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Symposium Article

Inhibition of Glutamate Release: A Possible Mechanism of Hypothermic Neuroprotection

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Summary: The neuroprotective properties of hypothermia are well recognized. For many years, the ability of hypothermia to decrease the neurologic morbidity associated with episodes of ischemia has been ascribed to the reduction in metabolic rate that accompanies decreases in temperature. More recently, evidence has accumulated that hypothermia may exert some of its neuroprotective effects by reducing the ischemia-induced release of excitatory amino acids. This attenuated release occurs even with the mild degrees of hypothermia that can easily be produced in operating rooms and intensive care units. Preliminary data suggest that mild hypothermia may be of benefit in surgical patients at risk for intraoperative cerebral ischemia and patients who have suffered traumatic brain injury. Because of the minimal risk associated with lowering body temperature to 34°C, additional outcome studies are in progress to ascertain the potential benefits of this mode of therapy. **Key Words:** Ischemia, brain—Glutamate—Hypothermia, induced—Neurotoxins—Excitatory amino acids.

Hypothermia possesses potent neuroprotective properties. This effect is robust and has been repeatedly demonstrated both in the laboratory and in clinical medicine. Despite decades of research, no pharmacologic agent has yet been found that can equal the neuroprotective properties of a decrease in brain temperature.

PROFOUND HYPOTHERMIA

In humans, the striking ability of hypothermia to prevent or attenuate neurologic injury is clearly demonstrated by the technique of deep hypothermic circulatory arrest. The use of hypothermia during circulatory arrest was first described in 1959 when Drew and Anderson (1) reported three cases of pediatric open heart surgery performed after cooling the patients to a temperature of 13° to 15°C. Circulatory arrest times ranged from 25 to 45 min and all patients reportedly made uneventful neurologic recoveries. Hypothermic circulatory arrest is currently performed using cardiopulmonary bypass with high-capacity heat exchangers. It is generally accepted that under conditions of profound hypothermia (body temperature of approximately 18°C), cerebral circulation in humans may be arrested for as long as an hour without evidence of neurologic injury (2). This technique has been employed at multiple centers during certain neurovascular procedures (e.g., large basilar aneurysms and cavern-

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ous sinus fistulas). Similar durations of circulatory arrest are also used in cardiac surgery patients undergoing complex procedures involving the heart and great vessels, including pulmonary thromboendarterectomy. Clearly, hypothermia can markedly increase the brain's tolerance for global ischemia.

Even more profound degrees of hypothermia combined with hemodilution or blood substitution are being investigated for use as neuroprotectants. Dogs have been maintained for as long as 3 h when perfused with an asanguinous fluid at 1.7°C with full neurologic recovery after rewarming and autotransfusion. Subsequent histologic examination failed to show any evidence of ischemic or hypoxic injury (3). In the future, it is possible that hemodilution combined with "ultraprofound" levels of hypothermia will allow longer circulatory arrest times than those currently tolerated.

Additional methods of producing selective cooling of the brain have been studied in animal models, including external cooling of the brain and administration of "cerebroplegia." In a recent study both external cooling and the intermittent administration of a nonblood-containing cerebroplegia solution resulted in improved neurologic outcome after 2 h of hypothermic circulatory arrest in sheep (4). In a similar study selective perfusion of the brain with chilled Ringer's lactate for 60 min resulted in a brain/body temperature differential of about 6°C (brain temp = 28° vs. body temp of 34°C). All animals survived and there was no evidence of ischemic injury on postmortem examination. In contrast, all animals in the normothermic control group died (5).

MILD HYPOTHERMIA

More intriguing and of greater immediate relevance than the use of profound degrees of hypothermia is that a mere 2° reduction in temperature can reduce neurologic injury. In rats subjected to 20 min of ischemia using the four-vessel occlusion technique, lowering brain temperature from 36° to 34°C produced a highly significant decrease in ischemic cell counts in the striatum as well as decreasing the amount of hippocampal damage (6). Subsequent studies have confirmed this finding of marked neuroprotection produced by mild degrees of hypothermia (7). These studies, if the results can be generalized to humans, are of considerable interest because they suggest that levels of hypothermia easily obtainable in the operating room may confer significant benefit. Lowering an anesthetized patient's body temperature by a few degrees happens spontaneously unless active efforts are made at warming. Further, the minor adverse effects of mild hypothermia can be mitigated by rewarming these patients before emergence from anesthesia. Even in the absence of controlled studies demonstrating a beneficial effect in humans, many neuroanesthetists seek to lower patients' body temperatures by 3° to 4°C before potential ischemic episodes.

MECHANISM OF HYPOTHERMIC NEUROPROTECTION

For decades, the cerebral protective properties of hypothermia have been ascribed to the decrease in cerebral metabolic rate associated with a fall in temperature. This seemed an eminently logical explanation for the ability of hypothermia to attenuate neurologic injury as ischemic damage was believed to represent a continued demand for metabolic substrates in the face of a curtailed supply. Because metabolism is known to decrease by about 7% for every 1°C decrement in temperature, one might predict that the brain would tolerate increased durations of ischemia under hypothermic conditions. The conviction that metabolic suppression was the mechanism of hypothermic neuroprotection is evidenced by the statement that, "The proven protective effects of hypothermia can be fully explained on a metabolic basis alone—no other mechanisms need be invoked, and none are known" (8).

Unfortunately, some metabolic studies have failed to demonstrate improved preservation of high-energy phosphates during hypothermic ischemia (6). In these studies, brain tissue levels of phosphocreatine, adenosine triphosphate (ATP), adenosine 5'-diphosphate (ADP), glucose, and glycogen were measured after 20 min of global ischemia at 39°, 36°, 34°, or 33°C. Ischemia resulted in a prompt and severe depletion of all high-energy compounds. Surprisingly, there was no difference in the levels of these high-energy compounds between the normothermic and the hypothermic groups. If markers of neuronal energy charge are not preserved during hypothermia, then one must reconsider the mechanism by which hypothermia provides protection.

POTENTIAL ROLE OF GLUTAMATE IN ISCHEMIC INJURY

Within the past 6 years, the role of glutamate as a mediator of ischemic neuronal injury has begun to be elucidated. Glutamate is an acidic amino acid found in most regions of the brain and spinal cord. Under normal conditions it functions as an excitatory neurotransmitter at a variety of postsynaptic membrane receptors. Activation of these receptors by glutamate results in the influx of sodium ions, calcium ions, and water. During episodes of ischemia, there may be a massive release of glutamate into the extracellular space. A significant proportion of this glutamate is derived from synaptosomal stores (9). This surge of glutamate results in depolarization of the postsynaptic neuron and an influx of water and calcium. In neuronal cell cultures, this influx is evidenced by an immediate swelling of first the dendritic processes and later the neuronal cell body. Subsequently, these neurons progress to cell death by means of calcium-induced activation of various intracellular proteases, endonucleases, and lipases. In vivo evidence of the neurotoxicity of glutamate may be seen after its direct injection into the hippocampus (10). Benveniste and colleagues (10) found a sixfold increase in the extracellular concentrations of hippocampal glutamate in response to 10 min of ischemia. To assess the neurotoxicity of these levels, they injected the same concentration of glutamate into the CA1 region and found that it was capable of destroying hippocampal neurons. Given the extensive in vitro and in vivo evidence from many investigators, it seems clear that excessive extracellular concentrations of glutamate constitute at least one of the mediators of ischemic neuronal damage.

Although the neurotoxic effects of glutamate can be blocked in cell culture studies by administration of either N-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazole propri-onic acid (AMPA) receptor antagonists, it has been difficult to convincingly demonstrate a beneficial effect in animal models of global ischemia (11,12). This failure to provide protection may result from a variety of factors, including suboptimal tissue concentrations of the glutamate antagonists, a multiplicity of glutamate receptor subtypes, and a lack of potency of the antagonists themselves. Clinical application of these potentially useful drugs has been further hindered by the fact that most of these compounds have potent anesthetic-like properties that may complicate the neurologic assessment of patients (13).

GLUTAMATE AND HYPOTHERMIA

Given the demonstrated neurotoxicity of glutamate, it was reasonable to study the effects of hypothermia on periischemic extracellular glutamate concentrations. If hypothermia were associated with lower levels of glutamate, this finding might provide an alternative explanation for the neuronal protective properties of hypothermia. Refinement of the technique of in vivo microdialysis made such investigations feasible, and in 1987 Busto and coauthors (6) reported the ability of mild levels of hypothermia to attenuate ischemia-induced release of excitatory amino acids, including glutamate and aspartate. These results were confirmed by a number of investigators using in vivo microdialysis in a variety of species (14). In each study mild levels of hypothermia were able to attenuate the release of glutamate. In those studies in which neurologic or histologic end points of ischemia were assessed hypothermia showed beneficial effects. A variety of putative neuroprotectants, including adenosine agonists and calcium-channel blockers, have been examined in terms of their ability to prevent or decrease periischemic glutamate levels. None, thus far, has been as potent as a mere 6° decrease in brain temperature.

CLINICAL USE OF HYPOTHERMIA

These positive studies have prompted renewed interest in the clinical use of hypothermia for brain protection. Intraoperatively, mild degrees of systemic hypothermia (3°C–4°C) can be easily produced in anesthetized patients with few adverse consequences. Although 4° of hypothermia may seem insignificant, animal studies predict that this level is sufficient to provide beneficial effects in terms of decreased periischemic levels of glutamate and improved neurologic outcome (6,15). With the advent of improved warming systems, patients can be cooled before a possible transient ischemic event and then returned to normothermia by the end of the surgical procedure after the risk of injury has passed. Use of transient mild hypothermia has been advocated during a variety of neurosurgical proce-

dures that may put the patient at risk of transient cerebral ischemia resulting from temporary occlusion of a cerebral vessel or retractor-induced ischemia.

There has been a resurgence of interest in the use of hypothermia in the intensive care unit (ICU). It is now widely acknowledged that patients with head trauma frequently suffer episodes of cerebral hypoperfusion leading to ischemic injury. Indeed, as many as 91% of traumatic brain injury patients have evidence of ischemic changes on postmortem examination. This high percentage of patients with evidence of ischemic injury was obtained even after excluding cases with necrosis and infarction related to contusions (16). Based on the distribution of the lesions, much of this ischemic damage appeared to be a result of decreased perfusion pressure. Because of the known beneficial effects of hypothermia on ischemic injury, a randomized trial of hypothermia (brain temperature of 32°–33°C for 24 h) has been initiated for patients presenting with closed head injuries and a Glasgow Coma Scale score of 3 to 7. Preliminary analysis of this ICU study suggests that lowering the patient's body temperature results in an improved outcome. Intracranial pressure was significantly lower in the hypothermic group, and 60% of the hypothermic group had moderate, mild, or no disability compared with 40% of those patients randomized to the normothermic group (17). Although the mechanism of this beneficial effect has not been examined, it is tempting to speculate that this degree of hypothermia is associated with lower levels of extracellular glutamate. A number of investigators are now using human in vivo microdialysis to examine this possibility.

In summary, it appears that hypothermia may exert its neuroprotective effects through several mechanisms. A reduction in the metabolic rate, particularly with profound levels of hypothermia, will preserve metabolic stores and increase the brain's tolerance for ischemic events. Hypothermia-induced reductions of periischemic glutamate levels may also contribute to improved neurologic outcome. It is clear that excessive extracellular concentrations of glutamate can be neurotoxic. The ability of hypothermia to attenuate ischemia-induced increases is a likely explanation for some of its neuroprotective properties. Preliminary data indicate that even mild degrees of hypothermia may be beneficial for patients at risk for ischemic events both in the operating room and in the ICU. Given the potential for significant benefit in the face of minimal risk, additional outcome studies are required to better define those situations in which hypothermia should be used.

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