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Cellular and Molecular Neurobiology of Brain Preconditioning

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Abstract The tolerant brain which is a consequence of adaptation to repeated nonlethal insults is accompanied by the upregulation of protective mechanisms and the down-regulation of prodegenerative pathways. During the past 20 years, evidence has accumulated to suggest that protective mechanisms include increased production of chaperones, trophic factors, and other antiapoptotic proteins. In contrast, preconditioning can cause substantial dampening of the organism's metabolic state and decreased expression of proapoptotic proteins. Recent microarray analyses have also helped to document a role of several molecular pathways in the induction of the brain refractory state. The present review highlights some of these findings and suggests that a better understanding of these mechanisms will inform treatment of a number of neuropsychiatric disorders.

Keywords Neuronal degeneration · Ischemia · Stroke · Tolerance · Brain preconditioning

Introduction

Effective neuroprotection against neurodegenerative insults is one of the most interesting and clinically important goals of research in neurology and psychiatry [1]. One of the approaches that have received a great deal of attention is the phenomenon whereby exposure to subtoxic or sublethal doses of toxins can result in protection against larger doses of the same toxins and other toxic events. The present state of affairs is different from ideas promulgated about three decades ago when it was thought that exposure to successive brief periods of ischemia had a cumulative effect leading to tissue necrosis and organ dysfunction [2]. These ideas were put to rest when it was demonstrated that animals exposed to four 5-min-long occlusions followed by 40 min occlusion showed smaller infarct size whereas animals exposed to the same pretreatment paradigm but to 3 h of occlusion did not show protection [3]. Experiments in canine models of myocardial ischemia showed that cardiac myocytes exposed to 10 min of ischemia would sustain loss of intracellular adenosine triphosphate (ATP) and adenine nucleotide depletion whereas cells exposed to a further 10-min ischemic insult after an intervening 20 min of reperfusion did not experience further ATP loss [4]. These findings led to suggestions that multiple brief ischemic episodes might provide some degree of protection against subsequent prolonged ischemic insults [3, 4]. This type of pretreatment-induced protection is not limited to cardiac cells because other tissues including skeletal muscle [5], liver [6, 7], and the brain [8] experience a similar phenomenon. This process has been called preconditioning [9].

Brain preconditioning is a phenomenon in which the brain protects itself against future injury by adapting to low doses of noxious insults [10]. Groups of investigators from diverse fields have used different approaches to show that stimuli such as anesthetic agents, hypothermia and hyperthermia, hypoxia/ischemia, as well as low doses of certain toxins can promote preconditioning-dependent protective responses. Elucidation of the basic mechanisms underlying these responses promises to significantly influence therapeutic approaches that will impact the course of a number of neurologic disorders. These include the acute and chronic treatment of cerebrovascular accidents and, possibly, the

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chronic treatment of neurological disorders that are characterized by chronic progressive courses. Thus, the purpose of this review is to provide a brief summary of the models of brain preconditioning and to discuss the cellular and molecular bases of the tolerant brain. Although there is evidence that post-conditioning might also provide protection against models of focal ischemia [11], discussion of this phenomenon is beyond the scope of the present paper.

Models of Neuronal Preconditioning

Cerebrovascular accidents are among the leading causes of morbidity and mortality in the world [12]. Strokes are subdivided into ischemic and hemorrhagic strokes, with ischemic strokes representing about 85% of all cases [13]. Ischemic strokes are the consequences of transient or permanent thromboembolic events and of systemic hypoperfusion which cause inefficient delivery or marked decreases in the delivery of oxygen and glucose to the brain [14, 15]. These ischemic events can result from partial or complete vascular occlusions and can cause variable degrees of neuroanatomical damage and associated neurological deficits. The decrease in blood flow and associated diminution of nutrients delivered to the brain is known to cause activation of cellular and molecular processes that cause neuronal death via excitotoxicity, pH and ionic imbalances, oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunctions, as well as protoxic inflammatory responses [14–16]. Even though some of these pathways are being investigated actively, much remains to be done in order to develop therapeutic approaches that might tamper the course of ischemia-induced injuries. Because ischemia-induced pathobiological processes appear to have complex and, sometimes, divergent time courses that can occur within minutes, hours, and/or days, specific time-dependent therapeutic manipulations might be needed to addresses the damage caused to the various cell types that are affected by hypoxic/ischemic injuries [17, 18]. Thus, specific interventions would have to counter the excitotoxic effects of severe restriction of blood flow that causes necrotic cell death within the central core of a stroke while other approaches would have to prevent ischemic effects in the penumbra [14]. Still other avenues of treatment would need to focus on remedying the effects of a stroke on individual cells that might be impacted by more delayed inflammatory processes [19]. These issues are important because, although controversial, it has been suggested that patients who experienced prior transient ischemic attacks might suffer from less severe strokes later on [20–22].

The accumulated evidence generated from models of cardiac preconditioning is influencing information-driven approaches to the treatment of myocardial injury [23]. Although research in brain preconditioning is lagging, somewhat, behind similar research in cardiology, it is possible that similar approaches might prove fruitful in the treatment of strokes. Indeed, as in cardiac preconditioning, the development of a tolerant brain appears to result from the activation of endogenous mechanisms of protection against brain injuries [10]. The mechanisms involved in this type of tolerance to ischemic brain injuries have been investigated using both in vivo and in vitro models. These are discussed below.

Gerbil Models

About three decades ago, Kirino [24] discussed a model in which Mongolian gerbils were subjected to bilateral carotid occlusion for 5 min followed by processing of their brains for light and electron microscopy. The author reported that there occurred rapid ischemic changes in the CA4 region, with development of slower abnormalities in the CA2 area and even still slower changes in the CA1 region [24]. Using that model, Kitagawa and others [25] showed that exposure to 2 min of ischemia caused depletion of high-energy phosphate compounds and perturbation of protein synthesis without any evidence of neuronal death. In addition, they found that single episodes of 2-min ischemia were able to provide protection against cell death caused by 5 min ischemic events performed 1 or 2 days after the pretreatment. Moreover, two 2-min ischemic pretreatments performed 1 day apart provided complete protection against cell death induced by 5 min of ischemia [25]. Kato et al. [26] also showed that preconditioning with 2 min of ischemia prevented damage to the hippocampal CA1 area following 3 min of ischemia induced 3 days later. In addition, Miyashita et al. [27] reported that pretreatment with unilateral occlusion of the middle cerebral artery (MCA) could induce ischemic tolerance in the hippocampal CA1 neurons of gerbils [27]. Moreover, Ohtsuki et al. [28] showed that a 2-min preconditioning ischemia also protected gerbil hippocampal neurons against damage caused by a 3.5-min ischemia performed 3 days later. Because previous investigators had focused their attention on younger animals and because most ischemic strokes occur in older individuals, Dowden and Corbett [29] used 18- to 20-month-old gerbils to test if they could observe preconditioning-induced protection in these animals. They found that two 1.5-min episodes of global ischemia separated by 24 h protected animals who underwent a 5-min occlusion of both carotid arteries 72 h later. They also reported normal microtubule-associated protein-2 expression in the hippocampal CA1 region of preconditioned animals in contrast to the complete loss of microtubule-associated protein-2 staining observed in ischemic animals. Kitagawa et al. [30] used the gerbil model to investigate if neurons adjacent to ischemic lesions would

acquire tolerance to subsequent ischemic insults. They reported that there was significant attenuation of structural damage in the hippocampus of these animals, a phenomenon which lasted up to 2 weeks after the initial preconditioning stimulus.

Rats

Liu et al. [31] used rats to test if preconditioning with sublethal ischemia could protect against neuronal death caused by subsequent lethal ischemic insults. They showed that forebrain ischemia for 3 min was able to protect against hippocampal CA1 neuronal damage caused by 6 and 8 min of ischemia but not against damage caused by 10 min of ischemia [31]. Simon et al. [32] also showed that two brief periods of global cerebral ischemia, separated by 24 h, which did not cause significant cell death in the brain was able to significantly reduce the size of an infarct caused by permanent MCA occlusion. Glazier et al. [33] also investigated the possibility that a brief period of focal ischemia could cause ischemic tolerance in the rat brain. They induced focal ischemia via the MCA occlusion for 20 min. Following a 24 h of recovery, the authors subjected the animals to a 10 min of forebrain ischemia and processed the brain for histopathological examination 3–4 days after surgery. They found substantial degrees of neuronal death in the cerebral cortex, striatum, hippocampus, and thalamus [33]. Matsushima and Hakim [34] also reported that global ischemia can provide substantial protection against strokes caused by MCA occlusion in the rat brain. Moreover, Belayev et al. [35] also investigated whether transient unilateral MCA occlusion for 2 h could induce bilateral ischemic tolerance to hippocampal damage caused by bilateral two-vessel occlusion (2-VO). They reported that animals subjected to MCA occlusion and the 2-VO procedure were protected against 2-VO-induced cell death in the hippocampus. In another very interesting study, Perez-Pinzon et al. [36] assessed the possibility that ischemic preconditioning done only 30 min before a lethal insult could still offer protection against histological damage in the rat brain. In that study, the conditioning ischemic insult lasted 2 min. After only 30 min of reperfusion, the rats were subjected to 10 min of ischemia. There were significant degrees of protection in the hippocampal CA1 subfield and in the cortex [36].

In Vitro Models

Cell culture systems have also been used extensively to investigate the effects of preconditioning. The model of combined oxygen and glucose deprivation (OGD) that recapitulates the cascades of changes that occur during ischemia-induced injuries in the mammalian brain [37, 38] has been used extensively to investigate potential bases of preconditioning-induced neuroprotection. In that model, transient deprivation of rodent cortical cell cultures of both oxygen and glucose causes neuronal swelling, followed by severe neuronal degeneration over a period of several hours even when the cells were returned to normal media [37]. Acute and delayed injuries were inhibited by combined removal of extracellular Ca^{2+} in conjunction with Na^{+} or Cl^{-} substitution. The toxic effects of OGD appear to be mediated by excitotoxic damage because it causes a large increase in the levels of extracellular glutamate and because *N*-methyl-D-aspartate (NMDA) receptor antagonists provided protection against both the early and the late damage caused by OGD. Bruer et al. [39] used the OGD model to study the effects of preconditioning in vitro. They found that cellular damage was significantly reduced in cells preexposed to 1.5 h of OGD with an intervening 48–72 h before a second exposure to 3 h of OGD. Khaspekov et al. [40] used mixed neuroglial hippocampal cell cultures in their study and found that a 60 min of OGD preconditioning could protect against 90 min of OGD performed 1 and 2 days later. Similarly, Grabb and Choi [41] used cells from cortices of fetal mice to test if exposure to periods of OGD (5–30 min) that were too brief to cause cell death could protect a subsequent exposure to lethal ODG (45–55 min) performed 24 h later. They also found that the preexposure did provide significant protection against the lethal exposure [41]. Neuroprotection was lost if the interval between the preconditioning and the severe insult was shortened to 7 h or lengthened to 72 h [41]. Interestingly, they also reported that the preconditioned cultures were not protected against NMDA, kainate-, or glutamate-induced neuronal death [41]. This is in contrast with the study by Tauskela et al. [42] who reported that subjecting rat cortical cultures to a preconditioning OGD paradigm provided cross-tolerance to NMDA-induced cell death. They also found that NMDA receptor blockade during preconditioning by OGD eliminated tolerance [42]. Another study comparing various preconditioning manipulations to OGD found that cycloheximide, heat stress, and MK801 were as protective as OGD preconditioning in both acute and delayed models of ischemia-induced neuronal death [43]. Similar results have been reported using hippocampal slice cultures [44], after combined deprivation of glucose and amino acids [45] and after exposure to neuronal cultures to various agents including anesthetic agents and diazoxide [46, 47], mild hypoxic insults [40], as well as stimulation of adenosine receptors [48]. Interestingly, Badaut et al. [49] used the OGD model in organotypic cultures of the hippocampus and demonstrated that preconditioning provided protection against cell death and the transient loss of evoked potential responses.

Other Models of Brain Tolerance

In addition to ischemia preconditioning, there are other pretreatment paradigms that have been shown to protect against ischemic injuries. These include hyperthermia [50], lipopolysaccharide (LPS) pretreatment [51, 52], and treatment with anesthetic agents [53, 54] among others. A small dose of LPS given systemically confers ischemic protection in the brain, a process that appears to involve activation of an inflammatory response before ischemia [51]. LPS preconditioning in the brain shares some hallmarks that are characteristic of ischemic preconditioning in the brain. These include delayed induction of tolerance after preconditioning and dependence on de novo protein synthesis [55]. The systemic route of LPS administration and the induction of some systemic changes are unique aspects of LPS preconditioning that might offer some clinical advantages. Other models of preconditioning that remain to be thoroughly investigated include hypoxic insults in a neonatal rat model [56, 57], spreading depression [58, 59], and the induction of seizures [60]. Of significant interest are recent studies that have demonstrated that exercise preconditioning might provide substantial benefit against ischemic damage [61–63] and heat strokes [64] in rodents.

Mechanisms of Brain Tolerance

Mechanistic studies of ischemic tolerance are very important to the development of strategies to treat ischemic strokes. These studies have focused on types of ischemic tolerance. The original or classic type of ischemic tolerance is a delayed type of tolerance that requires new protein synthesis and engenders neuronal protection 24–72 h after the preconditioning stimulus [65]. In contrast, rapid ischemic tolerance does not require new protein synthesis and produces neuroprotection within 1 h of the preconditioning event [36, 66]. Delayed tolerance develops over time, with protection against subsequent injury being present at 24 to 48 h after the preconditioning event, peaking at around 3 days and slowly disappearing over a week period. Rapid tolerance develops within a few minutes and lasts less than 3 days [36]. Understanding the cellular and molecular mechanisms that underlie the protective effects of preconditioning has significant clinical implications. These mechanisms appear to involve counteracting the pathways that cause ischemia-induced neuronal death. The fact that many strokes are preceded by transient ischemic attacks [12] suggests that it might be possible to develop approaches that are akin to preconditioning-induced protection in humans.

Glutamate Receptors and Excitotoxicity

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 [14, 15]. A role for NMDA receptor signaling in ischemic preconditioning in the CNS is supported by reports that NMDA receptor antagonists can alter the development of ischemic tolerance induced by brief repeated ischemic insults in gerbil brains [26], by OGD in mouse cortical cultures [41], and by hypoxia-induced damage in hippocampal slices [67]. Moreover, exposure to sublethal NMDA concentrations provided protection to neurons against lethal death stimuli applied at later time points [68]. Using the gerbil model, it has been shown that binding of [^{3}H]MK-801 and [^{3}H]α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) to excitatory NMDA and AMPA receptors were significantly decreased in the hippocampus of animals rendered tolerant to the degenerative effects of ischemic insults [69]. The expression of GluR2 protein is also significantly decreased in neuronal populations that survived in the tolerant gerbil brain [70]. The mRNA for glutamate receptor, ionotropic delta 2, was reported to be downregulated in the mouse brain after preconditioning [71]. In contrast, [^{3}H]muscimol binding to GABA(A) receptors in CA1 neurons was transiently but significantly increased after preconditioning but not after ischemic injuries [69]. These results suggest that preconditioning might cause a change in the balance between excitotoxic and inhibitory mechanisms in the brain in such a way that proinhibitory forces might be more prominent in the tolerant brain.

Lin et al. [72] used OGD model in cultured cortical neurons to investigate the mechanisms of protection conferred by glutamate preconditioning. Pretreatment of neurons with NMDA receptor antagonists prevented OGD-induced cell death whereas AMPA receptor and voltage-dependent Ca^{2+} channel blockers did not. Neurons preconditioned with glutamate exhibited resistance to damage induced by OGD, with the observed protection depending on the duration of preconditioning exposure and the interval between preconditioning exposure and test challenge. Protective efficacy was blocked by the NMDA or AMPA receptor antagonists.

Free Radical Mechanisms

Brain ischemia is known to induce the generation of oxygen- and nitrogen-based free radicals which are involved in causing much of the damage observed in the ischemic brain [73]. Superoxide can be generated through the actions of xanthine oxidase, leakage from the mitochondrial electron transport chain, and via the actions of other oxidases [74].

Nitric oxide can be generated by the action of nitric oxide synthases [75], whose activation during ischemic injuries is dependent on activation of glutamate receptors (see above). Nitric oxide and superoxide can interact to form the toxic peroxynitrite [75]. Some studies have suggested that preconditioning might exert its protective effects by preventing the production of free radicals or by inhibiting their toxic effects on the brain [76]. For example, Toyoda et al. [77] have reported that ischemic preconditioning which reduced the volume of cerebral infarction also caused significant increases in the activity of the antioxidant enzyme superoxide dismutase. Volatile anesthetics were also reported to exert their protective effects via inducible nitric oxide synthase-dependent mechanisms [78]. Heme oxygenase-1 (HO-1), the rate-limiting enzyme in the degradation of heme to produce bile pigments and carbon monoxide, is induced by a variety of factors such as heat, ischemia, and hydrogen peroxide [79]. Induction of HO-1 has been shown to exert protective activity against ischemic damage [80] and the protective effects of the anesthetic agent, isoflurane, have been reported to depend on increased HO-1 expression in OGD-induced neuronal injury [81].

Transduction Signaling Pathways

Activation of kinase pathways is thought to play integral role in the development of the tolerant heart [82]. Similarly, a few reports have suggested a role for the mitogen-activated protein kinase pathway in preconditioning-induced neuroprotection. For example, Shamloo et al. [83] had investigated the role of mitogen-activated protein kinase kinase (MEK)1/2 and extracellular signal-regulated kinase (ERK)1/2 in ischemic preconditioning. They found that 3 min of ischemic preconditioning caused increased expression of phosphorylated MEK and of phosphorylated ERK in the rat hippocampus. In addition, 9 min of lethal ischemia caused increases in phosphorylated ERK in both sham and preconditioned animals. However, the levels of phosphorylated ERK had returned to normal in the tolerant hippocampi whereas it stayed elevated in the damaged hippocampi 24 h after ischemic damage [83]. In vivo studies have also suggested that nitric oxide-dependent activation of the Ras–extracellular-regulated kinase cascade participates in the development of OGD tolerance in cortical neurons [84]. These findings have been replicated in the rat hippocampus where sustained increases in ERK phosphorylation have been found [85]. In the heart, ERK1/2 activation has been shown to participate in isoflurane preconditioning through the upregulation of hypoxia-inducible factor-1 α (HIF-1α) and vascular endothelial growth factor [86]. ERK5 activation also participates in the protective effects of ischemic preconditioning [87, 88]. Jia et al. [89] have also reported that ERK and novel protein kinase C (PKC) epsilon are involved in OGD-induced neuroprotection in hippocampal slices from mice. These results are consistent, in part, with previous reports that ischemia- and NMDA-induced tolerance involved activation of PKC epsilon in organotypic hippocampal slice cultures [90, 91]. The neuroprotective effects of PKC epsilon appear to involve phosphorylation of the mitochondrial K(+) ATP channel [92]. Other isoforms of PKC such as PKC alpha and delta have also been implicated in the mechanisms of ischemic preconditioning [93, 94].

Nishimura et al. [95] used the gerbil model and showed that, after 2-min preconditioning, there was enhanced p38 immunoreactivity in CA1 and CA3 neurons after 6 h of reperfusion. SB203580-induced inhibition of active p38 30 min before the 2-min ischemia reduced the ischemic tolerance effect. In the hippocampal CA1 region, preconditioning has also been reported to block MLK3 activation after lethal ischemia, an effect that is mediated by Akt1 activation through NMDA receptor stimulation [96]. Miao et al. [97] also reported that ischemic preconditioning caused downregulation of MLK3 and JNK1/2 in the rat brain in an Akt1-dependent fashion.

Transcription Factors

Ischemic injuries are associated with the activation of a number of transcription factors [98]. For example, adaptive responses to ischemic injuries in the brain have been shown to involve families of transcription factors including members of the activator protein 1 (AP-1) [97], cAMP response element-binding protein (CREB) [99], and hypoxia-inducible factors (HIFs) [100] among others. These are discussed below.

Dimeric AP-1 complexes are involved in the control of cell proliferation, differentiation, and death via the regulation of multiple gene families [101, 102]. Members of the AP-1 transcription factors include c-fos, fra-1, fra-2, fosB, c-jun, junB, and junD [103]. A number of studies have suggested that ischemic injuries are associated with significant changes in their expression. For example, Kiessling et al. [104] have reported that, after 5 min of ischemia, c-fos and Krox-24 mRNAs were increased in all hippocampal subpopulations peaking at 1 h after recirculation. Increased immediate-early gene proteins occurred in neuronal populations that were resistant to ischemia. An et al. [105] also reported on the expression of c-fos, c-jun, junB, and jun D in a rat model of ischemia for 90 min, which led to large cortical infarction, caused substantial increases in c-fos and junB expression in the brain, with c-jun showing less of an increase. Delayed expression of JunB was also reported to precede neuronal death after

global ischemia in the gerbil brain [106]. Yoneda et al. [107] used gel retardation electrophoresis and found that transient forebrain ischemia for 5 min caused potentiation of AP-1 binding in the hippocampal CA1 and CA3 regions of gerbils that had experienced ischemia for 2 min 2 days before, a process known to lead to the development of tolerance to subsequent severe ischemia. In contrast, ischemia for 5 min caused prolonged potentiation of AP-1 binding in the vulnerable CA1 subfield of the gerbils with prior ischemia for 5 min 14 days before, a process known to cause delayed death of pyramidal neurons in the CA1 area. These increases were not observed in the resistant CA3 subfield of the hippocampus [107]. Using a model of preconditioning which uses a sublethal ischemic stimulus (2.5 min), Kapinya et al. [108] measured DNA binding activity of the AP-1 transcription factor complex in the brain after severe ischemic injuries and in preconditioned gerbil brains. Ischemic tolerance was associated with short increases in AP-1 binding activity which peaked at 3 h. Similar changes occurred in cells that are destined to survive in the hippocampal CA1 areas. Dhodda et al. [109] found increases in c-jun during ischemic preconditioning. These authors also reported that preconditioning blocked ischemia-induced increases in c-fos, c-jun, and junB. In contrast, Sommer et al. [110] reported that there were selective increases in c-JUN expression in the gerbil hippocampus during and after the acquisition of the tolerant state. These discrepancies suggest that the role of AP-1 transcription factors in the development of ischemic tolerance is a complex issue that needs to be dissected further.

Ischemic injury is also associated with phosphorylation of CREB and increases in the expression of CREB-dependent genes in the brain [99, 111]. CREB is necessary for cellular proliferation, survival, and differentiation [112]. In the brain, CREB-induced gene expression participates in learning and memory, as well as in neuron survival and differentiation [113]. CREB is very important in stimulus-transcription coupling and in receptor-mediated changes in gene expression [112]. These changes are probably mediated via stimulation of glutamate receptors and subsequent increases in cytosolic calcium. This pathway is thought to also be involved in the manifestation of ischemic preconditioning because Mabuchi et al. [114] have reported that increased CREB phosphorylation might occur as a protective adaptation to ischemic injury. Of more direct relevance, Lee et al. [115] have reported that preconditioning is associated with CREB activation. Furthermore, inhibition of CREB-mediated gene transcription with either CRE-decoy oligonucleotide or protein kinase inhibitors interfered with the development of ischemic tolerance [116]. Rybnikova et al. [117] also recently reported prolonged expression of phosphorylated CREB after preconditioning. Because CREB is involved in the regulation of many genes, it will be important to identify which specific genes are involved in the actions of this transcription factor.

HIF-1 was cloned during a search for proteins that participate in the regulation of genes that are involved in adaptation to ischemic injuries [118]. HIF-1α can partner with HIF-1β and translocate to the nucleus to regulate the expression of genes involved in hypoxic adaptation [119]. This idea is supported by increases in HIF-1α immunoreactivity in hypoxic brains [120]. Treatment with desferrioxamine which protects against cell death in models of ischemia causes stabilization of HIF-1α in vitro and in vivo [119, 121].

Chaperones

Chaperones are known to be involved in protecting various systems against an array of insults including cerebral ischemia [122]. Several laboratories have studied the potential role of chaperones in both in vitro and in vivo models of the preconditioned response. Liu et al. [123] found that induction of heat shock protein 70 (HSP70) correlated with preconditioning-induced protection in the rat hippocampus. Similarly, Kato et al. [8] investigated the potential role of the low molecular weight stress proteins, 27-kDa heat shock protein (HSP27) and αB crystallin, and of HSP70 in ischemic tolerance in the rat brain. They found that the induction of both HSP27 and HSP70, but not of αB crystallin, had a good temporal correlation with the induction of ischemic tolerance [8]. Chen et al. [124] have conducted experiments to test the involvement of the inducible HSP70, glucose-regulated protein (GRP)75, and GRP78 in ischemic tolerance. They found that the expression of HSP70, but not that of GRP75 or GRP78, increased during times when ischemic tolerance was present but not after [124]. Tanaka et al. [125] also found increases in HSP40 in the hippocampus of tolerant mice. In addition, they observed parallel increases in HSP70 in these mice [125]. Microarray analyses have also found HSP70 and small heat shock protein HSPB2 to be upregulated in the mouse brain after preconditioning [71]. Dhodda et al. [109] have documented increases in the expression of HSP27, HSP70, and HSP90 using microarray and proteomic analyses. A study by Hayashi et al. [126] also reported significant increases in GRP78 at 2 days, a time which coincided with the time of tolerance in the rat hippocampus.

In addition to HSP40 and HSP70, Yagita et al. [127] assessed the expression profiles of all members of the HSP110 family including ischemia responsive protein 94, HSP110/105, and osp94/apg-1. They reported that the expression of HSP110/105 mRNA was increased from 4 to 24 h after a 6-min or longer ischemic period, with increased expression occurring first in the dentate gyrus

and, later, in the pyramidal layer. They also found that the changes in HSP110/105 expression after ischemia were similar to those of inducible HSP70, which was previously reported to be involved in preconditioning-related protection [8, 124]. Tolerant CA1 pyramidal neurons expressed both HSP70 and HSP110/105, thus suggesting that these two chaperones might work in concert to protect neurons against subsequent lethal damage [127]. When taken together, these studies indicate that HSPs might play an integral role in the protective effects observed after preconditioning. Some of these changes might occur in astrocytes that might be induced to release several factors to protect neurons against ischemic injuries (see Brown [122] for further discussion of the interactions of chaperones in the brain).

Neuroinflammatory Responses

Strokes are thought to be accompanied by inflammatory responses that might participate, in part, in the delayed effects of neuronal survival [128]. LPS preconditioning suppresses the cellular inflammatory response to ischemia in the brain and circulation. Diminished activation of cellular inflammatory responses that ordinarily exacerbate ischemic injury may contribute to neuroprotection induced by LPS preconditioning [51]. Glial cells, in particular astrocytes, have always been viewed as supporters of neuronal function. Only recently a very active role for glial cells has been emerging in physiology and pathophysiology. Specific pathways have been identified by which these cells can protect or even help to regenerate brain tissue after acute insults [129]. Ischemic damage is associated with reactive astrocytosis which is characterized by very obvious structural changes [130]. Astrocytes secrete cytokines and chemokines [131].

Microglia are resident macrophages that play immunocompetent and phagocytic functions in the brain [132]. In the case of ischemia, microglial cells undergo morphologic transformation and secrete a number of substances that participate in either protoxic or protective mechanisms [132, 133]. Minocycline, a tetracycline family antibiotic, has been reported to protect against ischemic damage by blocking microglial activation [134, 135]. It also should be noted that microglia/macrophages or their secreted factors may actually protect cells against damage caused by glutamate [136]. Thus, the role of microglia cells in the preconditioned brain needs to be further investigated.

Trophic Factors

Trophic factors are involved in the process of neurite outgrowth and in cell survival in the brain [137]. These neurotrophic factors which include brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are present in high concentrations in the hippocampal formation and cerebral cortex [138]. The intracellular pathways used by the neurotrophic factors are dependent on their interactions with high-affinity receptors (TrkA for nerve growth factor (NGF), TrkB for BDNF, and TrkC for NT-3) [138, 139]. Following cerebral ischemia or hypoglycemic coma, the levels of NGF and BDNF but not of NT-3 are decreased in hippocampal neurons [140–142]. However, the situation is different for the receptors, in that only the expression of TrkB is increased in the hippocampal formation [141]. These observations are consistent, in part, with the reports that NGF and BDNF can protect hippocampal CA1 neurons against ischemic cell damage [143–145]. Given these results, the potential role of trophic factors in ischemic preconditioning has been investigated [146]. It was found that increases in the levels of NGF and BDNF occurred within the first 6 h after ischemic preconditioning manipulations such as short-term global cerebral ischemia [147]. Preconditioning applied 3 days earlier was able to attenuate increases in the levels of BDNF mRNA observed at 12 h after reperfusion using a rat model [148]. More recently, Lee et al. [149] showed that NGF, BDNF, and their receptors recover better in tolerant hippocampal neurons. Because trophic factors are so important during development and participate so actively in neuroplastic and neuroprotective mechanisms in the brain, more investigations on their roles in the tolerant brain are warranted.

Other Mechanisms

Attempts have been made, using unbiased approaches, to identify genes that might be involved in the development of the tolerant brain [71, 150]. The facts that it takes several hours to induce delayed ischemic brain tolerance [25] and that protein synthesis is also necessary for its manifestation [65] have led to the suggestion that changes in the transcriptome might play a role in its appearance. On the other hand, short-term tolerance might be dependent on activation of the ubiquitin-proteasome pathway [151]. In order to test the role of gene expression in delayed ischemic preconditioning, Stenzel-Poore et al. [71] used unbiased microarray analyses and found that genes involved in responses to stress, metabolism, regulation of apoptosis, and signal transduction were upregulated by ischemia. In contrast, preconditioning of the brain causes downregulation of many genes involved in these pathways. Moreover, preconditioning prevented the upregulation of the genes that were induced by the lethal ischemic stimulus. Dhodda et al. [109] also used microarray analyses and reported that ischemic preconditioning was associated with increases in

Bcl-2, Bid, caspase-2, and LICE. The increases in Bcl-2 expression are probably mediated by CREB activation [152]. Glutamate preconditioning, using the OGD model, is also associated with increased Bcl-2 expression that was blocked by the calmodulin kinase inhibitor, KN93, by the protein kinase inhibitor, staurosporine, and by a CRE-decoy oligonucleotide [72]. Dhodda et al. [109] have also reported increases in SMAD1, SMAD7, TGF-α, and TGF-β after ischemic preconditioning. It is important to note that recent studies have suggested that Toll-like receptors (TLR), well known components of the innate immune system [153], might play a role in preconditioning [154]. Indeed, systemic administration of TLR ligands cause tolerance to ischemic injuries, a process that might involve increased expression of neuroprotective anti-inflammatory substances [155].

Concluding Remarks

The development of the preconditioning brain is dependent on the activation of several neuroprotective pathways. These changes are associated with both transcriptional and translational changes that bring the brain to another homeostatic state that allows it to be refractory to some lethal insults. In the case of ischemic strokes, there appear to be several triggers of the preconditioned states and these include heat, anesthetic agents, and seizures. Because there are substantial degrees of cross-tolerance between the various triggers of preconditioning, it is possible to suggest that elucidation of the mechanisms involved in anesthetic-induced preconditioning might impact significantly on the treatment of the delayed neurodegenerative changes that accompany ischemic strokes. The elucidation of these pathways might also impact on the treatment of older individuals who suffer from mild cognitive deficits. Although this review has focused mostly on the use of subtoxic insults to generate a tolerant state, these approaches might be fraught with ethical dilemma that might not be easily overcome. Approaches such as exercise preconditioning which has recently been shown to reduce brain damage might be less controversial and will need to be further investigated.

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