

Mechanisms of Brain Injury after Global Cerebral Ischemia

Izumi Harukuni, MD^a, Anish Bhardwaj, MD^{b,*}

^a*Department of Anesthesiology and Critical Care Medicine,
Johns Hopkins University School of Medicine, Division of Cardiac Anesthesiology, Tower 711,
Johns Hopkins Hospital, 600 N. Wolfe Street, Baltimore, MD 21287, USA*

^b*Departments of Neurology and Anesthesiology and Critical Care Medicine,
Johns Hopkins University School of Medicine, Neurosciences Critical Care Division,
Meyer 8-140, Johns Hopkins Hospital, 600 N. Wolfe Street, Baltimore, MD 21287, USA*

Global cerebral ischemia occurs commonly in patients who have a variety of clinical conditions including cardiac arrest (CA), shock, and asphyxia and in patients undergoing complex cardiac surgery [1–4]. In addition to injury to other organs from systemic hypoperfusion, neurologic sequelae from brain injury are varied and constitute a spectrum that includes coma, seizures, ischemic stroke, delirium, and neurocognitive impairment [5–7]. The commonest postulated mechanism for ischemic brain injury after CA (with subsequent resuscitation) is global cerebral ischemia from systemic hypoperfusion that can occur with or without pre-existing large-vessel occlusive disease. Embolism that arises from the heart, from aortic arch atheromas, or from extracorporeal circulation devices occurs more commonly in the perioperative period following complex cardiac surgery and less commonly during resuscitation following CA [7]. Irrespective of the etiology of cerebral ischemia, cellular and molecular processes trigger a cascade of events that culminate in a “final common pathway,” resulting in ischemic neuronal injury. Identification of these injury mediators and pathways in a variety of experimental animal models of global cerebral ischemia has led to investigation of target-specific cytoprotective strategies that are critical to clinical brain injury outcome. Although the authors have previously published on this subject [8], this article expands on the translational significance of many of the potential neuroprotective strategies that have

This work is supported in part by the US Public Health Service National Institutes of Health grant NS046379.

* Corresponding author.

E-mail address: abhardwa@jhmi.edu (A. Bhardwaj).

provided important insights into the mechanism or mechanisms of ischemic brain injury and might be of therapeutic benefit in the future.

Global versus focal cerebral ischemia

Ischemia is defined as diminution of cerebral blood flow (CBF) to a critical threshold that propagates brain damage involving the entire brain or a selective region. Global cerebral ischemia entails diminution in CBF over the entire brain, encountered clinically as sequelae during extracorporeal circulation following CA from ventricular fibrillation or asystole that lasts 5 to 10 minutes. Global ischemia from CA results in a predictable pattern of histologic injury in which specific neuronal populations are affected (selective ischemic necrosis) [9–11] (discussed later). Although reperfusion restores CBF, it can lead to secondary brain injury from influx of neutrophils and to increases in reactive oxygen species (ROS), cerebral edema, and hemorrhage. Elevated levels of ROS may lead to damage of intracellular proteins and DNA by way of oxidation and by activating a number of pathways that lead to cell death. Unlike global cerebral ischemia, focal cerebral ischemia entails reduction in regional CBF in a specific vascular territory and is usually encountered clinically as an “ischemic stroke” due to thromboembolic or arterothrombotic vaso-occlusive disease. Normal CBF ranges from 50 to 75 mL/100 g of brain tissue per minute and can differ between the white and gray brain matter. Ischemic depolarization occurs when CBF decreases to levels of approximately 18 mL/100 g of brain tissue per minute, and neuronal cell death ensues if CBF is less than 10 mL/100 g of brain tissue per minute. In focal ischemia, the ischemic vascular bed comprises an area with severe CBF reduction that consists of an “ischemic core” and a more distal “ischemic penumbra” and includes regions that are marginally perfused and might be served by collateral vascular channels. Histopathologic outcome following focal ischemia largely depends on ischemic severity and duration [8–10]. Increasing durations of depolarizing ischemia are associated with a spectrum of histopathologic correlates—from reversible injury to irreversible cerebral infarction.

Experimental models of global cerebral ischemia

Several animal models have been developed to simulate complete human global cerebral ischemia and have provided histologic evidence of and insight into mechanisms of brain injury. Rodent models (gerbil, mouse, and rat) provide the advantages of being inexpensive, of rendering consistent reproducibility of injury because they possess consistent cerebral vasculature, and of being homogeneous among strains, with transgenic counterparts allowing targeted mechanistic studies for delineating effects of specific gene deletion on ischemic brain injury. Monitoring of critical physiologic variables (pH, arterial blood pressure, arterial blood gases, core body and cranial

temperature, plasma glucose levels) during the peri-ischemic period, however, is suboptimal. Furthermore, assessment of functional neurologic outcomes with a comprehensive behavioral battery presents a challenge in small animal models. Because 50% of Mongolian gerbils possess an incomplete circle of Willis, bilateral carotid artery occlusion results in forebrain ischemia in these animals. The rat model of global cerebral ischemia includes (1) four-vessel occlusion (4-VO) by electrocoagulation of both vertebral arteries, with transient occlusion of both carotid arteries 24 hours later [12]; or (2) “two-vessel occlusion” by transient occlusion of both carotid arteries and accompanying reduction of arterial blood pressure to a level of 40 to 50 mm Hg by phlebotomy [13,14]. Both techniques yield high-grade ischemia of forebrain structures [15]. Larger animal models (rabbit, canine, feline, swine, nonhuman primates) allow for monitoring of critical physiologic variables during and following the ischemic insult and allow for a more accurate delineation of neurologic deficits following the insult; however, high cost and significant ethical concerns limit their use. The methods of brain injury in these larger-animal models include ventricular fibrillation [16], aortic occlusion [17], brachiocephalic or subclavian arterial occlusion in combination with hypotension, elevated intracranial pressure by neck-cuff insufflation, and intraventricular infusion [18]. In these animal models, specific neuronal populations in the brain, including CA1 pyramidal neurons of the hippocampus, medium-sized neurons of the striatum, and the Purkinje cells of the cerebellum [19], are susceptible to injury. The sensitivity of these neuronal populations is varied and dependent on duration and severity of ischemia, and a typical temporal profile is observed following CA. Neurons in the CA1 zone are the most sensitive to depolarizing ischemia (3–5 minutes), whereas the medium-sized neurons of the striatum are more resistant (15–20 minutes) [20]. Following successful resuscitation and cerebral recirculation, progression of irreversible neuronal injury also differs in these selective neuronal populations (eg, neuronal injury is observed within 3 hours of establishing recirculation in medium-sized neurons of the striatum but delayed up to 48–72 hours in CA1 hippocampal neurons, a phenomenon referred to as “delayed neuronal death”) [20].

Neuronal injury mechanisms: apoptosis versus necrosis

Over the past decade, research has demonstrated that consequences of cerebral ischemia result in two temporally distinct phases or processes of neuronal cell death, which in turn affect surrounding brain tissue. Each phase has characteristic defining morphologic and molecular features, and the distinction between the two processes is based on morphologic findings on electron microscopy [8,21]. Apoptosis or programmed cell death, a process associated with genomic fragmentation, is characterized by cell shrinkage, chromatin aggregation, and preservation of cell membrane integrity and mitochondria without inflammation and injury to surrounding tissue [21–25].

Conversely, necrosis, a process that is not “regulated or programmed,” is typically observed as a consequence of severe cerebral ischemia and characterized by disruption of cellular homeostasis from energy failure due to severe mitochondrial injury, which leads to cellular swelling, membrane lysis, inflammation, vascular damage, and edema formation. Although apoptosis and necrosis characteristically represent distinct modes of cellular injury, a large body of literature suggests that these processes represent a spectrum that coexists in a spatial distribution within injured tissue (neurons in the core being necrotic and neurons in the penumbra being apoptotic) and is temporally related to duration and severity of the ischemic insult. The accepted tenet is that the excitotoxic cascade triggered by exposure to excitatory amino acids (EAAs) plays a prominent role in neuronal necrosis. In vitro exposure to glutamate, however, results in apoptosis in neurons that survive the early necrotic phase and have partial recovery of mitochondrial function [26]. Therefore, maintenance of mitochondrial function is possibly the critical factor in determining the degree and progression of neuronal injury propagated by excitotoxicity [25]. A common pathway of stimuli leading to apoptosis has not yet been identified; however, a mitochondrial-dependent intrinsic pathway and a receptor-mediated extrinsic pathway are postulated [27,28]. In the intrinsic pathway, cerebral ischemia results in generation of a permeability transition pore in the inner mitochondrial membrane that leads to disruption of the outer mitochondrial membrane due to release of several proapoptotic factors (caspases, endonucleases, cytochrome c, and other proteases related to interleukin-1 β converting enzyme) [21,27,28]. These events ultimately lead to DNA fragmentation. The “mitochondrial-independent” or extrinsic pathway of apoptosis involves several death receptor families such as Fas. Bypassing the inhibitory effects of Bcl2-related proteins, which control proteolytic systems, has also been postulated to play an important role in apoptotic cell death, whereas other family members, such as Bax, augment apoptosis. Another family of protein-cleaving enzymes, caspases, is expressed at significant levels during cerebral ischemia, and caspase inhibitors produce resistance to ischemic damage [29].

Excitotoxic brain injury

The concept of excitotoxicity, introduced by Olney in 1969 [30], was based on a set of observations that included neuronal injury with local application of glutamate and other acidic amino acids (aspartate, *N*-methyl-D-aspartate [NMDA], homocysteine, cysteine). Since this description was published, other excitotoxic mediators have been delineated, including catecholamines (dopamine, norepinephrine), nitric oxide (NO), and related species. Glutamate, the most abundant EAA in the brain, serves a variety of important functions (metabolic, neurotrophic, and neurotransmitter) and is compartmentalized in neurons [19,31]. The healthy adult brain has the ability to clear extracellular glutamate by rapid uptake; however, under conditions in which energy stores

are depleted, such as in hypoxia-ischemia, glutamate efflux into the extracellular compartment due to cellular depolarization [32], coupled with its impaired uptake, results in increases in intracellular Ca^{2+} . Interference with cysteine uptake (causing depletion of cellular glutathione stores responsible for protecting against oxidative stress) results in neuronal injury [30,33]. Excitotoxic injury is characterized by its maximal effects on neuronal dendrites and soma, with relative sparing of axons, glia, and ependymal and endothelial cells, possibly due to differences in synaptic input, density, distribution of membrane glutamate receptors, and intrinsic defense mechanisms. Glutamate activates three major families of ionophore-linked receptors (NMDA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA], and kainate) and metabotropic receptors that activate second messenger systems [34]. Although glutamate release simultaneously stimulates NMDA and AMPA receptors, *in vitro* studies demonstrate that glutamate toxicity occurs in two distinct phases: (1) excitotoxicity is rapidly triggered by brief intense stimulation of NMDA receptors, which is critically dependent on the presence and influx of extracellular Ca^{2+} through the NMDA-gated receptor channel complex; and (2) a slowly triggered process by the prolonged stimulation of AMPA/kainate receptors that have limited Ca^{2+} channels [33]. Metabotropic glutamate receptors modify excitotoxic injury, rather than directly mediate the deleterious process.

The cascade of events responsible for glutamate excitotoxicity includes three distinct processes: (1) induction, whereby extracellular glutamate efflux is transduced by receptors on the neuronal membrane to cause intracellular Ca^{2+} overload, which leads to lethal intracellular derangements; (2) amplification of the derangement, with an increase in intensity and involvement of other neurons; and (3) expression of cell death triggered by cytotoxic cascades [33]. Excess release of Ca^{2+} and its intracellular influx is thought to be the primary trigger for a variety of complex, deleterious intracellular processes that result from activation of catabolic enzymes such as phospholipases (which lead to cell membrane breakdown, arachidonic acid, and free radical formation) and endonucleases (which lead to fragmentation of genomic DNA and energy failure due to mitochondrial dysfunction) (Fig. 1). Distinct neuronal populations are selectively vulnerable to excitotoxic injury, possibly from differences in excitatory synaptic inputs, density of glutamate receptors, or intrinsic defense mechanisms. Perhaps the most compelling evidence for the role of glutamate excitotoxicity following focal ischemia and hypoxic-ischemic brain injury from CA is the neuroprotection observed with antiexcitotoxic strategies including NMDA- or AMPA-receptor antagonists [35].

Acute efflux of dopamine and norepinephrine into the extracellular space following cerebral ischemia [36–39] possibly plays a role in the propagation of brain injury. Indirect evidence of the importance of their role in cerebral ischemia stems from amelioration of histopathologic injury following attenuation of ischemia-induced surges in extracellular dopamine pharmacologically

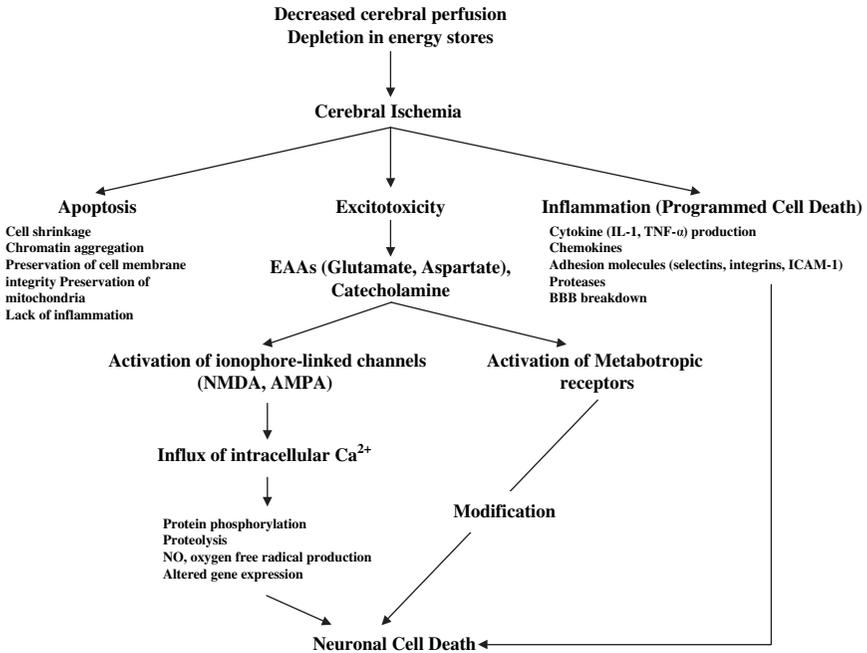


Fig. 1. Schematic diagram representing events leading to ischemic brain injury.

with barbiturates, isoflurane, and etomidate [40,41]. Furthermore, several lines of evidence suggest that catecholamine release and metabolism might underlie the selective vulnerability of striatal neurons to an ischemic insult [42,43]. For example, depletion of catecholamine stores by α -methyl-para-tyrosine exerts a strong protective effect on ischemic damage to nerve terminals [43], and reduction of striatal dopamine content by lesioning the nigrostriatal tract protects intrinsic striatal neurons from injury following global cerebral ischemia [44]. Although the precise mechanism of neuronal injury by dopamine is unclear, by-products of its metabolism, such as hydrogen peroxide, superoxide ion, and hydrogen radicals, have been implicated in this deleterious process [45].

NO, a free radical gas synthesized from the amino acid L-arginine by the enzyme NO synthase (NOS), is produced by a variety of sources (vascular endothelium, neurons, glia, macrophages, white blood cells) [46,47]. NO has several functions in the brain, including regulation of CBF, neurotransmission, and modification of inflammation [46,47]. At least three isoforms of NOS have been identified: the constitutively expressed neuronal and endothelial isoforms (encoded on chromosome 12 and 7) and the inducible isoform (encoded on chromosome 17) [48,49]. Neuronal NOS-containing neurons are widespread in the brain, including in the cerebral cortex, hippocampus, and striatum [47]. NO plays a dual role in ischemic neuropathol-

ogy—beneficial, in that it is a potent vasodilator, and cytotoxic, in that it inhibits important enzyme systems such as complexes I and II of the mitochondrial transport chain (Fig. 2). Formation of the oxidant peroxynitrite by combination of NO and superoxide anion is considered to be an important trigger in cytotoxicity. Peroxynitrite generation leads to formation of other ROS, hydroxyl free radicals, and nitrogen dioxide, resulting in nitrosylation of tyrosine residues in proteins. One way by which NO is thought to kill neurons is energy-dependent activation of the DNA repair enzyme poly(ADP-ribose) polymerase, leading to consumption of ATP, nicotinamide, and cell death [50].

In cerebral ischemia, constitutive NO activity (endothelial and neuronal isoforms) markedly increases as a consequence of NMDA, AMPA, and metabotropic glutamate receptor stimulation [51,52] culminating in a rise in intracellular Ca^{2+} , whereas more sustained levels of NO are expressed by the microglia and other inflammatory cells (Ca^{2+} -independent inducible isoform) 24 hours following the ischemic event [8,48]. Consequently, administration of NO donors and specific NOS inhibitors has yielded mixed results in experimental models of cerebral ischemia. Such varied results are explained by non-selectivity of NOS inhibitors and timing of such interventions in relation to ischemia. For example, infusion of the NO precursor and substrate L-arginine in the immediate period following experimental focal ischemia attenuates infarct volume by accentuating CBF by way of dilation of pial vessels [48,53]. In line with this evidence, male mutant mice that lack the endothelial isoform of NOS sustain larger ischemic injury than their wild-type counterparts following focal ischemia [54]. Infarct volume and functional outcomes are improved in wild-type mice following selective pharmacologic inhibition of neuronal NOS and in mutant mice that do not express genes for the neuronal or inducible isoforms [48,55]. Because vascular NO might favorably affect outcome and the neuronal isoform might adversely affect outcome, selective pharmacologic

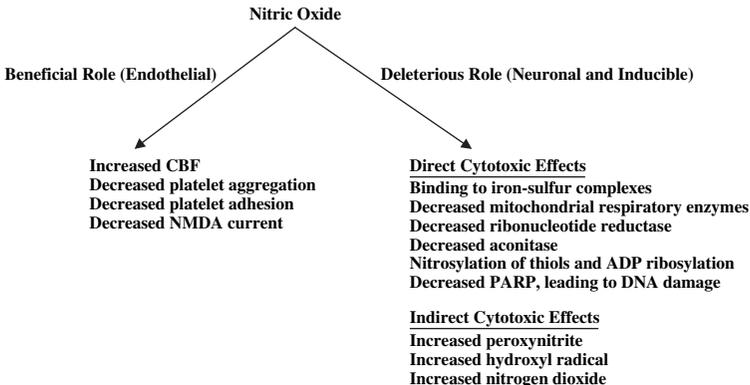


Fig. 2. Beneficial and deleterious effects of nitric oxide in cerebral ischemia. (Reproduced from Bhardwaj et al. [8] and adapted from Dalkara and Moskowitz [49], with permission.)

inhibition of the neuronal and inducible isoforms of NOS might provide a viable therapeutic strategy in cerebral ischemia.

Role of inflammation

Cerebral ischemia leads to inflammatory cell infiltrates from nonspecific immunologic reaction, migration of peripheral leukocytes into the brain, and activation of microglia [56]. Release of inflammatory cytokines (interleukin [IL]-1, tumor necrosis factor α [TNF- α]) by ischemic neurons and glia leads to generation of adhesion molecules (selectins, integrins, intercellular adhesion molecule 1) in the cerebral vasculature, which results in breakdown of the blood-brain barrier (BBB), culminating in edema formation [57,58]. Enhanced secretion of cytokines and proteases such as metalloproteinases causes further disruption of the extracellular matrix and the BBB. Although IL-1 is detrimental to ischemic brain injury, roles for IL-6, a proinflammatory cytokine, and IL-10, an anti-inflammatory cytokine, are less clear. TNF- α appears to have a dual role in the ischemic brain, in that it is involved in ischemic tolerance [59] and plays a role in propagating ischemic brain injury [60,61].

Glycemic control

A number of studies in animal models of traumatic brain injury [62], focal cerebral ischemia [63], and global cerebral ischemia [64] demonstrate that glycemic control is a critical factor in terms of outcome. Postulated mechanisms include accentuation of release of EAAs, attenuation of neuroinhibitory neurotransmitters [65], massive deposition of neutrophils [66], and early mitochondrial damage by way of activation of cytochrome c, caspase-9, and caspase-3 cleavage [67]. These mechanistic studies have led to clinical observations that poor glycemic control accentuates brain injury in ischemic stroke [68] and following cardiac surgery [69]. Glycemic control with insulin treatment has been demonstrated to improve neurologic outcome in critically ill patients [70] and in patients who undergo cardiac surgery [69]. Although insulin therapy has been shown to ameliorate damage in animal models of global cerebral ischemia [71], further clinical trials are warranted in the setting of aggressive glycemic control with insulin therapy in patients following CA.

Role of temperature

Experimental studies using animal models of focal and global cerebral ischemia have provided evidence for the importance of brain temperature on functional and histopathologic outcome [72]. Following cerebral ischemia, intraischemic hyperthermia leads to incomplete normalization of high-energy phosphate metabolites and the conversion of selective neuronal necrosis to infarction, increased microvascular injury, and edema, resulting in increased mortality [72]. Spontaneous elevations in body temperature

have been reported following experimental global and focal ischemia and are thought to be a consequence of brain injury [73]. Mild (34°C) to moderate (30°C) induced systemic hypothermia markedly attenuates ischemic brain injury following experimental CA [74]. Mechanisms of hypothermia-induced ischemic brain protection may be multifactorial and include mitigation of pre- and postsynaptic excitotoxic processes (attenuation of biosynthesis, release and uptake of EAAs), diminished hydroxyl radical production, protection of lipoprotein membranes, attenuation of intracellular acidosis, and reduction of oxygen demand by the injured brain [2]. Recent clinical trials have demonstrated improved neurologic outcome and decreased mortality in patients subjected to mild-to-moderate therapeutic hypothermia [1,2]. Future clinical trials should incorporate other pharmacologic neuroprotective strategies in combination with hypothermia to further enhance outcomes.

Ischemic tolerance and preconditioning

The concept of ischemic tolerance, introduced 2 decades ago and based on observations in the myocardium [75], was extended to ischemic brain injury, whereby brief ischemic insults protected the brain from subsequent and more severe ischemia [76]. Further experiments in a variety of animal models of focal and global cerebral ischemia confirmed these observations [77,78]. In addition to sublethal ischemia, other conditions such as hyperthermia [79], hypothermia [80], hypoglycemia [81], and pharmacologic agents (eg, antibiotics, erythropoietin, acetylsalicylic acid, volatile anesthetics) [82–85] have been shown to induce ischemic tolerance. The early phase of ischemic tolerance (within 30 minutes following sublethal insult) is thought to be due to flow-metabolism-mediated events, whereas delayed tolerance (> 24 hours) involves new gene induction and protein synthesis [86,87]. Molecules such as adenosine, hypoxia inducible factor 1 α , TNF- α , ROS, NO, and other receptor-linked events involving NMDA-receptor activation and downstream effects of intracellular calcium influx have been implicated in ischemic tolerance. Although the precise mechanisms of ischemic tolerance have not been elucidated completely, ischemic preconditioning provides a possible venue and therapeutic strategy in ameliorating brain injury in a few, select high-risk patients susceptible to ischemic brain injury.

Experimental pharmacologic neuroprotection and its translational significance

A comprehensive review of this topic is beyond the scope of this article; however, the interested reader is referred to a recent monograph by Weigl and colleagues [88]. Several pharmacologic agents have undergone investigation in animal models of global cerebral ischemia to directly or indirectly determine efficacy. Methods used to evaluate postischemic hyperemia or hypoperfusion include recovery of somatosensory-evoked potentials, recovery

of high-energy phosphates, functional neurologic recovery, and histologic injury [29,88]. The basis for the study of these agents is a logical extension of mechanistic studies in a variety of in vitro and in vivo systems of cerebral ischemia. In addition, results from neuroprotective studies with pharmacologic agents provide important insights into mechanisms because they entail disruption of a specific pathway or receptor blockade in the propagation of ischemic neuronal injury. Although many of these agents are still under investigation, data predominantly from studies using the focal ischemia paradigm underscore the future use of pharmacologic therapies following global cerebral ischemia.

N-methyl-D-aspartate-receptor antagonists

Pre- and post-treatment with dextrorphan, an NMDA-receptor antagonist, improves histologic injury in the hippocampus and the cortex in a 4-VO rat model and attenuates the reduction in loss of activity of calcium-dependent protein kinases (protein kinase C and calcium-dependent protein kinase II) [89]. Although the NMDA-receptor antagonist dizocipine (MK801) has been shown to provide significant histologic neuroprotection in animal models of global cerebral ischemia [90,91], its clinical use in ischemic stroke has been shown to produce significant undesirable side effects (delirium, psychosis, hallucinations, and so forth) [29].

Calcium channel antagonists

Because Ca^{2+} is the final common pathway in excitotoxic neuronal injury, nimodipine, a blocker of Ca^{2+} influx, has been studied in the experimental paradigm of global cerebral ischemia. Subcutaneous administration of nimodipine failed to demonstrate any histologic or functional neurologic improvement in the 4-VO rat model of global cerebral ischemia [92,93]; however, in a rabbit model, intravenous treatment with nimodipine reduced time of EEG recovery and attenuated the decrement in extracellular Ca^{2+} and disruption of the BBB. In this study, arterial blood pressure was maintained at 100 mm Hg following the ischemic insult, thereby offsetting the detrimental hypotensive effects of nimodipine. A prospective, randomized, double-blinded trial with nimodipine in patients who had out-of-hospital ventricular fibrillation failed to demonstrate any improvement in 1-year survival rate; however, it demonstrated some benefit in patients who had delayed resuscitation (>10 minutes) [94].

γ -Aminobutyric acid and γ -aminobutyric acid agonists

The premise of using γ -aminobutyric acid (GABA) or its agonists as neuroprotectants is based on their inhibitory properties by way of opening of the Cl^- channels [88]. Pretreatment with GABA attenuated histologic injury and improved neurobehavior in a gerbil model of global cerebral ischemia

[95]. Treatment following the insult failed to demonstrate any improvement in these parameters. Chlormethiazole, a GABA agonist with anticonvulsant, hypnotic, and sedative properties, failed to demonstrate any improvement in histologic injury or neurobehavior in a rat model of global cerebral ischemia [96]. Furthermore, local infusion of chlormethiazole by way of microdialysis did not alter ischemia-evoked release of dopamine, serotonin, or their metabolites in the ischemic striatum [96]. Intraperitoneal administration of γ -hydroxybutyrate improved histologic injury and neurobehavioral outcomes in a 4-VO rat model of global cerebral ischemia [97]. Tiagabine, a selective inhibitor of GABA reuptake, failed to demonstrate any improvement in histologic outcome in the gerbil model when given as a pretreatment [98].

Anticonvulsants

The basis for the use of anticonvulsants in ischemic neuroprotection is their ability to stabilize neurons by way of hyperpolarization of the membrane potential by blocking voltage-gated Na^+ channels [88]. Treatment with phenytoin attenuates accumulation of K^+ in cerebrospinal fluid in animals subjected to circulatory arrest. Some studies of phenytoin treatment have demonstrated attenuation of brain edema, increased Na^+/K^+ -ATPase activity, decreased intracellular Na^+ concentration, and attenuated accumulation of lactate and free fatty acids [99]. Lamotrigine attenuates ischemia-induced increases in extracellular glutamate levels and improves histologic outcomes in the gerbil and rat models of global cerebral ischemia [100].

Magnesium

Magnesium sulfate has multimodal actions. It is an NMDA receptor, a calcium antagonist, and a vasodilator. In a rat model of global cerebral ischemia, pre- and post-treatment with bolus intravenous magnesium sulfate followed by a continuous intravenous infusion attenuated injury to CA1 hippocampal neurons [101]. Other investigators have reported that neuroprotection is demonstrated only when magnesium sulfate is administered in combination with mild hypothermia [102].

Anesthetic agents

Barbiturates have long been known to exert neuroprotection by coupled decreases in cerebral metabolic rate of oxygen (CMRO_2) with CBF and by inhibiting agonist-induced cerebral vasoconstrictor responses, protein kinase C, ischemia-induced increases in free fatty acids, and EAA release [29]. In experimental global cerebral ischemia, however, results have been disappointing. Treatment with pentobarbital failed to improve survival in a dog model of global cerebral ischemia [103]. Clinical investigation with barbiturates has also proved to be disappointing, with lack of therapeutic benefit in the hypoxic-ischemic brain injury from CA and near-drowning [29]. Inhalational

anesthetics (halothane, isoflurane) have not been thoroughly investigated in the setting of global cerebral ischemia. The premise for their use is their ability to attenuate cerebral metabolic demand, enhance regional CBF, attenuate ischemia-evoked EAA and catecholamine efflux, and attenuate calcium influxes [29].

Cyclooxygenase inhibitors

Nimesulide, a cyclooxygenase-2 inhibitor, attenuated injury to the CA1 region of the hippocampus in a gerbil model when administered orally or intraperitoneally as a pre- or post-treatment (up to 24 hours) [104]. Further experimental studies in other animal models are needed to confirm these findings and bring these agents into the clinical paradigm.

Immunosuppressants

Tacrolimus (FK506) and cyclosporine are immunophilin and calcineurin inhibitors that attenuate apoptotic cell death. Chronic administration of both agents (for 3 days) before the ischemic insult demonstrated neuroprotection in the CA1 region at 7 days of reperfusion in a rat model of global cerebral ischemia [105]. These agents also attenuated calcineurin activity in the CA1, CA3, and dentate gyrus regions of the hippocampus up to 24 hours following the ischemic insult. Pretreatment with cyclosporin and FK506 inhibits dephosphorylation of the proapoptotic protein Bad. The inability of cyclosporine to cross an intact BBB is a significant therapeutic concern. These agents require more rigorous testing with treatment in the postischemia paradigm across different species and animal models of global cerebral ischemia [88].

Potential future of neuroprotective agents

Hormonal sex steroids

Evidence is mounting that outcome from cerebral ischemia is quantitatively different in adult male and female animals, reflecting patterns of some forms of human cerebrovascular disease [8,106]. Accordingly, it has become increasingly apparent that biologic sex is an important factor in pathophysiology and outcome following cerebral ischemia [107]. For example, when both sexes are studied, ischemic outcome in transgenic mice can be overtly sex dependent, even when the gene of interest (eg, inducible or neuronal NO) is not linked to sexual development [108–111]. These data suggest that molecular mechanisms of cell injury may not be the same or have the same impact in the male and the female brain. Furthermore, most experimental studies underscore the importance of sex steroids (predominantly estrogen) to outcome from focal ischemia [107,112]; recent studies have reported significant neuroprotection in the global cerebral ischemia paradigm [113].

Opioid-receptor agonists

Experimental research over the last 3 decades has implicated the opioidergic receptors in the brain to play an important pathophysiologic role in cerebral ischemia [114,115]. Opiate receptors in the central nervous system have been divided into three subtypes: mu (μ), kappa (κ), and delta (δ). Although the μ - and δ -receptor subtypes have been shown to play a role predominantly in antinociception, several experimental studies have demonstrated neuroprotective effects of κ -opioid-receptor agonists in models of global [116] and focal ischemia [117,118]. Differential time course and alterations in opioid-receptor binding after focal cerebral ischemia in mice indicate that κ -opioid-receptor binding sites are preserved much longer (12–48 hours) than other subtypes [119], suggesting a potentially longer therapeutic window with κ -opioid-receptor agonists. In vitro studies suggest that κ -opioid-receptor agonists modulate the excitotoxic action of glutamate, possibly by the presynaptic inhibition of its release by decreasing the entry of $^{45}\text{Ca}^{2+}$ into rat cortical synaptosomes [120,121]. It has been demonstrated that the selective κ -opioid-receptor agonist BRL 52,537 attenuates in situ NO production at doses that provide ischemic neuroprotection [122] that is receptor selective [123] but does not attenuate ischemia-evoked dopamine release in the ischemic striatum [124]. Furthermore, BRL 52537 provides significant ischemic neuroprotection when administered for up to 6 hours after the onset of focal cerebral ischemia [125], confirming a long therapeutic opportunity to afford ischemic neuroprotection with these compounds. It has recently been demonstrated that ischemic neuroprotection with BRL 52537 is sex specific; it confers neuroprotection only in male animals [126]. Thus, highly selective κ -opioid-receptor agonists might hold promise as neuroprotective agents in the future in the global cerebral ischemia paradigm.

Sigma-receptor agonists

Over the past 2 decades, nonopioid sigma (σ)-receptors have been considered to serve an important physiologic function [127]. A number of “atypical” antipsychotics are potent σ -receptor ligands—atypical, in that they are effective antipsychotic agents but have low propensity to induce extrapyramidal side effects [28]. Naturally occurring σ -receptor ligands include progesterone and neuropeptide Y [128]. Purification, molecular cloning, and high levels of expression of σ_1 -receptor binding sites in sterol-producing tissues have been demonstrated [129]. Subtyping of the σ -receptor into a σ_1 -receptor and σ_2 -receptor is based on important differences in regional distribution, enantomeric selectivity, molecular weights, and second messengers employed in signaling [130,131]. Until recently, specific σ_1 -receptor ligands or antagonists have been lacking. During evaluation as antipsychotic agents, several drugs known to be σ -receptor ligands were demonstrated to alter NMDA-receptor function [132]; however, it now appears that the effect

might be directly mediated through activity at the σ -receptor. The authors have demonstrated that the potent σ_1 -receptor ligand 4-phenyl-1-(4-phenylbutyl) piperidine (PPBP) provides robust ischemic neuroprotection in animal models of cerebral ischemia [133–135]. Although the exact *in vivo* mechanisms of neuroprotection by σ_1 -receptor ligands are not completely elucidated, several antiexcitotoxic mechanisms have been postulated, including inhibition of ischemia-induced presynaptic glutamate release, attenuation of postsynaptic glutamate-evoked Ca^{2+} influx, modulation of neuronal responses to NMDA-receptor stimulation, inhibition of dopamine neurotransmission, and prevention of cortical-spreading depression [133–135]. Recently, it was demonstrated that PPBP provides ischemic neuroprotection *in vivo* by way of attenuation of ischemia-evoked NO production [134] but does not alter ischemia-evoked dopamine efflux in the ischemic striatum [135]. Based on these data, σ_1 -receptor ligands hold promise for the future as neuroprotectants in the treatment of cerebral ischemia.

In addition to the above agents, a number of others that have been studied in the experimental paradigm of global cerebral ischemia hold promise for the future. These agents include anesthetics (etomidate, ketamine, propofol), sodium channel blockers (mexitine, lidocaine), α -receptor agonists (dexmedetomidine), and xanthine oxidase inhibitors (allopurinol). For a complete review of this subject, the reader is referred to the monograph by Weigl and colleagues [88].

Summary

Cerebral ischemia results in a rapid depletion of energy stores that triggers a complex cascade of cellular events such as cellular depolarization and Ca^{2+} influx, resulting in excitotoxic cell death. The critical determinant of severity of brain injury is the duration and severity of the ischemic insult and early restoration of CBF. Induced therapeutic hypothermia following CA is the only strategy that has demonstrated improvement in outcomes in prospective, randomized clinical trials. Although pharmacologic neuroprotection has been disappointing thus far in a variety of experimental animal models, further research efforts are directed at using some agents that demonstrate marginal or moderate efficacy in combination with hypothermia. Although the signal transduction pathways and intracellular molecular events during cerebral ischemia and reperfusion are complex, potential therapeutic neuroprotective strategies hold promise for the future.

Acknowledgments

The authors thank Tzipora Sofare, MA, for her editorial assistance in preparing this manuscript.

References

- [1] Bernard SA, Gray TW, Buist MD, et al. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med* 2002;346:557–63.
- [2] The Hypothermia After Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. *N Engl J Med* 2002;346:549–56.
- [3] Salazar JD, Wityk RJ, Grega MA, et al. Stroke after cardiac surgery: short- and long-term outcomes. *Ann Thorac Surg* 2001;72:1195–201.
- [4] Nussmeier NA. A review of risk factors for adverse neurologic outcome after cardiac surgery. *J Extra Corpor Technol* 2002;34:4–10.
- [5] Murkin JM. Etiology and incidence of brain dysfunction after cardiac surgery. *J Cardiothorac Vasc Anesth* 1999;13:12–37.
- [6] Llinas R, Barbut D, Caplan LR. Neurologic complications of cardiac surgery. *Prog Cardiovasc Dis* 2000;43:101–12.
- [7] Wityk RJ, Gloddsborough MA, Hillis A, et al. Diffusion- and perfusion-weighted brain magnetic resonance imaging in patients with neurologic complications after cardiac surgery. *Arch Neurol* 2001;58:571–6.
- [8] Bhardwaj A, Alkayed NJ, Kirsch JR, et al. Mechanisms of ischemic brain damage. *Curr Cardiol Rep* 2003;5:160–7.
- [9] Pulsinelli WA. Selective neuronal vulnerability: morphological and molecular characteristics. *Prog Brain Res* 1995;63:29–37.
- [10] Petito CK, Pulsinelli WA. Delayed neuronal recovery and neuronal death in rat hippocampus following severe cerebral ischemia: possible relationship to abnormalities in neuronal processes. *J Cereb Blood Flow Metab* 1984;4:194–205.
- [11] Kaplan B, Brint S, Tanabe J, et al. Temporal thresholds for neocortical infarction in rats subjected to irreversible focal cerebral ischemia. *Stroke* 1991;22:1032–9.
- [12] Pusinelli WA, Brierley JB, Plum F. Temporal profile of neuronal damage in a model of transient forebrain ischemia. *Ann Neurol* 1982;11:491–8.
- [13] Kagstorm E, Smith M-L, Siesjo BK. Local cerebral blood flow in the recovery period following complete cerebral ischemia in the rat. *J Cereb Blood Flow Metab* 1983;3:170–82.
- [14] Pulsinelli WA, Duffy DE. Regional energy balance in rat brain after transient forebrain ischemia. *J Neurochem* 1983;40:1500–3.
- [15] Ginsberg MD, Busto R. Rodent models of cerebral ischemia. *Stroke* 1989;20:1627–42.
- [16] Todd MM, Dunlop BJ, Shapiro HM, et al. Ventricular fibrillation in the cat: a model for global cerebral ischemia. *Stroke* 1981;12:808–15.
- [17] Koch KA, Jackson DL, Schmiedl M, et al. Total cerebral ischemia: effect of alterations in arterial PCO₂ on cerebral microcirculation. *J Cereb Blood Flow Metab* 1984;4:343–9.
- [18] Nishijima MK, Koehler RC, Hurn PD, et al. Postischemic recovery rate of cerebral ATP, phosphocreatine, pH, and evoked potentials. *Am J Physiol* 1989;257:H1860–70.
- [19] Fonnum F. Glutamate: a neurotransmitter in mammalian brain. *J Neurochem* 1984;42:1–11.
- [20] Koehler RC, Eleff SM, Traystman RJ. Global neuronal ischemia and reperfusion. In: Paradis NA, Halperin HR, Nowak RM, editors. *Cardiac arrest: the science and practice of resuscitation medicine*. Baltimore (MD): Williams and Wilkins; 1996. p. 113–45.
- [21] Leist M, Nicotera P. Cell death: apoptosis versus necrosis. In: Welch KMA, Caplan LR, Reis DJ, et al, editors. *Primer on cerebrovascular disease*. Philadelphia: Academic Press; 1997. p. 101–4.
- [22] Cohen JJ. Overview: mechanisms of apoptosis. *Immunol Today* 1993;14:126–30.
- [23] Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995;267:1456–62.
- [24] Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 1999;22:391–7.

- [25] Vaux L, Haecker G, Strasser A. An evolutionary perspective on apoptosis. *Cell* 1994;76:777–9.
- [26] Ankarcrona M, Dypbukt JM, Bonfoco E, et al. Necrosis and apoptosis elicited by glutamate in cerebellar granule cells: a temporal sequence depending on mitochondrial function. *Neuron* 1995;15:961–73.
- [27] Graham SH, Chen J. Programmed cell death in cerebral ischemia. *J Cereb Blood Flow Metab* 2001;21:99–109.
- [28] Sugawara T, Fujimura M, Noshita N, et al. Neuronal death/survival signaling pathways in cerebral ischemia. *NeuroRx* 2004;1:17–25.
- [29] Rossberg MI, Bhardwaj A, Hurn PD, et al. Principles of cerebroprotection. In: Murray MJ, Coursin DB, Pearl RG, et al, editors. *Critical care medicine: perioperative management*. Philadelphia: Lipincott Williams & Williams; 2002. p. 225–35.
- [30] Olney JW. Brain lesion, obesity and other disturbances in mice treated with monosodium glutamate. *Science* 1969;164:719–21.
- [31] Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1988;1:623–34.
- [32] Benveniste H, Drejer J, Schoushoe A, et al. Elevation of extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem* 1984;43:1369–74.
- [33] Choi DW. Excitotoxic cell death. *J Neurobiol* 1992;23:1261–76.
- [34] Sommer B, Seeburg PH. Glutamate receptor channels: novel properties and new clones. *Trends Pharmacol Sci* 1992;13:291–6.
- [35] Choi DW. Methods of antagonizing glutamate neurotoxicity. *Cerebrovasc Brain Metab Rev* 1990;2:105–47.
- [36] Bhardwaj A, Brannan T, Martinez-Tica J, et al. Ischemia in the dorsal hippocampus is associated with acute release of dopamine and norepinephrine. *J Neural Transm* 1990;80:195–201.
- [37] Bhardwaj A, Brannan T, Weinberger J. Pentobarbital inhibits extracellular release of dopamine in the ischemic striatum. *J Neural Transm* 1990;82:111–7.
- [38] Slivka A, Brannan TS, Weinberger J, et al. Increase in extracellular dopamine in the striatum during cerebral ischemia: a study utilizing cerebral microdialysis. *J Neurochem* 1988;50:1714–8.
- [39] Globus MY-T, Busto R, Dietrich WD, et al. Intraischemic extracellular release of dopamine and glutamate is associated with striatal vulnerability to ischemia. *Neurosci Lett* 1988;91:36–40.
- [40] Koorn R, Brannan TS, Martinez-Tica J, et al. Effect of etomidate on in vivo ischemia-induced dopamine release in the corpus striatum of the rat: a study using cerebral microdialysis. *Anesth Analg* 1994;78:73–9.
- [41] Koorn R, Kahn RA, Martinez-Tica J, et al. Effect of isoflurane and halothane on in vivo ischemia-induced dopamine release in the corpus striatum of the rat: a study utilizing cerebral microdialysis. *Anesthesiology* 1993;79:827–35.
- [42] Weinberger J, Cohen G, Nieves-Rosa J. Nerve terminal damage in cerebral ischemia: greater susceptibility of catecholamine nerve terminals relative to serotonin nerve terminals. *Stroke* 1983;14:986–9.
- [43] Weinberger J, Nieves-Rosa J, Cohen G. Nerve terminal damage in cerebral ischemia: protective effect of alpha-methyl-para-tyrosine. *Stroke* 1985;16:864–70.
- [44] Globus MY-T, Ginsberg MD, Dietrich WD, et al. Substantia nigra lesion protects against ischemic damage in the striatum. *Neurosci Lett* 1987;80:251–6.
- [45] Kahn RA, Weinberger J, Brannan T, et al. Nitric oxide modulates dopamine release during global temporary cerebral ischemia. *Anesth Analg* 1995;80:1116–21.
- [46] Dawson TM, Dawson VL, Snyder SH. A novel neuronal messenger in brain: the free radical, nitric oxide. *Ann Neurol* 1992;32:297–311.

- [47] Dawson TM, Dawson VL. Nitric oxide: actions and pathological roles. *Neuroscientist* 1995;1:7–18.
- [48] Samdani AF, Dawson TM, Dawson VL. Nitric oxide synthase in models of focal ischemia. *Stroke* 1997;28:1283–8.
- [49] Dalkara T, Moskowitz MA. The role of nitric oxide in cerebral ischemia. In: Welch KMA, Caplan LR, Reis DJ, et al, editors. *Primer on cerebrovascular disease*. Philadelphia: Academic Press; 1997. p. 207–8.
- [50] Goto S, Xue R, Sugo N, et al. Poly(ADP-ribose) polymerase impairs early and long-term experimental stroke recovery. *Stroke* 2002;33:1101–6.
- [51] Bhardwaj A, Northington FJ, Ichord RN, et al. Characterization of ionotropic glutamate receptor-mediated nitric oxide production in vivo in rats. *Stroke* 1997;28:850–6.
- [52] Bhardwaj A, Northington FJ, Martin LJ, et al. Characterization of metabotropic glutamate receptor-mediated nitric oxide production in vivo. *J Cereb Blood Flow Metab* 1997;17:153–60.
- [53] Dalkara T, Morikawa H, Panahian N, Moskowitz MA. Blood flow-dependent functional recovery in a rat model of cerebral ischemia. *Am J Physiol* 1994;267:H678–83.
- [54] Huang Z, Huang PL, Ma J, et al. Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. *J Cereb Blood Flow Metab* 1996;16:981–7.
- [55] Huang Z, Huang PL, Panahian N, et al. Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. *Science* 1994;265:1883–5.
- [56] Zheng Z, Lee JE, Yenari MA. Stroke: molecular mechanisms and potential targets for treatment. *Curr Mol Med* 2003;3:361–72.
- [57] Han HS, Yenari MA. Cellular targets of brain inflammation in stroke. *Curr Opin Investig Drugs* 2003;4:522–9.
- [58] Danton GH, Dietrich WD. Inflammatory mechanisms after ischemia and stroke. *J Neuro-pathol Exp Neurol* 2003;62:127–36.
- [59] Ginis I, Jaiswal R, Klimanis D, et al. TNF-alpha-induced tolerance to ischemic injury involves differential control of NF-kappaB transactivation: The role of NF-kappaB association with p300 adaptor. *J Cereb Blood Flow Metab* 2002;22:142–52.
- [60] Gary DS, Bruce-Keller AJ, Kindy MS, et al. Ischemic and excitotoxic brain injury is enhanced in mice lacking the p55 tumor necrosis factor receptor. *J Cereb Blood Flow Metab* 1998;18:1283–7.
- [61] Bruce AJ, Boling W, Kindy MS, et al. Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. *Nat Med* 1996;2:788–94.
- [62] Kinoshita K, Kraydieh S, Alonso O, et al. Effect of posttraumatic hyperglycemia on contusion volume and neutrophil accumulation after moderate fluid-percussion brain injury in rats. *J Neurotrauma* 2002;19:681–92.
- [63] Chew W, Kucharczyk J, Moseley M, et al. Hyperglycemia augments ischemic brain injury: in vivo MR imaging/spectroscopic study with nicardipine in cats with occluded middle cerebral arteries. *AJNR Am J Neuroradiol* 1991;12:603–9.
- [64] Conroy BP, Grafe MR, Jenkins LW, et al. Histopathologic consequences of hyperglycemic cerebral ischemia during hypothermic cardiopulmonary bypass in pigs. *Ann Thorac Surg* 2001;71:1325–34.
- [65] Guyot LL, Diaz FG, O'Regan MH, et al. The effect of streptozotocin-induced diabetes on the release of excitotoxic and other amino acids from the ischemic rat cerebral cortex. *Neurosurgery* 2001;48:385–90.
- [66] Lin B, Ginsberg MD, Busto R, et al. Hyperglycemia triggers massive neutrophil deposition in brain following transient ischemia in rats. *Neurosci Lett* 2000;278:1–4.
- [67] Ding C, He Q, Li PA. activation of cell death pathway after a brief period of global ischemia in diabetic and non-diabetic animals. *Exp Neurol* 2004;188:421–9.

- [68] Baird TA, Parsons MW, Phan T, et al. Persistent poststroke hyperglycemia is independently associated with infarct expansion and worse clinical outcome. *Stroke* 2003;34:2208–14.
- [69] Nuttall GA, Abel MD, Mullany CJ, et al. Intraoperative hyperglycemia and perioperative outcomes in cardiac surgery patients. *Mayo Clin Proc* 2005;80:862–6.
- [70] Van den Berghe G, Schoonheydt K, Bex P, et al. Insulin therapy protects the central and peripheral nervous system of intensive care patients. *Neurology* 2005;64(26):1348–53.
- [71] Auer RN. Non-pharmacologic (physiologic) neuroprotection in the treatment of brain ischemia. *Ann N Y Acad Sci* 2001;939:271–82.
- [72] Busto R, Dietrich WD, Globus MY, et al. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. *J Cereb Blood Flow Metab* 1987;7:729–38.
- [73] Zhao O, Memezawa H, Smith ML, et al. Hyperthermia complicates middle cerebral artery occlusion induced by an intraluminal filament. *Brain Res* 1994;649:253–9.
- [74] Minamisawa H, Smith ML, Siesjo BK. The effect of mild hyperthermia and hypothermia on brain damage following 5, 10 and 15 min of forebrain ischemia. *Ann Neurol* 1990;28:26–33.
- [75] Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1136.
- [76] Kitagawa K, Matsumoto M, Tagaya M. “Ischemic tolerance” phenomenon found in the brain. *Brain Res* 1990;528:21–4.
- [77] Liu Y, Kato H, Nakata N, et al. Protection of rat hippocampus against ischemic neuronal damage by pretreatment with sublethal ischemia. *Brain Res* 1992;586:121–4.
- [78] Nishi S, Taki W, Uemura Y, et al. Ischemic tolerance due to the induction of HSP70 in a rat ischemic recirculation model. *Brain Res* 1993;615(2):281–8.
- [79] Chopp M, Chen H, Ho KL, et al. Transient hyperthermia protects against subsequent forebrain ischemic cell damage in the rat. *Neurology* 1989;39:1396–8.
- [80] Nishio S, Yunoki M, Chen ZF, et al. Ischemic tolerance in the rat neocortex following hypothermic preconditioning. *J Neurosurg* 2000;93:845–51.
- [81] Bergstedt K, Hu BR, Wieloch T. Initiation of protein synthesis and heat-shock protein-72 expression in the rat brain following severe insulin-induced hypoglycemia. *Acta Neuropathol (Berl)* 1993;86:145–53.
- [82] Huber R, Kasischke K, Ludolph AC, et al. Increase of cellular hypoxic tolerance by erythromycin and other antibiotics. *Neuroreport* 1999;10:1543–6.
- [83] Riepe MW, Kasischke K, Raupach A. Acetylsalicylic acid increases tolerance against hypoxic and chemical hypoxia. *Stroke* 1997;28:2006–11.
- [84] Dawson TM. Preconditioning-mediated neuroprotection through erythropoietin? *Lancet* 2002;359:96–7.
- [85] Kapinya KJ, Lowl D, Futterer C, et al. Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. *Stroke* 2002;33:1889–98.
- [86] Stagliano NE, Perez-Pinzon MA, Moskowitz MA, et al. Focal ischemic preconditioning induces rapid tolerance to middle cerebral artery occlusion in mice. *J Cereb Blood Flow Metab* 1999;19:757–61.
- [87] Barone FC, White RF, Spera PA, et al. Ischemic preconditioning and brain tolerance: temporal histological and functional outcomes, protein synthesis requirement, and interleukin-1 receptor antagonist and early gene expression. *Stroke* 1998;29:1937–50.
- [88] Weigl M, Tenze G, Steinlechner B, et al. A systematic review of currently available pharmacological neuroprotective agents as a sole intervention before anticipated or induced cardiac arrest. *Resuscitation* 2005;65:21–39.
- [89] Arnowski J, Waxham MN, Grotta JC. Neuronal protection and preservation of calcium/calmodulin-dependent protein kinase II and protein kinase C activity by dextrorphan treatment in global ischemia. *J Cereb Blood Flow Metab* 1993;13:550–7.

- [90] Li MM, Payne RS, Reid KH, et al. Correlates of delayed neuronal damage and neuroprotection in a rat model of cardiac-arrest-induced cerebral ischemia. *Brain Res* 1999;826:44–52.
- [91] Dietrich WD, Lin B, Globus MY, et al. Effect of delayed MK-801 (dizocilpine) treatment with or without immediate postischemic hypothermia on chronic neuronal survival after global forebrain ischemia in rats. *J Cereb Blood Flow Metab* 1995;15:960–8.
- [92] Calle PA, Paridaens K, De Ridder LI, et al. Failure of nimodipine to prevent brain damage in a global brain ischemia model in the rat. *Resuscitation* 1993;25:59–71.
- [93] Lazarewicz JW, Pluta R, Puka M, et al. Diverse mechanisms of neuronal protection by nimodipine in experimental rabbit brain ischemia. *Stroke* 1990;21:108–10.
- [94] Roine RO, Kaste M, Kinnunen A, et al. Nimodipine after resuscitation from out-of-hospital ventricular fibrillation. A placebo-controlled, double-blind, randomized trial. *JAMA* 1990;264:3171–7.
- [95] Shuaib A, Murabit MA, Kanthan R, et al. The neuroprotective effects of gamma-vinyl GABA in transient global ischemia: a morphological study with early and delayed evaluations. *Neurosci Lett* 1996;204(2):1–4.
- [96] Thaminy S, Reymann JM, Heresbach N, et al. Is chlormethiazole neuroprotective in experimental global cerebral ischemia? A microdialysis and behavioral study. *Pharmacol Biochem Behav* 1997;56:737–45.
- [97] Vergoni AV, Ottani A, Botticelli AR, et al. Neuroprotective effect of gamma-hydroxybutyrate in transient global cerebral ischemia in the rat. *Eur J Pharmacol* 2000;397:75–84.
- [98] Iqbal S, Baziany A, Gordon S, et al. Neuroprotective effect of tiagabine in transient forebrain global ischemia: an in vivo microdialysis, behavioral, and histological study. *Brain Res* 2002;946:162–70.
- [99] Artru AA, Michenfelder JD. Anoxic cerebral potassium accumulation reduced by phenytoin: mechanism of cerebral protection? *Anesth Analg* 1981;60:41–5.
- [100] Imaizumi S, Kurosawa K, Kinouchi H, et al. Effect of phenytoin on cortical Na(+)-K(+)-ATPase activity in global ischemic rat brain. *J Neurotrauma* 1995;12:231–4.
- [101] Miles AN, Majda BT, Meloni BP, et al. Postischemic intravenous administration of magnesium sulfate inhibits hippocampal CA1 neuronal death after transient global ischemia in rats. *Neurosurgery* 2001;49:1443–50.
- [102] Zhu H, Meloni BP, Bojarski C, et al. Post-ischemic modest hypothermia (35 degrees C) combined with intravenous magnesium is more effective at reducing CA1 neuronal death than either treatment used alone following global cerebral ischemia in rats. *Exp Neurol* 2005;193:361–8.
- [103] Steen PA, Milde JH, Michenfelder JD. No barbiturate protection in a dog model of complete cerebral ischemia. *Ann Neurol* 1979;5:343–9.
- [104] Candelario-Jalil E, Alvarez D, Gonzalez-Falcon A, et al. Neuroprotective efficacy of nimesulide against hippocampal neuronal damage following transient forebrain ischemia. *Eur J Pharmacol* 2002;453:189–95.
- [105] Uchino H, Minamikawa-Tachino R, Kristian T, et al. Differential neuroprotection by cyclosporine A and FK506 following ischemia corresponds with differing abilities to inhibit calcineurin and the mitochondrial permeability transition. *Neurobiol Dis* 2002;10:219–33.
- [106] McCullough LD, Hurn PD. Estrogen and ischemic neuroprotection: an integrated view. *Trends Endocrinol Metab* 2003;14:228–35.
- [107] Hurn PD, Macrae IM. Estrogen as neuroprotectant in stroke. *J Cereb Blood Flow Metab* 2000;20:631–52.
- [108] Loihl AK, Asensio V, Campbell IL, et al. Expression of nitric oxide synthase (NOS)-2 following permanent focal ischemia and the role of nitric oxide in infarct generation in male, female and NOS-2 gene deficient mice. *Brain Res* 1999;830:155–64.
- [109] Sampei K, Mandir AS, Asano Y, et al. Stroke outcome in double mutant antioxidant transgenics. *Stroke* 2000;31:2685–91.

- [110] Kofler J, Otsuka T, Zhang Z, et al. Differential effect of PARP-2 deletion on brain injury after focal and global cerebral ischemia. *J Cereb Blood Flow Metab* 2005 [Epub ahead of print].
- [111] McCullough LD, Zeng Z, Blizzard KK, et al. Ischemic nitric oxide and poly(ADP-ribose) polymerase-1 in cerebral ischemia: male toxicity, female protection. *J Cereb Blood Flow Metab* 2005;25:502–12.
- [112] Garcia-Segura LM, Azcoitia I, DonCarlos LL. Neuroprotection by estradiol. *Prog Neurobiol* 2001;63:29–60.
- [113] Noppens RR, Kofler J, Hurn PD, et al. Dose-dependent neuroprotection by 17beta-estradiol after cardiac arrest and cardiopulmonary resuscitation. *Crit Care Med* 2005; 33:1595–602.
- [114] Fried RL, Nowak TS. Opioid peptide levels in gerbil brain after transient ischemia: lasting depletion of hippocampal dynorphin. *Stroke* 1987;18:765–70.
- [115] Zabramski JM, Spetzler RF, Selman WR, et al. Naloxone therapy during focal cerebral ischemia evaluation in a primate model. *Stroke* 1984;15:621–7.
- [116] Tang AH. Protection from cerebral ischemia by U-50,488E, a specific kappa opioid analgesic agent. *Life Sci* 1985;37:1475–82.
- [117] Mackay KB, Kusomoto K, Graham DI, et al. Effect of kappa-1 opioid agonist CI-977 on ischemic brain damage and cerebral blood flow after middle cerebral artery occlusion in the rat. *Brain Res* 1993;629:10–8.
- [118] Baskin DS, Widmayer MA, Browning JL, et al. Evaluation of delayed treatment of focal cerebral ischemia with three selective κ -opioid agonists in cats. *Stroke* 1994;25:2047–54.
- [119] Boutin H, Dauphin F, MacKenzie ET, et al. Differential time-course decreases in nonselective mu-, delta-, and kappa-opioid receptors after focal cerebral ischemia in mice. *Stroke* 1999;30:1271–7.
- [120] Bradford HF, Crowder JM, White EJ. Inhibitory actions of opioid compounds on calcium fluxes and neurotransmitter release from mammalian cerebral cortical slices. *Br J Pharmacol* 1986;88:87–93.
- [121] Xiang J-Z, Adamson P, Brammer MJ, et al. The kappa-opiate agonist U50488H decreases the entry of ^{45}Ca into rat cortical synaptosomes by inhibiting N- but not L-type calcium channels. *Neuropharmacol* 1990;29:439–44.
- [122] Goyagi T, Toung TJ, Kirsch JR, et al. Neuroprotective kappa-opioid receptor agonist BRL 52537 attenuates ischemia-evoked nitric oxide production in vivo in rats. *Stroke* 2003;34: 1533–8.
- [123] Zhang Z, Chen TY, Kirsch JR, et al. Kappa-opioid receptor selectivity for ischemic neuroprotection with BRL 52537 in rats. *Anesth Analg* 2003;97:1776–83.
- [124] Chen TY, Goyagi T, Toung TJ, et al. Prolonged opportunity for ischemic neuroprotection with selective kappa-opioid receptor agonist in rats. *Stroke* 2004;35:1180–5.
- [125] Chen CH, Toung TJ, Hurn PD, et al. Ischemic neuroprotection with selective kappa-opioid receptor agonist is gender specific. *Stroke* 2005;36:1557–61.
- [126] Zeynalov E, Nemoto M, Hurn PD, et al. Neuroprotective effect of selective kappa receptor agonist is gender specific and linked to reduced neuronal nitric oxide. *J Cereb Blood Flow Metab* 2005 July 27 [Epub ahead of print].
- [127] Vu TH, Weissmann AD, London ED. Pharmacological characteristics and distribution of σ - and phencyclidine receptors in the animal kingdom. *J Neurochem* 1990;54:598–604.
- [128] Su T-P, London ED, Jaffe JH. Steroid binding at sigma receptors suggests a link between endocrine, nervous, and immune systems. *Science* 1988;240:219–21.
- [129] Hanner M, Moebius FF, Flandorfer A, et al. Purification, molecular cloning, and expression of the mammalian σ_1 -binding site. *Proc Natl Acad Sci U S A* 1996;93:8072–7.
- [130] Su T-P. Delineating biochemical and functional properties of sigma receptors: emerging concepts. *Crit Rev Neurobiol* 1993;7:187–203.
- [131] Leitner ML, Hohmann AG, Patrick SL, et al. Regional variation in the ratio of σ_1 to σ_2 binding in rat brain. *Eur J Pharmacol* 1994;259:65–9.

- [132] Contreras PC, Gray NM, Ragan DM, et al. BMY-14802 protects against ischemia-induced neuronal damage in the gerbil. *Life Sci* 1992;51:1145–9.
- [133] Harukuni I, Bhardwaj A, Shaivitz AB, et al. Sigma₁-receptor ligand 4-phenyl-1-(4-phenylbutyl)-piperidine affords neuroprotection from focal ischemia with prolonged reperfusion. *Stroke* 2000;31:976–82.
- [134] Goyagi T, Goto S, Bhardwaj A, et al. Neuroprotective effect of σ_1 -receptor ligand, 4-phenyl-1-(4-phenylbutyl) piperidine (PPBP) is linked to reduced neuronal nitric oxide production. *Stroke* 2001;32:1613–20.
- [135] Goyagi T, Bhardwaj A, Koehler RC, et al. Potent sigma 1-receptor ligand 4-phenyl-1-(4-phenylbutyl) piperidine provides ischemic neuroprotection without altering dopamine accumulation in vivo in rats. *Anesth Analg* 2003;96:532–8.