Published in final edited form as:

Nat Neurosci. ; 14(11): 1363–1368. doi:10.1038/nn.2953.

Stroke research at a crossroad: Asking the brain for directions

Costantino Iadecola and Josef Anrather

Division of Neurobiology, Department of Neurology and Neuroscience, Weill Cornell Medical College, New York, NY 10065

Abstract

Ischemic stroke remains a vexing public health problem. While progress has been made in prevention and supportive care, efforts to protect the brain from ischemic cell death have failed. Thus, no new treatment has made it from bench to bedside since tissue plasminogen activator was introduced in 1996. The brain has a remarkable capacity for self-preservation, illustrated by the protective responses induced by ischemia, preconditioning and exercise. Here we describe the mechanisms underlying brain self-protection, with the goal of identifying features that could provide insight into stroke therapy. Unlike traditional therapeutic approaches based on counteracting selected pathways of the ischemic cascade, endogenous neuroprotection relies on coordinated neurovascular programs that support cerebral perfusion, mitigate the harmful effects of cerebral ischemia and promote tissue restoration. Learning how the brain triggers and implements these protective measures may advance our quest to treat stroke and open a new era in stroke therapeutics.

Introduction

Stroke is a leading cause of brain injury that strikes approximately 800,000 people per year in the US alone, killing about 150,000¹. Significant progress has been made to mitigate the enormous public health impact of stroke, the majority of which is caused by occlusion of a cerebral artery (ischemic stroke). Efforts in prevention have reduced stroke incidence and mortality, whereas the introduction of specialized intensive care units has improved the functional outcome of stroke victims². However, limited advances have been made in developing therapies to counter the deleterious effects of cerebral ischemia, the only treatment available being thrombolysis with tissue plasminogen activator (tPA). Unfortunately, due to the narrow therapeutic window (<4.5 hours) and safety concerns, less than 5% of patients are treated with tPA and the majority of stroke victims receive only supportive care³. Most therapeutic approaches developed in the laboratory have focused on protecting neurons from the main pathogenic mechanisms causing ischemic injury, such as excitotoxicity, oxidative stress, inflammation or apoptosis⁴ (fig. 1). These experimental treatments have failed in large clinical trials, an outcome that has sparked a lively debate about the promise of neuroprotection in stroke therapy⁵. Therefore, translational stroke research is at a crossroad, and moving forward will require a reevaluation of traditional approaches and the development of a new conceptual framework to guide therapy. In this context, there is much to be gained by learning how the brain protects itself. The brain has a well-developed capacity for self-preservation and understanding how these protective measures are engaged and implemented provides useful lessons on how to best counteract ischemic brain injury. This article will briefly review the different modalities by which the

brain protects itself, aiming to provide a synthesis of the different mechanisms and highlighting their potential relevance for the future of stroke therapy.

The Janus face of ischemic injury: Balancing life and death

Interruption of blood flow cuts off the supply of oxygen and glucose and prevents the brain from generating the ATP needed to support its considerable energy demands⁴. After focal ischemia, this energy deficit is most severe in areas with the lowest residual flow (ischemic core), wherein cell death occurs rapidly⁶. In areas of less severe ischemia (ischemic penumbra), waves of depolarization (peri-infarct depolarizations) lead to neurotransmitter release, which, in concert with impairment of glial reuptake, generates toxic concentration of extracellular glutamate and other excitatory neurotransmitters (fig. 1). The resulting activation of glutamate receptors (excitotoxicity) leads to accumulation of intracellular Ca^{2+} , which, in turn, sets off deleterious events including activation of lytic enzymes, mitochondrial dysfunction, as well as oxidative and nitrosative stress⁴. At the same time, ischemia triggers inflammatory signaling, which leads to intravascular and parenchymal accumulation of leukocytes⁷. These inflammatory cells damage the brain by generating cytotoxic mediators⁷. In addition, inflammation, mitochondrial dysfunction, and oxidative stress activate programmed cell death which also contribute to tissue damage⁴.

These deleterious events are counteracted by local and remote protective mechanisms intended to mitigate tissue damage and re-establish homeostasis. Cerebral ischemia increases arterial pressure through sympathetic activation and hormonal release⁸. The increase in blood pressure promotes the delivery of blood flow to the ischemic area through anastomotic vessels arising from adjacent arterial territories that are normally perfused (collateral circulation)⁹. The delivery of flow to the ischemic area is also facilitated by the local release of potent vasodilators, including adenosine, vasoactive ions (K^+ and H^+), and nitric oxide⁶. Local hypoxia prevents the degradation of the transcription factor hypoxia inducible factor 1 (HIF1), which may promote tissue oxygen and glucose delivery¹⁰. The impact of the energy deficit is dampened by reducing the energy demands of the ischemic brain through suppression of neural activity and protein synthesis^{11,12}. After initial activation, NMDA receptors become desensitized, while release of inhibitory neurotransmitters suppresses synaptic activity and tends to limit the deleterious consequences of excitotoxicity¹³ {Dirnagl, 2003 #142}. Post-ischemic oxidative stress induces an antioxidant response mediated by the transcriptional activator nuclear factor-erythroid 2-related factor 2 (Nrf2)¹³. In parallel, the anti-apoptotic factors inhibitor of apoptosis (IAP) and B-cell lymphoma 2 (Bcl2), and heat shock proteins are upregulated, while activation of the pro-survival kinase Akt dampens the pro-apoptotic signaling triggered by ischemia¹⁴. The anti-inflammatory and neuroprotective cytokines interleukin-10 and transforming growth factor- β , produced in part by regulatory lymphocytes, attempt to limit leukocyte invasion and to suppress innate and adaptive immune responses, while protecting surviving neurons⁷. In concert with these central events, there is a marked suppression of systemic immunity, which may also be beneficial by limiting innate and adaptive immune responses initiated by tissue damage⁷. The protective nature of these central and peripheral changes triggered by ischemia is demonstrated by the fact that their inhibition exacerbates ischemic damage. However, some of these seemingly protective measures can also be damaging. For example excessive blood pressure elevation can lead to brain hemorrhages and exacerbate cerebral edema {Liebeskind, 2010 #14}, whereas post-stroke immunosuppression is associated with potentially fatal systemic infections⁷.

Protective pathways, operating in the late stages of the ischemic cascade, promote repair processes in the damaged brain (fig. 1). Microglia, macrophages, neurons, astrocytes and

vascular cells secrete growth factors (fig. 1), some of which, like erythropoietin (EPO) and insulin-like growth factor-1 (IGF1), are also produced by peripheral organs and enter the brain via the cerebral vasculature^{15–17}. Glutamatergic synaptic activity induces brain derived neurotrophic factor (BDNF) expression through activation of the transcription factor cAMP response element-binding (CREB)¹⁸. Surviving neurons sprout new processes in an attempt to reconstitute damaged neuronal connections¹⁹. Neural precursors invade the damaged area²⁰, whereas bone marrow-derived progenitor cells contribute to the reconstruction of the brain's microvascular network²¹. These processes attempt to re-constitute tissue homeostasis by reorganizing the extracellular matrix, replacing damage cells, and re-establishing neuronal networks.

These findings, collectively, suggest that ischemic injury, while activating destructive pathways that lead to cell death, also triggers local and systemic protective mechanisms aimed at counteracting the development of the injury. Although the damaging effectors in the end prevail, the evidence suggests that concomitant self-protective mechanisms limit the resulting damage and set the stage for tissue repair and reorganization.

Preconditioning: “*What does not kill me makes me stronger*”

The phenomenon of ischemic tolerance (IT) or preconditioning (PC) aptly demonstrates the potential for self-protection of the brain. A mild cerebral ischemic insult not sufficient to produce extensive damage protects the brain from subsequent damaging ischemia. In addition to ischemia, IT can also be induced by hypoxia, inflammatory mediators, metabolic blockers, anesthetics, cortical spreading depression, and seizures²². PC stimuli induce “early” and “delayed” protective time windows. Early PC induces tolerance within minutes, lasts for a few hours and is protein synthesis independent, whereas delayed PC typically develops after 12–72 hours, requires protein synthesis, and persists for days or weeks²². Notably, tolerance-inducing stimuli can protect the brain even when applied during or after the ischemic event (per- and post-conditioning)²², raising the possibility that induction of IT could be used not only as a preventive strategy in high risk individuals, but also as a treatment for acute stroke (see section on stroke therapeutics).

PC stimuli activate “triggers” that initiate a series of intermediate molecular events and lead to the expression of effectors that are responsible for tissue protection (fig. 2). In early PC, mediators act directly on effectors, which, in turn, mediate the protection, whereas in late PC transcription factors and epigenetic mechanisms reprogram gene expression (Box 1) and set the stage for the delayed protective effect (fig. 2). PC is observed at the cellular, tissue, organ and organism level. Thus, purified neuronal, glial, or endothelial cell cultures, as well as brain slices, can be preconditioned, suggesting that cells and tissue have the intrinsic molecular machinery needed to mount the protective response (Supplementary Table 1). Furthermore, transient ischemia in one organ induces IT in other organs (remote PC)²³, exemplifying how a threat to one organ evokes a systemic defensive response involving the entire organism.

Once ischemia strikes, the preconditioned brain is protected through multifunctional and coordinated biological programs targeting not only neurons, but also glial cells, the vasculature, the circulating blood, and the immune system. PC has a profound impact on neurons leading to protection of oxidative phosphorylation, membrane potential preservation, activation of hypoxia responsive genes, and induction of anti-apoptotic genes²² (Supplementary Table 1). Upregulation of growth factors increases the potential for structural and functional brain recovery. PC also exerts beneficial effect on cerebral blood vessels, which improve the perfusion of the penumbra by preserving neurovascular coupling and endothelium-dependent vasodilation²⁴. PC inhibits the formation of intravascular

platelet-leukocyte aggregates²⁵, a major cause of post-ischemic microvascular occlusions. In addition, PC promotes endothelial cell survival during ischemia by Akt dependent mechanisms²⁶ and protects the blood-brain barrier (BBB), possibly through astrocytes²⁷. PC also suppresses inflammation by dampening post-ischemic expression of adhesion molecule, leukocyte infiltration and microglial activation^{28,29}.

These observations in PC highlight the remarkable capacity for self-protection of the brain, which manifests itself at the cellular, tissue, organ and organism levels by converging protective mechanisms based on non-transcriptional, transcriptional and epigenetic effectors.

Exercise and brain protection: “*Mens sana in corpore sano*”

Exercise exerts a number of protective effects that increase the resistance of the brain to ischemic injury and neurodegeneration³⁰. Thus, 2 or 3 weeks of exercise reduces ischemic injury in rodents, whereas moderate exercise reduces stroke risk and improves recovery after stroke in humans³¹. These beneficial effects are linked to the multiple actions of physical activity on the brain and its vessels, and on peripheral organs. Exercise upregulates vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS) and endothelial progenitor cells (EPC), while enhancing post-ischemic cerebral perfusion and protecting the BBB³¹. Physical activity promotes hippocampal neurogenesis, and increases BDNF, fibroblast growth factor-2 and IGF1, factors involved in the associated increased resistance to brain injury and improved recovery of function^{32,33}. Mice lacking glial maturation factor- β do not exhibit BDNF mRNA increases after physical activity, suggesting the participation of astrocytes in exercise-induced growth factor production³⁴. The beneficial actions of exercise are also attributable to suppression of post-ischemic inflammation and apoptosis³⁵.

A key feature of the protective effects of exercise is the participation of systemic organs in its implementation. For example, afferent feedback from contracting muscles is needed for the full expression of the cardiovascular, neurohumoral and metabolic effects of exercise³⁶. Furthermore, some growth factors, like IGF1, are also produced in the periphery and gain access to the brain through the BBB¹⁶. Similarly, the “myokine” interleukin-6 is released from the skeletal muscle and its immunomodulatory effects have been linked to the suppression of inflammation associated with exercise³⁷. Conversely, brain-derived growth factors, such as BDNF, are released from the brain during physical activity and may have regulatory effects on systemic metabolism³⁸.

These findings indicate that the cerebroprotective effects of physical activity are based on maintaining vascular integrity, improving blood flow, suppressing the damaging effects of inflammation and apoptosis, and enhancing post-ischemic repair processes. These beneficial actions are implemented through a highly coordinated response involving multiple organs and are driven by the neurohumoral cross talk between the brain and the periphery.

Endogenous cytoprotection and preservation of tissue homeostasis

Several common threads emerge by cross-examining the mechanisms of endogenous neuroprotection and may be instructive to highlight some of their features (fig. 3). First, the potential for self-preservation exists at the cellular level (Supplementary Table 1)(Box 1) (fig. 2). Superimposed on these intracellular protective pathways, are multicellular processes directed at preserving the integrity of the whole tissue (fig. 3). These mechanisms are based on reciprocal trophic interactions, such that, if one cell type is threatened, a coordinated multicellular response emerges to maintain tissue homeostasis. Finally, a coordinated systemic defensive program aims to preserve the homeostasis of the whole brain by sustaining the circulation of blood, enhancing brain oxygen delivery, releasing growth factors and modulating the immune system to reduce tissue damage and foster repair

processes (fig. 3). These actions rely on neural and humoral signals derived from the injured brain and directed towards peripheral organs, which, in turn, generate other neurohumoral mediators that feed back on the brain.

Stroke therapeutics: What can we learn from the brain?

A mainstay of the treatment of stroke has been attempting to reperfuse the brain as quickly as possible after the ischemic event. Anticoagulants and, more recently, thrombolytics have been used to prevent clot formation or dissolve existing clots in cerebral arteries⁹. While anticoagulants were shown not to be effective in acute ischemic stroke, thrombolysis with intravenous tPA has been successful in reducing brain damage and neurological deficits³. Furthermore, acute carotid endarterectomy and endovascular reperfusion strategies may benefit selected patients⁹. Therefore, reperfusion therapy remains the first-line intervention in stroke patients who qualify. However, there still is a great need to develop therapies for those patients, representing the vast majority, in whom tPA or interventional approaches are not feasible or contraindicated³. Most therapeutic attempts to date have targeted individual pathogenic components of the ischemic cascade (fig. 1). This approach is not consistent with the way the brain protects itself, which relies on multifaceted central and peripheral protective programs targeted at maintaining tissue homeostasis. Acute stroke treatment could benefit from similar coordinated and multifunctional therapeutic approaches. Therefore, elucidating the triggers and effectors of endogenous neuroprotection and developing new therapies based their mechanism of action would be a welcome step forward in stroke therapeutics. Here we provide examples of treatments currently in clinical trials, which, like endogenous neuroprotection, engage multiple neuroprotective pathways and may promote repair processes.

- *Minocycline*, an agent with powerful anti-apoptotic, anti-inflammatory and antiexcitotoxic properties, protects the animal brain with a wide therapeutic window³⁹. Although not effective in females in experimental models, minocycline has shown promise in small clinical trials and a large phase III trial is in the planning stages³⁹. Short-term administration may not lead to the complications associated with long-term administration³⁹. Importantly, minocycline extends the therapeutic window and reduces hemorrhages when administered with tPA³⁹, suggesting that it could provide added benefit in patients receiving tPA. It is unclear whether minocycline acts by engaging endogenous neuroprotective pathways, but it is mentioned here because it represents an example of a multifunctional approach that mimics selected features of endogenous neuroprotection.
- *Hematopoietic growth factors*, including EPO, granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF), are broadly neuroprotective and are involved in PC^{40,17}. Like endogenous neuroprotection, these treatments engage both central and peripheral protective mechanisms, and enhance brain repair⁴⁰. EPO was efficacious in stroke patients in a small trial, but proved to be ineffective or damaging in a larger clinical trial, especially when used in conjunction with tPA⁴¹. Therefore, EPO may not be suitable for acute stroke treatment, but non-hematopoietic EPO analogs have shown promise in experimental studies and their safety in stroke patients is being tested (ClinicalTrials.gov Identifier: NCT00756249; NCT00870844). G-CSF has shown similar promise in pre-clinical studies⁴⁰, and a clinical trial is under way (ClinicalTrials.gov Identifier: NCT00927836).
- *Hypothermia* is another potent neuroprotective strategy that engages central and peripheral mechanisms leading to a marked reduction in ischemic brain injury in

experimental stroke⁴². Although the relative importance of the multiple physiological effects of hypothermia in neuroprotection remains unclear, hypothermia improves neurological outcome in patients with cardiac arrest and in children with hypoxic-ischemic brain injury⁴². Like endogenous neuroprotection, hypothermia counteracts excitotoxicity, inflammation and apoptosis, and promotes tissue homeostasis by producing growth factors and “cold shock proteins” endowed with cytoprotective and repair-promoting properties⁴². Mild hypothermia (33 °C) in stroke patients receiving tPA is feasible, but did not improve outcome⁴³, possibly, because too few patients were studied and cooling was started relatively late after stroke. Furthermore,

- hypothermia has also potentially detrimental complications, for example pneumonia and malignant cerebral edema during rewarming, which need to be controlled. Therefore, additional studies and new strategies to minimize the complications of cooling and rewarming are needed⁴³.
- *Remote preconditioning* by limb ischemia has shown promise in cardiac ischemia²³. Remote PC has been tested in a phase I trial of subarachnoid hemorrhage to protect the brain from delayed ischemic lesions, a major cause of morbidity and mortality in this stroke type, and was found to be safe and well tolerated⁴⁴. Importantly, remote PC is also effective in reducing brain and retinal ischemic injury after it has occurred (post-conditioning)^{45,46}, and is being tested in clinical trials (ClinicalTrials.gov identifier: NCT00975962). Remote post-conditioning is attractive because of its safety, simplicity and low cost, but its feasibility, effectiveness and potential downsides in ischemic stroke need to be established.

Conclusions

The brain is endowed with a rich complement of central and peripheral defense mechanisms that are unveiled by acute injury, PC stimuli or exercise (fig. 3). Pharmacological interventions or other therapeutic approaches that reproduce or mobilize these coordinated neuroprotective programs could have a transformative impact on the treatment of ischemic stroke, which has remained stagnant for almost two decades. This would represent a paradigm shift in stroke therapy: from interventions targeting individual pathogenic mechanisms to protect neurons, to interventions that engage multifunctional genetic and epigenetic programs directed at maintaining the homeostasis of the brain tissue as a whole. Therapies with hematopoietic growth factors, minocycline, hypothermia or remote post-conditioning are steps in this direction, but their efficacy in human stroke remains to be proven. Recently identified factors that contribute to the genomic response that confers IT, such as polycomb proteins⁴⁷ and sirtuins⁴⁸ raise the possibility of using epigenetic approaches to induce tolerance to cerebral ischemia. Although these treatments are still in the early preclinical stage, they offer the opportunity to enrich our armamentarium for the fight against stroke. Learning how to mimic or engage endogenous neuroprotective mechanisms may provide new directions in stroke research and open new avenues in the treatment of this devastating disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by NIH grants R37-NS34179 and NS35806. We apologize that the broad scope of the topic prevented us from citing many important studies.

References

1. Roger VL, et al. Heart disease and stroke statistics--2011 update: a report from the American Heart Association. *Circulation*. 2011; 123:e18–e209. [PubMed: 21160056]
2. Hachinski V, et al. Stroke: working toward a prioritized world agenda. *Stroke*. 2010; 41:1084–1099. [PubMed: 20498453]
3. Fonarow GC, et al. Timeliness of Tissue-Type Plasminogen Activator Therapy in Acute Ischemic Stroke: Patient Characteristics, Hospital Factors, and Outcomes Associated With Door-to-Needle Times Within 60 Minutes. *Circulation*. 2011; 123:750–758. [PubMed: 21311083]
4. Moskowitz MA, Lo EH, Iadecola C. The science of stroke: mechanisms in search of treatments. *Neuron*. 2010; 67:181–198. [PubMed: 20670828]
5. Moskowitz MA. Brain protection: maybe yes, maybe no. *Stroke*. 2010; 41:S85–S86. [PubMed: 20876513]
6. Kunz A, Iadecola C. Chapter 14 Cerebral vascular dysregulation in the ischemic brain. *Handbook of clinical neurology* / edited by P.J. Vinken and G.W. 2008; 92:283–305.
7. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med*. 2011; 17:796–808. [PubMed: 21738161]
8. Johansson A, Ahrén B, Näsman B, Carlström K, Olsson T. Cortisol axis abnormalities early after stroke--relationships to cytokines and leptin. *J Intern Med*. 2000; 247:179–187. [PubMed: 10692080]
9. Liebeskind DS. Reperfusion for acute ischemic stroke: arterial revascularization and collateral therapeutics. *Current opinion in neurology*. 2010; 23:36–45. [PubMed: 19926989]
10. Sharp FR, Bergeron M, Bernaudin M. Hypoxia-inducible factor in brain. *Adv Exp Med Biol*. 2001; 502:273–291. [PubMed: 11950144]
11. Dirnagl U, Simon RP, Hallenbeck JM. Ischemic tolerance and endogenous neuroprotection. *Trends Neurosci*. 2003; 26:248–254. [PubMed: 12744841]
12. Paschen W. Shutdown of translation: lethal or protective? Unfolded protein response versus apoptosis. *J Cereb Blood Flow Metab*. 2003; 23:773–779. [PubMed: 12843781]
13. Hardingham GE, Lipton SA. Regulation of Neuronal Oxidative and Nitrosative Stress by Endogenous Protective Pathways and Disease Processes. *Antioxid Redox Signal*. 2011; 14:1421–1424. [PubMed: 20977364]
14. Yenari MA. Heat shock proteins and neuroprotection. *Adv Exp Med Biol*. 2002; 513:281–299. [PubMed: 12575825]
15. Greenberg DA, Jin K. Growth factors and stroke. *NeuroRx*. 2006; 3:458–465. [PubMed: 17012059]
16. Torres-Aleman I. Toward a comprehensive neurobiology of IGF-I. *Dev Neurobiol*. 2010; 70:384–396. [PubMed: 20186710]
17. Digicaylioglu M. Erythropoietin in stroke: quo vadis. *Expert Opin Biol Ther*. 2010; 10:937–949. [PubMed: 20394473]
18. Kitagawa K. CREB and cAMP response element-mediated gene expression in the ischemic brain. *Febs J*. 2007; 274:3210–3217. [PubMed: 17565598]
19. Clarkson AN, Huang BS, Macisaac SE, Mody I, Carmichael ST. Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. *Nature*. 2010; 468:305–309. [PubMed: 21048709]
20. Kernie SG, Parent JM. Forebrain neurogenesis after focal Ischemic and traumatic brain injury. *Neurobiol Dis*. 2010; 37:267–274. [PubMed: 19909815]
21. Zhang ZG, Zhang L, Jiang Q, Chopp M. Bone marrow-derived endothelial progenitor cells participate in cerebral neovascularization after focal cerebral ischemia in the adult mouse. *Circ Res*. 2002; 90:284–288. [PubMed: 11861416]

22. Dirnagl U, Becker K, Meisel A. Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. *Lancet neurology*. 2009; 8:398–412. [PubMed: 19296922]
23. Saxena P, Newman MAJ, Shehatha JS, Redington AN, Konstantinov IE. Remote ischemic conditioning: evolution of the concept, mechanisms, and clinical application. *J. Card. Surg.* 2010; 25:127–134. [PubMed: 19549044]
24. Kunz A, et al. Neurovascular protection by ischemic tolerance: role of nitric oxide and reactive oxygen species. *J Neurosci*. 2007; 27:7083–7093. [PubMed: 17611261]
25. Pedersen CM, et al. Remote ischemic preconditioning prevents systemic platelet activation associated with ischemia-reperfusion injury in humans. *Journal of thrombosis and haemostasis*. 2011; 9:404–407. [PubMed: 21083644]
26. Zhang Y, Park TS, Gidday JM. Hypoxic preconditioning protects human brain endothelium from ischemic apoptosis by Akt-dependent survivin activation. *Am J Physiol Heart Circ Physiol*. 2007; 292:H2573–2581. [PubMed: 17337592]
27. Gesuete R, et al. Glial Cells Drive Preconditioning-Induced Blood-Brain Barrier Protection. *Stroke*. 2011; 42:1445–1453.. [PubMed: 21474800]
28. Andjelkovic AV, Stamatovic SM, Keep RF. The protective effects of preconditioning on cerebral endothelial cells in vitro. *J Cereb Blood Flow Metab*. 2003; 23:1348–1355. [PubMed: 14600442]
29. Huang SS, Wei FC, Hung LM. Ischemic preconditioning attenuates postischemic leukocyte--endothelial cell interactions: role of nitric oxide and protein kinase C. *Circ J*. 2006; 70:1070–1075. [PubMed: 16864944]
30. Cotman CW, Berchtold NC, Christie L-A. Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci*. 2007; 30:464–472. [PubMed: 17765329]
31. Endres M, et al. Mechanisms of stroke protection by physical activity. *Ann Neurol*. 2003; 54:582–590. [PubMed: 14595647]
32. Llorens-Martín M, Torres-Alemán I, Trejo JL. Exercise modulates insulinlike growth factor 1-dependent and -independent effects on adult hippocampal neurogenesis and behaviour. *Mol Cell Neurosci*. 2010; 44:109–117. [PubMed: 20206269]
33. Gómez-Pinilla F, Dao L, So V. Physical exercise induces FGF-2 and its mRNA in the hippocampus. *Brain Res*. 1997; 764:1–8. [PubMed: 9295187]
34. Zaheer A, et al. GMF-knockout mice are unable to induce brain-derived neurotrophic factor after exercise. *Neurochem. Res*. 2006; 31:579–584. [PubMed: 16758368]
35. Zhang F, Wu Y, Jia J. Exercise preconditioning and brain ischemic tolerance. *Neuroscience*. 2011; 177:170–176. [PubMed: 21241780]
36. Dishman RK, et al. Neurobiology of exercise. *Obesity (Silver Spring)*. 2006; 14:345–356. [PubMed: 16648603]
37. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev*. 2008; 88:1379–1406. [PubMed: 18923185]
38. Rasmussen P, et al. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Exp Physiol*. 2009; 94:1062–1069. [PubMed: 19666694]
39. Fagan S, Cronin LE, Hess DC. Minocycline development for acute ischemic stroke. *Trans Stroke Res*. 2011; 2:202–208.
40. Bath PM, Sprigg N. Colony stimulating factors (including erythropoietin, granulocyte colony stimulating factor and analogues) for stroke. *Cochrane database of systematic reviews (Online)*. 2007:CD005207. [PubMed: 17443577]
41. Ehrenreich H, et al. Recombinant human erythropoietin in the treatment of acute ischemic stroke. *Stroke*. 2009; 40:e647–656. [PubMed: 19834012]
42. Tang XN, Yenari MA. Hypothermia as a cytoprotective strategy in ischemic tissue injury. *Ageing Res Rev*. 2010; 9:61–68. [PubMed: 19833233]
43. Hemmen TM, et al. Intravenous thrombolysis plus hypothermia for acute treatment of ischemic stroke (ICTuS-L): final results. *Stroke*. 2010; 41:2265–2270. [PubMed: 20724711]
44. Koch S, Katsnelson M, Dong C, Perez-Pinzon M. Remote Ischemic Limb Preconditioning After Subarachnoid Hemorrhage: A Phase Ib Study of Safety and Feasibility. *Stroke*. 2011; 42:1387–1391. [PubMed: 21415404]

45. Ren C, et al. Limb remote ischemic postconditioning protects against focal ischemia in rats. *Brain Res.* 2009; 1288:88–94. [PubMed: 19631625]
46. Dreixler JC, et al. Delayed post-ischemic conditioning significantly improves the outcome after retinal ischemia. *Exp. Eye Res.* 2011; 92:521–527. [PubMed: 21501608]
47. Stapels M, et al. Polycomb group proteins as epigenetic mediators of neuroprotection in ischemic tolerance. *Sci Signal.* 2010; 3:ra15. [PubMed: 20197544]
48. Morris KC, Lin HW, Thompson JW, Perez-Pinzon MA. Pathways for ischemic cytoprotection: Role of sirtuins in caloric restriction, resveratrol, and ischemic preconditioning. *J. Cereb. Blood Flow Metab.* 2011; 31:1003–1019. [PubMed: 21224864]
49. Stenzel-Poore MP, et al. Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. *Lancet.* 2003; 362:1028–1037. [PubMed: 14522533]
50. Marsh B, et al. Systemic lipopolysaccharide protects the brain from ischemic injury by reprogramming the response of the brain to stroke: a critical role for IRF3. *J Neurosci.* 2009; 29:9839–9849. [PubMed: 19657036]

Box 1: The genetic and epigenetic landscape of the tolerant brain

Genomic and proteomic studies comparing the changes in gene expression induced by PC and by focal ischemia in preconditioned or naïve brains have provided insight into the genetic programs underlying IT^{47,49,50}. PC stimuli and ischemia without PC lead preferentially to gene up-regulation, but with markedly different patterns. PC ischemia increases the expression of genes regulating metabolic function and cell cycle, while injurious ischemia upregulates genes involved predominantly in immune function and host defenses⁴⁹. These observations indicate that the gene expression pattern underlying the endogenous protection program accompanying ischemic injury (fig. 1) is distinct from that induced by PC. Surprisingly, the gene expression profile induced by ischemia in preconditioned brains is characterized by transcriptional suppression, 77% of differentially regulated genes being downregulated and only 23% increased⁴⁹. The suppressed genes are involved in metabolic function, synaptic activity, ion transport and cell cycle, a pattern consistent with suppression of cellular activity. In contrast, in animals preconditioned with the proinflammatory mediator lipopolysaccharide (LPS) and subjected to ischemia, the gene expression profile is characterized by enhanced transcription, 84% of regulated genes being increased and consisting mainly of NF- κ B and IFN type I-dependent genes⁵⁰. Therefore, the genetic underpinnings of the tolerant phenotype can be markedly different despite similar cytoprotective effects. It is noteworthy that PC with LPS increases gene expression at 3 and 24 hours, immune and inflammatory genes making up the bulk of the response. However, very few genes are still upregulated at 72 hours when focal ischemia was induced⁵⁰. This raises the question of how the “memory” of the PC event was preserved if the attendant mRNA response had already subsided. One possibility is that the protein products of the subsided transcriptional activity are still present at the time of induction of ischemia, which could have conditioned the response to injury toward protection. Another possibility is that the transcriptional response to PC induces longer lasting epigenetic changes that shape the genetic response to subsequent injurious ischemia. Epigenetic changes occur in models of ischemic PC, in which upregulation of gene repressor proteins, namely histone H2A and H2B variants and polycomb group proteins, has been reported⁴⁷. Therefore, it is conceivable that such “epigenetic memory” may play a role in developing a tolerant phenotype to ischemia in brain and other organs. These genomic and proteomic data, collectively, reveal a diversity of the transcriptional responses underlying the induction of IT, and suggest that the protection conferred by diverse PC stimuli does not follow a final common genetic pathway, but relies on multiple genetic and epigenetic programs that may have translational relevance⁴⁷.

Figure 1. Protective pathways activated by cerebral ischemia

Cerebral ischemia, while activating damaging processes, also triggers a coordinated response that attempts to counteract tissue damage. The reduction in blood flow produced by the arterial occlusion is opposed by an increase in blood pressure, by the production of vasoactive mediators in the ischemic brain and by the activation of eNOS, which increase perfusion pressure and reduce vascular resistance in collateral vessels supplying the ischemic territory. Hypoxia activates HIF1 leading to a transcriptional response that promotes oxygen and glucose delivery to the tissue. The energy deficit associated with ischemia is countered by suppression of protein synthesis and neuronal activity (spike arrest and channel closure), which reduce energy expenditures. Post-ischemic oxidative stress triggers an antioxidant response via the transcription factor Nrf2, while inhibitory neurotransmitters and glutamate transporters (GLT1/EAAT2) counterbalance the excitotoxicity associated with glutamate receptor activation. The deleterious effects of post-ischemic apoptosis are antagonized by expression of anti-apoptotic factors (Bcl2, IAP), HSP and activation of the protective kinase Akt. Inflammation is mitigated by production of anti-inflammatory cytokines and neurotransmitters, as well as an influx of lymphocytes with anti-inflammatory properties (T_{reg}, B_{reg}). Systemic immunosuppression limits the development of adaptive and innate immune responses that may induce tissue damage.

Ischemia is also associated with expression of CREB-dependent prosurvival genes, including growth factors, and with proliferation of neural and vascular progenitor cells that participate in tissue repair. These endogenous protective pathways limit the extent of ischemic brain injury as shown by studies in which their inhibition enhances the damage, e.g.^{10,14}.

Figure 2. Intracellular events leading to ischemic tolerance

PC triggers act through G-coupled receptors dependent phospholipase C (PLC) activation leading to hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) and generation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃), which acts on smooth endoplasmic reticulum (ER) Ca²⁺ channels to mobilize intracellular Ca²⁺ stores leading to PKC activation. PIP₂ is also phosphorylated by phosphatidylinositol 3-kinase (PI3K) resulting in phosphatidylinositol 3-phosphate (PIP₃) generation and Akt activation. Ca²⁺ influx through glutamate receptors activates NOS. NO increases guanylate cyclase activity (GC) resulting in protein kinase G (PKG) activation. Together these early mediators enhance the activity of mK_{ATP} channels and inhibit pro-apoptotic signaling and opening of the

mitochondrial permeability transition pore (mPTP). At the same time, transcription factors activated by these signaling cascades as well as reduced oxygen levels, reactive oxygen species (ROS), and ATP deficit lead to the expression of pro-survival genes, like the anti-apoptotic factor Bcl2, HSP, and the antioxidant enzymes MnSOD and HO-1. Genes are also expressed that help the tissue operate under reduced oxygen and glucose availability, like the glucose transporter GLUT-1, the pro-angiogenic growth factor VEGF, and the hematopoietic and cytoprotective factor EPO. DAMPs released from stressed cells activate Toll-like receptors (TLR) leading to NF- κ B and type-I interferon response. Epigenetic factors are also likely to contribute to the reprogramming of post-ischemic gene expression and may include the epigenetic modifiers Polycomb Group proteins (PcG) and Sirtuin class histone deacetylases (SIRT).

Figure 3. Local and remote mechanisms of endogenous neuroprotection

In brain, there are protective interactions among neurons, astrocytes, microglia and cerebral blood vessels. These are mediated by cell-cell contact, by the uptake of excessive glutamate, and by the release of growth factors and cytokines. These interactions are directed at preserving tissue homeostasis by maintaining CBF, suppressing excitotoxicity, reducing energy use, dampening inflammation and apoptosis, and boosting repair mechanisms. Central signals (red arrows) through neurohumoral pathways act on peripheral organs to support the cardiovascular system, release growth factors and cytokines, and mobilize protective cells, such as T_{reg} and B_{reg} lymphocytes and EPC. Peripheral signals (blue arrows) generated by the systemic response, in turn, may feed back on the brain and exert protective effects.