Mol Neurobiol (2016) 53:2579–2593
DOI 10.1007/s12035-015-9278-z

Cerebral Ischemic Preconditioning: the Road So Far…

Thushara Vijayakumar N[1] · Amit Sangwan[1] · Bhargy Sharma[1] · Arshad Majid[2] · Rajanikant GK[1]

Received: 26 November 2014 / Accepted: 2 June 2015 / Published online: 17 June 2015

Abstract Cerebral preconditioning constitutes the brain's adaptation to lethal ischemia when first exposed to mild doses of a subtoxic stressor. The phenomenon of preconditioning has been largely studied in the heart, and data from in vivo and in vitro models from past 2–3 decades have provided sufficient evidence that similar machinery exists in the brain as well. Since preconditioning results in a transient protective phenotype labeled as ischemic tolerance, it can open many doors in the medical warfare against stroke, a debilitating cerebrovascular disorder that kills or cripples thousands of people worldwide every year. Preconditioning can be induced by a variety of stimuli from hypoxia to pharmacological anesthetics, and each, in turn, induces tolerance by activating a multitude of proteins, enzymes, receptors, transcription factors, and other biomolecules eventually leading to genomic reprogramming. The intracellular signaling pathways and molecular cascades behind preconditioning are extensively being investigated, and several first-rate papers have come out in the last few years centered on the topic of cerebral ischemic tolerance. However, translating the experimental knowledge into the clinical scaffold still evades practicality and faces several challenges. Of the various preconditioning strategies, remote ischemic preconditioning and pharmacological preconditioning appears to be more clinically relevant for the management of ischemic stroke. In this review, we discuss current developments in the field of cerebral preconditioning and then examine the potential of various preconditioning agents to confer neuroprotection in the brain.

Keywords Preconditioning · Cerebral ischemia · Ischemic tolerance · Neuroprotection · Genomic reprogramming · Epigenetics

Abbreviations

IT	Ischemic tolerance
IPC	Ischemic preconditioning
CPC	Cerebral preconditioning
HPC	Hypoxic preconditioning
RIPC	Remote ischemic preconditioning
PPC	Pharmacological preconditioning
ADK	Adenosine kinase
NCX	Na^+/Ca^{2+} exchanger
TACE	Tumor necrosis factor-α converting enzyme
HRE	Hypoxia-responsive elements
EPO	Erythropoietin
VEGF	Vascular endothelial growth factor
SIP	Sphingosine-1-phosphate
εPKC	Epsilon protein kinase C
CCL	Chemokine (C–C motif) ligand
SPK	Sphingosine kinase
COX	Cyclooxygenase
TLR	Toll-like receptor
CNS	Central nervous system
LPS	Lipopolysaccharide
TNF-α	Tumor necrosis factor-α
IL	Interleukin
ROS	Reactive oxygen species
SAH	Subarachnoid haemorrhage
iNOS	Inducible nitric oxide synthase
nNOS	Neuronal nitric oxide synthase

✉ Rajanikant GK
rajanikant@nitc.ac.in

[1] School of Biotechnology, DBT—Centre for Bioinformatics, National Institute of Technology Calicut, Calicut 673601, India

[2] Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, UK

eNOS	Endothelial nitric oxide synthase
NOS	Nitric oxide synthase
NO	Nitric oxide
cAMP	Cyclic adenosine monophosphate
AMP	Adenosine monophosphate
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPK	Adenosine 5′-monophosphate-activated protein kinase
JNK	c-Jun N-terminal kinase
ERK	Extracellular signal-regulated kinase
NF-κB	Nuclear factor-κB
NMDA	N-methyl-D-aspartate
EA	Electroacupuncture
3-NPA	3-Nitropropionic acid
CREB	cAMP responsive element binding
HIF	Hypoxia inducible factor
HBO	Hyperbaric oxygenation
ATP	Adenosine triphosphate
MCAO	Middle cerebral artery occlusion
OGD	Oxygen–glucose deprivation
tPA	Tissue plasminogen activator
IPost	Ischemic post-conditioning
TGF	Transforming growth factor
TGF-α	Transforming growth factor alpha
HSP	Heat shock protein
MeCP2	Methyl-CpG-binding protein 2
IGF	Insulin-like growth factor
PcG	Polycomb group
TrxG	Trithorax group
TIA	Transient ischemic attack
IRI	Ischemia-reperfusion injury
IR	Ionizing radiation
HCA	Hypothermic circulatory arrest
BAIPC	Bilateral arm ischemic preconditioning
IAS	Intracranial arterial stenosis
AKT	Protein kinase B
RHP	Repetitive hypoxic preconditioning
p53	Tumor protein p53

Introduction

Beginning of the twenty-first century saw cerebrovascular diseases, more specifically stroke, as the chief perpetrators of about 10 % of all deaths round the globe (roughly six million deaths worldwide) [1]. An episode of stroke usually results in severe disability and neuronal impairment. More often than not, the traumatized patients are forced to depend on others for their survival [2]. Ischemic stroke results from the occlusion of blood vessels to the brain and accounts for 80–85 % of stroke cases in most parts of the world. When the cerebral circulation is completely arrested following ischemic stroke, it leads to weakening of the energy state and ion homeostasis. This results in the depletion of high-energy phosphates, membrane depolarization, efflux of cellular potassium, and influx of sodium, chloride, and water [3]. Irreversible tissue damage occurs by a host of mechanisms including ionic perturbations, free radical production, excitotoxicity, inflammation, and ultimately, cell death [4]. The thrombolytic tissue plasminogen activator (tPA) therapy for acute ischemic stroke has shown favorable outcomes in patients, but it has a narrow therapeutic window and may not always be clinically feasible [4–6].

Because of the ever-present risk of cerebral ischemic injury, the brain is thought to have developed a backup machinery to brace itself against ischemic attacks and survive in the event of an injury. Specific regenerative processes in living organisms have evolved over time that can safeguard the body to an extent from tissue injury or organ damage [5, 7, 8]. Preconditioning (PC) is one such phenomenon that can exploit the fundamental characteristic of adaptability in organisms. A noxious stimulus strong enough to initiate a response but not so much as to cause permanent tissue damage is provided. The aim is to eventually condition the body against subsequent attacks from a lethal stressor. The targeted tissue or organism after successful PC tends to show an increased level of tolerance against ensuing ischemic attacks. PC works by altering signaling reactions and molecular pathways to lessen or reverse the injury and possibly bring on genetic reprogramming so that the "tolerant" phenotype can be protective and long lasting at the same time.

Even though a lot of time and effort has been invested in deciphering the phenomenon of preconditioning and vast amounts of data that have been generated, it has not yet hit the clinical platform on a groundbreaking level as envisaged. This review aims to illustrate the remarkable potential of cerebral preconditioning (CPC) in the management of ischemic stroke. First, a deeper comprehension of the various preconditioning stimuli and the degree of induced tolerance acquired using them is essential. We have tried to enable the exposition of some of the underlying endogenous repair mechanisms that take place during preconditioning. Additionally, we have also attempted to put together a repertoire of current and previous works that have been carried out through the years in this promising field.

Understanding Cerebral Preconditioning

Ischemic tolerance (IT) of the brain is a transient protective phenotype brought about by the application of sublethal stimuli (hypoxia, chemicals, etc.), which can increase the resistance of cells or tissues against a subsequent, more rigorous ischemic event. This form of adaptive status induced by preconditioning is an inherent attribute of certain living tissues,

ensuring survival and protection from harmful stressors [8, 9]. Preconditioning paradigms in cells are varied and take place in different windows of time [10]. Acute tolerance is developed within minutes of exposure to the preconditioning stimuli and offers only short-term protection; this is called early or rapid preconditioning. Formed normally through changes in post-translational modifications, the window of protection is very small and recedes after a few hours. Delayed or classical preconditioning prevails due to genetic alterations and protein synthesis and hence is of more significance within the ischemic region [11, 12]. It is longer in duration, from a few hours to several days but usually less than a week. Between the early and delayed IT, there is usually an unprotected window with little or no tolerance [11]. These two phases of IT brought about by preconditioning possibly work by diverse mechanisms and in varied time frames in different tissues of the brain [12]. Factors that may affect the scope of CPC include age, gender, and strain of the animals used as models for cerebral ischemia [11, 13].

An interesting aspect of preconditioning mechanisms is the apparent lack of specificity to the injury. Most degenerative or defensive pathways show some level of integration in processes like cell death or repair [7]. Neurodegenerative and cerebrovascular diseases follow similar pathophysiology like inflammation, calcium overload, and apoptosis. Even if the underlying causes and symptoms are varied, this feature could be exploited in therapeutic applications. The means to precondition the ischemic brain may be varied, whether it is rapid or delayed PC. When preconditioning is induced by drugs like anesthetics, which can pulse protective signals within the brain, it is referred to as pharmacological preconditioning (PPC) [14–16]. Sometimes, unrelated stressors are capable of producing similar adaptive mechanisms and may reiterate parts of ischemia (hypoxia or inhibitor molecules) or other unrelated conditions like depression and heat stress; this form of PC characterizes cross-tolerance [12]. Ischemic post-conditioning (IPost) is the method of inducing tolerance after occurrence of the event. It works by the periodic applications of brief ischemic stress following reperfusion and helps in cerebrovascular regeneration and protection [17].

Over the Years

The concept of preconditioning was gestated during the early 1960s, when researchers stumbled upon cases of brain adaptability [12]. In 1964, for example, the work by Dahl and Balfour revealed that anoxic preexposure in female rats for 30 s increased the survival time to 90 s in a second spell of anoxia (compared to 60 s in non-conditioned rats), quite possibly through enhanced rates of anaerobic glycolysis and increased pyruvate concentration in the brain [18]. In 1976, Vanucci and Duffy documented an increased tolerance in fetal rats toward anoxia compared to neonates, hinting at lower cerebral energy requirements for prolonged survival [19].

The term "ischemic preconditioning (IPC)" was first described for cardiac tissue by Murry et al. in 1986. They had applied brief periods of simultaneous coronary occlusions and reperfusions in dogs followed by a longer spell of cardiac occlusion and found that instead of worsening the insult, the sublethal strokes had surprisingly resulted in an adaptive conformation in the cardiac tissue [20]. This documentation, although not sensational at the time, was later instrumental in breaching a largely unexplored and novel avenue for research into the potential of IT and anti-infarct techniques [21]. A paper on the adaptability of rat brain tissue to anoxia using an in vitro model of hippocampal slices was also published the same year [22]. In 1989, it was documented that brief hypothermia contributed toward cerebral protection [23].

In 1990, Kitagawa et al. showed that pretreating adult gerbils with mild ischemia resulted in delayed neuronal death in the CA1 region of the hippocampus and conferred an outstanding degree of protection against neuronal death [24]. They were also the first to propose the existence of the "ischemic tolerance" phenomenon in the brain, and their work soon became the benchmark for preconditioning related work in animal models. Hypoxic preconditioning (HPC) joined the fray soon after, when Gidday et al. studied hypoxia-induced protection for the first time in a neonatal rat model, though hypoxia was already being used in brain research [25–27]. During the late 1990s, a considerable amount of data was gathered on IPC-induced tolerance in animal models of focal and global cerebral ischemia (CI), signifying the recognition of region-specific induction of the preconditioning stimuli for ensuing neuroprotection [28, 29].

The idea of possible genetic reprogramming following preconditioning gained popularity only in the twenty-first century [30]. Now, researchers seek to decipher the genomic profile of the ischemic-tolerant brain and understand the other biochemical reactions induced by PC [31–33].

Experimental Models

CPC and IT have been studied extensively in a variety of reliable experimental models. Primary neuronal cells (usually murine cell lines and human neuroblastoma cells) or organotypic slice cultures are commonly used to mimic PC in vitro [34–37]. Mammalian models using mice, rats, gerbils, pigs, and genetic model systems like *Drosophila* strains have shown a certain line of defense against injury by preconditioning in vivo [24, 38–41]. Rodents are routinely employed experimental models for both focal and global cerebral ischemia to enable a clearer understanding of the scope and duration of stress required to induce PC.

In focal preconditioning, PC is induced through the occlusion of the middle cerebral artery for a few minutes with equally spaced reperfusions in between. In contrast, global preconditioning is brought about by a single, shorter (<5 min) occlusion, in all the four cerebral vessels of the fore brain. Subsequently, permanent ischemia of a longer duration is established. While the sublethal ischemia may last for an hour or more in focal models, it is generally in the order of a few minutes for global IPC. The degree of protection against ischemia has a distinctive time frame: heightened protection for a few minutes with subsequent decline and remote protection that sets in after a day and quite possibly lasts a week [17, 42].

Varying durations of focal and global ischemia together produce different experimental setups for better comprehension of IT paradigms.

Global-Global Four-vessel occlusion in rats and two-vessel occlusion in rats, gerbils, and mice before final ischemia have been described [24, 43–45].

Global-Focal Four-vessel occlusion with hypotension, followed by permanent focal ischemia in hippocampal neurons and astroglial cells, induced neuroprotection in rats [46].

Focal-Focal Transient middle cerebral artery occlusion (MCAO) followed by permanent MCAO in rats [47].

Focal-Global MCAO at distal site followed by global ischemia in rats or unilateral MCAO before transient forebrain ischemia in gerbils [29, 48].

Key Players in Cerebral Preconditioning

Preconditioning stimuli triggers protective responses through various sensors and signaling molecules and thus generate a protective phenotype within the brain. IT mechanisms involve interconnected biological pathways that minimize neuronal damage and promote restorative cascades [12]. These cascades are typically specific to the applied stimulus and determined by its duration. A PC mechanism engages both neuronal and non-neuronal pathways [10].

Glutamate Pathway Glutamate excitotoxicity is a chief culprit behind nerve cell injury following stroke [49, 50]. Glutamate receptors are affected when adenosine triphosphate (ATP) levels drop following oxygen deprivation during ischemia. This results in impaired synaptic plasticity and accumulation of glutamate [51]. High levels of glutamate, in turn, overactivate N-methyl-D-aspartate (NMDA) receptors, which leads to increased calcium influx in a series of events ending in excitotoxic neuronal death [49]. Mild activation of NMDA receptors is required for induction of IT, possibly through an adaptive pathway involving nuclear factor-κB (NF-κB) and tumor necrosis factor-α (TNF-α) [10, 52]. NMDA preconditioning involves exposure to small doses of NMDA before the final insult [53, 54]. NMDA activation was reported to confer neuroprotection by inhibition of stress-activated c-Jun N-terminal kinase (JNK), activation of extracellular signal-regulated kinase (ERK½) and protein kinase B (Akt1), and regulation of normal cyclin adenosine monophosphate (cAMP) responsive element binding (CREB) activity [54, 55]. In neuronal cortical cultures, tolerance was achieved with glutamate preconditioning and blocked by NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists [54, 56]. It has been found that upregulation of glial glutamate transporter-1 (GLT-1) expression assists in inducing IT [57].

Nitric Oxide (NO) Synthase The other popular player in the NMDA stimulation cascade is nitric oxide (NO) [58, 59]. That NO is a crucial member is evident from both in vivo and in vitro models of PC, but the exact mode of action remains unclear. In a newborn rat model, tolerance due to HPC was chiefly attributed to endothelial NOS (eNOS) led NO mediation, rather than neuronal NOS (nNOS) [60]. Similarly, rat hippocampal slices exposed to anoxia displayed protection from a final anoxic insult, and the tolerance disappeared in the presence of NOS inhibitor (7-nitroindazole) [61]. In a focal ischemia model using eNOS and nNOS knockout mice, no reduction in infarct volume was observed after rapid IPC, compared to their preconditioned wild-type counterpart [62]. Inducible NOS (iNOS) was found to be involved in isoflurane PC-induced neuroprotection in in vivo and in vitro conditions, as well as during IPC [16, 63, 64]. eNOS has been recently implicated as a mediator of neurovascular protection against subarachnoid hemorrhage (SAH)-induced vasospasm, through HPC in mice, showing for the first time that PC is beneficial for other forms of stroke as well [65].

Immune System A stroke-like event can trigger the innate immune system. A series of inflammatory cells like leukocytes, microglia, etc. are recruited into the infarct zone in a time-dependent manner and eventually damage the brain tissue [66, 67]. The immune response is setup by a signaling pathway initiated by non-catalytic toll-like receptors (TLRs) that recognize foreign molecules and lead to the induction of transcription factor NF-κB. The nuclear factor then leads to the transcription of cytokines and chemokines to establish the inflammatory cascade [68, 69]. In the early phases of post-ischemia, reactive oxygen species (ROS) are rapidly released from the cells and microglia activate pro-inflammatory cytokines like IL-6, IL-1β, and TNF-α that worsen the injury [67]. However, these very cytokines also serve as mediators of IT in brain [70]. PC pathways could either promote anti-

inflammatory cascades or inhibition of pro-inflammatory molecules. Genomic reprogramming of TLR signaling can bring about tolerance by controlling the inflammatory response and mediating anti-inflammatory mechanisms [71, 72]. Endotoxin preconditioning with 1.0 mg/kg lipopolysaccharide (LPS) for four consecutive days in mice showed that LPS receptor (TLR4) was required for the activation of central nervous system (CNS) microglia, which could significantly reduce neuronal death and impart protection to neurons [73]. Interestingly, while LPS functioned by suppressing TNF-α signaling, in endotoxin PC, the same TNF-α was required for the activation of preconditioning effect of LPS, suggesting divergent roles for this cytokine in neuroprotection [74]. LPS induced activation of TLR4 and pretreatment with IL-6 and IL-1, and post-treatment with IL-10 after ischemia is also associated with reduced neuronal injury in various in vivo and in vitro settings [70, 75–78].

Enzymes and Receptors Enzymes, like stress-activated kinases, are the other group of proteins activated in response to a sublethal insult in the brain. It has been reported that the activation of anti-apoptotic factor Akt/protein kinaseB can suppress the action of JNK signaling during IPC [45]. Cyclooxygenase-2 (COX-2), an isoform of the cyclooxygenase enzyme required for the oxidation of arachidonic acid into prostanoids, participates in the neuroinflammatory cascade during ischemic injury [79]. COX-2 messenger RNA (mRNA) levels were upregulated in rats following MCAO, indicative of its role in mediating delayed neuronal death [79]. Obstruction of the COX-2 pathway has been proposed as a therapeutic strategy in case of global cerebral ischemia after Sprague Dawley rats subjected to HBO showed reduced COX-2 expression. Also, addition of the selective COX-2 inhibitor NS-398 abrogated the protection observed before [80]. Downregulation of COX-2 following the induction of IT was previously reported in gerbils as well [81]. Interestingly, COX-1 is seen to have a protective function during cerebral injury, signifying opposing roles for the two isoforms [82].

The lipid kinase sphingosine kinase 2 (SPK-2) is an important mediator of IT, as studied in mice preconditioned with isoflurane and hypoxia [83]. A mechanism has recently been proposed in which preconditioning with cobalt or hypoxia promotes SPK-2 catalytic activity, producing the signaling molecule sphingosine-1-phosphate (SIP). SIP upregulates chemokine (C–C motif) ligand 2 (CCL2) to bring about tolerance post-ischemia [84].

Epsilon protein kinase C (εPKC) facilitates the localization of regulatory enzyme SIRT1 (a known IT mediator) to the neuronal mitochondria, rendering direct protection to the organelle during the delayed phase of IPC induced tolerance in vivo [85, 86]. The adenosine surge following stroke is thought to be part of an integral neuroprotective strategy of the brain and upregulation of adenosine receptors were observed after ICP [87]. Adenosine kinase (ADK) negatively regulates the nucleoside adenosine and is a central molecule in the augmentation of brain injury. Underexpression of cerebral ADK in transgenic mice induced cortical protection and overexpression resulted in its abolishment. Therefore, this enzyme is being projected as a promising target for developing a stroke therapeutic. ADK knockdown by a viral system was also found to be defensive against stroke in mice [88].

Other probable targets for PC strategies include the membrane transporter, Na^+/Ca^{2+} exchanger (NCX) and its isoforms NCX1 and NCX3, and adenosine 5′-monophosphate-activated protein kinase (AMPK). NCX1 is upregulated by hypoxia inducible factor (HIF-1) and NCX3 mediated by pAKT (serine/threonine protein kinase) signaling [89, 90]. MCAO in male mice led to a spike in pAMPK levels 4 h after IPC and reduction after 72 h, suggesting that downregulation of AMPK contributes toward delayed PC [91]. Finally, the roles of many key players like HIF-2α, SIRT1, and CREB suspected of being part of the preconditioning cascade are now established [85, 92, 93].

Transcriptional Regulation Another method by which preconditioning leads to IT is through the activation of transcription factors. Recent research on intracellular enzyme and protein levels after exposure to various stressors has shed light on the mechanisms by which modifications at gene level are brought about during PC. For instance, exposure to prolonged and intermittent normobaric hyperoxia in rats at different time frames resulted in tolerance induced by activation of NF-κB [94]. NF-κB is the transcription factor for TNF-α, which is triggered by the tumor necrosis factor-α converting enzyme (TACE). In this study, increased expression of TACE and serum TNF-α was observed along with greatly reduced infarct volume [94]. Another central player in the development of IT in the brain is the HIF-1, a transcription factor activated in response to low oxygen concentration in the cell. One of more popular molecules to be linked to HPC, HIF-1 enables the upregulation of survival genes in oxygen-depressed environment. HIF is a heterodimer composed of two proteins HIF-1α and HIF-1β. Under hypoxic stress, HIF-1α binds to HIF-1β, and together, they attach to the hypoxia-responsive elements (HRE) present on the HIF target genes [95]. This transcriptional complex now promotes the transcription of protective genes like erythropoietin (EPO), vascular endothelial growth factor (VEGF), and glucose transporters [95–98]. Another member of the HIF family of transcription factors is the HIF-2α subunit, which has been found to mediate the transcriptional upregulation of EPO mRNA in astrocytes [92]. Although its role for a possible preconditioning signaling cascade has not been deeply explored, the HIF-2α-

mediated transcriptional regulation provides an interesting feature of tissue-specific protective mechanisms in the cell and may contribute to IT in astrocytes [92, 99]. Other modulators for transcriptional regulation during CPC include JNK and Akt/FoxO signaling pathways [41, 100, 101]. Early activation of transcription factor AP-1 with increased DNA binding affinity was implicated in the induction of IT in vivo [102]. AP-1 is a dimeric protein consisting of important components c-Jun and c-Fos which functions in both neuroprotection and cell death. In a transgenic MCAO mouse model, c-Jun was associated with increased AP-1 DNA binding activity [103].

Bone morphogenic protein-7 (BMP-7) has been found to mediate IT through IPC in rats [104]. In an MCAO model of preconditioning involving BMP-7, neuroprotection was made possible through the activation of p38/MAPK signaling pathway [105].

Genomic Reprogramming PC-induced transcriptional response is now responsible for an altered genomic profile in the tolerant organism, distinct from stroke models without preconditioning. Identification of genes involved and expressed in the neuroprotective phenotype of IT becomes mandatory for the complete understanding of IT [30].

"Genomic reprogramming" not only involves activation of genes for neuronal protection and regeneration but also suppression of those that are directly involved in the degenerative pathway during stroke. The genetic response is tailored according to the stimulus, whether it is a PC triggering agent or an ischemic event following PC. Different regulatory molecules such as transcription factors, transducers, sensors, and effectors, as well as numerous post-translational modifications, all contribute toward "reprogramming" of the genetic architecture after a PC incentive [12]. A multitude of genes from different families participate in the ischemic response which is different from a PC reaction. Genetic repression is also equally important in the preconditioned brain [8, 12].

Genomic upregulation during IPC was studied in adult rats using GeneChip technology and subsequent protein synthesis of molecules inducing tolerance, like heat shock proteins (e.g., HSP70) and transforming growth factor (TGF-α) was confirmed [31]. DNA microarray technology has been an invaluable tool for resolution of the genetic profile of IT. Microarrays were used to study differential gene expression patterns in oxygen–glucose deprived rat hippocampal slices and microRNA expression and regulation of its target MeCP2 in IPC-stimulated mouse cortex [32, 106]. It was also found to be beneficial in an adult mouse model of HPC, where upregulation of cell survival genes like HIF, insulin-like growth factor (IGF), etc., and region-specific expression patterns within the same brain were made apparent [33].

Preconditioning Agents

PC agents are characterized by their effectiveness in mimicking an ischemic environment without permanent neuronal damage. IPC is normally seen as the prototypical PC stimuli since its first application in cardiac tissue in 1986 [20] and later in the brain [24]. IPC is now widely used in different rodent models of focal and global ischemia, which has enabled a deeper comprehension of the quality and duration of stress required to induce PC in the brain [11].

Cross-tolerance, brought about by stressors other than ischemia, like oxidative stress, cortical spreading depression, heat shock, etc., could be the frontrunner in CPC studies, quite naturally because of the risk involved in inducing ischemia, however mild, in stroke patients [10, 12]. Also, many of these "non-ischemic" agents appear to function through an overlapping array of molecular pathways that could imply at some sort of common response evoked when the brain is under stress [12].

Hypoxic Preconditioning (HPC) HPC is among the more frequently used preconditioning stimuli for in vivo models of IT. Even though neurons are highly sensitive to hypoxia, they have over time, evolved certain protective mechanisms that helps the brain, the most crucial organ in the body, survive during extreme conditions. That a large number of animals like many amphibians and few mammals, display varying degrees of hypoxic resistance is a proof of this trait [9]. The clue here is the differential rates of ion metabolism and ATP turnover that is enhanced in hypoxia-sensitive mammals, compared to naturally low states in tolerant species of certain fishes and turtles [107, 108]. Mention should be made of the 1994 work by Gidday et al. in perinatal rats exposed to a 3 h hypoxic (8 % oxygen) episode followed by hypoxia ischemia after 24 h that revealed no damage to the neurons of the "conditioned" animals; this later helped establish a time-framed regime for CPC in animal models of stroke [25]. Similarly, when adult rats were placed in a chamber of normobaric hypoxia (8 % O_2 during 1, 3, or 6 h) and subjected to focal permanent ischemia after an interval of 24 h, they showed a 30 % reduction in infarct volume and tolerance lasting 3 days [97]. Observations from rodent models have suggested that exposure to hypobaric hypoxia can lead to the reversal of GLT-1 protein downregulation caused by global brain ischemia [57]. A recent study has revealed that HPC may even play a role in angiogenesis following acute cerebral infarction, which could potentially explain its neuroprotective conduit [109].

Oxygen–Glucose Deprivation (OGD) Murine cortical cultures exposed to short periods of oxygen–glucose deprivation (OGD) exhibited 30–50 % less neuronal death than controls after exposure to a longer period of OGD, although the

window of protection existed only between a 7- to 72-h gap (from preconditioning to final insult) and depended on the duration of PC [110]. Ischemia modeled in vitro by OGD is particularly useful for studying the mechanism of IT in neuronal cell cultures and brain tissue and has helped ascertain that PC does not affect systemic flow following stroke [56, 110]. It has been shown that OGD-induced tolerance in cortical cultures may induce a signaling cascade involving neuronal nitric oxide synthase (NOS) activation for subsequent neuroprotection [58].

Hyperoxic Preconditioning Preconditioning with periodic exposure to normobaric hyperoxia (95 % O_2) in rodents for a prolonged period was observed to subdue neurologic damage and reduce infarct volume [94]. Hyperoxia has been shown to induce IT in MCAO models of rats and mice, possibly through a combination of cellular reactions and biochemical changes brought about by genetic reprogramming [111].

Hyperbaric oxygenation (HBO) also functions as stimuli; in a global ischemia model, Sprague Dawley rats were pressurized in a hyperbaric chamber for an hour for each treatment and after different patterns of administration showed reduced cellular apoptosis [112]. HBO-PC was found to induce tolerance against MCAO through the mediation of SIRT-1 proteins [113] and resulted in the downregulation of COX-2 in a murine model of global cerebral ischemia [80].

Hypothermia and Hyperthermia Hypothermia is seen to be safe and practical in surgical procedures based on data from randomized clinical trials [114, 115]. Brief periods of hypothermia can confer rapid tolerance in focal ischemic models, though there is no perceivable impact of increasing the duration of the stimulus [116]. The underlying cause of delayed tolerance conferred through hypothermic preconditioning is different and supposedly depends on de novo protein synthesis when stimulus is prolonged [116, 117]. It has been shown in rodent models that elevated temperatures can also bring about a certain degree of protection against ischemic injury. Seven-day-old Wistar rats were partially submerged in a hot water bath, and brain temperatures increased to 41.5–42 °C, measured using a digital thermometer. Twenty-four hours post-conditioning, the newborns subjected to a 2-h hypoxic-ischemic insult showed mitigation of neuronal damage after the stroke [118]. In mouse astrocytes, 6 h of hyperthermia (38–40 °C) rendered protection from ischemia/reperfusion injury (IRI) [119].

Chemical/Pharmacological Preconditioning (PPC) Exogenously delivered agents that diminish disruption of energy metabolism induct chemical preconditioning [12]. Inhibition of oxidative phosphorylation in the CA1 region of rat hippocampal slices led to the reduction in oxygen-free radicals post-hypoxia, in what is considered to be the first instance of chemical preconditioning (1997) [120]. Many chemical PC agents, including inhalational anesthetics like isoflurane, act on adenosine receptors [121]. A short ischemic event leads to the release of adenosine, which is instrumental in the activation of ATP-sensitive K^+ channels in the brain. PPC with adenosine receptor agonist can confer adenosine-mediated neuroprotection, though it was marginally less than IPC [122].

Isoflurane, halothane, and other inhalational anesthetics could promote the antagonism of NMDA and AMPA receptors, leading to a subsequent protective phenotype during PPC [121]. The neuroprotective mechanism of such chemicals and their potential role as preclinical and clinical PC agents are steadily being uncovered [121, 123–126]. Isoflurane preconditioning in the instances of OGD, glutamate-induced cell death, and NMDA or AMPA neurotoxicity, has mitigated the effects of ischemic injury [127]. Isoflurane preconditioning with 2 % isoflurane for half an hour in adult male rats reduced brain infarct sizes after permanent focal ischemia [128]. Resveratrol is an effectual activator of Sirtuin proteins [129]. This naturally occurring phytoalexin has been found to mimic IPC in vitro through seemingly converging cellular pathways [15]. Sevoflurane is considered to possess neuroprotective potential in both focal and global ischemic models [127]. Male Wistar rats subjected to sevoflurane treatment both before focal cerebral ischemia and at the start of reperfusion displayed reduced brain damage as assessed by smaller infarct size and better motor coordination. This in vivo model also demonstrated the preconditioning and early post-conditioning effect of sevoflurane to confer neuroprotection [126]. Halothane could be potentially neuroprotective but has no clinical feasibility due to the likelihood of hepatotoxicity and other systemic side effects [127]. An alternative would be to use combined inhaled anesthetics for extended neuroprotection [130].

3-Nitropropionic acid (3-NPA) has been reported to reduce infarct volume in rats subjected to focal ischemia [131]. LPS preconditioning involves injecting low doses of this potent bacterial endotoxin in rodents before the final insult (hypoxia ischemia, MCAO), which later imparts IT in the brain [75, 132]. Yet, another mode of CPC involves estrogen preconditioning [36]. Nitrous oxide (N_2O) administration in focal models of ischemia has shown little neuroprotective effect, and it seems to be almost ineffective in case of global ischemia. Moreover, when used in conjugation with other inhalational anaesthetics, it may even repress the neuroprotective effects of such compounds [127].

Remote Ischemic Preconditioning (RIPC) In RIPC, a short ischemic spell is carried out in a different limb or organ; thus,

IT is induced remotely in the brain [133, 134]. This mechanism offers an edge over other methods in terms of clinical applications because it drastically reduces the risk involved with cerebral occlusion, through an elementary route for PC delivery. PPC is second to RIPC, because using drugs may not generate the necessary degree of resilience and may produce adverse side effects in patients if left unchecked. Reduced IL-17 expression and increased tolerance was observed in a MCAO rat model after limb ischemic preconditioning possibly through anti-inflammatory mechanisms [135]. In a porcine model of hypothermic circulatory arrest (HCA), RIPC of the hindlimb was found to protect the neurons from IRI [133]. Similar results were observed in murine models as well [136]. RIPC has been shown to prevent decline of postoperative cognitive function in patients undergoing cardiac surgery [137]. But, one of the most significant findings in recent years is the safety and feasibility of inducing RIPC in critically ill patients without any adverse effects or danger, implying that at least one type of PC strategy is a step closer to clinical trials, even useful for other forms of stroke as well [138].

Other Agents of PC Many random players elicit IT in animal models. Consecutive electroacupuncture in mice for 20 min daily for 3 days at acupoints GV20 and GV14 exhibited reduction in infarct volume and improved neurological and motor function after focal ischemia [139]. Other IT inducing stimuli include exercise and cortical spreading depression as demonstrated in rats [140, 141].

Epigenetic Regulation

Regulators of epigenetic modifications and their role in ischemic brain have been studied to assess the prospects of generating a tolerant phenotype through genomic reprogramming [142]. As one of the main epigenetic mechanisms, DNA methylation has been thought to enhance cerebral damage following ischemia or traumatic brain injury [143, 144]. Expression of HDAC9 mediates increased risk of large vessel ischemic stroke [145]. Neuroprotection can be conferred by pharmacological inhibitors of epigenetic modulation [143, 146]. Inhibition of DNA methyltransferases is neuroprotective in focal models of ischemia [142]. Histone modifications also have an impact on the ischemic brain. Preconditioning by inhibition of histone acetyltransferase and histone deacetylase (HDAC) in mice models of focal ischemia has shown mitigation of damage [142]. HDAC inhibitors maintain histone acetylation levels and alter the transcriptional activity. Transcriptional repression is now understood to be a characteristic of an IT brain [147]. Deacetylation of NF-κB and tumor protein p53 by SIRT-1 provides neuroprotection, probably by inhibiting inflammatory and apoptotic pathways [148]. The effects of acetylation of histones on neuroprotection are, however, yet to be confirmed. The abundance of epigenetic modulators like histones and polycomb group (PcG) proteins were found to be increased following MCAO in vivo [147]. This opens up a new perspective of preconditioning-induced IT, involving epigenetic regulation in response to brain injury. PcG action is countered by the activation of trithorax group (TrxG) proteins. Hence, studies on PcG/TrxG system and its role in neuroprotection might prove to be beneficial in the future [149]. Similarly, microRNA (miRNA) regulates the expression of epigenetic mediators like PcG [150]. miRNAs are the best studied examples of ncRNAs, which constitute another player in the epigenomic pathway [142]. In IPC models, there is a tendency for upregulation of miRNAs, but their expression is selectively regulated [151]. SUMO-I conjugation levels were hiked in cells preconditioned for tolerance against neuronal damage through OGD [184]. SUMOylation regulates the recruitment of HADC to promoters and augments the deacetylase activity of SIRT-1 during post-translational changes. In another study, it was found that preconditioning induced by cortical spreading depression (CSD) can also epigenetically regulate retrotransposable elements through histone modifications [152].

Recent Developments in CPC

Needless to say, CPC like any other field of scientific research is now in an era of advanced technology and innovative possibilities. This is made clear from the large number of publications that come out every year illustrating newer and improved paradigms for CPC in stroke research. We describe a few of the noteworthy developments in CPC in the past few years. Repetitive hypoxic preconditioning (RHP) is an upgraded version of HPC which was found to produce long-term protection in mice through repeated exposure to hypoxia followed by focal ischemia; the benefits were seen to last for weeks after the final insult [153]. Similarly, repetitive IPC was found to be beneficial in adult rats exposed to both IPC and/or MCAO [154]. Transplanting hypoxia-preconditioned stem cells into rats helped enhance various survival and regenerative mechanisms [155]. Metformin, a well-known anti-diabetic drug, has been used to induce PC in rats with promising results, further expanding the scope of PPC [156]. The effects of anti-inflammatory drugs like indomethacin and PC methods like ionizing radiation (IR) have also been newly investigated in the context of CPC [157, 158]. Limb preconditioning was seen to be well tolerated in patients with unilateral middle cerebral artery stenosis as well as healthy volunteers [159]. Particularly encouraging is a recent commentary that endorses RIPC as holding the key for stroke treatment. However, it warns of the shortage of sufficient preclinical trials and other complications that might hinder progress and must be dealt with before this vision becomes a reality [160].

Clinical Concerns and Future Prospects

From its largely flourishing trials in the experimental scenario, CPC has the potential to transform stroke research in humans, provided all the issues and impracticalities are effectively addressed. In spite of its apparent popularity and the extensive research that is currently underway, CPC is yet to be launched on an effective scale for human clinical trials.

Recent studies have provided evidence that some mechanism analogous to PC might already be functioning in the human brain. Transient ischemic attacks or "warning strokes," are ephemeral episodes of focal ischemic attacks. TIAs are characterized by a short and non-lethal blockage of blood supply to the brain without infarction which, although share the same symptoms as stroke, do not cause permanent damage. TIAs are clinically relevant because they are thought of as preindicators of a more severe ischemic event [161]. TIAs can confer some degree of IT, as understood from the few clinical studies in stroke patients. One of the earliest works in this field was carried out in a German case-control study in stroke patients, where an association was found between previous incidence of TIA and reduced severity in subsequent stroke [162]. Around the same period, another study in stroke patients with and without prior transient attacks demonstrated that ipsilateral TIAs of 10–20 min duration before cerebral ischemia produced a positive outcome [163]. In another retrospective study involving 65 patients, smaller lesions and reduced infarct volumes within 12 h after onset of stroke were linked to those patients with prodromal TIAs [164]. There are several more reports of IT like status after TIAs in different case control scenarios, which might be probed further to understand their preconditioning effects [165–167]. A contradictory study based in Northern California using a cohort of more than 1000 stroke patients, however, showed no association between TIA and disability from stroke. They also reported higher disability (instead of delayed preconditioning) in some patients in whose cases stroke occurred 1–7 days after TIA, underlying some of the limitations of medication-induced PC after TIA [168]. Heterogeneity among patients and diversity among the causes of TIAs and stroke constitute the limitations in understanding TIA-induced IPC. Exact time frames from the onset of TIA to the final insult, medications that can interfere with or trigger TIAs, extending survival periods for more than 1 week (as is usually the case), and the ever present threat of sudden ischemia are some of the other problems that are to be tackled before full-fledged clinical adaptation. Identification of novel biomarkers of TIA and PC can help in detecting IT in stroke patients. Heat shock proteins (HSPs) are a class of stress proteins already implicated as mediators of CNS injury, brain ischemia, and hypoxia, characterized by their rapid response in tissues exposed to near lethal stressors [169]. Increased expression of Hsp70 has been observed in a rat model of focal cerebral ischemia [28]. Similarly, upregulation of Hsp70, Hsp27, and Hsp90 was noted in adult rats preconditioned with a 10-min transient middle cerebral artery occlusion [31]. Other candidates for preconditioning research include TNF-α/IL-6, VEGF, and EPO [92, 170–172].

It is not easy nor always realistic to choose one particular stimuli from the many as the best agent of preconditioning. Every PC agent has its characteristic set of advantages as well as limitations and must be evaluated based on scenarios where they work best. From a clinical point of view, RIPC is sometimes seen as a more promising PC strategy, because of improved safety (being generally non-invasive) and better tolerance of the organs to IRI [134, 173]. Many even believe that this PC mechanism might be the future choice of clinical ischemic treatment [160]. Recently, bilateral arm ischemic preconditioning (BAIPC) was found to reduce the occurrence of stroke in patients with symptomatic atherosclerotic intracranial arterial stenosis (IAS) [174]. Limb preconditioning was found to be safe in patients with subarachnoid hemorrhage (SAH), and in a phase I clinical trial of RIPC-SAH, patients reported protection from ischemia for up to 2 days following RIPC [138, 175]. It has already proved beneficial in the heart and, hopefully with more research, can be mimicked in the brain as well [176, 177]. Similarly, inhalational anesthetics are worth further scrutiny, being already in use in surgical settings. Extensive research is required particularly on the use of volatile anesthetics like sevoflurane, isoflurane, etc., keeping in mind the ongoing debate among many experts on whether these chemicals are more neurotoxic than neuroprotective [178].

Despite the promising strategies, CIPC is not yet universally popular and experts must address the Janus-faced position that it holds. Since preconditioning involves application of non-lethal but noxious stressors, one can question whether it is wise to create one pathological problem to solve another [179]. It is important to analyze whether complete elucidation of the internal repair mechanisms and molecular pathways of PC can guarantee therapeutic benefits in the immediate future [180]. Research in the area of ischemic neuroprotection is challenging and not always successful, and some maintain that preclinical trials need to be perfected first before actual adaptation into the clinical context [181]. PC strategies have worked well in murine models and tissue cultures, but the question remains of their safety index in humans. The next big challenge is to choose the most effective method from the multitude of PC agents, in terms of its protective margin and correct dosage for use in clinical settings. More detailed examination of different facets of CPC is required, like possible side effects of pharmacological agents used in PPC and long-term tissue damage during limb IPC [182]. Mitochondrial preconditioning is another promising strategy worth further exploration [183]. CPC can be an answer to many cerebral maladies, not just stroke, once all the issues are resolved.

Conclusion

Cerebral IPC constitutes the ischemia-tolerant phenotype of the brain achieved by episodes of brief, sublethal ischemia, administered before the onset of longer and more severe events of stroke. The end result is the induction of IT, a state of transient neuroprotection in the brain. Outcomes of various preconditioning experiments have provided us with multiple possibilities through which neuroprotection can be conferred prior to a possibly lethal episode of ischemia. This knowledge base is invaluable not only in the treatment of stroke but also for other cerebrovascular diseases and traumatic brain injuries. Though there are many methodologies to attain IT, there is a need for combinational studies, which compares the impact of various preconditioning stimuli and any adverse or unwanted side effects they may have on a healthy brain. By reviewing current and previous work in the field, we can easily deduce that the outcomes of different PC mediators vary significantly based on the severity and duration of the stimuli introduced, dosage of various chemical modulators, and the locales of an injury. Differences in temporal separation between subsequent stimuli have also given multiple results in many rodent and mammalian models. CPC strategies should be scientifically anatomized in terms of the type and nature of preconditioning regime. With each PC agent, various factors, such as the endogenous mechanisms of repair and signaling pathways for neuronal survival, the duration of tolerance and exact window of protection after the final insult, the safety margin and possible side effects in humans, should be carefully analyzed. Of all PC techniques, RIPC and PPC require special mention, as they seem to hold more promise for a clinical setting. More studies with broader span and different combinations of stimuli should be conducted over the next few years to establish a paradigm to use CPC as a prime therapeutic approach for combating stroke and related neurological disorders.

Disclosure All authors have approved of the final article.

Funding Source No specific funding was received for this study.

Conflict of Interest The author(s) confirm that this article content has no conflicts of interests.

References

1. Mackay J, Mensah G (2004) The Atlas of Heart Disease and Stroke. Organization, World Health
2. Mendis S, Puska P, Norrving B (2011) Global Atlas on cardiovascular disease prevention and control. Organization, World Health
3. Astrup J, Siesjö BK, Symon L (1981) Thresholds in cerebral ischemia—the ischemic penumbra. Stroke 12:723–5. doi:10.1161/01.STR.12.6.723
4. Bramlett HM, Dietrich WD (2004) Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. J Cereb Blood Flow Metab 24:133–150. doi:10.1097/01.WCB.0000111614.19196.04
5. Iadecola C, Anrather J (2011) Stroke research at a crossroad: asking the brain for directions. Nat Neurosci 14:1363–1368. doi:10.1038/nn.2953
6. Albers GW, Bates VE, Clark WM, Bell R, Verro P, Hamilton SA (2000) Intravenous tissue-type plasminogen activator for treatment of acute stroke: the Standard Treatment with Alteplase to Reverse Stroke (STARS) study. JAMA 283(9):1145–1150
7. Dirnagl U, Becker K, Meisel A (2009) Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. Lancet Neurol 8:398–412. doi:10.1016/S1474-4422(09)70054-7
8. Obrenovitch TP (2008) Molecular physiology of preconditioning-induced brain tolerance to ischemia. Physiol Rev 88:211–247. doi:10.1152/physrev.00039.2006
9. Bickler PE, Donohoe PH (2002) Adaptive responses of vertebrate neurons to hypoxia. J Exp Biol 205:3579–3586
10. Shpargel KB et al (2008) Preconditioning paradigms and pathways in the brain. Cleve Clin J Med 75(Suppl 2):S77–82
11. Durukan A, Tatlisumak T (2010) Preconditioning-induced ischemic tolerance: a window into endogenous gearing for cerebroprotection. Exp Transl Stroke Med 2:2. doi:10.1186/2040-7378-2-2
12. Gidday JM (2006) Cerebral preconditioning and ischaemic tolerance. Nat Rev Neurosci 7:437–448. doi:10.1038/nrn1927
13. Della-Morte D et al (2013) Age-related reduction of cerebral ischemic preconditioning: myth or reality? Clin Interv Aging 8:1055–1061. doi:10.2147/CIA.S47462
14. Wang L, Traystman RJ, Murphy SJ (2008) Inhalational anesthetics as preconditioning agents in ischemic brain. Curr Opin Pharmacol 8:104–110. doi:10.1016/j.coph.2007.09.005
15. Raval AP, Dave KR, Pérez-Pinzón MA (2006) Resveratrol mimics ischemic preconditioning in the brain. J Cereb Blood Flow Metab 26:1141–1147. doi:10.1038/sj.jcbfm.9600262
16. Kapinya KJ et al (2002) Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. Stroke 33:1889–1898. doi:10.1161/01.STR.0000020092.41820.58
17. Dezfulian C (2013) Clinical Cerebral Preconditioning and Postconditioning. In: Gidday JM, Perez-Pinzon MA, Zhang JH (eds) Innate tolerance in the CNS. Springer, New York, pp 553–566
18. Dahl NA, Balfour WM (1964) Prolonged anoxic survival due to anoxia pre-exposure: brain ATP, lactate, and pyruvate. Am J Physiol 207:452–456
19. Vannucci RC, Duffy TE (1976) Carbohydrate metabolism in fetal and neonatal rat brain during anoxia and recovery. Am J Physiol 230:1269–1275
20. Murry CE, Jennings RB, Reimer KA (1986) Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74:1124–1136. doi:10.1161/01.CIR.74.5.1124
21. Cohen MV, Yang XM, Downey JM (1994) Conscious rabbits become tolerant to multiple episodes of ischemic preconditioning. Circ Res 74:998–1004. doi:10.1161/01.RES.74.5.998
22. Schurr A et al (1986) Adaptation of adult brain tissue to anoxia and hypoxia in vitro. Brain Res 374(2):244–248. doi:10.1016/0006-8993(86)90418-X
23. Chopp M et al (1989) Transient hyperthermia protects against subsequent forebrain ischemic cell damage in the rat. Neurology 39:1396–1396. doi:10.1212/WNL.39.10.1396
24. Kitagawa K et al (1990) 'Ischemic tolerance' phenomenon found in the brain. Brain Res 528:21–24. doi:10.1016/0006-8993(90)90189-I

25. Gidday JM, Fitzgibbons JC, Shah AR, Park TS (1994) Neuroprotection from ischemic brain injury by hypoxic preconditioning in the neonatal rat. Neurosci Lett 168:221–224. doi:10.1016/0304-3940(94)90455-3
26. Adolph EF (1971) Physiological adaptation to hypoxia in newborn rats. Am J Physiol 221:123–127
27. Schurr A et al (1988) Lactic acidosis and recovery of neuronal function following cerebral hypoxia in vitro. Brain Res 438:311–314. doi:10.1016/0006-8993(88)91354-6
28. Chen J, Graham SH, Zhu RL, Simon RP (1996) Stress proteins and tolerance to focal cerebral ischemia. J Cereb Blood Flow Metab 16:566–77. doi:10.1097/00004647-199607000-00006
29. Glazier SS, O'Rourke DM, Graham DI, Welsh FA (1994) Induction of ischemic tolerance following brief focal ischemia in rat brain. J Cereb Blood Flow Metab 14:545–553. doi:10.1038/jcbfm.1994.68
30. Stenzel-Poore MP et al (2003) Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. Lancet 362:1028–1037. doi:10.1016/S0140-6736(03)14412-1
31. Dhodda VK, Sailor KA, Bowen KK, Vemuganti R (2004) Putative endogenous mediators of preconditioning-induced ischemic tolerance in rat brain identified by genomic and proteomic analysis. J Neurochem 89:73–89. doi:10.1111/j.1471-4159.2004.02316.x
32. Benardete EA, Bergold PJ (2009) Genomic analysis of ischemic preconditioning in adult rat hippocampal slice cultures. Brain Res 1292:107–122. doi:10.1016/j.brainres.2009.07.027
33. Xu H, Lu A, Sharp FR (2011) Regional genome transcriptional response of adult mouse brain to hypoxia. BMC Genomics 12:499. doi:10.1186/1471-2164-12-499
34. Kim E et al (2007) Ischemic preconditioning via epsilon PKC activation requires cyclooxygenase-2 activation in vitro. Neuroscience 145:931–941. doi:10.1016/j.neuroscience.2006.12.063
35. Xu GP et al (2002) Improvement in neuronal survival after ischemic preconditioning in hippocampal slice cultures. Brain Res 952(2):153–158. doi:10.1016/S0006-8993(02)02988-8
36. Raval AP, Bramlett H, Perez-Pinzon MA (2006) Estrogen preconditioning protects the hippocampal CA1 against ischemia. Neuroscience 141:1721–1730. doi:10.1016/j.neuroscience.2006.05.016
37. Egashira Y et al (2012) The conditioned medium of murine and human adipose-derived stem cells exerts neuroprotective effects against experimental stroke model. Brain Res 1461:87–95. doi:10.1016/j.brainres.2012.04.033
38. Ara J et al (2011) Hypoxic-preconditioning induces neuroprotection against hypoxia–ischemia in newborn piglet brain. Neurobiol Dis 43:473–485. doi:10.1016/j.nbd.2011.04.021
39. Cantagrel S et al (2003) Hypoxic preconditioning reduces apoptosis in a rat model of immature brain hypoxia-ischaemia. Neurosci Lett 347:106–110. doi:10.1016/S0304-3940(03)00525-1
40. Azad P, Haddad GG (2013) Genetic animal models of preconditioning. Transl Stroke Res 4:51–55. doi:10.1007/s12975-012-0218-1
41. Zhang N et al (2007) Neuron-specific phosphorylation of c-Jun N-terminal kinase increased in the brain of hypoxic preconditioned mice. Neurosci Lett 423:219–224. doi:10.1016/j.neulet.2007.07.028
42. Traystman RJ (2003) Animal models of focal and global cerebral ischemia. ILAR J 44(2):85–95
43. Liu C, Chen S, Kamme F, Hu BR (2005) Ischemic preconditioning prevents protein aggregation after transient cerebral ischemia. Neuroscience 134:69–80. doi:10.1016/j.neuroscience.2005.03.036
44. Wu C et al (2001) A forebrain ischemic preconditioning model established in C57Black/Crj6 mice. J Neurosci Methods 107:101–106. doi:10.1016/S0165-0270(01)00356-9
45. Zhang QG et al (2006) Ischemic preconditioning negatively regulates plenty of SH3s–mixed lineage kinase 3–Rac1 complex and c-Jun N-terminal kinase 3 signaling via activation of Akt. Neuroscience 143:431–444. doi:10.1016/j.neuroscience.2006.07.049
46. Geddes JW et al (1996) Permanent focal and transient global cerebral ischemia increase glial and neuronal expression of heme oxygenase-1, but not heme oxygenase-2, protein in rat brain. Neurosci Lett 210:205–208. doi:10.1016/0304-3940(96)12703-8
47. Masada T et al (2001) Attenuation of ischemic brain edema and cerebrovascular injury after ischemic preconditioning in the rat. J Cereb Blood Flow Metab 21:22–33. doi:10.1097/00004647-200101000-00004
48. Miyashita K et al (1994) Induction of ischaemic tolerance in gerbil hippocampus by pretreatment with focal ischaemia. Neuroreport 6:46–48
49. Mark LP et al (2001) Pictorial review of glutamate excitotoxicity: fundamental concepts for neuroimaging. AJNR Am J Neuroradiol 22:1813–1824
50. Hazell AS (2007) Excitotoxic mechanisms in stroke: an update of concepts and treatment strategies. Neurochem Int 50:941–953. doi:10.1016/j.neuint.2007.04.026
51. Paschen W (1996) Glutamate excitotoxicity in transient global cerebral ischemia. Acta Neurobiol Exp (Wars) 56:313–322
52. Watters O, O'Connor JJ (2011) A role for tumor necrosis factor-α in ischemia and ischemic preconditioning. J Neuroinflammation 8:87. doi:10.1186/1742-2094-8-87
53. Navon H, Bromberg Y, Sperling O, Shani E (2012) Neuroprotection by NMDA preconditioning against glutamate cytotoxicity is mediated through activation of ERK 1/2, inactivation of JNK, and by prevention of glutamate-induced CREB inactivation. J Mol Neurosci 46:100–108. doi:10.1007/s12031-011-9532-4
54. Soriano FX et al (2006) Preconditioning doses of NMDA promote neuroprotection by enhancing neuronal excitability. J Neurosci 26:4509–4518. doi:10.1523/JNEUROSCI.0455-06.2006
55. Miao B et al (2005) Neuroprotective effects of preconditioning ischemia on ischemic brain injury through down-regulating activation of JNK1/2 via N-methyl-D-aspartate receptor-mediated Akt1 activation. J Biol Chem 280:21693–21699. doi:10.1074/jbc.M500003200
56. Lin CH, Chen PS, Gean PW (2008) Glutamate preconditioning prevents neuronal death induced by combined oxygen–glucose deprivation in cultured cortical neurons. Eur J Pharmacol 589(1-3):85–93. doi:10.1016/j.ejphar.2008.05.047
57. Gong SJ et al (2012) Intermittent hypobaric hypoxia preconditioning induced brain ischemic tolerance by up-regulating glial glutamate transporter-1 in rats. Neurochem Res 37(3):527–537
58. Scorziello A et al (2007) NO-induced neuroprotection in ischemic preconditioning stimulates mitochondrial Mn-SOD activity and expression via RAS/ERK1/2 pathway. J Neurochem 103(4):1472–1480
59. Kawahara K et al (2004) Nitric oxide produced during ischemia is toxic but crucial to preconditioning-induced ischemic tolerance of neurons in culture. Neurochem Res 29:797–804. doi:10.1023/B:NERE.0000018853.30131.4d
60. Gidday JM (1999) Nitric oxide mediates cerebral ischemic tolerance in a neonatal rat model of hypoxic preconditioning. J Cereb Blood Flow Metab 19(3):331–340. doi:10.1097/00004647-199903000-00011
61. Centeno JM et al (1999) Nitric oxide is involved in anoxic preconditioning neuroprotection in rat hippocampal slices. Brain Res 836(1-2):62–69. doi:10.1016/S0006-8993(99)01610-8

62. Atochin DN et al (2003) Rapid cerebral ischemic preconditioning in mice deficient in endothelial and neuronal nitric oxide synthases. Stroke 34(5):1299–1303. doi:10.1161/01.STR.0000066870.70976.57
63. Zhao P, Zuo Z (2004) Isoflurane preconditioning induces neuroprotection that is inducible nitric oxide synthase–dependent in neonatal rats. Anesthesiology 101(3):695–703
64. Cho S (2005) Obligatory role of inducible nitric oxide synthase in ischemic preconditioning. J Cereb Blood Flow Metab 25(4):493–501. doi:10.1038/sj.jcbfm.9600058
65. Vellimana et al (2011) Endothelial nitric oxide synthase mediates endogenous protection against subarachnoid hemorrhage-induced cerebral vasospasm. Stroke 42(3):776–782. doi:10.1161/STROKEAHA.110.607200
66. Wang Q, Tang XN, Yenari MA (2007) The inflammatory response in stroke. J Neuroimmunol 184:53–68. doi:10.1016/j.jneuroim.2006.11.014
67. Jin R, Yang G, Li G (2010) Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. J Leukoc Biol 87:779–789. doi:10.1189/jlb.1109766
68. Stevens SL, Stenzel-Poore MP (2006) Toll-like receptors and tolerance to ischaemic injury in the brain. Biochem Soc Trans 34:1352–1355. doi:10.1042/BST0341352
69. Stevens SL et al (2008) Toll-like receptor 9: a new target of ischemic preconditioning in the brain. J Cereb Blood Flow Metab 28:1040–1047. doi:10.1038/sj.jcbfm.9600606
70. Karikó K, Weissman D, Welsh FA (2004) Inhibition of toll-like receptor and cytokine signaling—a unifying theme in ischemic tolerance. J Cereb Blood Flow Metab 24:1288–1304. doi:10.1097/01.WCB.0000145666.68576.71
71. Pradillo JM et al (2009) Toll-like receptor 4 is involved in neuroprotection afforded by ischemic preconditioning. J Neurochem 109:287–294. doi:10.1111/j.1471-4159.2009.05972.x
72. Marsh BJ, Williams-Karnesky RL, Stenzel-Poore MP (2009) Toll-like receptor signaling in endogenous neuroprotection and stroke. Neuroscience 158:1007–1020. doi:10.1016/j.neuroscience.2008.07.067
73. Chen Z et al (2012) Lipopolysaccharide-induced microglial activation and neuroprotection against experimental brain injury is independent of hematogenous TLR4. J Neurosci 32:11706–11715. doi:10.1523/JNEUROSCI.0730-12.2012
74. Rosenzweig HL et al (2007) Endotoxin preconditioning protects against the cytotoxic effects of TNFα after stroke: a novel role for TNFα in LPS-ischemic tolerance. J Cereb Blood Flow Metab 27:1663–1674. doi:10.1038/sj.jcbfm.9600464
75. Vartanian KB et al (2011) LPS preconditioning redirects TLR signaling following stroke: TRIF-IRF3 plays a seminal role in mediating tolerance to ischemic injury. J Neuroinflammation 8:140. doi:10.1186/1742-2094-8-140
76. Sakata H et al (2012) Interleukin 6-preconditioned neural stem cells reduce ischaemic injury in stroke mice. Brain 135:3298–3310. doi:10.1093/brain/aws259
77. Ohtsuki T, Ruetzler CA, Tasaki K, Hallenbeck JM (1996) Interleukin-1 mediates induction of tolerance to global ischemia in gerbil hippocampal CA1 neurons. J Cereb Blood Flow Metab 16:1137–1142. doi:10.1097/00004647-199611000-00007
78. Spera PA, Ellison JA, Feuerstein GZ, Barone FC (1998) IL-10 reduces rat brain injury following focal stroke. Neurosci Lett 251:189–192
79. Nogawa S, Zhang F, Ross ME, Iadecola C (1997) Cyclo-oxygenase-2 gene expression in neurons contributes to ischemic brain damage. J Neurosci 17:2746–2755
80. Cheng O et al (2011) Cyclooxygenase-2 mediates hyperbaric oxygen preconditioning in the rat model of transient global cerebral ischemia. Stroke 42:484–490. doi:10.1161/STROKEAHA.110.604421
81. Colangelo V et al (2004) Downregulation of COX-2 and JNK expression after induction of ischemic tolerance in the gerbil brain. Brain Res 1016:195–200. doi:10.1016/j.brainres.2004.05.017
82. Iadecola C et al (2001) Increased susceptibility to ischemic brain injury in cyclooxygenase-1–deficient mice. J Cereb Blood Flow Metab 21:1436–1441. doi:10.1097/00004647-200112000-00008
83. Yung LM et al (2012) Sphingosine kinase 2 mediates cerebral preconditioning and protects the mouse brain against ischemic injury. Stroke 43:199–204. doi:10.1161/STROKEAHA.111.626911
84. Wacker BK, Perfater JL, Gidday JM (2012) Hypoxic preconditioning induces stroke tolerance in mice via a cascading HIF, sphingosine kinase, and CCL2 signaling pathway. J Neurochem 123:954–962. doi:10.1111/jnc.12047
85. Yan W et al (2013) SirT1 mediates hyperbaric oxygen preconditioning-induced ischemic tolerance in rat brain. J Cereb Blood Flow Metab 33:396–406. doi:10.1038/jcbfm.2012.179
86. Thompson JW et al (2013) Epsilon PKC increases brain mitochondrial SIRT1 protein levels via heat shock protein 90 following ischemic preconditioning in rats. PLoS One 8:e75753. doi:10.1371/journal.pone.0075753
87. Zhou AM et al (2004) A short cerebral ischemic preconditioning upregulates adenosine receptors in the hippocampal CA1 region of rats. Neurosci Res 48:397–404. doi:10.1016/j.neures.2003.12.010
88. Shen HY et al (2011) Adenosine kinase determines the degree of brain injury after ischemic stroke in mice. J Cereb Blood Flow Metab 31:1648–1659. doi:10.1038/jcbfm.2011.30
89. Pignataro G et al (2012) NCX1 and NCX3: two new effectors of delayed preconditioning in brain ischemia. Neurobiol Dis 45:616–623. doi:10.1016/j.nbd.2011.10.007
90. Valsecchi V et al (2011) NCX1 is a novel target gene for hypoxia-inducible factor-1 in ischemic brain preconditioning. Stroke 42:754–763. doi:10.1161/STROKEAHA.110.597583
91. Venna VR, Li J, Benashski SE, Tarabishy S, McCullough LD (2012) Preconditioning induces sustained neuroprotection by downregulation of AMPK. Neuroscience 201:280–287. doi:10.1016/j.neuroscience.2011.11.014
92. Chavez JC, Baranova O, Lin J, Pichiule P (2006) The transcriptional activator hypoxia inducible factor 2 (HIF-2/EPAS-1) regulates the oxygen-dependent expression of erythropoietin in cortical astrocytes. J Neurosci 26:9471–9481. doi:10.1523/JNEUROSCI.2838-06.2006
93. Terasaki Y et al (2010) Activation of NR2A receptors induces ischemic tolerance through CREB signaling. J Cereb Blood Flow Metab 30:1441–1449. doi:10.1038/jcbfm.2010.18
94. Bigdeli MR, Khoshbaten A (2008) *In vivo* preconditioning with normobaric hyperoxia induces ischemic tolerance partly by triggering tumor necrosis factor-α converting enzyme/tumor necrosis factor-α/nuclear factor-κB. Neuroscience 153:671–678. doi:10.1016/j.neuroscience.2008.02.064
95. Sharp FR et al (2004) Hypoxic preconditioning protects against ischemic brain injury. NeuroRx 1:26–35. doi:10.1602/neurorx.1.1.26
96. Ruscher K et al (2002) Erythropoietin is a paracrine mediator of ischemic tolerance in the brain: evidence from an in vitro model. J Neurosci 22:10291–10301
97. Bernaudin M et al (2002) Normobaric hypoxia induces tolerance to focal permanent cerebral ischemia in association with an increased expression of hypoxia-inducible factor-1 and its target genes, erythropoietin and VEGF, in the adult mouse brain. J Cereb Blood Flow Metab 22:393–403. doi:10.1097/00004647-200204000-00003
98. Jones NM, Bergeron M (2001) Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain. J Cereb Blood

Flow Metab 21:1105–1114. doi:10.1097/00004647-200109000-00008
99. Vangeison G, Rempe DA (2009) The Janus-faced effects of hypoxia on astrocyte function. Neuroscientist 15:579–588. doi:10.1177/1073858409332405
100. Zhan L et al (2010) Activation of Akt/FoxO signaling pathway contributes to induction of neuroprotection against transient global cerebral ischemia by hypoxic pre-conditioning in adult rats. J Neurochem 114:897–908. doi:10.1111/j.1471-4159.2010.06816.x
101. Wang LW, Tu YF, Huang CC, Ho CJ (2012) JNK signaling is the shared pathway linking neuroinflammation, blood–brain barrier disruption, and oligodendroglial apoptosis in the white matter injury of the immature brain. J Neuroinflammation 9:175. doi:10.1186/1742-2094-9-175
102. Kapinya K, Penzel R, Sommer C, Kiessling M (2000) Temporary changes of the AP-1 transcription factor binding activity in the gerbil hippocampus after transient global ischemia, and ischemic tolerance induction. Brain Res 872:282–293. doi:10.1016/S0006-8993(00)02503-8
103. Huang CY et al (2001) Overexpression of copper-zinc superoxide dismutase attenuates acute activation of activator protein-1 after transient focal cerebral ischemia in mice. Stroke 32:741–747. doi:10.1161/01.STR.32.3.741
104. Guan J (2013) Bone morphogenetic protein-7 (BMP-7) mediates ischemic preconditioning-induced ischemic tolerance via attenuating apoptosis in rat brain. Biochem Biophys Res Commun 441(3):560–566. doi:10.1016/j.bbrc.2013.10.121
105. Guan J et al (2014) Bone morphogenic protein-7 contributes to cerebral ischemic preconditioning induced-ischemic tolerance by activating p38 mitogen-activated protein kinase signaling pathway. Inflammation 37(4):1289–1296. doi:10.1007/s10753-014-9856-7
106. Lusardi TA et al (2010) Ischemic preconditioning regulates expression of microRNAs and a predicted target, MeCP2, in mouse cortex. J Cereb Blood Flow Metab 30:744–756. doi:10.1038/jcbfm.2009.253
107. Vannucci RC, Towfighi J, Vannucci SJ (1998) Hypoxic preconditioning and hypoxic-ischemic brain damage in the immature rat: pathologic and metabolic correlates. J Neurochem 71:1215–1220. doi:10.1046/j.1471-4159.1998.71031215.x
108. Bickler PE, Buck LT (1998) Adaptations of vertebrate neurons to hypoxia and anoxia: maintaining critical Ca2+ concentrations. J Exp Biol 201(8):1141–1152
109. Li S et al (2013) Hypoxic preconditioning stimulates angiogenesis in ischemic penumbra after acute cerebral infarction. Neural Regen Res 8(31):2895–2903
110. Grabb MC, Choi DW (1999) Ischemic tolerance in murine cortical cell culture: critical role for NMDA receptors. J Neurosci 19(5):1657–1662
111. Bigdeli MR (2011) Neuroprotection caused by hyperoxia preconditioning in animal stroke models. ScientificWorldJournal 11:403–421. doi:10.1100/tsw.2011.23
112. Ostrowski RP et al (2008) The hyperbaric oxygen preconditioning-induced brain protection is mediated by a reduction of early apoptosis after transient global cerebral ischemia. Neurobiol Dis 29(1):1–13. doi:10.1016/j.nbd.2007.07.020
113. Yan W (2013) SirT1 mediates hyperbaric oxygen preconditioning-induced ischemic tolerance in rat brain. J Cereb Blood Flow Metab 33(3):396–406. doi:10.1038/jcbfm.2012.179
114. Hemmen TM et al (2010) Intravenous thrombolysis plus hypothermia for acute treatment of ischemic stroke (ICTuS-L) final results. Stroke 41(10):2265–2270. doi:10.1161/STROKEAHA.110.592295
115. Yenari MA, Hemmen TM (2010) Therapeutic hypothermia for brain ischemia: where have we come and where do we go? Stroke 41(10 Suppl):S72–S74. doi:10.1161/STROKEAHA.110.595371
116. Yunoki M et al (2003) Hypothermic preconditioning induces rapid tolerance to focal ischemic injury in the rat. Exp Neurol 181(2):291–300. doi:10.1016/S0014-4886(03)00056-6
117. Nishio S et al (2000) Ischemic tolerance in the rat neocortex following hypothermic preconditioning. J Neurosurg 93(5):845–851. doi:10.3171/jns.2000.93.5.0845
118. Ikeda T, Xia XY, Xia YX, Ikenoue T (1999) Hyperthermic preconditioning prevents blood–brain barrier disruption produced by hypoxia–ischemia in newborn rat. Brain Res Dev Brain Res 117(1):53–58. doi:10.1016/S0165-3806(99)00097-8
119. Du F et al (2010) Hyperthermic preconditioning protects astrocytes from ischemia/reperfusion injury by up-regulation of HIF-1 alpha expression and binding activity. Biochim Biophys Acta 1802(11):1048–1053. doi:10.1016/j.bbadis.2010.06.013
120. Riepe MW et al (1997) Increased hypoxic tolerance by chemical inhibition of oxidative phosphorylation: "chemical preconditioning". J Cereb Blood Flow Metab 17(3):257–264. doi:10.1097/00004647-199703000-00002
121. Kitano H, Kirsch JR, Hurn PD, Murphy SJ (2007) Inhalational anesthetics as neuroprotectants or chemical preconditioning agents in ischemic brain. J Cereb Blood Flow Metab 27(6):1108–1128. doi:10.1038/sj.jcbfm.9600410
122. Heurteaux C, Lauritzen I, Widmann C, Lazdunski M (1995) Essential role of adenosine, adenosine Al receptors, and ATP-sensitive K+ channels in cerebral ischemic preconditioning. Pharmacology 92(10):4666–4670. doi:10.1073/pnas.92.10.4666
123. Wang H et al (2011) Sevoflurane preconditioning confers neuroprotection via anti-inflammatory effects. Front Biosci (Elite Ed) 3:604–615
124. Li L, Zuo Z (2009) Isoflurane preconditioning improves short-term and long-term neurological outcome after focal brain ischemia in adult rats. Neuroscience 164(2):497–506. doi:10.1016/j.neuroscience.2009.08.011
125. Statler KD et al (2006) Isoflurane exerts neuroprotective actions at or near the time of severe traumatic brain injury. Brain Res 1076(1):216–224. doi:10.1016/j.brainres.2005.12.106
126. Adamczyk S et al (2010) Sevoflurane pre- and post-conditioning protect the brain via the mitochondrial KATP channel. Br J Anaesth 104(2):191–200. doi:10.1093/bja/aep365
127. Kitano H, Kirsch JR, Hurn PD, Murphy SJ (2007) Inhalational anesthetics as neuroprotectants or chemical preconditioning agents in ischemic brain. J Cereb Blood Flow Metab 27:1108–1128. doi:10.1038/sj.jcbfm.9600410
128. Zheng S, Zuo Z (2004) Isoflurane preconditioning induces neuroprotection against ischemia via activation of P38 mitogen-activated protein kinases. Mol Pharmacol 65(5):1172–1180. doi:10.1124/mol.65.5.1172
129. Morris KC et al (2011) Pathways for ischemic cytoprotection: role of sirtuins in caloric restriction, resveratrol, and ischemic preconditioning. J Cereb Blood Flow Metab 31(4):1003–1019. doi:10.1038/jcbfm.2010.229
130. Nunes RR et al (2013) Anesthetics, cerebral protection and preconditioning. Braz J Anesthesiol 63(1):119–138. doi:10.1016/S0034-7094(13)70204-6
131. Zhu H, Sun S, Li H, Xu Y (2006) Cerebral ischemic tolerance induced by 3-nitropropionic acid is associated with increased expression of erythropoietin in rats. J Huazhong Univ Sci Technolog Med Sci 26(4):440–443. doi:10.1007/s11596-006-0416-8
132. Lin HY, Wu CL, Huang CC (2010) The Akt-endothelial nitric oxide synthase pathway in lipopolysaccharide preconditioning-induced hypoxic-ischemic tolerance in the neonatal rat brain. Stroke 41(7):1543–1551. doi:10.1161/STROKEAHA.109.574004

133. Jensen HA et al (2011) Remote ischemic preconditioning protects the brain against injury after hypothermic circulatory arrest. Circulation 123(7):714–721. doi:10.1161/CIRCULATIONAHA.110.986497
134. Moskowitz MA, Waeber C (2011) Remote ischemic preconditioning: making the brain more tolerant, safely and inexpensively. Circulation 123(7):709–711. doi:10.1161/CIRCULATIONAHA.110.009688
135. Wang W et al (2014) Limb ischemic preconditioning attenuates cerebral ischemic injury in rat model. Perfusion 29(3):210–218
136. Ren C, Gao X, Steinberg GK, Zhao H (2008) Limb remote-preconditioning protects against focal ischemia in rats and contradicts the dogma of therapeutic time windows for preconditioning. Neuroscience 151(4):1099–1103. doi:10.1016/j.neuroscience.2007.11.056
137. Hudetz JA et al (2014) Remote ischemic preconditioning prevents deterioration of short-term postoperative cognitive function after cardiac surgery using cardiopulmonary bypass: results of a Pilot Investigation. J Cardiothorac Vasc Anesth 29(2):382–388
138. Koch S, Katsnelson M, Dong C, Perez-Pinzon M (2011) Remote ischemic limb preconditioning after subarachnoid hemorrhage a phase Ib study of safety and feasibility. Stroke 42(5):1387–1391
139. Kim JH et al (2013) Electroacupuncture preconditioning reduces cerebral ischemic injury via BDNF and SDF-1α in mice. BMC Complement Altern Med 13:22. doi:10.1186/1472-6882-13-22
140. Douen AG (2000) Preconditioning with cortical spreading depression decreases intraischemic cerebral glutamate levels and down-regulates excitatory amino acid transporters EAAT1 and EAAT2 from rat cerebal cortex plasma membranes. J Neurochem 75(2):812–818. doi:10.1046/j.1471-4159.2000.0750812.x
141. Liebelt B et al (2010) Exercise preconditioning reduces neuronal apoptosis in stroke by up-regulating heat shock protein-70 (heat shock protein-72) and extracellular-signal-regulated-kinase 1/2. Neuroscience 166(4):1091–1100. doi:10.1016/j.neuroscience.2009.12.067
142. Thompson JW, Dave KR, Young JI, Perez-Pinzon MA (2013) Ischemic preconditioning alters the epigenetic profile of the brain from ischemic intolerance to ischemic tolerance. Neurotherapeutics 10(4):789–797. doi:10.1007/s13311-013-0202-9
143. Endres M et al (2000) DNA methyltransferase contributes to delayed ischemic brain injury. J Neurosci 20(9):3175–3181
144. Lundberg J, Karimi M, von Gertten C, Holmin S, Ekström TJ, Sandberg-Nordqvist AC (2009) Traumatic brain injury induces relocalization of DNA-methyltransferase 1. Neurosci Lett 457:8–11. doi:10.1016/j.neulet.2009.03.10
145. Markus HS et al (2013) Evidence HDAC9 genetic variant associated with ischemic stroke increases risk via promoting carotid atherosclerosis. Stroke 44(5):1220–1225. doi:10.1161/STROKEAHA.111.000217
146. Langley B, Gensert JM, Beal MF, Ratan RR (2005) Remodeling chromatin and stress resistance in the central nervous system: histone deacetylase inhibitors as novel and broadly effective neuroprotective agents. Curr Drug Targets CNS Neurol Disord 4(1):41–50. doi:10.2174/1568007053005091
147. Stapels M et al (2010) Polycomb group proteins as epigenetic mediators of neuroprotection in ischemic tolerance. Sci Signal 3(111):ra15. doi:10.1126/scisignal.2000502
148. Hernández-Jiménez M et al (2013) Silent information regulator 1 protects the brain against cerebral ischemic damage. Stroke 44(8):2333–2337. doi:10.1161/STROKEAHA.113.001715
149. Simon RP, Meller R, Zhou A, Henshall D (2012) Can genes modify stroke outcome and by what mechanisms? Stroke 43(1):286–291. doi:10.1161/STROKEAHA.111.622225
150. Kim DH, Sætrom P, Snøve O, Rossi JJ (2008) MicroRNA-directed transcriptional gene silencing in mammalian cells. Proc Natl Acad Sci U S A 105(42):16230–16235. doi:10.1073/pnas.0808830105
151. Lee ST et al (2010) MicroRNAs induced during ischemic preconditioning. Stroke 41(8):1646–1651. doi:10.1161/STROKEAHA.110.579649
152. Lee YJ et al (2009) SUMOylation participates in induction of ischemic tolerance. J Neurochem 109(1):257–267
153. Tu XK et al (2014) Repetitive ischemic preconditioning attenuates inflammatory reaction and brain damage after focal cerebral ischemia in rats: involvement of PI3K/Akt and ERK1/2 signaling pathway. J Mol Neurosci 55(4):912–922. doi:10.1007/s12031-014-0446-9, **Epub 2014 Oct 22**
154. Jiang T et al (2014) Acute metformin preconditioning confers neuroprotection against focal cerebral ischaemia by pre-activation of AMPK-dependent autophagy. Br J Pharmacol 171(13):3146–3157. doi:10.1111/bph.12655
155. Wei L et al (2012) Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. Neurobiol Dis 46(3):635–645
156. Li S et al (2015) Safety and feasibility of remote limb ischemic preconditioning in patients with unilateral middle cerebral artery stenosis and healthy volunteers. Cell Transplant. doi:10.3727/096368914X683520
157. Kokošová N, Danielisova V, Smajda B, Burda J (2014) Ionizing radiation as preconditioning against transient cerebral ischemia in rats. Gen Physiol Biophys 33(4):403–410
158. Rana G et al (2012) Cortical spreading depression differentially affects lysine methylation of H3 histone at neuroprotective genes and retrotransposon sequences. Brain Res 1467:113–119
159. Lee JY, et al (2015) Indomethacin preconditioning induces ischemic tolerance by modifying zinc availability in the brain. Neurobiol Dis (14)00390-8. doi: 10.1016/j.nbd.2014.12.019
160. Keep RF et al (2014) Full steam ahead with remote ischemic conditioning for stroke. Transl Stroke Res 5(5):535–537
161. Lovett JK et al (2003) Very early risk of stroke after a first transient ischemic attack. Stroke 34(8):e138–e140. doi:10.1161/01.STR.0000080935.01264.91
162. Weih M et al (1999) Attenuated stroke severity after prodromal TIA: a role for ischemic tolerance in the brain? Stroke 30(9):1851–1854. doi:10.1161/01.STR.30.9.1851
163. Moncayo J et al (2000) Do transient ischemic attacks have a neuroprotective effect? Neurology 54(11):2089–2094. doi:10.1212/WNL.54.11.2089
164. Wegener et al (2004) Transient ischemic attacks before ischemic stroke: preconditioning the human brain?: a multicenter magnetic resonance imaging study. Stroke 35(3):616–621. doi:10.1161/01.STR.0000115767.17923.6A
165. Sitzer M et al (2004) Transient ischaemic attack preceding anterior circulation infarction is independently associated with favourable outcome. J Neurol Neurosurg Psychiatry 75(4):659–660
166. Schaller B (2005) Ischemic preconditioning as induction of ischemic tolerance after transient ischemic attacks in human brain: its clinical relevance. Neurosci Lett 377(3):206–211. doi:10.1016/j.neulet.2004.12.004
167. Zsuga J et al (2008) Prior transient ischemic attack is independently associated with lesser in-hospital case fatality in acute stroke. Psychiatry Clin Neurosci 62(6):705–712. doi:10.1111/j.1440-1819.2008.01874.x
168. Johnston SC (2004) Ischemic preconditioning from transient ischemic attacks? Data from the Northern California TIA Study. Stroke 35(11 Suppl 1):2680–2682. doi:10.1161/01.STR.0000143322.20491.0f
169. Hecker JG, McGarvey M (2011) Heat shock proteins as biomarkers for the rapid detection of brain and spinal cord ischemia: a review and comparison to other methods of detection in thoracic

aneurysm repair. Cell Stress Chaperones 16(2):119–131. doi:10.1007/s12192-010-0224-8
170. Castillo J et al (2003) The release of tumor necrosis factor–α is associated with ischemic tolerance in human stroke. Ann Neurol 54(6):811–819. doi:10.1002/ana.10765
171. Laudenbach V et al (2007) Neonatal hypoxic preconditioning involves vascular endothelial growth factor. Neurobiol Dis 26(1):243–252. doi:10.1016/j.nbd.2006.12.020
172. Matsuo R et al (2013) Clinical significance of plasma VEGF value in ischemic stroke- research for biomarkers in ischemic stroke (REBIOS) study. BMC Neurol 13:32. doi:10.1186/1471-2377-13-32
173. Vasdekis SN, Athanasiadis D, Lazaris A et al (2013) The role of remote ischemic preconditioning in the treatment of atherosclerotic diseases. Brain Behav 3(6):606–616. doi:10.1002/brb3.161
174. Meng R et al (2012) Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis. Neurology 79(18):1853–1861. doi:10.1212/WNL.0b013e318271f76a
175. Gonzalez NR et al (2013) Cerebral hemodynamic and metabolic effects of remote ischemic preconditioning in patients with subarachnoid hemorrhage, Cerebral Vasospasm: Neurovascular Events After Subarachnoid Hemorrhage. Springer, Vienna, pp 193–198
176. Hausenloy DJ et al (2007) Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomised controlled trial. Lancet 370(9587):575–579. doi:10.1016/S0140-6736(07)61296-3
177. Thielmann et al (2010) Remote ischemic preconditioning reduces myocardial injury after coronary artery bypass surgery with crystalloid cardioplegic arrest. Basic Res Cardiol 105(5):657–664. doi:10.1007/s00395-010-0104-5
178. Zuo Z (2012) Are volatile anesthetics neuroprotective or neurotoxic. Med Gas Res 2(1):10
179. Caplan LR (2013) Creating one problem to try and fix another: the saga of ischemic preconditioning. Brain Behav 3(6):603–605. doi:10.1002/brb3.170
180. Schaller B, Graf R (2002) Cerebral ischemic preconditioning. J Neurol 249(11):1503–1511. doi:10.1007/s00415-002-0933-8
181. Mergenthaler P, Dirnagl U (2011) Protective conditioning of the brain: expressway or roadblock? J Physiol 589(17):4147–4155. doi:10.1113/jphysiol.2011.209718
182. Koch S (2010) Preconditioning the human brain: practical considerations for proving cerebral protection. Transl Stroke Res 1(3):161–169. doi:10.1007/s12975-010-0025-5
183. Correia et al (2010) Mitochondrial preconditioning: a potential neuroprotective strategy. Front Aging Neurosci: 138. doi: 10.3389/fnagi.2010.00138