior putamen and ventrolateral thalamus, as well as in a the peri-Rolandic cerebral cortex, but after several months, the same areas lose their T1 enhancement and have increaed signal on T2 weighted images. A very similar pattern of basal ganglia and thalamic injury as well as altered expression and phosphorylation of NMDA receptors have been noted in the corpus striatum (16, 35, 36). Infants and children with this pattern generally have disabling cerebral palsy with dystonia, rigidity and athetosis, although intelligence is relatively spared.

As shown in the schematic in Figure 5, the pattern of injury seen following near total asphyxia could result from excessive excitation within the reciprocal excitatory connections between the thalamus and cortex and the cortico-striatal excitatory connections to the putamen (55). The exact mechanisms for this very selective injury are not known, but one factor could be the stage of development of these particular excitatory pathways at the time of birth. Although the thalamus is also vulnerable to injury in premature infants, this characteristic pattern of injury on MRI is strongly associated with term infants (33, 46). It is noteworthy that the globus pallidus is usually spared in this type of asphyxial injury, and this may be due to the fact that sustained excitatory activity in the putamen is likely to inhibit the globus pallidus. In contrast, other disorders that can damage the perinatal brain, such as kernicterus and genetic mitochondrial disorders, selectively damage the globus pallidus while sparing the thalamus and putamen (26). These disorders may produce an injury over a more prolonged period to mitochondrial energy metabolism in the globus pallidus that results in neuronal depolarization and passive opening of the NMDA glutamate channel (52). In addition, the globus pallidus in the newborn period possesses a transient glutamate innervation that is not present in the mature brain (15). NMDA antagonists can provide protection against neuronal degeneration in experimental models of kernicterus (42).

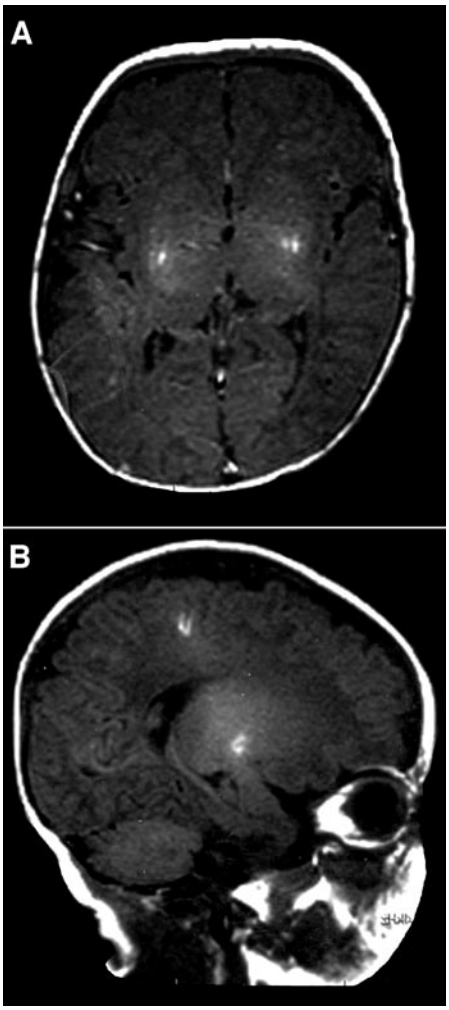

Figure 4. T1-weighted magnetic resonance images of a full term infant at 10 days after an episode of severe, near-total asphyxia from umbilical cord compression and severe metabolic acidosis. Panel **A** shows increased signal in the posterior putamen and thalamus, while panel **B** shows increased signal in the peri-rolandic sulcus and thalamus on lateral view. These areas are selectively vulnerable to injury from this form of asphyxia and other areas of the brain are relatively spared. This selective vulnerability may be related to excitotoxic injury in areas of the developing brain that have developed functional glutamate circuits that mediate motor function, control of breathing and other brainstem functions.

While the mature brainstem is relatively resistant to hypoxia-ischemia, the inferior olive, griseum pontis, inferior colliculus

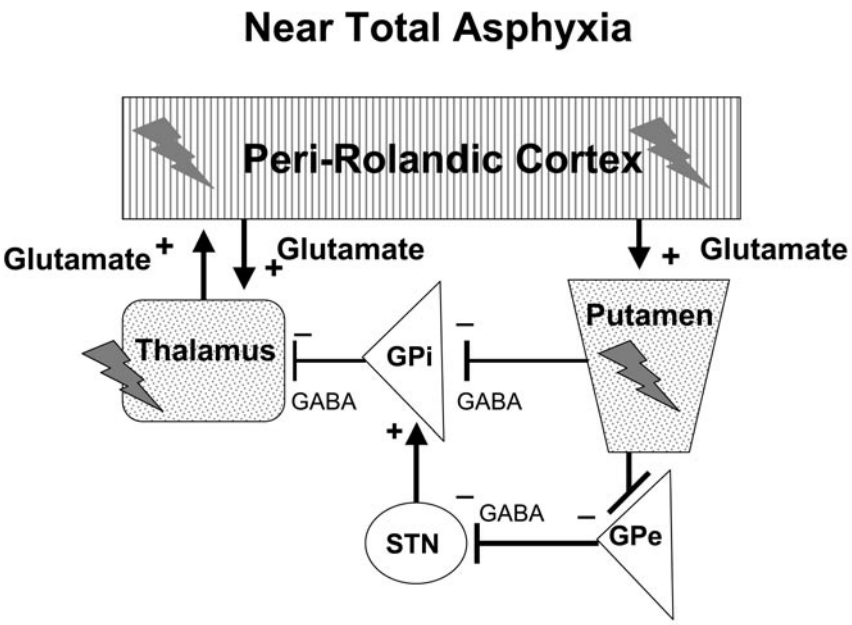

Figure 5. Proposed pathogenesis of selective injury in the peri-rolandic cortex, putamen and thalamus from near-total asphyxia in term infants, shown schematically. The vulnerable regions are connected by glutamate containing excitatory circuits, while areas such as the globus pallidus, which are usually spared in this type of injury, are downstream to GABAergic inhibitory synapses. On the other hand, subacute disorders such as kernicterus and mitochondrial disorders often target the globus pallidus and subthalamic nucleus. It is hypothesized that mitochondrial energy failure leads to passive opening of NMDA channels in these disorders (26). Abbreviations: GABA: γ-aminobutyric acid; GPi: internal segment globus pallidus; GPe: globus pallidus external segment; STN: subthalamic nucleus.

and reticular formation are vulnerable to injury in the perinatal period (Figure 4). In contrast to the probable role that NMDA receptors play in perinatal damage to the cerebral cortex, thalamus and basal ganglia, AMPA and kainate receptors are implicated most strongly in perinatal damage to the brainstem. Autoradiographic studies in human postmortem tissue indicate that AMPA and/or kainate receptor binding is elevated in these vulnerable regions in the midgestation fetus and neonate, and then declines at later ages, while NMDA receptor binding is undetectable at midgestation and then matures in the postnatal period (56, 57). Elevated levels of AMPA/kainate receptors in the griseum pontis at midgestation and early infancy may be relevant to pontosubicular necrosis from hypoxia-ischemia during the last trimester and early infancy (56). AMPA receptors probably mediate the stimulus of breathing movements via the nucleus of the solitary tract during the fetal period, while NMDA receptors likely mediate stimulation in response to hypoxia and sustained ventilation in the newborn and infant (56). This suggests that the vulnerability of these brainstem structures to injury is related to the adaptive roles that the different types of glutamate receptors play in normal neuronal development and plasticity.

EXCITOTOXICITY AND WHITE MATTER INJURY

Despite the fact that there are few synapses in white matter, receptors for glutamate have also been shown to play an important role in the pathogenesis of white matter injury in the immature brain (12, 38). This may occur because hypoxia-ischemia or other insults release glutamate from axons within the white matter or that glutamate diffuses into white matter from astroglia and synapses in adjacent neuron-rich areas. Immature oligodendrocytes express AMPA receptors during a window of time when they are vulnerable to excitotoxic and hypoxic-ischemic injury on postnatal day 7 in the immature rat. In cultured oligodendrocytes, a similar window of vulnerability to AMPA mediated injury occurs during the developmental stage before immature oligodendrocytes express myelin. AMPA/kainate receptors are also expressed on developing human oligodendrocytes present in fetal white matter at 23- to 32-week gestation, the period of highest risk for periventricular leukomalacia (53) (PVL). The AMPA-kainate receptor antagonists 6-nitro-7-sulfamoylbenzo-(f)quinoxaline-2,3-dione (NBQX) and clinically used anticonvulsant topiramate have been shown to block AMPA-kainate cell death and calcium influx in immature oligodendrocytes in vitro, as well as damage from hypoxia-ischemia in the 7-day-old rat model (11, 12). One possible physiologic role of these glutamate receptors on immature oligodendrocytes is to receive glutamate signals from electrically active axons, providing a link between neuronal activity and myelination. This hypothesis suggests that glutamate receptors and channels may provide activity-dependent trophic stimulation for immature oligodendrocytes that can become excessive and injurious at times of stress. Drugs that block non-NMDA glutamate receptors or events downstream in the neurotoxic cascade activated by these receptors, such as oxygen free radicals, may be useful clinically for reducing the incidence and severity of white matter injury.

In contrast to neurons, there is little evidence that immature oligodendrocytes express NMDA receptors or that activating NMDA receptors causes direct injury to oligodendrocytes (67). However, direct injection of the NMDA and glutamate metabotropic receptor agonist ibotenate has been shown to produce cystic white matter lesions in rodents, and activation of microglia appears to mediate this effect (67). In vitro studies indicate that microglia activated by ibotenate release soluble factors such as cytokines, free radicals, nitric oxide, glutamate and metalloproteins that kill cultured astrocytes. Growth factors such as vasoactive intestinal peptide (VIP) and brain derived neurotrophic factor (BDNF) have been shown to prevent cell death in this model of white matter injury (22). Recent work in this model also suggests that interleukin-9 release associated with microglial activation stimulates release of transforming growth factor β1 (TGF-β1), which in turn can activate mast cells to release histamine and exacerbate excitotoxic injury (47). This NMDA-mediated pathway for white matter injury links the classic glutamate and ion channel pathway with the inflammatory pathway that has also been shown to be important in the pathogenesis of PVL.

CONCLUSION

Excitotoxicity is one of the important themes in the pathogenesis of perinatal brain injuries. Most neurons as well as many oligodendrocytes and astroglia possess receptors for glutamate, which is the most prominent neurotransmitter in the brain. Glutamate is important for classic neurotransmission, as well as for activity-dependent plasticity during development. There may also be a link between electrical activity in axons and stimulation of myelination via glutamate receptors on immature oligodendroglia. Commensurate with their importance for normal development, these systems are enhanced during the perinatal period. However, dysfunction of these powerful excitatory pathways in the brain in response to stresses in the perinatal period can lead to damage that resembles a "power surge" in a computer. Specific patterns of perinatal pathology can be linked to developmental programs for expression of these excitatory systems in the developing brain.

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