Metabolic Downregulation:

A Key to Successful Neuroprotection?

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Abstract

Background and Purpose—The search for effective neuroprotectants remains frustrating, particularly with regard to specific pharmaceuticals. However, laboratory studies have consistently shown remarkable neuroprotection with 2 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 neurological outcome from cardiac arrest.

Methods—A systematic review of both topics is presented in terms of underlying pathophysiological mechanisms and application at the clinical level.

Results—Although the mechanisms of protection for both therapeutic strategies are multifold, both share features of downregulating metabolism. Both therapeutic strategies are robust neuroprotectants, but translating them to the clinical arena is challenging, though not impossible, and clinical studies have shown or suggest benefits of both treatments.

Conclusions—The strategy of metabolic downregulation should be further explored to identify effective neuroprotectants that can be easily applied clinically.

Keywords

cerebral ischemia; ischemic tolerance; hypothermia

Although the search for effective neuroprotectants for use in acute ischemic stroke remains frustrating, insights from laboratory studies have identified 2 different interventions that consistently result in neuroprotection across multiple labs and paradigms. They are therapeutic hypothermia, or cooling the brain to ranges between 28 to 34°C, and ischemic preconditioning preconditioning, the phenomenon that brief periods of sublethal ischemia can protect against a subsequent lethal one. Recent work has indicated that both are associated with downregulation of cellular metabolism and gene transcription. Understanding the mechanism of protection could identify important therapeutic targets and eventual translation of these

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neuroprotective strategies to humans. This review will cover the basic science underpinnings of these concepts and their potential translation for use in humans.

Therapeutic Hypothermia

Brain cooling is well known to limit injury attributable to ischemic insults. Deep hypothermia (<25°C) has long been used during high-risk surgery to protect the brain from ischemic insults. Mild to moderate hypothermia (cooling in the range of 28 to 34°C) provides similar improvement in neurological outcome with fewer deleterious side effects. Numerous laboratory studies have consistently shown that mild hypothermia protects against ischemic injury (see reviews [1, 2]).

Mechanisms of Hypothermic Protection

Hypothermia and Its Effect on Metabolism and Cerebral Blood Flow—Cerebral metabolism, reflected in estimates of oxygen consumption, glucose utilization, and lactate levels, is temperature-dependent, with hypothermia decreasing each of these parameters (see reviews [3, 4]). On average, hypothermia reduces brain oxygen consumption by approximately 5% per degree Celsius fall in body temperature in the range of 22 to 37°C, and in anesthetized animals CMRO2 decreases linearly between 8 to 37°C. The Q10 for CMRO2, or the ratio of metabolism at 10C intervals, has been estimated at 2.5. The Q10 is remarkably consistent between various laboratory reports and appears to be independent of age and species.

Under injury conditions, hypothermia preserves losses of high-energy organic phosphates and slows the rates of metabolite consumption and lactic acid accumulation and reduces CMRO2, while improving glucose utilization. Hypothermia was originally thought to protect the brain by reducing cerebral metabolism during conditions of reduced substrate and shift to anaerobic glycolysis. However, hypothermia slows but does not completely prevent the eventual depletion of ATP, and several other studies suggest that metabolism is not significantly altered in the face of remarkable neuroprotection. For example, rodents subjected to 20 minutes of forebrain ischemia showed marked protection, but brain levels of various metabolites were no different from normothermic ischemic controls. Thus, the influence of hypothermia on cerebral metabolism probably does not fully explain its protective effect. Cerebral metabolic rate should be reduced by ≈20% to 25% at a brain temperature of 33°C, but the extent of protection as documented by several labs (on the order of 50% to 80%[2]) greatly exceeds this. Therefore, it is likely that hypothermia influences other factors in such a way as to lead to neuroprotection.

The effect of hypothermia on cerebral metabolism in humans under injury states is similar to that reported in laboratory studies. The most widely studied clinical condition in which changes in cerebral metabolism attributable to hypothermia have been documented is traumatic brain injury. Most studies report a substantial decrease in CMRO2 during mild-to-moderate (32 to 34°C) hypothermia compared to normothermia. Therapeutic hypothermia (32 to 33°C) in patients who suffered brain trauma led to decreased CMRO2 by about 45%, which further decreased to 27% after 24 hours.

Cooling the brain can also lead to decreases in cerebral blood flow (CBF). In noninjury conditions, CBF decreases linearly between temperatures of 18 to 37°C, and coupling between CBF and brain metabolism was preserved at different temperatures. Focal and global ischemia lead to initial decreases in CBF, but on reperfusion, hyperemia occurs followed by gradual CBF decline. However, data regarding the influence of hypothermia on CBF are conflicting. Some studies indicate that hypothermia actually increases CBF during the period of ischemia, whereas other reports note reduced CBF or no effect.[4] Mild hypothermia to 33°C also appears to blunt the early postreperfusion hyperemia and prevent the gradual decrease in CBF during reperfusion. The reasons for these differences are not entirely clear but may be attributable to

experimental variables such as the type of anesthesia used, inherent difficulties in detecting regional differences using laser Doppler flowmetry, and the time points studied.

Like the data reported in experimental studies, reports of hypothermia on cerebral blood flow at the clinical level have also been inconsistent. During cardiopulmonary bypass, cerebral blood flow declines as temperature is lowered in some reports but increased in other studies.[1,5] In patients with severe traumatic brain injury, mild-to-moderate (32 to 34°C) hypothermia decreases CBF in most studies,[6,7] although some studies have reported no change.[8] Such differences may be explained in part by the severity of injury or the timing of measurements. With the renewed interest in therapeutic hypothermia, it is likely that more clinical data will become available in the future.

Hypothermia and Reduction of Glutamate Release—Several studies have now correlated hypothermic protection with the suppression of glutamate accumulation or release (reviewed by Liu et al[5]). ATP loss occurring during ischemia leads to ions flowing down their concentration gradients, and eventual efflux of potassium and influx of sodium and calcium. Calcium influxes lead to direct neurotoxicity as well as extracellular accumulation of glutamate, which are well known to be damaging. Consistent with the notion that hypothermia downregulates metabolism, experimental studies have shown that mild to moderate hypothermia attenuates the initial and delayed rise of extracellular potassium (Ke) and prevents intracellular calcium accumulation, thus leading to decreased glutamate efflux. However, hypothermia, particularly delayed hypothermia, may protect by other mechanisms, because protective effects do not always correlate to glutamate release.

Hypothermia, Oxidative Stress, and Blood-Brain Barrier Effects—Numerous studies have shown salutary effects of hypothermia on reducing the generation of reactive oxygen species, reduction of brain edema, and prevention of blood-brain barrier (BBB) breakdown (reviewed recently[5]). One potential mechanism is that hypothermia inhibits matrix metalloproteinases and preserves basal lamina proteins after stroke.[9-11]

Hypothermia and Gene Regulation, Inflammation, and Apoptosis—The effect of hypothermia has also been correlated to favorable changes in gene expression, antiinflammatory, and antiapoptotic effects with enhancement of recovery processes. Hypothermia has been documented by numerous investigators to alter gene expression normally observed after brain ischemia. Whereas a majority of genes are downregulated by hypothermia, a number of genes are also upregulated.[12] Interestingly, many genes thought to be detrimental to ischemic injury (proinflammatory, proapoptotic) tend to be downregulated, [13-15] whereas those that may play a beneficial role (cell survival and trophic genes) seem to be upregulated.[16-20] Certainly, this model is naïve, as protective genes are also decreased by hypothermia, such as some stress proteins.[21] The reasons for differential gene expression are unclear but could be attributable to a temperature dependence of transcription factor(s)[22,23] or upregulation of cold shock proteins (see recent review [24]).

A recent study using gene profiling in an experimental stroke model showed that many genes downregulated by hypothermia appear to be related to inflammation.[12] Consistent with these observations, hypothermia has been shown to inhibit activation of the inflammatory transcription factor nuclear factor kappa B (NFkB) via temperature-dependent inhibition of its inhibitor protein's kinase, IKK.[23] Other studies indicate that hypothermia has antiapoptotic effects such as reduction of cytochrome C release, and inhibition of caspases and proapoptotic genes.[25-29] Interestingly, although hypothermia downregulates a majority of cell death pathways, it also seems to upregulate cell survival pathways such as Akt[16] or ERK[22,30] and increase trophic factor secretion.[17-20]

Conditions for Optimal Hypothermic Protection

For hypothermia to be reliably applied at the clinical level, it is critical to understand the depth and duration of cooling, as well as whether cooling can be delayed and whether it is effective against ischemia without reperfusion (see reviews by Krieger and Yenari[2,31]). In general, hypothermia appears to be effective whether the brain is cooled to 33°C or 28°C, but temperatures on the lower end appeared to be most effective according to a recent meta-analysis of the experimental literature.[31] Protection is similar if cooling is maintained for at least 1 hour and not delayed more than 3 hours after stroke onset. However, whereas cooling applied during both the ischemic period and shortly into the reperfusion period produces long lasting neuroprotection, neuroprotection from cooling initiated only during reperfusion was transient. Interestingly, long-term (6 months) protection could be seen in a global cerebral ischemia model provided cooling to 32 to 33°C was maintained for 24 hours, and protection was still observed if cooling was delayed 6 hours and maintained for 48 hours. Conflicting data exist as to whether hypothermia is also effective against ischemia without reperfusion, with some reports showing a lack of efficacy and others showing only modest effects.

Combination Therapy With rt-PA

A few studies have addressed whether hypothermia could be used in combination with thrombolytics, as these are the only treatments available to patients with acute ischemic stroke. Whereas an in vitro study suggested that the thrombolytic efficacy of rt-PA is directly proportional to incubation temperature,[32] 2 studies have attempted to address this question using in vivo stroke model. However, neither have convincingly shown that combination is superior to either treatment alone.[33,34] Nevertheless, together with the notion that hypothermia is less or ineffective against ischemia without reperfusion, it is clear that to obtain a maximal effect at the clinical level, hypothermia should be combined with recanulization.

Furthermore, because hypothermia is known to reduce edema and BBB disruption, it has the potential to reduce intracerebral hemorrhage after thrombolytic therapy for acute ischemic stroke. One study of hemorrhagic transformation in the mouse showed that hypothermia prevented rt-PA–induced exacerbated BBB disruption and gross cerebral bleeds.[35]

Clinical Application of Hypothermia

Clinical Trials of Therapeutic Hypothermia—Recently, 2 randomized controlled trials of therapeutic hypothermia in patients suffering cardiac arrest were published simultaneously (see reviews [1,36]). Both studies showed that therapeutic hypothermia in this patient population was associated with better survival and neurological outcome. The main difference in the studies is that the Australian group allowed cooling to commence in the field, rather than waiting until patients arrived to the hospital. Thus, the average time required to reach target temperature was shorter in the Australian study (2 hours)[37] compared to the European study (8 hours).[38] These 2 trials show that delayed mild hypothermia improves mortality and neurological outcome in patients who suffer cardiac arrest. As a result of these trials, therapeutic hypothermia is becoming a standard of care for victims of cardiac arrest in several centers.

Clinical trials of therapeutic hypothermia in neonates with hypoxic encephalopathy also suggest a benefit in this patient population. To date, there have been two large multicenter randomized studies of newborn infants with hypoxic ischemic encephalopathy. One trial showed that term infants with moderate but not severe neonatal encephalopathy and abnormal EEGs subjected to head cooling for 72 hours (rectal temperature 34 to 35°C) within 6 hours of birth showed a benefit that persisted at 18 months of life.[39] In another trial, whole-body cooling to 33.5°C for 72 hours in infants with moderate or severe encephalopathy reduced the risk of death or moderate or severe disability assessed at 18 to 22 months.[40]

Patients with brain trauma do not appear to benefit from cooling. In a large phase III trial, patients with brain trauma who were cooled within 6 hours of injury to a body temperature of 33°C for 48 hours did no better than those maintained at normal temperatures.[41] However, there was suggestion that patients who were already hypothermic at the time of presentation, and were kept cool had a better neurological outcome than those who were either rewarmed or presented normothermic and were later cooled.

Whether hypothermia is effective in human stroke patients is unknown, but a few pilot studies of hypothermia for stroke have been published (reviewed recently [1, 42]). In essence, these studies have collectively shown that mild therapeutic hypothermia is feasible, though not completely without complications. Some side effects seen with very deep cooling (lower than 33°C), include thrombocytopenia, bradycardia, moderate hypotension, hypokalemia, pneumonia, pancreatitis, coagulopathy, and cardiac failure. A significant challenge in applying hypothermia to stroke patients is that unlike other neurological conditions, stroke patients are generally awake and do not tolerate cooling. Similar to problems encountered in the cardiac arrest and brain injury studies, attaining target temperature and maintaining it, especially in adult humans, is challenging. Another issue in applying hypothermia to patients with stroke is the rebound increased intracranial pressure experienced during rewarming, a phenomenon not well studied in laboratory models.[43]

Two clinical studies have used intravascular cooling devices to cool acute stroke patients. COOL-AID[44] was a randomized controlled study of 40 patients presenting within 12 hours of symptom onset, of which 18 patients received cooling and 22 received standard medical management. Most patients tolerated hypothermia, and clinical outcomes were similar in both groups although there was a suggestion of reduced lesion growth on diffusion-weighted imaging (DWI). ICTuS, another nonrandomized clinical trial using a different intravascular cooling device, studied 18 acute stroke patients cooled within 12 hours of symptom onset.[45] Patients could also receive intravenous rt-PA, if appropriate. Overall, these patients also tolerated cooling, and the incidence of cerebral hemorrhage did not appear increased among the small number of patients (n=5) given rt-PA. ICTuS-L, a study to examine hypothermia and rt-PA treatment in stroke patients with a longer time window for initiation of rt-PA (6 hours), is ongoing.[46] Preliminary results do not suggest any serious safety issues in this study.

Cooling Methods—Surface cooling is a widely used method of cooling in many clinical trials. These methods include convective air blankets, water mattresses, alcohol bathing, and ice packing, but these strategies have been limited by a slow rate of temperature change, target temperature overshoot, poor control at the target temperature, and cumbersome administration.[1,42] Awake patients also do not tolerate surface cooling because of shivering and general discomfort. To overcome the problems with surface cooling and other techniques, several groups proposed endovascular, or internal cooling which reduces shivering as simultaneous surface warming is possible.[47] Chilled saline infusions promote rapid reduction of core temperatures in anesthetized patients but require large infusion volumes. Experimental studies have also explored the possibility of retrograde perfusion of cooled blood through the internal jugular in pigs.[48] This latter approach led to rapid reduction of ipsilateral brain temperature which could be controlled by adjusting perfusate temperature; however, this has not been studied yet in humans. Recently, 3 endovascular catheter-based devices have been used in preliminary safety trials to cool patients internally. Cooling rates of 1.5C/h have been achieved using these devices, with greater patient comfort provided modest sedation with meperidine is used. The ICTuS study[45] established an effective protocol to manage shivering in patients cooling endovascularly using meperidine and buspirone.

In the infant hypoxic encephalopathy studies, whole-body cooling or selective head cooling was feasible, possibly because of the smaller body size. Comparison of whole-body cooling or selective head cooling showed similar decreases in MRI-detected brain lesions.[49]

There is also a body of literature reporting pharmacological approaches to cooling. Rather than subjecting patients to mechanical devices, it may be possible to administer a potentially effective drug to decrease temperature. Neurotensin is an endogenous peptide involved in circadian temperature regulation, and analogues have been developed that penetrate the BBB. Single intraperitoneal injections to rats decreased body temperatures by about 5°C within 1 hour, and cooling could be maintained for about 7 hours.[50-52] Neurotensin analogues reduced oxygen consumption and appeared to be well tolerated as estimated by behavior assays. Furthermore, the neurotensin analogue NT77 was shown to provide comparable neuroprotection as surface cooling in a rodent model of cardiac arrest.[53] 3-iodothyronamine (T1AM), a naturally occurring derivative of thyroxine, rapidly induced hypothermia in rodents.[54] Mice were given thyronamines IP, and rapid (minutes) decreases in body temperature to as low as 28°C were observed. Temperature decreases persisted as long as 3 to 4 hours after a single injection. However, T1AM was not without side effects, as significant bradycardia and reduced cardiac output was also observed. A recent report showed that T1AM and thryonamine (T0AM) given before the onset of ischemia could reduce body temperature in mice from 37°C to 31°C for several hours, and led to ≈30% reduction in infarct size.[55] Hydrogen sulfide (H2S), possibly by buffering unrestricted oxygen consumption, has also been reported to reduce body temperature to as low as 15°C within 6 hours (30°C in less than 1 hour) and reduce metabolic rate by 90%. It also appears to prolong survival in mice exposed to lethal hypoxia.[56] Whether any of these approaches could be applied in humans has yet to be addressed, but are clearly worthy areas of investigation.

Tolerance/Preconditioning and Metabolic Depression

The phenomenon of ischemic preconditioning (IPC) is that a sublethal insult, in this case brief periods of ischemia, lead to protection against subsequent lethal insults. The field of IPC is highly complex and involves a great diversity of metabolic pathways. The objective of this section is to review the field of ischemic tolerance in terms of metabolic depression. For more comprehensive reviews of the field of ischemic preconditioning, the reader is invited to read more specialized reviews.[57-61] Metabolic depression has been suggested to play a role in the phenomena of IPC or ischemic tolerance.[62] In fact, it is plausible that the field of IPC emerged from studies describing adaptations to extreme environmental conditions found in nature. Hypoxic or anoxic tolerance is found ubiquitously in nature, and hibernation is another natural adaptation where extreme low-blood flow perfusion occurs. A common feature in most anoxic tolerant species or during hibernation is a pronounced metabolic depression.

The field of "ischemic preconditioning" (IPC) or "ischemic tolerance" has exploded, and the induction of tolerance against ischemia resulting from sublethal ischemic or anoxic insults has gained attention as a robust neuroprotective mechanism against conditions of extreme metabolic stress such as anoxia/ischemia in heart and brain (see review [63]). There are a large number of different paradigms used to induce ischemic tolerance. For example, sublethal global[64] or focal cerebral ischemia,[65] cortical spreading depression,[66,67] chronic hypoxia,[68] hypothermia,[69] and different pharmacological agents[70-72] have been used to induce ischemic tolerance.

Different IPC Windows

Since 1986, studies have established a "rapid" or "acute" window. This type of window occurs when the sublethal insult precedes the lethal ischemic insult within 1 hour. A second window was first identified in brain when Kitagawa et al[64] demonstrated that a sublethal insult could

precede the lethal insult by as much as 24 hours. In fact it is now clear that this 2nd window is still active over several days and is thus termed as "delayed" window. Interestingly, many of the same triggering pathways that activate the 1st window also activate the 2nd window (for review see [60]). A distinct difference of this 2nd window is that at least in brain neuroprotection against cerebral ischemia is more lasting. For example, "rapid" IPC provided significant neuroprotection against histopathology for at least 3 days after global cerebral ischemia, but that protection nearly disappeared after 7 days.[73]

A major effort in this field is the elucidation of the mechanisms that induce ischemic tolerance. One question that arises in this field is whether IPC induces metabolic depression as it is known to occur in anoxic tolerance in lower vertebrate species or during hibernation. As noted earlier, it is known that turtle brains undergo major metabolic depression by reducing electric activity. Though there is no direct evidence that electric activity in mammalian brain is particularly depressed after IPC, there is evidence that inhibitory pathways are enhanced. Many groups have demonstrated that IPC promotes tolerance by ameliorating different aspects of neuronal excitotoxicity. For example, glutamate release during ischemia was ameliorated by several forms of preconditioning.[74-76] IPC appears to inhibit excitatory pathways by downregulating AMPA[77] and NMDA receptors.[78] Decreases in glutamate release were accompanied by increases in GABA release. This enhanced GABA release was suggested to be neuroprotective[75,76] and linked to downregulation of the glial glutamate transporter isoforms EAAT2 and EAAT1[74] and upregulation of $GABA_A$ receptors.[79] These results are indicative of a relative shift from excitatory to inhibitory neurotransmission after IPC that may promote ischemic tolerance. These results also suggest that IPC promotes a downregulation in brain metabolic rate and thus reduce energy consuming pathways.

A key in neuroprotection in anoxic tolerance is the increase in anoxic energy producing pathways (ie, Pasteur Effect). Although this is not possible during ischemia because of substrate limitation, 2 alternative pathways have been described in IPC. First, IPC is known to preserve normal mitochondrial function after "test" ischemia,[80] indicating that mitochondria have altered their normal functional state after IPC. Secondly, IPC also promotes some signaling pathways that induce upregulation of genes involved in energy metabolism.

For a comprehensive review of the signaling pathways activated by different types of preconditioning, see the review by Gidday.[60] In particular, one signaling pathway directly involved in glucose metabolism and angiogenesis, two potential mechanisms geared to enhance energy production, is that of the hypoxia-inducible factor (HIF).[68,81-83] Here, IPC stimulates anaerobic glycolysis[84] and preserves mitochondrial function.[85] Hypoxic preconditioning induces tolerance to hypoxic-ischemic injury in neonatal rat brain and was associated with changes in gene expression that result in increased the expression of GLUT-1 mRNA and protein, and of aldolase, phosphofructokinase, and lactate dehydrogenase proteins but not mRNA.[86]

IPC also promotes mitochondrial protection. IPC was correlated with mitochondrial protection against the deficits in respiration affecting complexes I to IV.[80] Many studies have demonstrated that reactive oxygen species (ROS) and the resulting oxidative stress play a pivotal role in neuronal cell death.[87,88] There are two major regions in the electron transport chain where ROS are produced. One is complex I and the other is complex III. Because oxidative stress is implicated in the pathophysiology that ensues after cerebral and cardiac ischemia,[89] one can surmise that a key mechanism by which IPC in the second window protects hippocampus against delayed neuronal cell death is by protecting mitochondrial oxidative phosphorylation.

Clinical Application of Tolerance

The most direct and significant application of understanding the mechanisms of ischemic preconditioning is therapeutic access to this protective state, especially during cardiopulmonary bypass surgery, cardiac transplantation and other types of brain surgeries, where necessary periods of ischemia are provoked in healthy tissue to repair other cardiac or CNS anomalies.[57] Another potential therapy is preemptive treatment of an identified subpopulation at risk for stroke (eg, metabolic syndrome) as defined in the AHA/ASA Guidelines.[90] A major effort in this field is the identification of a drug(s) that can emulate ischemic preconditioning and can be given either acutely (for surgical procedures) or chronically for a subpopulation at risk of stroke.

TIAs and Ischemic Tolerance—Another question that has emerged in the clinical arena is whether transient ischemic attacks are in fact a type of ischemic preconditioning. TIA is defined as a brief episode of neurological dysfunction caused by focal brain or retinal ischemia, with clinical symptoms typically lasting less than 1 hour, and without evidence of acute infarction. A number of studies support that ischemic tolerance may play a role in patients with TIA who have a subsequent ischemic stroke. In 1999, Weih et al[91] analyzed 148 ischemic stroke patients with TIA (n=37) and without TIA (n=111) in terms of stroke severity and outcome in a retrospective case–control study. They showed that TIA before stroke was an independent predictor of mild stroke after correction for other cardiovascular risk factors. Since then, 5 reports published all supported the notion that TIA has a neuroprotective effect in stroke patients. In Lausanne Stroke Registry, Moncayo et al[92] divided a total of 2490 patients admitted for first ever cerebral infarction in the anterior circulation into 2 groups on the basis of the presence or absence of prior ipsilateral TIAs. Overall, patients with previous TIAs (n=263) had a more favorable outcome than those without TIA (n=2086; 67% versus 58%, *P*=0.004). In particular, TIAs lasting 10 to 20 minutes were associated with favorable outcome after adjusting for confounding variables. Furthermore, they showed that patients who experienced TIAs less than 1 week before the cerebral infarct have a higher proportion of favorable outcome (73%) than those with a TIA 1 to 4 weeks before cerebral infarct (66%) and with a TIA more than 4 weeks before (53%). Castillo et al[93] studied 283 patients with ischemic stroke including atherothrombotic (n=134) and cardioembolic infarcts (n=149) and divided those patients with and without relevant TIA. The infarct volume measured on CT on days 4 to 7 and the frequency of poor outcome at 3 months in both atherothrombotic and cardioembolic infarcts groups were significantly lower in patients with relevant TIA. Wegener et al[94] investigated 65 patients with ischemic territorial stroke that received diffusion- (DWI) and perfusion-weighted MRI (PWI) within 12 hours of symptoms onset. They compared the size of lesion volume for DWI, mean transit time, cerebral blood flow, cerebral blood volume for PWI, and infarct volume fro T2-weighted images at 3 to 7 days between patients with prodromal TIA (n=16) and without TIA (n=49). Although patients both with and without TIA show similar size and severity of the perfusion deficits, initial diffusion lesions tended to be smaller and final infarct volume were significantly smaller in patients with TIA than in those without TIA, which was even more pronounced when only TIA <4 weeks were analyzed. Arboix et al[95] analyzed 1753 patients with ischemic stroke admitted to the hospital within 48 hours. Among them, 221 patients had experienced a previous TIA. The percentage of nonlacunar stroke patients (n=1269) with favorable putcome was significantly higher in those with a history of TIA (21.7%) than that without TIA (15.0%). However, in the lacunar stroke group (n=484), the percentage with favorable outcome was similar between those with TIA (27.3%) and without TIA (27.0%). Recently, Schaeller[96] investigated 130 patients treated with local intraarterial thrombolysis after ischemic stroke attributable to occlusion of a main cerebral artery in a retrospective case–control study. Eleven patients (8%) demonstrated prestroke ipsilateral TIA in the same vascular territory. Despite no significant differences in baseline characteristics such as NIHSS on admission and recanalization, patients with prestroke TIA

demonstrated a more favorable outcome and modified Rankin score compared to patients without TIA.

Based on the findings above, it seems that TIAs induce ischemic tolerance in the human brain. The protective effect of TIA is observed in patients with nonlacunar cerebral infarction and when TIAs occur weeks before cerebral infarction and lasts for more than 10 minutes. However, it should be noted that in a cohort study of more than 1700 patients presenting with TIA in Northern California failed to show a correlation between the occurrence of TIA and reduced disability attributable to subsequent stroke.[97] The reasons for these discrepancies are unclear, but it is difficult to control certain factors in clinical settings such as the duration and timing of TIAs relative to the occurrence of stroke.

Rapid Ischemic Preconditioning in the Heart—Reduced ATP Utilization as a Key Factor—In the heart, there is a "rapid" or "first" window of preconditioning, and there was a reduction in ATP utilization which could be responsible for delaying ischemic cell death, suggesting metabolic downregulation as a key factor for "rapid" ischemic preconditioning.[98] In humans, numerous studies have shown that preinfarction angina is associated with smaller infarcts, [99,100] and although patients with and without preinfarction angina had similar areas at risk, infarct size was smaller in patients with preinfarction angina.[101] With respect to percutaneous transluminal coronary angioplasty, cardiac protection was conferred with attenuated ischemia on subsequent balloon inflations, suggesting another human model for preconditioning. By studying specimens from patients undergoing coronary artery bypass surgery, Yellon et al[102] showed that the rate of ATP depletion slowed during ischemia when patients underwent preconditioning with 2 3-minute periods of cross-clamping. Both animal and human findings indicate that reduced ATP utilization, and thus, metabolic downregulation, was a key factor for "rapid" ischemic preconditioning in the heart.

Conclusions

Clearly, hypothermia and ischemic tolerance have been shown to have the most consistent and robust effects in the laboratory with promising results at the clinical level. Although seemingly disparate approaches, recent work has suggested that both may have the overlapping mechanism of downregulating metabolism. This theme of downregulation should therefore stimulate more research toward finding an effective neuroprotectant.

Acknowledgements

References

1. Lyden P, Krieger DW, Yenari MA, Dietrich WD. Therapeutic hypothermia for acute stroke. Int J Stroke 2006;1:9–19. [PubMed: 18706063]

2. Krieger DW, Yenari MA. Therapeutic hypothermia for acute ischemic stroke: What do laboratory studies teach us? Stroke 2004;35:1482–1489. [PubMed: 15073396]

3. Erecinska M, Thoresen M, Silver IA. Effects of hypothermia on energy metabolism in mammalian central nervous system. J Cereb Blood Flow Metab 2003;23:513–530. [PubMed: 12771566]

4. Yenari, M.; Wijman, C.; Steinberg, G. Effects of hypothermia on cerebral metabolism, blood flow and autoregulation. In: Mayer, S.; Sessler, D., editors. Hypothermia in Neurocritical Care. Marcel Dekker, Inc; New York: 2004. p. 141-178.

5. Liu L, Yenari MA. Therapeutic hypothermia: Neuroprotective mechanisms. Front Biosci 2007;12:816–825. [PubMed: 17127332]

6. Marion DW, Penrod LE, Kelsey SF, Obrist WD, Kochanek PM, Palmer AM, Wisniewski SR, DeKosky ST. Treatment of traumatic brain injury with moderate hypothermia. N Engl J Med 1997;336:540–546. [PubMed: 9023090]

7. Vigue B, Ract C, Zlotine N, Leblanc PE, Samii K, Bissonnette B. Relationship between intracranial pressure, mild hypothermia and temperature-corrected paco2 in patients with traumatic brain injury. Intensive Care Med 2000;26:722–728. [PubMed: 10945389]

8. Metz C, Holzschuh M, Bein T, Woertgen C, Frey A, Frey I, Taeger K, Brawanski A. Moderate hypothermia in patients with severe head injury: Cerebral and extracerebral effects. J Neurosurg 1996;85:533–541. [PubMed: 8814152]

9. Wagner S, Nagel S, Kluge B, Schwab S, Heiland S, Koziol J, Gardner H, Hacke W. Topographically graded postischemic presence of metalloproteinases is inhibited by hypothermia. Brain Res 2003;984:63–75. [PubMed: 12932840]

10. Hamann GF, Burggraf D, Martens HK, Liebetrau M, Jager G, Wunderlich N, DeGeorgia M, Krieger DW. Mild to moderate hypothermia prevents microvascular basal lamina antigen loss in experimental focal cerebral ischemia. Stroke 2004;35:764–769. [PubMed: 14976330]

11. Lee JE, Yoon YJ, Moseley ME, Yenari MA. Reduction in levels of matrix metalloproteinases and increased expression of tissue inhibitor of metalloproteinase-2 in response to mild hypothermia therapy in experimental stroke. J Neurosurg 2005;103:289–297. [PubMed: 16175859]

12. Ohta H, Terao Y, Shintani Y, Kiyota Y. Therapeutic time window of post-ischemic mild hypothermia and the gene expression associated with the neuroprotection in rat focal cerebral ischemia. Neurosci Res 2007;57:424–433. [PubMed: 17212971]

13. Han HS, Qiao Y, Karabiyikoglu M, Giffard RG, Yenari MA. Influence of mild hypothermia on inducible nitric oxide synthase expression and reactive nitrogen production in experimental stroke and inflammation. J Neurosci 2002;22:3921–3928. [PubMed: 12019311]

14. Xu L, Yenari MA, Steinberg GK, Giffard RG. Mild hypothermia reduces apoptosis of mouse neurons in vitro early in the cascade. J Cereb Blood Flow Metab 2002;22:21–28. [PubMed: 11807390]

15. Van Hemelrijck A, Hachimi-Idrissi S, Sarre S, Ebinger G, Michotte Y. Post-ischaemic mild hypothermia inhibits apoptosis in the penumbral region by reducing neuronal nitric oxide synthase activity and thereby preventing endothelin-1-induced hydroxyl radical formation. Eur J Neurosci 2005;22:1327–1337. [PubMed: 16190888]

16. Zhao H, Shimohata T, Wang JQ, Sun G, Schaal DW, Sapolsky RM, Steinberg GK. Akt contributes to neuroprotection by hypothermia against cerebral ischemia in rats. J Neurosci 2005;25:9794–9806. [PubMed: 16237183]

17. D'Cruz BJ, Fertig KC, Filiano AJ, Hicks SD, DeFranco DB, Callaway CW. Hypothermic reperfusion after cardiac arrest augments brain-derived neurotrophic factor activation. J Cereb Blood Flow Metab 2002;22:843–851. [PubMed: 12142569]

18. Schmidt KM, Repine MJ, Hicks SD, DeFranco DB, Callaway CW. Regional changes in glial cell line-derived neurotrophic factor after cardiac arrest and hypothermia in rats. Neurosci Lett 2004;368:135–139. [PubMed: 15351435]

19. Boris-Moller F, Kamme F, Wieloch T. The effect of hypothermia on the expression of neurotrophin mrna in the hippocampus following transient cerebral ischemia in the rat. Brain Res Mol Brain Res 1998;63:163–173. [PubMed: 9838092]

20. Vosler PS, Logue ES, Repine MJ, Callaway CW. Delayed hypothermia preferentially increases expression of brain-derived neurotrophic factor exon iii in rat hippocampus after asphyxial cardiac arrest. Brain Res Mol Brain Res 2005;135:21–29. [PubMed: 15857665]

21. Hicks SD, DeFranco DB, Callaway CW. Hypothermia during reper-fusion after asphyxial cardiac arrest improves functional recovery and selectively alters stress-induced protein expression. J Cereb Blood Flow Metab 2000;20:520–530. [PubMed: 10724117]

22. Hicks SD, Parmele KT, DeFranco DB, Klann E, Callaway CW. Hypothermia differentially increases extracellular signal-regulated kinase and stress-activated protein kinase/c-jun terminal kinase activation in the hippocampus during reperfusion after asphyxial cardiac arrest. Neuroscience 2000;98:677–685. [PubMed: 10891611]

23. Han HS, Karabiyikoglu M, Kelly S, Sobel RA, Yenari MA. Mild hypothermia inhibits nuclear factor-kappab translocation in experimental stroke. J Cereb Blood Flow Metab 2003;23:589–598. [PubMed: 12771574]

24. Han HS, Yenari MA. Effect of gene expression by therapeutic hypothermia in cerebral ischemia. Future Neurology 2007;2:435–440.

25. Zhao H, Yenari MA, Cheng D, Sapolsky RM, Steinberg GK. Biphasic cytochrome c release after transient global ischemia and its inhibition by hypothermia. J Cereb Blood Flow Metab 2005;25:1119–1129. [PubMed: 15789032]

26. Zhang Z, Sobel RA, Cheng D, Steinberg GK, Yenari MA. Mild hypothermia increases bcl-2 protein expression following global cerebral ischemia. Brain Res Mol Brain Res 2001;95:75–85. [PubMed: 11687278]

27. Yenari MA, Iwayama S, Cheng D, Sun GH, Fujimura M, Morita-Fujimura Y, Chan PH, Steinberg GK. Mild hypothermia attenuates cytochrome C release but does not alter bcl-2 expression or caspase activation after experimental stroke. J Cereb Blood Flow Metab 2002;22:29–38. [PubMed: 11807391]

28. Inamasu J, Suga S, Sato S, Horiguchi T, Akaji K, Mayanagi K, Kawase T. Postischemic hypothermia attenuates apoptotic cell death in transient focal ischemia in rats. Acta Neurochir Suppl 2000;76:525–527. [PubMed: 11450083]

29. Prakasa Babu P, Yoshida Y, Su M, Segura M, Kawamura S, Yasui N. Immunohistochemical expression of bcl-2, bax and cytochrome c following focal cerebral ischemia and effect of hypothermia in rat. Neurosci Lett 2000;291:196–200. [PubMed: 10984640]

30. D'Cruz BJ, Logue ES, Falke E, DeFranco DB, Callaway CW. Hypothermia and ERK activation after cardiac arrest. Brain Res 2005;1064:108–118. [PubMed: 16289484]

31. 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 2007;130:3063–3074. [PubMed: 17478443]

32. Yenari MA, Palmer JT, Bracci PM, Steinberg GK. Thrombolysis with tissue plasminogen activator (tPA) is temperature dependent. Thromb Res 1995;77:475–481. [PubMed: 7778062]

33. Meden P, Overgaard K, Pedersen H, Boysen G. The influence of body temperature on infarct volume and thrombolytic therapy in a rat embolic stroke model. Brain Res 1994;647:131–138. [PubMed: 8069695]

34. Kollmar R, Henninger N, Bardutzky J, Schellinger PD, Schabitz WR, Schwab S. Combination therapy of moderate hypothermia and thrombolysis in experimental thromboembolic stroke: an MRI study. Exp Neurol 2004;190:204–212. [PubMed: 15473993]

35. Liu L, XN T, MA Y. Mild hypothermia reduces hemorrhage and blood brain barrier (BBB) disruption following tissue plasminogen activator treatment in experimental stroke. Neurology 2006;66:A242.

36. Bernard SA, Buist M. Induced hypothermia in critical care medicine: A review. Crit Care Med 2003;31:2041–2051. [PubMed: 12847402]

37. Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, Smith K. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. N Engl J Med 2002;346:557–563. [PubMed: 11856794]

38. Hypothermia after Cardiac Arrest Study Group T. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. N Engl J Med 2002;346:549–556. [PubMed: 11856793]

39. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, Polin RA, Robertson CM, Thoresen M, Whitelaw A, Gunn AJ. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: Multicentre randomised trial. Lancet 2005;365:663–670. [PubMed: 15721471]

40. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, Fanaroff AA, Poole WK, Wright LL, Higgins RD, Finer NN, Carlo WA, Duara S, Oh W, Cotten CM, Stevenson DK, Stoll BJ, Lemons JA, Guillet R, Jobe AH. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. N Engl J Med 2005;353:1574–1584. [PubMed: 16221780]

41. Clifton GL, Miller ER, Choi SC, Levin HS, McCauley S, Smith KR Jr, Muizelaar JP, Wagner FC Jr, Marion DW, Luerssen TG, Chesnut RM, Schwartz M. Lack of effect of induction of hypothermia after acute brain injury. N Engl J Med 2001;344:556–563. [PubMed: 11207351]

42. Hemmen TM, Lyden PD. Induced hypothermia for acute stroke. Stroke 2007;38:794–799. [PubMed: 17261741]

43. Schwab S, Georgiadis D, Berrouschot J, Schellinger PD, Graffagnino C, Mayer SA. Feasibility and safety of moderate hypothermia after massive hemispheric infarction. Stroke 2001;32:2033–2035. [PubMed: 11546893]

44. De Georgia MA, Krieger DW, Abou-Chebl A, Devlin TG, Jauss M, Davis SM, Koroshetz WJ, Rordorf G, Warach S. Cooling for acute ischemic brain damage (cool aid): A feasibility trial of endovascular cooling. Neurology 2004;63:312–317. [PubMed: 15277626]

45. Lyden PD, Allgren RL, Ng K, Akins P, Meyer B, Al-Sanani F, Lutsep H, Dobak J, Matsubara BS, Zivin J. Intravascular cooling in the treatment of stroke (ICTUS): Early clinical experience. J Stroke Cerebrovas Dis 2005;14:107–114.

46. Guluma KZ, Hemmen TM, Olsen SE, Rapp KS, Lyden PD. A trial of therapeutic hypothermia via endovascular approach in awake patients with acute ischemic stroke: Methodology. Acad Emerg Med 2006;13:820–827. [PubMed: 16766740]

47. Georgiadis D, Schwarz S, Kollmar R, Schwab S. Endovascular cooling for moderate hypothermia in patients with acute stroke: First results of a novel approach. Stroke 2001;32:2550–2553. [PubMed: 11692015]

48. Allers M, Boris-Moller F, Lunderquist A, Wieloch T. A new method of selective, rapid cooling of the brain: An experimental study. Cardiovasc Intervent Radiol 2006;29:260–263. [PubMed: 16362468]

49. Rutherford MA, Azzopardi D, Whitelaw A, Cowan F, Renowden S, Edwards AD, Thoresen M. Mild hypothermia and the distribution of cerebral lesions in neonates with hypoxic-ischemic encephalopathy. Pediatrics 2005;116:1001–1006. [PubMed: 16199715]

50. Gordon CJ, McMahon B, Richelson E, Padnos B, Katz L. Neurotensin analog nt77 induces regulated hypothermia in the rat. Life Sci 2003;73:2611–2623. [PubMed: 12967685]

51. Tyler-McMahon BM, Stewart JA, Farinas F, McCormick DJ, Richelson E. Highly potent neurotensin analog that causes hypothermia and antinociception. Eur J Pharmacol 2000;390:107–111. [PubMed: 10708713]

52. Katz LM, Young AS, Frank JE, Wang Y, Park K. Regulated hypothermia reduces brain oxidative stress after hypoxic-ischemia. Brain Res 2004;1017:85–91. [PubMed: 15261103]

53. Katz LM, Young A, Frank JE, Wang Y, Park K. Neurotensin-induced hypothermia improves neurologic outcome after hypoxic-ischemia. Crit Care Med 2004;32:806–810. [PubMed: 15090966]

54. Scanlan TS, Suchland KL, Hart ME, Chiellini G, Huang Y, Kruzich PJ, Frascarelli S, Crossley DA, Bunzow JR, Ronca-Testoni S, Lin ET, Hatton D, Zucchi R, Grandy DK. 3-iodothyronamine is an endogenous and rapid-acting derivative of thyroid hormone. Nat Med 2004;10:638–642. [PubMed: 15146179]

55. Doyle KP, Suchland KL, Ciesielski TM, Lessov NS, Grandy DK, Scanlan TS, Stenzel-Poore MP. Novel thyroxine derivatives, thyronamine and 3-iodothyronamine, induce transient hypothermia and marked neuroprotection against stroke injury. Stroke 2007;38:2569–2576. [PubMed: 17690312]

56. Blackstone E, Roth MB. Suspended animation-like state protects mice from lethal hypoxia. Shock 2007;27:370–372. [PubMed: 17414418]

57. Dirnagl U, Simon RP, Hallenbeck JM. Ischemic tolerance and endogenous neuroprotection. Trends Neurosci 2003;26:248–254. [PubMed: 12744841]

58. Kirino T. Ischemic tolerance. J Cereb Blood Flow Metab 2002;22:1283–1296. [PubMed: 12439285]

59. Chen J, Simon R. Ischemic tolerance in the brain. Neurology 1997;48:306–311. [PubMed: 9040711]

60. Gidday JM. Cerebral preconditioning and ischaemic tolerance. Nat Rev Neurosci 2006;7:437–448. [PubMed: 16715053]

61. Nandagopal K, Dawson TM, Dawson VL. Critical role for nitric oxide signaling in cardiac and neuronal ischemic preconditioning and tolerance. J Pharmacol Exp Ther 2001;297:474–478. [PubMed: 11303032]

62. 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: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. Lancet 2003;362:1028–1037. [PubMed: 14522533]

63. Perez-Pinzon MA. Mechanisms of neuroprotection during ischemic preconditioning: lessons from anoxic tolerance. Comp Biochem Physiol A Mol Integr Physiol 2007;147:291–299. [PubMed: 17045830]

64. Kitagawa K, Matsumoto M, Tagaya M, Hata R, Ueda H, Niinobe M, Handa N, Fukunaga R, Kimura K, Mikoshiba K, Kamada T. Ischemic tolerance phenomenon found in the brain. Brain Res 1990;528:21–24. [PubMed: 2245337]

65. Stagliano NE, Perez-Pinzon MA, Moskowitz MA, Huang PL. Focal ischemic preconditioning induces rapid tolerance to middle cerebral artery occlusion in mice. J Cereb Blood Flow Metab 1999;19:757–761. [PubMed: 10413030]

66. Kraig, R.; Kunkler, P. Spreading depression: A teleological means for self-protection from brain ischemia. In: Chan, P., editor. Princeton Conference. Cambridge University Press; Cambridge: 2001. p. 142-157.

67. Kobayashi S, Harris VA, Welsh FA. Spreading depression induces tolerance of cortical neurons to ischemia in rat brain. J Cereb Blood Flow Metab 1995;15:721–727. [PubMed: 7673367]

68. Sharp FR, Bernaudin M. Hif1 and oxygen sensing in the brain. Nat Rev Neurosci 2004;5:437–448. [PubMed: 15152194]

69. Nishio S, Yunoki M, Chen ZF, Anzivino MJ, Lee KS. Ischemic tolerance in the rat neocortex following hypothermic preconditioning. J Neurosurg 2000;93:845–851. [PubMed: 11059667]

70. Riepe MW, Kasischke K, Gericke CA, Lowe A, Hellweg R. Increase of hypoxic tolerance in rat hippocampal slices following 3-nitropropionic acid is not mediated by endogenous nerve growth factor. Neurosci Lett 1996;211:9–12. [PubMed: 8809835]

71. Raval AP, Dave KR, Perez-Pinzon MA. Resveratrol mimics ischemic preconditioning in the brain. J Cereb Blood Flow Metab 2006;26:1141–1147. [PubMed: 16395277]

72. Ruscher K, Freyer D, Karsch M, Isaev N, Megow D, Sawitzki B, Priller J, Dirnagl U, Meisel A. Erythropoietin is a paracrine mediator of ischemic tolerance in the brain: Evidence from an in vitro model. J Neurosci 2002;22:10291–10301. [PubMed: 12451129]

73. Perez-Pinzon 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 Cereb Blood Flow Metab 1997;17:175–182. [PubMed: 9040497]

74. 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 2000;75:812–818. [PubMed: 10899959]

75. Dave KR, Lange-Asschenfeldt C, Raval AP, Prado R, Busto R, Saul I, Perez-Pinzon MA. Ischemic preconditioning ameliorates excitotoxicity by shifting glutamate/gamma-aminobutyric acid release and biosynthesis. J Neurosci Res 2005;82:665–673. [PubMed: 16247804]

76. Johns L, Sinclair AJ, Davies JA. Hypoxia/hypoglycemia-induced amino acid release is decreased in vitro by preconditioning. Biochem Biophys Res Commun 2000;276:134–136. [PubMed: 11006095]

77. Tanaka H, Calderone A, Jover T, Grooms SY, Yokota H, Zukin RS, Bennett MV. Ischemic preconditioning acts upstream of glur2 down-regulation to afford neuroprotection in the hippocampal ca1. Proc Natl Acad Sci U S A 2002;99:2362–2367. [PubMed: 11842229]

78. Aizenman E, Sinor JD, Brimecombe JC, Herin GA. Alterations of N-methyl-D-aspartate receptor properties after chemical ischemia. J Pharmacol Exp Ther 2000;295:572–577. [PubMed: 11046090]

79. Sommer C, Fahrner A, Kiessling M. [3h]muscimol binding to gamma-aminobutyric acid(a) receptors is upregulated in Ca1 neurons of the gerbil hippocampus in the ischemia-tolerant state. Stroke 2002;33:1698–1705. [PubMed: 12053014]

80. Dave KR, Saul I, Busto R, Ginsberg MD, Sick TJ, Perez-Pinzon MA. Ischemic preconditioning preserves mitochondrial function after global cerebral ischemia in rat hippocampus. J Cereb Blood Flow Metab 2001;21:1401–1410. [PubMed: 11740201]

81. Sharp FR, Ran R, Lu A, Tang Y, Strauss KI, Glass T, Ardizzone T, Bernaudin M. Hypoxic preconditioning protects against ischemic brain injury. NeuroRx 2004;1:26–35. [PubMed: 15717005]

82. Bergeron M, Gidday JM, Yu AY, Semenza GL, Ferriero DM, Sharp FR. Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. Ann Neurol 2000;48:285–296. [PubMed: 10976634]

83. Liu M, Alkayed NJ. Hypoxic preconditioning and tolerance via hypoxia inducible factor (HIF) 1alpha-linked induction of p450 2c11 epoxygenase in astrocytes. J Cereb Blood Flow Metab 2005;25:939–948. [PubMed: 15729289]

84. Janier MF, Vanoverschelde JL, Bergmann SR. Ischemic preconditioning stimulates anaerobic glycolysis in the isolated rabbit heart. Am J Physiol 1994;267:H1353–H1360. [PubMed: 7943381]

85. Yabe K, Nasa Y, Sato M, Iijima R, Takeo S. Preconditioning preserves mitochondrial function and glycolytic flux during an early period of reperfusion in perfused rat hearts. Cardiovasc Res 1997;33:677–685. [PubMed: 9093539]

86. Jones NM, Bergeron M. Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain. J Cereb Blood Flow Metab 2001;21:1105–1114. [PubMed: 11524615]

87. Hall ED, Braughler JM. Free radicals in CNS injury. Res Publ Assoc Res Nerv Ment Dis 1993;71:81–105. [PubMed: 8380240]

88. Vlessis AA, Widener LL, Bartos D. Effect of peroxide, sodium, and calcium on brain mitochondrial respiration in vitro: Potential role in cerebral ischemia and reperfusion. J Neurochem 1990;54:1412–1418. [PubMed: 2313294]

89. Kannan K, Jain SK. Oxidative stress and apoptosis. Pathophysiology 2000;7:153–163. [PubMed: 10996508]

90. Goldstein LB, Adams R, Alberts MJ, Appel LJ, Brass LM, Bushnell CD, Culebras A, Degraba TJ, Gorelick PB, Guyton JR, Hart RG, Howard G, Kelly-Hayes M, Nixon JV, Sacco RL. Primary prevention of ischemic stroke: A guideline from the American Heart Association/American Stroke Association Stroke Council: Cosponsored by the Atherosclerotic Peripheral Vascular Disease Interdisciplinary Working Group; Cardiovascular Nursing Council; Clinical Cardiology Council; Nutrition, Physical Activity, and Metabolism couNcil; and the Quality of Care and Outcomes Research Interdisciplinary Working Group: The American Academy of Neurology affirms the value of this guideline. Stroke 2006;37:1583–1633. [PubMed: 16675728]

91. Weih M, Kallenberg K, Bergk A, Dirnagl U, Harms L, Wernecke KD, Einhaupl KM. Attenuated stroke severity after prodromal tia: A role for ischemic tolerance in the brain? Stroke 1999;30:1851–1854. [PubMed: 10471435]

92. Moncayo J, de Freitas GR, Bogousslavsky J, Altieri M, van Melle G. Do transient ischemic attacks have a neuroprotective effect? Neurology 2000;54:2089–2094. [PubMed: 10851368]

93. Castillo J, Moro MA, Blanco M, Leira R, Serena J, Lizasoain I, Davalos A. The release of tumor necrosis factor-alpha is associated with ischemic tolerance in human stroke. Ann Neurol 2003;54:811–819. [PubMed: 14681891]

94. Wegener S, Gottschalk B, Jovanovic V, Knab R, Fiebach JB, Schellinger PD, Kucinski T, Jungehulsing GJ, Brunecker P, Muller B, Banasik A, Amberger N, Wernecke KD, Siebler M, Rother J, Villringer A, Weih M. Transient ischemic attacks before ischemic stroke: Preconditioning the human brain? A multicenter magnetic resonance imaging study. Stroke 2004;35:616–621. [PubMed: 14963288]

95. Arboix A, Cabeza N, Garcia-Eroles L, Massons J, Oliveres M, Targa C, Balcells M. Relevance of transient ischemic attack to early neurological recovery after nonlacunar ischemic stroke. Cerebrovasc Dis 2004;18:304–311. [PubMed: 15331877]

96. Schaller B. Ischemic preconditioning as induction of ischemic tolerance after transient ischemic attacks in human brain: Its clinical relevance. Neurosci Lett 2005;377:206–211. [PubMed: 15755527]

97. Johnston SC. Ischemic preconditioning from transient ischemic attacks? Data from the northern california tia study. Stroke 2004;35:2680–2682. [PubMed: 15388902]

98. Murry CE, Richard VJ, Reimer KA, Jennings RB. Ischemic preconditioning slows energy metabolism and delays ultrastructural damage during a sustained ischemic episode. Circ Res 1990;66:913–931. [PubMed: 2317895]

99. Kloner RA, Jennings RB. Consequences of brief ischemia: Stunning, preconditioning, and their clinical implications: Part 1. Circulation 2001;104:2981–2989. [PubMed: 11739316]

100. Kloner RA, Jennings RB. Consequences of brief ischemia: Stunning, preconditioning, and their clinical implications: Part 2. Circulation 2001;104:3158–3167. [PubMed: 11748117]

101. Yamagishi H, Akioka K, Hirata K, Sakanoue Y, Toda I, Yoshiyama M, Teragaki M, Takeuchi K, Yoshikawa J, Ochi H. Effects of preinfarction angina on myocardial injury in patients with acute myocardial infarction: A study with resting 123i-bmipp and 201t1 myocardial spect. J Nucl Med 2000;41:830–836. [PubMed: 10809199]

102. Yellon DM, Alkhulaifi AM, Pugsley WB. Preconditioning the human myocardium. Lancet 1993;342:276–277. [PubMed: 8101304]