Neuroproctective Effects of Ischemic Tolerance (Preconditioning) and Postconditioning

Önkoşullama ve Ardkoşullamanın Nöroprotektif Etkileri

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ABSTRACT

Elucidation of the endogenous cell survival pathways involved in ischemic tolerance (preconditioning) and postconditioning has significant clinical implications for preventing neuronal damage in susceptible patients. Ischemic tolerance is a phenomenon in which the brain protects itself against future injury by adapting to low doses of noxious insults. Ischemic postconditioning is defined as brief periods of reperfusion alternating with re-occlusion applied during the very early minutes of reperfusion that mechanically alters the hydrodynamics of reperfusion. Similar pathways and molecules play a role in pre-and postconditioning but their roles and timing are different in each conditioning. Understanding the neuroprotective effects of mechanisms underlying conditionings has been elusive, but NMDA receptor activation, nitric oxide, inflammatory cytokines, and suppression of the innate immune system appear to have a role. Reactive oxygen species and classical ligand stimuli play a role in postconditioning with KATP channels and protein kinase C pathways acting as mediators.

KEYWORDS: Preconditioning, Postconditioning, Neuronal protection

ÖZ

Organizmalar potensiyel olarak tekrar oluşacak darbelere koruyucu mekanizmalarla karşılık verirler. Janoff bu fenomeni iskemik tolerans (İT) ve iskemik preconditioning (İP) olarak tanımlamıştır. Pratik olarak nöral travmaya neden olan herhangi bir uyarı, harabiyete neden olabilecek doz eşiğine yakın fakat harabiyete neden olmayacak derecede uygulandığı zaman SSS'ni daha sonraki iskemiye, travmaya karşı dayanıklılığı arttıran kuvvetli endojen yolları upregüle ederek korur. İT/İP potensiyel olarak; iskemi, hipoksi veya inflamasyon gibi zararlı uyarılara akut veya kronik reaksiyon olarak tanımlanabilir. Serebral İP, ilk defa iskemiye karşı 24 saat sonra tolerans oluşturmak amacıyla global serebral iskemi modelinde iki dakikalık geçici iskemi oluşturulduktan sonra demonstre edilmiştir. Kısa süreli global iskemi sonrası oluşan serebral korunmanın %20-91 oranında oluştuğu saptanmıştır. İskemik önkoşullama ve ardkoşullama, endojen nöral korunma mekanizmalarından sorumlu moleküler mekanizmalara yeni bir bakış açısı getirmekte ve beyin hücrelerinin iskemik travmaya karşı daha dayanıklı hale getirilebilmeleri için yeni stratejilerin geliştirilmesi gerekliliğini göstermektedir.

ANAHTAR SÖZCÜKLER: Önkoşullama, Ardkoşullama, Nöronal korunma

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INTRODUCTION

Elucidation of the endogenous cell survival pathways involved in ischemic tolerance (preconditioning) and postconditioning has significant clinical implications for preventing neuronal damage in susceptible patients. Ischemic tolerance is a phenomenon in which the brain protects itself against future injury by adapting to low doses of noxious insults. Ischemic postconditioning is defined as brief periods of reperfusion alternating with re-occlusion applied during the very early minutes of reperfusion that mechanically alters the hydrodynamics of reperfusion.

Similar pathways and molecules take place in pre-and postconditioning but their roles and timing are different in each conditioning. Understanding the neuroprotective effects of mechanisms underlying conditionings has been elusive, but NMDA receptor activation, nitric oxide, inflammatory cytokines, and suppression of the innate immune system appear to have a role. Reactive oxygen species and classical ligand stimuli play a role in postconditioning with KATP channels and protein kinase C pathways acting as mediators.

Following cerebral ischemia a complex and dynamic interaction of vascular cells, glial cells, and neurons determines the extent of the ensuing lesion. Traditionally, the focus has been on mechanisms and severity of damage, while recently it has become clear that endogenous mechanisms of protection are equally important for the final outcome.

Over recent years, neuroscientists have acquired a considerable body of evidence to support the fact that the mammalian brain can adapt to injurious insults such as cerebral ischemia, thus increasing the chances of survival from subsequent injury (12). Adaptation is one of physiology's fundamental tenets, operating not only at the level of species, as Darwin proposed, but also at the level of tissues, cells, molecules and perhaps genes.

Ischemic pre-and postconditionings provide a new insight into molecular mechanisms responsible for endogenous neuronal protection and this indicates a necessity for new strategies to increase the durability of brain cells to ischemic insult (20,43,47,50). Ischemic tolerance (preconditioning) is taken to mean a short ischemic episode subfatal to cells that activates the protective endogenous

mechanisms that ensure tolerance of further longer and more severe episodes of ischemia by the organ or tissue. Preconditioning is a phenomenon whereby low doses of noxious insults shield the brain from future insults rather than inflicting damage (12). Preconditioning by ischemic tolerance was first identified in the heart by Murry et al (30), and was subsequently found to occur in the brain (20) and a variety of organs including the liver, intestine, kidney and lung (20). Preconditioning stimuli can be cross-tolerant, safeguarding against other types of injury. For example, endotoxin preconditioning can also protect against subsequent ischemia and vice versa (38).

Brain cells, even without preconditioning, try to decrease cellular damage and death by using their own defense systems against ischemia (6). Any stimulus capable of causing neural trauma may protect the central nervous system by upregulating endogenous pathways that will increase endurance to ischemia or trauma (37).

Postconditioning, defined as brief periods of reperfusion alternating with re-occlusion applied during the very early minutes of reperfusion, mechanically alters the hydrodynamics of early reperfusion. Reperfusion injury is a complex process involving endothelial and microvascular dysfunction, impaired blood flow, metabolic dysfunction, cellular necrosis and apoptosis (1,52,47,49). While cerebral ischemic preconditioning has been known to protect against strokes for some time, postconditioning has recently been shown to reduce ischemic damage (52)

Similar pathways and molecules take place in pre and postconditioning but their roles and timing are different in each condition (42). Reperfusion has the potential to introduce additional injury that is not evident at the end of ischemia per se, i.e. reperfusion injury. Reperfusion injury is expressed as endothelial and microvascular dysfunction, impaired blood flow, metabolic dysfunction, cellular necrosis, and apoptosis. There is an impressive array of mechanisms contributing to reperfusion injury. Postconditioning also stimulates endogenous mechanisms that attenuate the multiple manifestations of reperfusion injury. These mechanisms include ligands, such as adenosine and opioids that act as proximal triggers to stimulate molecular pathways involving mediators such as protein kinase C, mitochondrial ATP-sensitive

potassium channels, and survival kinases. Postconditioning has been shown to inhibit the mitochondrial permeability transition pore. Postconditioning may also inhibit deleterious pathways such as p38 and JNK mitogen-activated protein (MAP) kinases and attenuate the damage to endothelial cells and cardiomyocytes from oxidants, cytokines, proteases, and inflammatory cells. Hence, postconditioning marshals a variety of endogenous mechanisms that operate at numerous levels and target a broad range of pathological mechanisms. A recent clinical study in patients with acute myocardial infarction has demonstrated that postconditioning was effective in reducing infarct size (40). Postconditioning indirectly supports the concept of reperfusion injury in animal models of ischemia-reperfusion and in patients, and exerts cardio protection that is equivalent to that of ischemic preconditioning.

Preconditioning stimuli include but are not limited to transient global and focal ischemia (8,24,39), hypoxia (2), cortical spreading depression (17,21,46), brief episodes of seizure, exposure to anesthetic inhalants (21,46), low doses of endotoxin (54), hypothermia and hyperthermia (32,34) and 3-nitropropionic acid treatment (44). Depending on the specific preconditioning stimulus, a state of neuronal tolerance can be established in at least two temporal profiles: one in which the trigger induces protection within minutes (rapid or acute tolerance) (35) and one in which the protected state develops after a delay of several hours to days (delayed tolerance) (20). Some preconditioning paradigms induce both phases of ischemic tolerance, while others can induce only the acute phase or only the delayed phase (41). The acute phase is most likely due to rapid posttranslational modifications of proteins (31). In contrast, the delayed phase is dependent on de novo protein synthesis (19).

Understanding of the mechanisms underlying preconditioning has been elusive, but NMDA receptor activation, nitric oxide, inflammatory cytokines, and suppression of the innate immune system appear to have a role. Ischemia, hypoxia, hypothermia/hyperthermia, hyperbaric oxygenation and metabolic inhibitors may be preconditioning stimuli (2,12,19,43).

Ischemia

Preconditioning-induced neuroprotection is observed not only in terms of infarct volume (4,20,47) but in some studies also in terms of neurological and behavior studies.

Experimental studies have also demonstrated that ischemic postconditioning reduces cerebral ischemic/reperfusion injury (I/R) (1,49,52). An interval of 30 seconds was referred to in the study by Zhao et al. Whether postconditioning played its role in an "on-off" or a "dose dependent" manner was not fully elucidated in Zhao's study (52).

It is well known that generation of excessive ROS during reperfusion plays a major role in brain injury associated with stroke. Because of the brain's low activities of antioxidative enzymes, it is very vulnerable to ROS induced by I/R injury, which causes oxidative damage to brain lipids, proteins, and DNA, leading to brain dysfunction and cell death (7). Postconditioning treatment was found to decrease the level of MDA and increase superoxide dismutase activity, suggesting there was attenuated lipid peroxidation and reduced generation of superoxide anions in cerebral I/R (49). Abas et al., in 2006 and recently Xing et al., showed that postconditioning significantly inhibited apoptosis of cortical neurons caused by I/R injury, which was proved by DNA fragmentation and activated caspase-3. To further clarify the mechanism of postconditioning protection, Xing et al., (49) studied the expressions of key apoptosis-related molecules. It was shown that postconditioning increased the level of antiapoptotic Bcl-2 protein in the mitochondria, inhibited Bax translocation to the mitochondria, and inhibited cytochrome c release from the mitochondria to the cytosol. Therefore, the aforementioned mechanism led to a decrease in the activation of caspase. It is known that cytochrome c is released from the mitochondria to the cytosol and plays a key role in the initiation of apoptosis through the activation of the caspase cascade (27). By regulating the Bcl-2 (antiapoptosis)/Bax (proapoptosis) balance, the Bcl-2 family maintains mitochondrial stabilization (14). Previous work by Tsuchia et al., has shown that overexpression of HSP70 is associated with a reduction of cytochrome c release from the mitochondria (48). Xing's study also revealed that postconditioning increased the level of HSP70 in the cortex during cerebral ischemic/reperfusion injury (49).

Hypoxia

Hypoxic events are common in newborns but

their consequences on brain development have not been demonstrated. It has been reported that short-term hypoxia before the insult completely prevented brain damage in newborn animal models of cerebral hypoxic-ischemic insult (2). The mechanisms of this brain tolerance are not yet fully understood. Preconditioning by a sublethal stimulus induces tolerance to a subsequent, and otherwise lethal insult. A relatively convenient method for preconditioning animals is hypoxic exposure. Animals are put in a chamber in which oxygen and nitrogen proportions can be controlled. The oxygen concentration usually ranges from 8% to 13% with normobaric pressure. Exposure time ranges from 1 to 6 hours. Twenty-four to 72 hours later, transient or permanent focal stroke is induced in the animals (6). Hypoxia-preconditioned neuroprotection usually starts at 1 to 3 days with a significant reduction of infarct size (2).

Hypothermia and hyperthermia

Hypothermia (32,33,51) is a well-known neuroprotective procedure used during and after cerebral surgery.

The neuroprotective effectiveness of postischemic hypothermia is typically viewed with skepticism because of conflicting experimental data. However, recent experimental data have revealed that a protected reduction in brain temperature can provide sustained behavioral and histological neural protection (22,50).

Cortical spreading depression

Cortical spreading depression is defined as the electrophysiological phenomenon of slowly propagating transient depolarization waves across the cortex and induces a prolonged phase of ischemic tolerance that lasts 1 to 7 days (17,18,46).

Anaesthetic agents

Exposure to anesthetics such as isoflurane and halothane at pharmacologic concentration ranges also confers delayed-phase ischemic tolerance of the brain (5,29).

NEURONAL CONDITIONING PATHWAYS IN BRAIN

Cellular preconditioning can be subdivided into intrinsic neuronal pathways (preventing excitotoxic damage, signaling through anti-apoptotic molecules, and treatment by neurotrophic factors) or extrinsic nonneuronal pathways (peripheral cytokine production, microglial activation, and regulation of the cerebrovascular system) (38).

NMDA receptor activation and excitotoxity protection

In neurons, ischemic tolerance is mediated largely by the activation of the N-methyl-D-aspartate (NMDA) glutamate receptors through increases in intracellular calcium (13,15,16).

Preconditioning with cortical spreading depression results in the downregulation of the excitatory amino acid transporters from cerebral cortex plasma membranes (11). Although these transporters are normally involved in glutamate uptake, it has been suggested that the influx of sodium that occurs during excitotoxicity may cause their reversal and result in additional glutamate release. Downregulating these transporters may thus contribute to ischemic tolerance.

Nitric oxide

Nitric oxide (NO) may play a key role as a mediator of the neuronal ischemic preconditioning response, either in conjunction with or independent of NMDA receptor activation (3,9).

Ischemia generated by occlusion of the middle cerebral artery causes defects in cerebrovascular function for not only the infarcted area but also the surrounding ischemic region. LPS preconditioning has been reported in some cases to increase this regional cerebral blood flow both before and after ischemia (53).

Inflammatory cytokines and the innate immune system

LPS, a component of the gram-negative bacterial cell wall, can illicit a potent innate immune response. While this systemic inflammatory response can be destructive, tolerable LPS doses render the brain transiently resistant to subsequent ischemic injury (45).

Glial cells, in particular astrocytes, have always been viewed as supporters of neuronal function. Microglia is the resident central nervous system component of the innate immune system. Microglia and macrophages become activated with ischemia in the infarcted and surrounded area (28). Upon activation in ischemia, microglia will become phagocytic and secrete a multitude of noxious chemokines and cytokines (25). Preconditioning the brain with LPS ameliorates microglial activation,

neutrophil infiltration, and circulating monocyte activation following MCAo (10,36). Alternatively, microglia can exhibit neuroprotective properties within the brain. In fact, greater ischemic damage from longer periods of MCAO is correlated with fewer proliferating microglia, suggesting a protective microglial role. Consistently, ablation of proliferating microglia increases the infarction area following MCAO (26). Therefore, microglia can be protective in ischemia.

Pre-/postconditioning represent adaptive responses to prime the brain for protection against future injury. Elucidation of these endogenous cell survival pathways has significant clinical implications for preventing neuronal damage in susceptible patients. For this reason, understanding the underlying mechanisms in establishing a tolerant state will be a critical step in adapting pre/postconditioning for safe patient applications. Only with a more thorough understanding of conditioning mechanisms can we adapt these pathways for the most efficient and protective treatments.

CONCLUSION

Refinement of various conditioning models is of great clinical significance. Neurosurgical vascular procedures such as aneurysm surgery have a negative impact on brain function due to stoppage of blood flow during clipping. As a result, it is of premier importance to develop strategies to protect the brain either prior to vascular surgeries or in patients at high risk of stroke. While it would be dangerous and impractical to precondition at-risk patients with ischemia, the identification of underlying conditioning mechanisms may lead to safer therapeutic factors that can be administered before surgery. The most direct and significant application of understanding the mechanism of ischemic tolerance is therapeutic access to this protective state, especially during cardiac bypass surgery, cardiac transplantation and neurosurgical procedures.

Pre-/Postconditioning may prevent oncoming cerebral ischemia due to vasospasm. For example, producing a short period of intermittent occlusion and reperfusion to the main trunk a few times during early aneurysm surgery might provide protection from ischemia due to subsequently developing vasospasm/ischemia injury or

developing infarction after prolonged temporary artery occlusion during STA–MCA anastomosis, two potential areas for application of post conditioning. Postconditioning after neuro-interventional treatment may also protect from cerebral ischemia and similarly post conditioning after coronary angioplasty and stenting has been shown to protect the heart during a myocardial infarct (40). Abaş et al. (1) and recently Xing et al. (49), showed in their experimental studies that postconditioning reduces infarct size, probably by blocking apoptosis and free radical generation (1).

The endogenous survival mechanisms activated in response to preconditioning do not depend on differences in drug pharmacokinetics or administration protocols that can confound the translation of neuroprotective strategies from rodents to humans. Therefore, the identification of intrinsic cell survival pathways should provide more direct opportunities for translational neuroprotection trials. Although primarily focused on stroke at present, we might find that the innate regulatory schemes that underlie tolerance to ischaemia are also applicable to protecting the brain from other acute and chronic neurodegenerative disorders.

The search for effective neuroprotectants remains frustrating, particularly with regard to specific pharmaceuticals. However, laboratory studies have consistently shown remarkable neuroprotection with two nonpharmacological strategies - therapeutic hypothermia and ischemic preconditioning. Recent studies have shown that the mechanism of protection underlying both of these treatments is correlated to downregulation of cellular and tissue metabolism. Thus, understanding the mechanisms underlying such robust protective effects could lead to appropriate translation at the clinical level. In fact, hypothermia is already being used at many centers to improve the neurological outcome from cardiac arrest.

REFERENCES

1. Abas F: Effects of ischemic postconditioning on lipid peroxidation and apoptosis after focal cerebral ischemia/reperfusion injury in the rat. Department of Neurosurgery, Faculty of Medicine, Uludag University, Thesis 2006

2. Alkan T, Gören B, Vatansever E, Sarandöl E: Effects of hypoxic preconditioning in antioxidant enzyme activities in hypoxic-ischemic brain damage in immature rats. Turkish Neurosurgery 18(2):165-171, 2008

3. Atochin DN, Clark J, Demchenko IT, Moskowitz MA, Huang PL: Rapid cerebral ischemic preconditioning in mice deficient in endothelial and neuronal nitric oxide synthases. Stroke 34(5):1299-1303, 2003

4. Barone FC, White RF, Spera PA, Ellison J, Currie RW, Wang X, Feuerstein GZ: Ischemic preconditioning and brain tolerance: Temporal histological and functional outcomes, protein synthesis requirement, and interleukin-1 receptor antagonist and early gene expression. Stroke 29:1937–1950, 1998

5. Baughman VL, Hoffman WE, Miletich DJ, Albrecht RF, Thomas C: Neurologic outcome in rats following incomplete cerebral ischemia during halothane, isoflurane, or N2O. Anesthesiology 69(2):192-198, 1988

6. Bernaudin M, Nedelec AS, Divoux D, MacKenzie ET, Petit E, Schumann-Bard P: 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 Cerebr Blood F Met 22:393–403, 2002

7. Chan PH: Role of oxidants in ischemic brain damage. Stroke 27(6):1124-1129, 1996

8. Chen J, Simon R: Ischemic tolerance in the brain. Neurology 48:306–311, 1997

9. Cho S, Park EM, Zhou P, Frys K, Ross ME, Iadecola C: Obligatory role of inducible nitric oxide synthase in ischemic preconditioning. J Cerebr Blood F Met 25:493–501; 2005

10. Denes A, Vidyasagar R, Feng J, Narvainen J, McColl BW, Kauppinen RA, Allan SM: Proliferating resident microglia after focal cerebral ischaemia in mice. J Cerebr Blood F Met 27:1941–1953, 2007

11. Douen AG, Akiyama K, Hogan MJ, Wang F, Dong L, Chow AK, Hakim A: 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 75:812–818; 2000

12. Gidday JM: Cerebral preconditioning and ischaemic tolerance. Nat Rev Neurosci 7(6):437-448, 2006

13. Grabb MC, Choi DW: Ischemic tolerance in murine cortical cell culture: critical role for NMDA receptors. J Neurosci 19:1657–1662, 1999

14. Hu XL, Olsson T, Johansson IM: Dynamic changes of the anti- and pro-apoptotic proteins Bcl-w, Bcl-2, and Bax with Smac/Diablo mitochondrial release after photothrombotic ring stroke in rats. EurJ Neurosci 20:1177–1188, 2004

15. Kasischke K, Ludolph AC, Riepe MW: NMDA-antagonists reverse increased hypoxic tolerance by preceding chemical hypoxia. Neurosci Lett 214:175–178, 1996

16. Kato H, Liu Y, Araki T, Kogure K: MK-801, but not anisomycin, inhibits the induction of tolerance to ischemia in the gerbil hippocampus. Neurosci Lett 139:118–121, 1992

17. Kawahara N, Ruetzler CA, Klatzo I: Protective effect of spreading depression against neuronal damage following cardiac arrest cerebral ischaemia. Neurol Res 17(1):9-16, 1995

18. Kawahara N, Ruetzler CA, Mies G, Klatzo I: Cortical spreading depression increases protein synthesis and upregulates basic fibroblast growth factor. Exp Neurol 158(1):27-36, 1999

19. Kirino T: Ischemic tolerance. J Cerebr Blood F Met 22:1283–1296, 2002

20. Kitagawa K, Matsumoto M, Kuwabara K, Tagaya M, Ohtsuki T, Hata R, Ueda H, Handa N, Kimura K, Kamada T: 'Ischemic tolerance' phenomenon found in the brain. Brain Res 528:21–24, 1990

21. Kobayashi S, Harris VA, Welsh FA.Spreading depression induces tolerance of cortical neurons to ischemia in rat brain. J Cerebr Blood F Met 15(5):721-727, 1995

22. Krieger DW, Yenari MA: Therapeutic hypothermia for acute ischemic stroke: What do laboratory studies teach us? Stroke 35: 1482–1489, 2004

23. Kulinskii VI, Minakina LN, Gavrilina TV: Neuroprotective effect of hypoxic preconditioning: Phenomenon and mechanisms. Exp Biol Med 133:202–204, 2002

24. Kunz A, Park L, Abe T, Gallo EF, Anrather J, Zhou P, Iadecola C: Neurovascular protection by ischemic tolerance: Role of nitric oxide and reactive oxygen species. J Neurosci 4;27(27):7083-7093, 2007

25. Lai AY, Todd KG: Microglia in cerebral ischemia: Molecular actions and interactions. Can J Physiol Pharm 84:49–59, 2006

26. Lalancette-Hebert M, Gowing G, Simard A, Weng YC, Kriz J: Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. J Neurosci 27:2596–2605, 2007

27. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X: Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 14;91(4):479-489, 1997

28. Mabuchi T, Kitagawa K, Ohtsuki T, Kuwabara K, Yagita Y, Yanagihara T, Hori M, Matsumoto M: Contribution of microglia/ macrophages to expansion of infarction and response of oligodendrocytes after focal cerebral ischemia in rats. Stroke 31:1735–1743, 2000

29. McAuliffe JJ, Joseph B, Vorhees CV: Isoflurane-delayed preconditioning reduces immediate mortality and improves striatal function in adult mice after neonatal hypoxia-ischemia. Anesth Analg 104(5):1066-1077, 2007

30. Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. Circulation 74(5):1124-1136, 1986

31. Nakase H, Heimann A, Uranishi R, Riepe MW, Kempski O: Early-onset tolerance in rat global cerebral ischemia induced by a mitochondrial inhibitor. Neurosci Lett 290:105–108, 2000

32. Nishio S, Yunoki M, Chen ZF, Anzivino MJ, Lee KS: Ischemic tolerance in the rat neocortex following hypothermic preconditioning. J Neurosurg 93:845–851, 2000

33. Nishio S, Chen ZF, Yunoki M, Toyoda T, Anzivino M, Lee KS: Hypothermia-induced ischemic tolerance. Ann NY Acad Sci 890:26–41, 1999

34. Ota A, Ikeda T, Xia XY, Xia YX, Ikenoue T: Hypoxic-ischemic tolerance induced by hyperthermic pretreatment in newborn rats. J Soc Gynec Invest 7:102–105, 2000

35. Pérez-Pinzón MA, Xu GP, Dietrich WD, Rosenthal M, Sick TJ: Rapid preconditioning protects rats against ischemic neuronal damage after 3 but not 7 days of reperfusion following global cerebral ischemia. J Cerebr Blood F Met 17(2):175-182, 1997

36. Rosenzweig HL, Lessov NS, Henshall DC, Minami M, Simon RP, Stenzel-Poore MP: Endotoxin pre-conditioning prevents cellular inflammatory response during ischemic neuroprotection in mice. Stroke 35:2576–2581, 2004

37. Sapolsky RM: Cellular defenses against excitotoxic insults. J Neurochem 76(6):1601-1611, 2001

38. Shpargel KB, Jalabi W, Jin Y, Dadabayev A, Penn MS, Trapp BD: Preconditioning paradigms and pathways in the brain. Clev Clin J Med 75 Suppl 2:77-82, 2008

39. Simon RP, Cho H, Gwinn R, Lowenstein DH: The temporal profile of 72-kDa heat-shock protein expression following global ischemia. J Neurosci 11(3):881-889, 1991

40. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, Aupetit JF, Bonnefoy E, Finet G, André-Fouët X, Ovize M: Postconditioning the human heart. Circulation. 4;112(14): 2143-2148, 2005

41. Stagliano NE, Perez-Pinzon MA, Moskowitz MA, Huang PL: Focal ischemic preconditioning induces rapid tolerance to middle cerebral artery occlusion in mice. J Cerebr Blood F Met 19:757–761, 1999

42. Stenzel-Poore MP, Stevens SL, Xiong Z, Lessov NS, Harrington CA, Mori M, Meller R, Rosenzweig HL, Tobar E, Shaw TE, Chu X, Simon RP: Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similari- ty to neuroprotective strategies in hibernation and hypoxia-tolerant states. Lancet 362:1028–1037, 2003

43. Stenzel-Poore MP, Stevens SL, King JS, Simon RP: Preconditioning reprograms the response to ischemic injury and primes the emergence of unique endogenous neuroprotective phenotypes: A speculative synthesis. Stroke 38:680-685, 2007

44. Sugino T, Nozaki K, Takagi Y, Hashimoto N: 3-Nitropropionic acid induces ischemic tolerance in gerbil hippocampus in vivo. Neurosci Lett 259:9–12, 1999

45. Tasaki K, Ruetzler CA, Ohtsuki T, Martin D, Nawashiro H, Hallenbeck JM: Lipopolysaccharide pre-treatment induces resistance against subsequent focal cerebral ischemic damage in spontaneously hypertensive rats. Brain Res 748:267–270, 1997

46. Taga K, Patel PM, Drummond JC, Cole DJ, Kelly PJ: Transient neuronal depolarization induces tolerance to subsequent forebrain ischemia in rats. Anesthesiology. 87(4):918-925, 1997

47. Taskapilioglu MO, Alkan T, Goren B, Tureyen K, Sahin S, Taskapilioglu O, Korfali E: Neuronal protective effects of focal ischemic pre- and/or postconditioning on the model of transient focal cerebral ischemia in rats. J Clin Neurosci 16:693-697, 2009

48. Tsuchiya D, Hong S, Matsumori Y, Shiina H, Kayama T, Swanson RA, Dillman WH, Liu J, Panter SS, Weinstein PR: Overexpression of rat heat shock protein 70 is associated with reduction of early mitochondrial cytochrome C release and subsequent DNA fragmentation after permanent focal ischemia. J Cerebr Blood F Met 23(6):718-727, 2003

49. Xing B, Chen H, Zhang M, Zhao D, Jiang R, Liu X, Zhang S: Ischemic postconditioning inhibits apoptosis after focal cerebral ischemia/reperfusion injury in the rat. Stroke 39(8):2362-2369, 2008

50. van der Worp HB, Sena ES, Donnan GA, Howells DW, Macleod MR: Hypothermia in animal models of acute ischaemic stroke: A systematic review and meta-analysis. Brain 130:3063–3074, 2007

51. Yunoki M, Nishio S, Ukita N, Anzivino MJ, Lee KS: Hypothermic preconditioning induces rapid tolerance to focal ischemic injury in the rat. Exp Neurol 181:291–300, 2003

52. Zhao H, Sapolsky RM, Steinberg GK: Interrupting reperfusion as a stroke therapy: ischemic postconditioning reduces infarct size after focal ischemia in rats. J Cerebr Blood F Met 26(9):1114-1121, 2006

53. Zhao L, Nowak TS Jr: CBF changes associated with focal ischemic preconditioning in the spontaneously hypertensive rat. J Cerebr Blood F Met 26:1128–1140, 2006

54. Zheng S, Zuo Z: Isoflurane preconditioning induces neuroprotection against ischemia via activation of P38 mitogen-activated protein kinases. Mol Pharmacol 65:1172–1180, 2004