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Preconditioning paradigms and pathways in the brain

■ ABSTRACT

Preconditioning is a phenomenon in which the brain protects itself against future injury by adapting to low doses of noxious insults. Preconditioning stimuli include ischemia, low doses of endotoxin, hypoxia, hypothermia and hyperthermia, cortical spreading depression, anesthetics, and 3-nitropropionic acid, among others. Understanding of the mechanisms underlying preconditioning has been elusive, but NMDA receptor activation, nitric oxide, inflammatory cytokines, and suppression of the innate immune system appear to have a role. Elucidation of the endogenous cell survival pathways involved in preconditioning has significant clinical implications for preventing neuronal damage in susceptible patients.

The brain relies upon internal defense mechanisms for protection from injurious stimuli. Preconditioning is a phenomenon whereby low doses of these noxious insults shield the brain from future insults rather than inflicting damage. Preconditioning stimuli include but are not limited to transient global and focal ischemia,¹⁻⁴ cortical spreading depression,⁵⁻⁷ brief episodes of seizure, exposure to anesthetic inhalants,⁸⁻¹⁰ low doses of endotoxin (lipopolysaccharide [LPS]),^{11,12} hypothermia and hyperthermia,^{13,14} and 3-nitropropionic acid treatment.^{15,16}

Depending on the specific preconditioning stimulus, a state of neuronal tolerance can be established in at least two temporal profiles: one in which the trigger induces protection within minutes (rapid or acute tolerance),¹⁷ and one in which the protected state develops after a delay of several hours to days (delayed tolerance).⁴ Some preconditioning paradigms induce both

phases of ischemic tolerance, while others can induce only the acute phase or only the delayed phase.¹⁸⁻²¹ The acute phase is most likely due to rapid posttranslational modifications of proteins.^{22,23} In contrast, the delayed phase is dependent on de novo protein synthesis.^{24,25}

Preconditioning by ischemic tolerance was first identified in the heart by Murry et al,²⁶ and was subsequently found to occur in the brain^{4,27} and a variety of organs including the liver, intestine, kidney, and lung. Preconditioning stimuli can be cross-tolerant, safeguarding against other types of injury. For example, endotoxin preconditioning can protect against subsequent ischemia and vice versa. Thus, there may be some overlapping mechanisms in preconditioning, and unraveling these pathways may uncover an arsenal of neuroprotective therapeutic targets. In this review, we will compare different preconditioning paradigms and discuss potential mechanisms in initiating brain ischemic tolerance.

■ PARADIGMS TO ESTABLISH PRECONDITIONING

Refinement of various preconditioning models is of great clinical significance. Cardiovascular or cerebrovascular surgery has a negative impact on brain function due to stoppage of blood flow during surgery. In fact, more than 25% of patients who receive coronary artery bypass surgery suffer from temporary or permanent memory loss.^{28,29} As a result, it is of premier importance to develop strategies to protect the brain either prior to vascular surgeries or in patients at high risk of stroke. While it would be dangerous and impractical to precondition at-risk patients with ischemia, the identification of underlying preconditioning mechanisms may lead to safer therapeutic factors that can be administered before surgery.

Ischemia

Global ischemic preconditioning in the brain is accomplished by occlusion of the bilateral common carotid

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arteries. In contrast, in focal ischemic preconditioning, occlusion of one side of the middle cerebral artery is induced for about 1 to 20 minutes, depending on methods and animal species.^{4,30-32} Twenty-four hours after ischemic preconditioning, stroke is induced in these animals. Preconditioning-induced neuroprotection is observed not only in terms of infarct volume but also in terms of neurological scores and behavior studies.

Lipopolysaccharide

Tolerance to ischemic injury can also be induced by a small dose of LPS injected into the peritoneal cavity. Dosages vary from 0.05 to 1 mg/kg body weight in small rodents such as mice and rats.^{11,33-36} This dose of LPS usually does not bring abnormal signs and symptoms to the animals. The ischemic protection yields a reduction of infarct volume of approximately 30%. This tolerant state can be sustained for about 1 week, with maximum protection occurring around 2 to 3 days after injection of LPS.

Hypoxia

A relatively convenient method for preconditioning animals is hypoxic exposure. Animals are put in a chamber in which oxygen and nitrogen proportions can be controlled. Oxygen concentration usually ranges from 8% to 13% with normobaric pressure. Exposure time ranges from 1 to 6 hours. Twenty-four to 72 hours later, transient or permanent focal stroke is induced in the animals.³⁷⁻⁴⁰ Hypoxia-preconditioned neuroprotection usually starts at 1 to 3 days with a significant reduction of infarct size. Hypoxic preconditioning has also been demonstrated for in vitro neuron culture models using oxygen-glucose deprivation injury.⁴¹

3-Nitropropionic acid

3-Nitropropionic acid (3-NP) is an irreversible inhibitor of succinate dehydrogenase, an enzyme required for oxidative phosphorylation and adenosine triphosphate production. When applied at low doses 1 to 4 days before ischemia, 3-NP can lead to ischemic tolerance in the forebrain of gerbils and rats.^{16,42,43} The dose ranges from 1 to 20 mg/kg body weight.¹⁶ Such treatment significantly improves neurological behavior and increases neuronal survival in the CA1 region of hippocampus. In addition, 3-NP preconditioning induces tolerance to hypoxia in hippocampal slice preparations.^{15,44}

Hypothermia and hyperthermia

Hypothermia is a well-characterized protective procedure used during and after cerebral surgery. It is also reported that brief hypothermic or hyperthermic exposure can also lead to ischemic tolerance. The tempera-

tures adopted range from 25°C to 32°C^{13,45,46} in hypothermia and from 42°C to 43°C in hyperthermia.¹⁴

Cortical spreading depression

Cortical spreading depression is defined as the electrophysiologic phenomenon of slowly propagating transient depolarization waves across the cortex. Usually 5 M of potassium chloride is infused into the cortex, or a cotton pad soaked with the solution is put on the surface of dura mater, which results in depolarization, firing of neurons, and cortical spreading depression. Cortical spreading depression induces a prolonged phase of ischemic tolerance that lasts 1 to 7 days.^{5,6,47,48}

Anesthetics

Exposure to volatile anesthetics such as isoflurane and halothane within pharmacologic concentration ranges also confers delayed-phase ischemic tolerance of the brain.^{8-10,49}

■ MOLECULAR PRECONDITIONING PATHWAYS

Mechanistically, cellular preconditioning can be subdivided into intrinsic neuronal pathways (preventing excitotoxic damage, signaling through anti-apoptotic molecules, and treatment by neurotrophic factors) or extrinsic nonneuronal pathways (peripheral cytokine production, microglial activation, and regulation of the cerebrovascular system). Several neuroprotective molecules are expressed and signal through multiple cell types both within and peripheral to the brain, so that assigning an exact source and paradigm for preconditioning pathways has proven difficult.

NMDA receptor activation and excitotoxicity protection

In neurons, ischemic tolerance is mediated largely by the activation of the *N*-methyl-D-aspartate (NMDA) glutamate receptors through increases in intracellular calcium.⁵⁰⁻⁵² Although glutamate receptor activation is generally believed to be responsible for much of the neuronal damage caused by excitotoxicity, it appears to also be implicated in the establishment of preconditioning. One study demonstrated that exposure of cortical cell cultures to low levels of glutamate activated NMDA receptors in preconditioning.⁵⁰ In addition, preconditioning by oxygen-glucose deprivation was blocked when an NMDA antagonist was applied. NMDA receptor activation can induce a tolerant state through rapid adaptation of the voltage-dependent calcium flux. In addition, activation of NMDA receptors leads to rapid release of brain-derived neurotrophic factor, which then binds to and activates its cognate receptor, receptor tyrosine kinase B. Both NMDA and tyrosine kinase B receptors activate nuclear factor- κ B (NF κ B), a transcription factor involved

in protecting neurons against insults. In sublethal ischemic preconditioning, activation of NF κ B and its translocation from the cytosol to the nucleus was required for the development of late cerebral protection against severe ischemia or epilepsy.⁵³ Other key mediators involved in synaptic NMDA receptor-dependent neuroprotection are phosphatidylinositol 3-kinase (PI3K), Akt, and glycogen synthase kinase 3- β .⁵⁴

Preconditioning with cortical spreading depression results in the downregulation of the excitatory amino acid transporters EAAT1 and EAAT2 from cerebral cortex plasma membranes.⁵⁵ Although these transporters are normally involved in glutamate uptake, it has been suggested that the influx of sodium that occurs during excitotoxicity may cause their reversal and result in additional glutamate release. Downregulating these transporters may thus contribute to ischemic tolerance.

Nitric oxide

Nitric oxide (NO) may play a key role as a mediator of the neuronal ischemic preconditioning response, either in conjunction with or independent of NMDA receptor activation. Both the inhibition of nitric oxide synthase (NOS) and the scavenging of NO during preconditioning significantly attenuated the induced neuronal tolerance, and neither endothelial NOS nor neuronal NOS knockout mice showed protection from rapid ischemic preconditioning.^{56,57} Treatment with the inducible NOS (iNOS) inhibitor aminoguanidine abolished the induced protection. The mechanisms responsible for NO-induced tolerance are not clear. Downregulation of the glutamate transporter GLT-1 might play a role.⁵⁸ A common link to NMDA receptor activation and NO is p21^{ras} (Ras). Preconditioning induces p21^{ras} activation in an NMDA- and NO-dependent manner and leads to the downstream activation of Raf kinase, mitogen-activated protein kinases, and extracellular regulated kinase.⁵⁹ Inhibition of these kinases attenuates subsequent protection from ischemia.^{60,61} Pharmacologic inhibition of Ras, as well as a dominant negative Ras mutant, blocked preconditioning, whereas a constitutively active form of Ras promoted neuroprotection against lethal insults. An important consideration regarding NO is also that preconditioning by volatile anesthetics appears to involve NO pathways.⁹

NO and reactive oxygen species (ROS) are also implicated in regulating the peripheral cerebrovascular system. Ischemia generated by occlusion of the middle cerebral artery causes defects in cerebrovascular function for not only the infarcted area but also the surrounding ischemic region. LPS preconditioning has been reported in some cases to increase this

regional cerebral blood flow both before and after ischemia.^{1,21,36,62–64} LPS also improves microvascular perfusion.^{33,64} It was recently reported that LPS-stimulated cerebral blood flow is induced through reactive oxygen and nitrogen species (ROS or NO).¹ Mouse knockouts of iNOS (NO production) or of the nox2 subunit of NADPH oxidase (ROS production) eliminated the LPS-upregulated cerebrovascular activity. Furthermore, blockage of these ROS and NO pathways reduced the preconditioning effect of LPS. Therefore, LPS may play a more direct role in preventing ischemic damage by increasing blood availability to the affected brain region.

Inflammatory cytokines and the innate immune system

LPS, a component of the gram-negative bacterial cell wall, can illicit a potent innate immune response. While this systemic inflammatory response can be destructive (at doses of 5 mg/kg),⁶⁵ tolerable LPS doses of 0.05 to 1 mg/kg injected intraperitoneally render the brain,¹¹ heart,^{66,67} liver,^{68,69} kidneys,⁷⁰ and pancreas⁷¹ transiently resistant to subsequent ischemic injury. This preconditioning paradigm relies on the ability of a peripheral signal to cross into multiple organ systems. LPS injected into the gut can signal through peritoneal macrophages and circulating monocytes. Toll-like receptor 4 is a pattern-recognition receptor that binds to pathogen-associated molecular patterns in LPS and initiates a signaling cascade through the NF κ B pathway. This pathway culminates in the expression and secretion of several proinflammatory cytokines to fight off the infection and anti-inflammatory cytokines to control the immune response.

The major output of LPS signaling is innate production of proinflammatory cytokines to fight infection and clear cellular debris. Central cytokines, including tumor necrosis factor- α (TNF α), interleukin-6 (IL-6), and interleukin-1 β (IL-1 β), can be neurodestructive if administered after ischemia. TNF α administration by cerebroventricular injection after ischemia augmented the extent of injury, and blockage of TNF α signaling proved neuroprotective.^{11,72,73} However, in LPS preconditioning, cytokine production precedes ischemia. Intracisternal injections of TNF α before middle cerebral artery occlusion (MCAO) were protective in reducing the infarct size of pretreated mice.⁷⁴ Furthermore, intracisternal injection of ceramide analog, a downstream component of the TNF α signaling pathway, was also capable of reducing the MCAO infarct area.⁷⁵ Preischemic treatment with IL-6 and IL-1 also reduced neuronal damage.^{76,77} TNF α knockout mice eliminated the LPS

protective phenotype,⁷² demonstrating that cytokine production is a critical feature of LPS preconditioning in ischemia. Additionally, ischemic damage in the absence of LPS preconditioning was exacerbated in TNF α receptor 1 knockout mice.^{78,79} Consistently, TNF α protein levels are upregulated after LPS treatment but are downregulated following LPS-preconditioned MCAO.⁷² A unifying theme in LPS preconditioning comprises early activation of the innate immune system with ensuing suppression in ischemia.

As a potential mechanism, the initial inflammatory response induced by LPS appears to render the innate immune system hyporesponsive to subsequent insults such as ischemia. This may occur by persistence of anti-inflammatory cytokines produced by the primary insult. These molecules are expressed in tandem with proinflammatory cytokines to control the innate immune response, but may also play a role in delayed preconditioning. For instance, intravenous or intracerebroventricular IL-10 injection can reduce the infarct size with MCAO.⁸⁰ Alternatively, several proinflammatory cytokine signaling pathways may be downregulated by negative feedback inhibition.^{20,81} This inhibition may occur extracellularly, using soluble cytokine receptors, decoy receptors, or receptor antagonists. For example, intravenous injection of IL-1 receptor antagonist can provide neuroprotection against ischemic injury from MCAO.^{82,83} Cytokine feedback inhibitors that act intracellularly are also induced with the innate immune response. Intracellular inhibition may involve direct downregulation of cytokine transcription (peroxisome proliferator-activated receptor gamma [PPAR- γ]) or inhibition of intracellular signaling pathways that promote cytokine production (suppressor of cytokine signaling [SOCS] and PI3K). Antisense mRNA knockdown of SOCS-3 exacerbates ischemic injury from MCAO.⁸⁴ The MCAO infarction area is increased after treatment with PPAR- γ antagonists and decreased by PPAR- γ agonists.^{85,86} Administration of compounds that increase PI3K signaling is also capable of reducing ischemic damage.⁸⁷ Thus, several defense mechanisms designed to suppress the innate immune response may play an active role in LPS ischemic preconditioning.

Role of microglia in ischemic preconditioning

Microglia represent the resident central nervous system (CNS) component of the innate immune system. Microglia and macrophages become activated with ischemia in the infarcted and surrounded area.⁸⁸ Upon activation in ischemia, microglia will become phagocytic and secrete a multitude of noxious chemokines and cytokines.⁸⁹ Accordingly, anti-inflammatory anti-

biotics such as doxycycline and minocycline reduce microglial activation and diminish the ischemic infarction area.⁹⁰ Preconditioning the brain with LPS ameliorates microglial activation, neutrophil infiltration, and circulating monocyte activation following MCAO.³⁵ However, primary ischemic damage is not correlated with CNS infiltration of peripheral leukocytes but rather with an increase in proliferating resident microglial cells.⁹¹ Alternatively, microglia can exhibit neuroprotective properties within the brain.⁹² In fact, greater ischemic damage from longer periods of MCAO is correlated with fewer proliferating microglia, suggesting a protective microglial role.⁹¹ Consistently, ablation of proliferating microglia increases the infarction area following MCAO.⁹³ Therefore, microglia can be protective in ischemia, and preconditioning with LPS may render microglia more capable of reacting to ischemic conditions.

CONCLUSIONS

Preconditioning represents an adaptive response to prime the brain for protection against future injury. Elucidation of these endogenous cell survival pathways has significant clinical implications for preventing neuronal damage in susceptible patients. For this reason, understanding the underlying mechanisms in establishing a tolerant state will be a critical step in adapting preconditioning for safe patient applications. The field of ischemic research has made great strides in deciphering causative preconditioning factors but has been hampered by the complex, multifactorial nature of preconditioning paradigms. The study of tolerance is further complicated by the fact that signaling takes place both peripheral to and within the brain in multiple cell types. Future research will require the exploration of interactions between multiple pathways and roles of individual cell types in establishing ischemic tolerance. Only with a more thorough understanding of preconditioning mechanisms can we adapt these pathways for the most efficient and protective treatments.

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