Preface

Stroke is a global health problem affecting approximately 750,000 people annually in the United States alone and ranks as the third leading cause of death and the most common cause of disability in most developed countries. Traumatic brain injury (TBI) accounts for an estimated 34% of all injury-related deaths in the United States. Stroke and TBI can produce both focal and widespread damage to the brain, which can yield acute and chronic impairments of sensory, motor, and cognitive functions. Because of their enormous medical and socioeconomic impact, a tremendous research investment is being made in the treatment and prevention of stroke and TBI.

Strategies for reducing adverse neurologic outcomes after ischemic or TBI have led to the development of a wide range of neuroprotective agents. However, despite promising results in animal models of stroke and TBI, and extensive testing in randomized clinical trials, no neuroprotective drug has yet proven effective in humans.

In recent years, there has been a resurgence of interest in mild hypothermia as a method of cerebral protection. Although deep hypothermia (below 30°C) is known to be neuroprotective, clinically the benefit is offset by the risks of cardiac arrhythmias and coagulopathies, and by the extensive resources necessary to achieve deep hypothermia, including cardiopulmonary bypass. Alternatively, small decreases in brain temperature (2–5°C below normal brain temperature) are well-tolerated and confer significant neuroprotection in animal models of cerebral ischemia. Indeed, mild hypothermia is one of the most effective neuroprotective therapies in experimental ischemia models, and the feasibility of using mild hypothermia to treat stroke and TBI patients is currently being evaluated in clinical trials. Recently, two prospective, randomized controlled studies demonstrated improved neurologic outcome with mild hypothermic treatment for patients with cardiac arrest from ventricular fibrillation.

Increased understanding of the mechanisms by which mild hypothermia exerts its neuroprotective effects has allowed basic scientists and clinicians to optimize the use of mild hypothermia as a therapeutic strategy. New technological advances are now facilitating the imple-

mentation of mild hypothermia in the clinical setting. Knowledge and experience gained from clinical trials around the world have helped develop guidelines for the intraoperative and intensive care management of patients undergoing mild hypothermic treatment.

There is also interest in combining hypothermia with other therapeutic strategies. The rationale for this combination approach is that mild hypothermia could prolong the therapeutic window for neuroprotective agents. Using hypothermia in conjunction with other pharmacological agents for the treatment of acute cerebral ischemia is also discussed in this book, along with future directions in both basic and clinical research.

Hypothermia and Cerebral Ischemia: Mechanisms and Clinical Applications is intended to provide a comprehensive review of mild hypothermia's therapeutic potential, its limitations, and recent developments in both basic and clinical research. We hope that this volume serves to educate clinicians, other health professionals, and basic scientists, as well as promote interest in the study and implementation of mild hypothermia for the treatment of stroke and TBI.

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2 The Effects of Hypothermia and Hyperthermia in Global Cerebral Ischemia

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INTRODUCTION

The colorful early history of therapeutic hypothermia has been reviewed elsewhere *(1)*. Its first documented application was as a local anesthetic during surgical procedures. Early in the 20th century, head injury, tumors, and other conditions were treated using local and generalized cooling *(2,3)*. Clinical descriptions note retrograde amnesia at temperatures below 34°C, dysarthria at 34°C, aphasia at 27°C, loss of the pupillary light reflex at 26°C, and the common occurrence of sudden cardiac failure, perhaps attributable to ventricular fibrillation *(4)*. During World War II, the Nazis exposed concentration camp victims to inhumane hypothermia experimentation; these brutal atrocities have been reviewed recently *(5)*.

In cardiac surgical procedures requiring the interruption of circulation, hypothermia was introduced to confer protection against cerebral ischemia and was eventually applied via pump oxygenation and extracorporeal cooling *(6)*. Hypothermia was also used to mitigate cerebral ischemia resulting from vascular occlusion or hypotension in the course of cerebral aneurysm clipping or the resection of arteriovenous malformations *(7)*. Temperatures as low as 4–15°C were used in cardiac bypass procedures *(8)*. Profound systemic hypothermia, however, was typically associated with severe medical complications including ventricular fibrillation and other cardiac arrhythmias, hypotension, acidosis, coagulopathies, and suppression of immunological function *(9,10)*.

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Nonetheless, the enthusiasm for profound hypothermia was bolstered by experimental studies revealing progressive linear declines in cerebral oxygen utilization with decreasing temperature *(11,12)*, and by demonstrations that profound hypothermia was neuroprotective in canine models of ischemia and cerebral contusion *(13,14)*. Selective brain-cooling methods such as cold carotid artery perfusion were considered as a means of avoiding systemic complications, but mortality remained high *(15)*. This might have been attributable in part to alterations of vascular tone provoked by the perfusate *(16)*. Nonetheless, remarkable experimental results emerged from selective brain cooling to 14–15°C: monkeys with 45 min of complete cerebral circulatory arrest recovered without deficits when so cooled *(17)*.

MODULATION OF ISCHEMIC INJURY BY MILD TO MODERATE HYPOTHERMIA

In studies conducted in the 1960s in a model of complete brain ischemia in rabbits, it was shown that the duration of tolerable ischemia compatible with complete functional and histological recovery increased continuously with declining temperature, and could be extended fivefold by reducing temperature from 37°C to 25°C *(18)*. Other workers showed that a decrease of body temperature of only 1–3°C reduced the degree of brain energy metabolite depletion and acidosis in a model of carotid ligation and hypoxia *(19)*.

These observations emphasize the necessity for stringent control of body temperature in the conduct of animal ischemia experiments. The more specific need to monitor and regulate brain temperature, however, was not initially appreciated. Experimental hypoxia–ischemia studies conducted prior to the mid-1980s typically controlled the rectal temperature of their animal preparations but failed to monitor or regulate brain temperature. This situation changed when our group serendipitously observed that rats with global ischemia had less severe neuropathology when their scalp tissues had been reflected for electroencephalographic (EEG) monitoring than did animals with intact scalps—suggesting that the former setting might have led to cranial cooling (study described in Vibulsresth et al. *[20]*). Indeed, by separately monitoring rectal, temporalis muscle, and brain (striatal) temperature, we documented spontaneous, variable declines in cranial and brain temperature during a 20-min global ischemic insult *(21)*. These observations, we hypothesized, accounted for much of the variability

noted in previously published animal studies of global ischemia (e.g., Smith et al. *[22]*) and emphasized the need for independent control of both rectal and cranial temperatures.

To study this problem carefully, we conducted experiments in which anesthetized Wistar rats received a 20-min global forebrain ischemic insult by a modification of the four-vessel occlusion method *(23)*. Brain temperature was monitored by a thermocouple inserted stereotaxically into the striatum, and striatal temperature was regulated by a high-intensity lamp positioned over the head *(21)*. In all animals, rectal temperature was separately regulated at 37°C. Brain temperature, however, was intentionally thermostated during the ischemic insult at 33, 34, 36, or 39°C. Following 3-d survival, histopathology in the normothermic group (36°C intraischemic brain temperature) revealed prominent ischemic neuronal pathology in the dorsolateral and central striatum, as well as a marked loss of CA1 pyramidal neurons of the hippocampus (**Fig. 1**). In animals with intraischemic brain cooling to 33–34°C, the extent of these neuronal changes was markedly reduced in both hippocampus and striatum; animals with intraischemic brain temperatures of 30°C had even more pronounced neuroprotection *(21)*. This study thus established that effective hypothermic neuroprotection was possible by intraischemic brain temperature reductions as small as 2°C, and it emphasized that brain temperature is a crucial determinant of ischemic injury and must be separately monitored and regulated to obtain interpretable data from animal experiments. As temporalis muscle temperature correlated highly with brain temperature *(24)*, it could be monitored in lieu of a direct brain thermocouple insertion provided that calibration curves were specifically established under the conditions of each experiment *(25)*.

Other laboratories have repeatedly confirmed the determinative influence of small decrements of brain temperature on the histological outcome of global ischemia. Thus, a 2°C body temperature reduction in ischemic gerbils led to complete protection of CA1 hippocampal neurons *(26)*. In the rat model of bilateral carotid artery occlusions and hypotension, temperature reductions to 35°C protected against selective neuronal necrosis in vulnerable regions *(27,28)*. Selective brain cooling during and following a prolonged (30-min) period of global ischemia in rats reduced cortical damage *(29)*. In studies of ischemic insults in which the temperature was not controlled and a spontaneous decline of brain temperature was permitted, protection of hippocampal

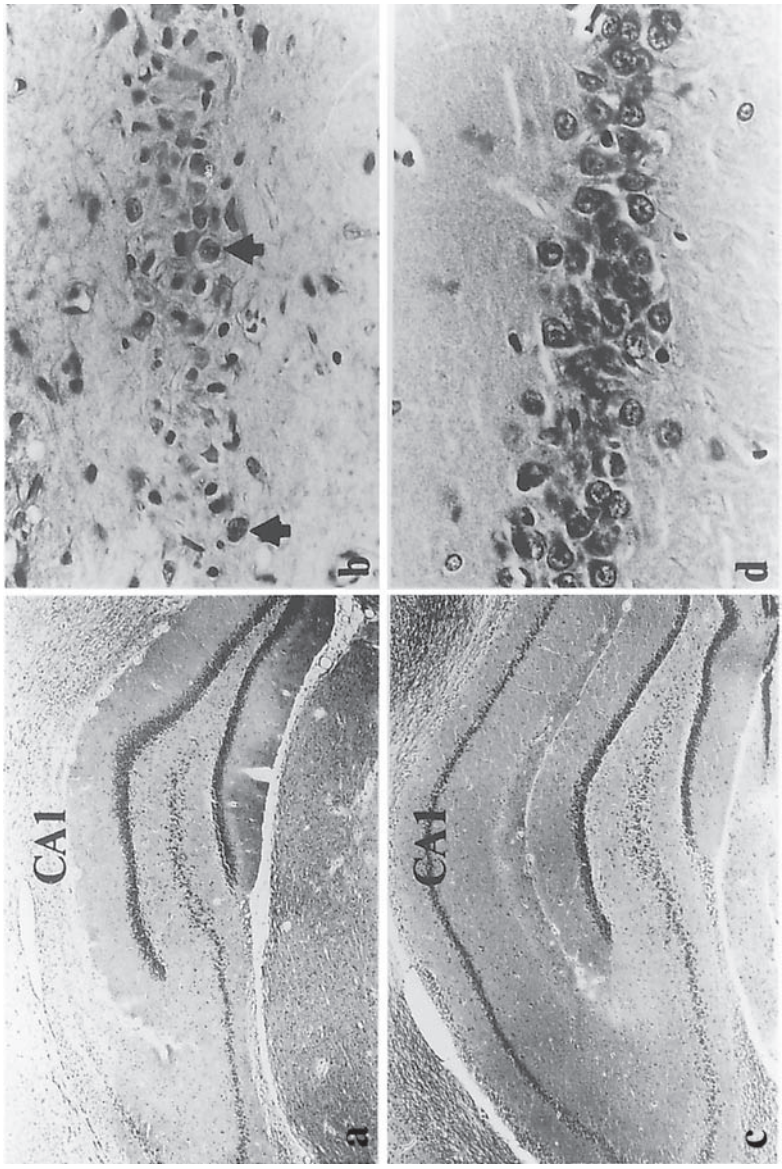

Fig. 1.

neurons was demonstrated relative to the situation in which the brain was held at preischemic, normothermic levels *(27,30,31)*. Moderate hypothermia (32–33°C) was also able to increase survival, abolish seizures, and diminish the extent of pathological alterations in the setting of global ischemia compounded by hyperglycemia *(32)*.

Moderate hypothermia has also proven neuroprotective in animal models of cardiac arrest and cardiopulmonary bypass. In a canine cardiac arrest model, hypothermia to 34°C produced by ice water immersion of the head during the period of arrest and 1 h thereafter led to histopathological improvement *(33)*. These results were confirmed by a later study in which dogs were cooled for 1 h postarrest to 30–34°C; in that study, however, deeper hypothermia (15°C) worsened outcome *(9)*. If cooling was delayed by 15 min after reperfusion, the beneficial effect of resuscitative hypothermia after cardiac arrest in dogs was lost *(34)*. In another canine study, a temperature change of even 1–2°C within the 37–39°C range significantly altered neurological outcome and histopathology in a model of complete cerebral ischemia produced by arterial hypotension plus intracranial hypertension *(35)*. Selective brain cooling to 25–30°C reduced neuronal injury in a cat model of cardiac arrest and resuscitation *(36)*. In a pig cardiopulmonary bypass study, neuronal degeneration and astrocytic swelling were prevented by moderate hypothermia (27°C) *(37)*.

STUDIES OF POSTISCHEMIC HYPOTHERMIA IN GLOBAL ISCHEMIA

The salutary influence of mild to moderate degrees of intraischemic hypothermia in models of global ischemia raised the clinically relevant issue of whether *postischemic* cooling to the same degree would also

Fig. 1. *(previous page)* Paraffin-embedded coronal brain sections stained with hematoxylin and eosin from normothermic–ischemic (a, b) and hypothermic–ischemic (c, d) rats. (a) Two months after 12.5 min of normothermic (37°C) global ischemia, severe necrosis of CA1 hippocampus is evident (×120). (b) Higher magnification of injured CA1 sector illustrates only two viable neurons (*arrowheads*) among reactive astrocytes and microglia (×1100). (c) In contrast to the normothermic results, a relatively intact CA1 sector is present in a rat that had undergone hypothermic (30°C) global ischemia (×120). (d) Higher magnification of CA1 demonstrates many viable neurons containing a distinct nucleus and nucleolus (×1100). [Reproduced with permission from Green E. J., et al. (1992) *Brain Research* **580,** 197–204. Elsevier Science Publishers, B. V.]

protect. Several questions arose: How quickly must hypothermia be initiated after ischemia to be neuroprotective? For how long and to what depth should it be administered? Is the resulting neuroprotection temporary or permanent? Using a model of 10-min normothermic global forebrain ischemia by the two-vessel occlusion model, we compared the neuropathological outcome when cranial cooling to 30°C was begun at either 5 or 30 min following the ischemic insult, and was maintained for 3 h *(38)*. In these initial studies, in which only a 3-d survival was permitted, 50% protection of hippocampal CA1 pyramidal neurons was achieved when cooling was begun at 5 min postischemia, but cooling was ineffective if deferred until 30 min. Other workers showed that cooling to 34°C for 2 h following normothermic ischemia provided partial histological protection of hippocampal neurons if the insult was only 8 min in duration but failed to protect if the ischemic insult lasted 12 min *(30,39)*. In a gerbil study of 5-min global ischemia at 38°C (a severe insult), followed by prompt temperature reduction to 33°C for 1 h or to 23°C for 2 h, hippocampal CA1 injury was extensive despite the cooling *(40)*. In other studies examining the effect of postischemic cooling duration, 6 h of immediate postischemic hypothermia conferred histological protection whereas a 1-h cooling period did not *(41)*. Postischemic hypothermia of approx 33°C begun at 2 h postischemia conferred hippocampal protection if continued for 5 h *(42)*.

A crucial, clinically relevant issue is whether postischemic cooling confers *permanent* neuroprotection. Certain observations described above suggested that this might not be the case *(40)*. By contrast, our studies of *intraischemic* hypothermia had shown enduring neuroprotection out to 2 mo (**Fig. 1**) *(38,43)*. This issue was tested in a study in which rats were subjected to 10 min of normothermic ischemia followed by a 3-h period of cranial hypothermia (30°C) and survival for either 3 d, 7 d, or 2 mo *(44)*. In hypothermic animals with 3-d survival, significant protection of CA1 pyramidal neurons was observed, but this protective effect declined in rats surviving for 7 d and was lost in rats surviving for 2 mo. This result contrasted with the permanent protection seen at 2 mo in a similar insult under conditions of intraischemic hypothermia. This study thus indicated that, in contrast to intraischemic cooling, postischemic hypothermia is capable of *delaying* the onset of ischemic cell change but may not permanently protect, and it emphasized the importance of chronic survival studies in assessing neuroprotection. These results also raised the possibility that the cellular and

molecular mechanisms of intra- vs postischemic cooling might differ from one another.

The delay in onset of ischemic cell change with postischemic cooling raised the possibility that this therapy might, in fact, extend the therapeutic window for intervention with other (e.g., pharmacological) neuroprotectants. This proved to be the case in a study in which rats receiving 10-min of global ischemia followed by 3-h postischemic cooling to 30°C were subsequently treated with the noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist dizocilpine administered on postischemic d 3, 5, and 7. Partial permanent hippocampal neuroprotection resulted from the combined therapy *(45)*. Similarly, postischemic hypothermia combined with delayed administration of *n-tert*-butyl-α-phenylnitrone (PBN), a free radical spin-trap agent, led to long-term cognitive improvement *(46)*. In rats with 12.5 min of normothermic ischemia, 4 h of postischemic hypothermia (33–34°C) alone failed to protect the CA1 hippocampus 2 mo later, whereas the combination of hypothermia plus interleukin-10 administration conferred long-lasting partial protection *(47)*. The potential clinical implications of these findings are obvious.

Studies in other laboratories have substantiated that more prolonged periods of postischemic cooling alone do, in fact, confer a more permanent degree of neuroprotection. In gerbil studies with 30-d survival, when cooling to 32°C was instituted for 12 h beginning 1 h after a global ischemic insult, substantially reduced CA1 necrosis resulted when the insult was mild (3-min insult), but this effect was only partial when a 5-min insult had been used *(48)*. When the cooling period was prolonged to 24 h, the 5-min ischemic animals exhibited much greater degrees of neuroprotection at 30 d. A subsequent study extended the survival time to 6 mo and showed enduring neuroprotection with prolonged postischemic cooling *(49)*. A still more recent study explored an analogous paradigm in rats with 10-min of severe forebrain ischemia exposed to a 48-h period of mild hypothermia starting 6 h after the insult. A 28-d survival was permitted. Robust, enduring CA1 protection was obtained *(50)*. Postischemic hypothermia is considered in detail elsewhere in this volume, and the subject has been recently reviewed extensively *(51)*.

An important implication of the aforementioned findings is the possibility that neuroprotective effects might be falsely ascribed to pharmacological agents that acted by inadvertently producing hypothermia.

This proved to be the case for the NMDA antagonist dizocilpine (MK-801), which had initially been reported to be highly neuroprotective in some (but not all) global ischemia studies *(52,53)*. Further investigation revealed that this neuroprotective effect was associated with prolonged hypothermia *(54)*, which, when prevented, abrogated the "protective" effect of the drug. It is highly likely that the putative neuroprotective effect ascribed to other pharmacological agents (e.g., barbiturates) may, in retrospect, also have been explicable on the basis of unnoticed hypothermia.

THE OBVERSE OF HYPOTHERMIA: THE DELETERIOUS EFFECT OF HYPERTHERMIA

Ten years ago, it was observed in a model of 5-min global ischemia in gerbils that the expected severe hippocampal neuronal loss could be markedly attenuated by prolonging the period of halothane anesthesia, which blunted the mild postischemic hyperthermia (approx 1.5°C) that would otherwise occur *(55)*. This effect was not specific to halothane but rather could be duplicated by warming the head by a similar amount in its absence. This study established the marked sensitivity of the postischemic brain to even mild hyperthermia.

Early in our studies of hypothermia, we observed that warming the brain to 39°C during a 20-min global ischemic insult accentuated ischemic changes in cortex, hippocampus, and subcortical structures *(21)*. A careful study comparing the sequelae of 20-min global ischemia in rats under conditions of intraischemic temperatures of either 37°C or 39°C revealed both an acceleration and marked accentuation of injury in the hyperthermic group, in which evidence of severe neuronal injury was present at even 1-d survival *(21,56)*. Severe ischemic changes were present in striatum and hippocampus; laminar necrosis was seen in the cortex; focal thalamic infarction occurred, and widespread ischemic changes involved other structures. This deleterious effect of hyperthermia was confirmed in the rat *(28)* and gerbil *(57)*.

We subsequently used a rat model of 5- or 7-min global ischemia by two-vessel occlusion to study whether delayed hyperthermia instituted after an ischemic insult would exacerbate injury. Twenty-four hours after ischemia, rats of one group were exposed to elevated ambient temperature so as to raise rectal temperature to 39–40°C for 3 h *(58)*. Following an 8-d survival, histopathological examination in rats with 7-min ischemia revealed that delayed hyperthermia led to an approxi-

mately 2.6-fold increase in numbers of ischemic neurons in the hippocampal CA1 sector; a similar but nonsignificant trend was seen in the 5-min ischemic group *(58)*. A comparable study was conducted in a model of 60-min transient focal ischemia—an insult ordinarily giving rise to a small subcortical infarction. When body temperature was elevated to 40°C for 3 h 1 d after middle cerebral artery occlusion, infarct volume was dramatically increased *(59)*.

Interestingly, these observations appear to have important clinical relevance. Direct monitoring of intracranial temperature in neurosurgical patients with head injury and other conditions has revealed that brain temperatures commonly exceed rectal temperatures and, in the injured brain, this gradient may be as high as 2.5°C *(60)*. Thus, it might be expected that systemic fever of a given degree would result in even greater elevations of brain temperature in the injured brain. Similarly, during rewarming following hypothermic cardiac surgery, cerebral temperature (as reflected in cerebral venous temperature) may quickly rise to 39°C or higher *(61)*. In patients with acute stroke, several studies have now shown a correlation between elevated temperature and poor outcome *(62,63)*. Fever of 38°C or above proved to be an independent factor predicting worse prognosis and higher mortality *(62)*. A 1°C difference in body temperature increased the relative risk of poor outcome by 2.2-fold *(63)*. In 260 patients with acute hemispheric ischemic stroke in whom body temperature was recorded every 2 h, axillary temperature >37.5°C during the first 24 h after stroke onset was highly correlated to larger infarct volumes at 3 mo *(64)*.

Hyperthermia appears to act through several mechanisms to worsen cerebral ischemia. Intraischemic hyperthermia accentuates and prolongs the release of extracellular glutamate; it also accentuates the release of γ-aminobutyric acid and glycine and markedly increases the so-called "excitotoxic index"—a composite measure of neurotransmitter release *(65,66)*. Comparable results were observed in a model of focal ischemia *(67)*. In patients with acute stroke, cerebrospinal fluid (CSF) concentrations of glutamate and glycine correlated with increased body temperature, suggesting that excitotoxic mechanisms may contribute to the hyperthermia-associated worsening *(68)*.

Postischemic oxygen radical production is also accentuated by hyperthermia. Microdialysis studies sampling the brain's extracellular fluid for a signal of hydroxyl radical production have revealed two- to threefold elevations after normothermic global ischemia, but four- to

fivefold elevations after mildly hyperthermic (39°C) ischemia *(69,70)*. Ischemia-induced blood–brain barrier (BBB) opening is also highly sensitive to brain temperature. Following mild intraischemic hyperthermia (39°C), marked accentuation of BBB breakdown has been described *(71,72)*. Magnetic resonance spectroscopy studies have revealed enhanced intracellular acidosis and impaired recovery of cerebral energy metabolites in cats with global cerebral ischemia under hyperthermic conditions *(73)*. Similar findings were obtained by direct assay of energy metabolites *(74)*.

Hyperthermia affects a number of intracellular processes. For example, temperatures of 39°C during ischemia accentuate the inhibition of calcium/calmodulin-dependent protein kinase II induced by brief global ischemia *(57)*. Patterns of protein kinase C alterations induced by global ischemia are also significantly influenced by hyperthermia *(75)*. Mild intraischemic hyperthermia during global ischemia in gerbils aggravated the decreases in calmodulin and microtubule-associated protein 2 (MAP2) immunoreactivities in hippocampus *(76)*.

These observations (as well as corroborative evidence in focal ischemia, not reviewed here) have led us to offer the strong recommendation that *body temperature be maintained in a safe normothermic range (e.g., 36.7–37°C) for at least the first several days after the onset of acute stroke or head injury*, and that caution should be taken to avoid rewarming following hypothermic cardiopulmonary bypass *(77)*.

MECHANISMS OF HYPOTHERMIC NEUROPROTECTION

Cerebral Blood Flow and Metabolism

The effect of hypothermia on cerebral perfusion appears to vary according to the method of cooling (systemic vs local) and the level of temperature reduction. Both increases and decreases of cerebral blood flow (CBF) have been reported. During a global ischemic insult itself, direct measurements have shown that the degree of CBF reduction during ischemia is unaffected by the intraischemic temperature level *(21,78)*. In like manner, assays of brain energy metabolites following global ischemia have shown that the magnitudes of high-energy phosphate depletion and lactate elevation are similar, irrespective of intraischemic temperature over the range of 30–39°C *(74)*. Although intraischemic hypothermia does not appear to act via an alteration of energy metabolite levels, other studies suggest that the *initial* rate of

ATP depletion during global ischemia is retarded by mild hypothermia *(79–81)*. This slowing of the rate of ATP depletion may contribute significantly to the protective effect of hypothermia. Conflicting data appear in the literature as to whether hypothermia alters cerebral lactate accumulation, with both negative findings *(82)* and reduced acidosis *(83)* being reported. Magnetic resonance spectroscopy studies in rats with forebrain ischemia have shown that the postischemic intracellular alkalosis present with normothermia is abolished in hypothermic animals *(84)*. In an extensive study of regional brain energy metabolites by direct sampling following 20-min global ischemia at intraischemic cranial temperatures of 30°C, 37°C, or 39°C *(74)*, somewhat less complete recovery of ATP levels and the sum of adenylates was observed in the hyperthermic group.

Studies assessing local cerebral glucose utilization (lCMRglu) and blood flow (lCBF) in the postischemic state in rats with 20-min global ischemia have shown significantly greater recovery of lCMRglu throughout cortical and subcortical structures of rats with intraischemic hypothermia compared to normothermic animals *(85)*. Autoradiographic studies have also revealed improved metabolic activation in response to peripheral stimuli following global ischemia conducted under hypothermic compared to normothermic circumstances *(86)*.

Neurotransmitter Release

A major mechanism of ischemic injury is thought to involve the release and extracellular accumulation of excitatory amino acids in ischemia, leading to excessive activation of postsynaptic glutamate receptors, increases in intracellular free calcium ion concentration, and a subsequent cascade of complex events leading to cell death *(87–89)*. Multiple neurotransmitters and neuromodulators are massively released in ischemia, including dopamine, norepinephrine, serotonin, and others *(90–92)*. Hypothermic temperatures tend to inhibit the biosynthesis, release, and/or reuptake of these various neurotransmitters *(93,94)*. Our laboratory has shown that in rats with 20-min global ischemia by two-vessel occlusion, mild intraischemic hypothermia markedly diminishes the extent of glutamate release in the striatum *(78)*. During normothermia, there is a sevenfold surge of glutamate above baseline levels; this is completely inhibited by brain temperature reductions to 33°C or 30°C *(78,90,95)*. Similarly, the 500-fold release of dopamine in normothermic ischemia is attenuated by approximately 60% at hypothermic

temperatures of 33°C or 30°C. Microdialysis studies in other laboratories have confirmed these findings *(96)*. In rabbits with 10-min global ischemia, marked attenuations of glutamate, aspartate, and glycine release were reported at epidural temperature reductions of 20°C *(97)*. Other studies of transient global ischemia in hypothermic rabbits have confirmed profoundly reduced levels of hippocampal glutamate and glycine *(98,99)*. The hypothermic inhibition of glutamate increase has been demonstrated also under conditions of ischemia complicated by hyperglycemia *(100)*. By means of a real-time method for monitoring extracellular glutamate levels, it was shown that hypothermia appears to enhance postischemic glutamate reuptake *(101)*.

The excitotoxic index was developed by our group as a composite descriptor of excitatory/inhibitory amino acid neurotransmitter balance as measured by microdialysis in the brain's extracellular space *(66,102)*. This index is defined as:

$$\text{Excitotoxic index} = [\text{glutamate}] \times [\text{glycine}]/[\text{GABA}]$$

Our group was able to show that 12.5 min of normothermic global ischemia led to significant, 7- to 12-fold increases in the striatal excitotoxic index that persisted for 3–4 h. By contrast, animals with postischemic hypothermia (30°C for 3 h) showed no changes in the excitotoxic index during recirculation *(103)*.

The preceding observations are obviously relevant to the neuroprotective effect of *intraischemic* hypothermia. Late increases in extracellular glutamate and aspartate levels have also been reported after ischemia *(104)* and following multiple ischemic insults *(105)*. It is possible that prolonged postischemic hypothermia may affect these late processes, although this has not been established.

Intracellular Messengers and Mediators

Hypothermia has been shown to affect a variety of intracellular mediators, although an integrated synthesis has not yet emerged. Studies in our laboratory have shown that inositol 1,4,5-trisphosphate (IP_3) decreases significantly in cortex and subcortical structures during normothermic global ischemia, but these declines are partially mitigated by intraischemic hypothermia *(106)*. Protein kinase C (PKC) is a calcium-dependent enzyme activated by diacylglycerol and produced in the course of inositol phospholipid hydrolysis. Its activation involves a translocation from cytosol to the cell membrane. Translocation and

inhibition of PKC occur during ischemia *(107,108)*. One study reported an absence of PKC translocation and a lack of its inhibition with intraischemic hypothermia *(109)*. In another study, intraischemic temperature highly influenced PKC activity during recirculation *(75)*. In normothermic rats, significantly reduced PKC activity was observed at all recirculation time points, but in the hypothermic group normal PKC levels were observed during ischemia and reperfusion. Conversely, *hyperthermia* significantly decreased PKC activity in both controls and ischemic animals *(75)*. Similar findings were obtained in a global forebrain ischemia model in which mild hypothermia applied intraischemically and during reperfusion (60 min) inhibited translocation of PKC-α, β, γ isoforms as well as fodrin proteolysis *(110)*.

Calcium/calmodulin-dependent protein kinase II (CaM kinase II) is a mediator of synaptic and cytoskeletal function as well as neurotransmitter release. The reduced CaM kinase II activity observed following normothermic ischemia is not seen under conditions of intraischemic hypothermia *(57,110)*. Ubiquitin, a small protein involved in the catabolism of other abnormal proteins, is decreased following ischemia; this may lead to an accumulation of abnormal proteins that affect cell function. Intraischemic hypothermia induces a significant restitution of ubiquitin compared to the normothermic condition *(111)*.

BBB Breakdown

The extent to which the BBB is influenced by ischemia is highly temperature dependent. Early BBB breakdown to protein tracers is demonstrable after normothermic global ischemia but is suppressed by mild to moderate hypothermia and is greatly accentuated by intraischemic hyperthermia *(71,112)*. Similarly, postischemic edema following global ischemia is reduced by moderate hypothermia *(113)*.

Reactive Oxygen Species

Oxygen free radicals are elaborated during ischemia and reperfusion and have been strongly implicated in the pathophysiology of ischemic brain injury *(114)*. These reactions may lead to oxidative injury to cellular lipids, proteins, and nucleic acids. Evidence for free radical elaboration in ischemia is obtainable by means of a microdialysis method in which administered salicylate is converted, in the presence of hydroxyl radical, to dihydroxybenzoic acid (DHBA) species—stable adducts detectable by chromatographic methods. A study from our laboratory of

20-min global ischemia documented substantial early elevations of the DHBA signal from striatum following 20 min of normothermic global ischemia *(69)*. These elevations were strikingly exaggerated following hyperthermic (39°C) ischemia and, conversely, were completely attenuated following hypothermic (30°C) ischemia of similar duration.

Inflammatory mechanisms involving polymorphonuclear leukocytes may, in part, mediate radical-induced pathology in ischemia. In focal ischemia, intraischemic hypothermia was shown to attenuate neutrophil infiltration *(115)*.

Gene Expression and Protein Synthesis

A generalized depression of protein synthesis occurs following global ischemia and may affect the translation of messages such as those for immediate early genes, which are rapidly transcribed after ischemia. Moderate hypothermia (30°C) during ischemia reverses the postischemic inhibition of protein synthesis *(116)*. Detailed studies *(117)* suggest that intraischemic hypothermia affects transcriptional events in a temporally and spatially complex fashion. Hypothermia appears to enhance the expression of certain immediate early genes following ischemia; this may be consistent with the promotion of cell survival. In a model of 10-min forebrain ischemia in the gerbil, intraischemic hypothermia (30°C) hastened the time course of expression of the immediate early genes c-*fos* and *fos*-B in hippocampal regions, again suggesting a possible recovery-associated mechanism *(118)*.

Similarly, following 10 min of forebrain ischemia, mild hypothermia applied for 3 h attenuated apoptotic death in hippocampal neurons 72 h postinsult *(119)*. The neuroprotection appeared to be correlated with increased expression of Bcl-2, an antiapoptotic protein.

While moderate *intra*ischemic hypothermia (33°C) failed to increase the expression of mRNAs for the neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), or TrkB in ischemia-sensitive hippocampal subregions, hypothermia did induce neurotrophin mRNA alterations in the ischemia-resistant dentate gyrus; it was speculated that this might confer protection *(120)*. In contrast, *post*ischemic hypothermia (33°C) potentiated the increase in BDNF at 24 h postcardiac arrest (8-min duration) and increased tissue levels of and tyrosine phosphorylation of TrkB *(121)*. In this study it was suggested that increased activation of BDNF signaling could be a possible mechanism by which mild hypothermia reduces neuronal injury after global cerebral ischemia.

CONCLUSIONS

Investigations from laboratories throughout the world accrued over the past dozen years have established with certitude the neuroprotective influence of mild to moderate degrees of brain hypothermia in the setting of global (and focal) cerebral ischemia. Conversely, mild hyperthermia has emerged as an important factor exacerbating ischemic brain injury. The avoidance of fever in patients with acute brain injury should now be part of routine clinical practice. Randomized trials of therapeutic hypothermia will be required to establish efficacy in patients with brain ischemia; in the modern intensive care setting, they are eminently feasible.

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