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Postischemic Hypothermia

A Critical Appraisal with Implications for Clinical Treatment

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Abstract

The use of hypothermia to mitigate cerebral ischemic injury is not new. From early studies, it has been clear that cooling is remarkably neuroprotective when applied during global or focal ischemia. In contrast, the value of postischemic cooling is typically viewed with skepticism because of early clinical difficulties and conflicting animal data. However, more recent rodent experiments have shown that a protracted reduction in temperature of only a few degrees Celsius can provide sustained behavioral and histological neuroprotection. Conversely, brief or very mild hypothermia may only delay neuronal damage. Accordingly, protracted hypothermia of 32–34°C may be beneficial following acute clinical stroke.

A thorough mechanistic understanding of postischemic hypothermia would lead to a more selective and effective therapy. Unfortunately, few studies have investigated the mechanisms by which postischemic cooling conveys its beneficial effect. The purpose of this article is to evaluate critically the effects of postischemic temperature changes with a comparison to some current drug therapies. This article will stimulate new research into the mechanisms of lengthy postischemic hypothermia and its potential as a therapy for stroke patients.

Index Entries: Ischemia; postischemic hypothermia; maturation phenomenon; CA1, memory; review.

Introduction

The use of induced hypothermia in medicine has a long and diverse history. Hypothermia has been utilized for the treatment of many diseases, ranging from an analgesic for limb amputation to the treatment of cancer (Fay, 1941) and schizophrenia (Talbott and Tillotson, 1941). More recently the possibility has been explored that hypothermia may decrease

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ischemic brain injury, which is the topic of this article. Although the neuroprotective value of intraischemic (preservative) hypothermia is experimentally well established, postischemic (resuscitative) hypothermia is often viewed with skepticism. Talbott (1941) clearly realized the likely possibility that hypothermia would be abandoned if it were not carefully studied when he wrote: "If a hazardous procedure such as this were mishandled, it might well fall into unwarranted disrepute before the facts and fallacies accompanying it were clearly defined." Review of clinical studies suggests that his prediction came true. After more than 50 years of research, this article attempts to delineate the true value of hypothermia as an anti-ischemic agent. This hypothesis is based largely on data obtained from studies in the rodent. As such, the conclusions are limited by the models used.

Ischemia Models

Brief global ischemia, such as from cardiac arrest, results in selective neuronal loss restricted to vulnerable populations, such as the hippocampal CA1 sector, the dorsolateral striatum, and mid neocortical layers. With prolonged ischemia, injury extends outside of these selectively vulnerable regions, and can also include glia and endothelial cells (Pulsinelli et al., 1982a; Smith et al., 1984). Although some cells die during ischemia, many undergo a delayed neuronal death. The slowest to die and most widely studied are the hippocampal CA1 neurons, which characteristically succumb 24–72 h postischemia. This maturation phenomenon was originally described in gerbil by Ito et al. (1975), and subsequently by Kirino (1982) in gerbil, Pulsinelli et al. (1982a) in rat, and Petito et al. (1987) in human. Ischemic CA1 loss in humans results in anterograde amnesia, an inability to form new declarative memories (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996). Similarly, deficits in working memory (Ordy et al., 1988; Volpe et al., 1988, 1992, Hogan and Beaughard, 1990; Imamura et al., 1991; Colbourne and Corbett, 1995) and habituation (Wang and Corbett, 1990; Babcock et al., 1993; Colbourne and Corbett, 1995) are evident in rats and gerbils following CA1 injury.

In rat, global ischemia is usually produced by either two (2VO) or four vessel occlusion (4VO) (Eklöf and Siesjö 1972; Pulsinelli and Brierley, 1979). In the 2VO model, ischemia is produced by transient occlusion of the common carotid arteries plus systemic hypotension, whereas the 4VO involves cauterizing of the two vertebral arteries followed later by temporary bilateral common carotid artery occlusion. In these models, ischemia durations of 10–20 min produce near-complete CA1 loss. The gerbil, which has an incomplete circle of Willis (Levine and Sohn, 1969, Levy and Brierley, 1974; Ito et al., 1975), is also widely used. Accordingly, the model is much simpler, and brief (3–5 min) bilateral carotid occlusion alone produces dense forebrain ischemia with severe CA1 loss (Mitani et al., 1991; Andou et al., 1992; Colbourne et al., 1993; Colbourne and Corbett, 1995).

Focal ischemia, such as from a middle cerebral artery occlusion (MCAO), produces an ischemic core associated with a marked reduction in cerebral blood flow (CBF) and a surrounding penumbral region that suffers a mild to moderate perfusion impairment. If ischemia is of sufficient duration, pan necrosis (infarction) occurs in the core with a slower-evolving selective neuronal injury in the penumbral region. Ischemic injury typically evolves over the first day (Garcia et al., 1993), but with short-duration ischemic insults, injury may be delayed for several days (Du et al., 1996). In rodents, forelimb reaching deficits and memory impairment result from MCAO (Yamamoto et al., 1988, Tominaga and Ohnishi, 1989; Markgraf et al., 1992; Wahl et al., 1992; Grabowski et al., 1993; Okada et al., 1995a,b). At present, the most common focal ischemia models are in the rat, and involve either direct MCAO via craniectomy (Tamura et al., 1981; Baderson et al., 1986) or the intraluminal model where a suture is advanced through the internal carotid artery until it effectively occludes the middle cerebral artery (MCA) (Koizumi et al., 1986, Longa et al., 1989).

Although a critique of ischemia models (Ginsberg and Busto, 1989; Karpiak et al., 1989; Millikan, 1992) is not the central purpose of this paper, it is important to note several important variations and/or limitations to which all animal models are subject. These factors are, at least, a partial explanation for some of the discrepant findings to be discussed.

There are species, sex, and strain differences in tolerance to ischemia (Payan and Conrad, 1977; Brint et al., 1988; Hall et al., 1991; Barone et al., 1993; Oliff et al., 1995), and species differences in tolerance to hypothermia (Horvath et al., 1948; Adolph, 1951; Hervey, 1973). Lack of protection in one model, therefore, does not necessarily mean lack of protection in other models or in humans.

Within the literature, there is also variability in the degree of physiological regulation (e.g., temperature, glucose, blood pressure). For example, small changes in brain temperature during ischemia can markedly alter injury (Busto et al., 1987). Surprisingly, many investigators continue to use rectal and/or skull temperature measures even though they do not reliably reflect brain temperature (Busto et al., 1987; Minamisawa et al., 1990; Colbourne et al., 1993b). Thus, unknown variations in brain temperature makes the quantification of the insult severity difficult. Careful physiological control (e.g., blood gasses, shivering) during hypothermia is also important. However, few studies closely mimic the manner in which hypothermia is produced in humans. This is especially true in rodents because of the technical limitations (e.g., limited blood sampling) of prolonged physiological control under anesthesia.

Outcome evaluation varies widely. Even though it is unrealistic to expect similar results in all brain regions (e.g., therapeutic windows may be different), the majority of investigators focus on CA1 exclusively. Additionally, many studies rely solely on histopathology, such as CA1 cell counts or infarct volume measurements, to gage a therapy's efficacy even though functional outcome is a more clinically relevant end point. Notably, several factors complicate the relationship between histopathology and behavior, such as innate variation in performance, presence of undetected damage, selective protection by a treatment, and recovery of function. Neurons may also appear normal in Nissl stains, but not function properly (Bothe et al., 1986; Hori and Carpenter, 1994). Function thus cannot be precisely gaged by histology alone. Finally, outcome is critically dependent on the time of assessment (e.g., survival time), as will be discussed.

Many experiments use young adult animals, whereas most strokes occur in the middle-aged to elderly. In fact, young animals have been shown to be more resistant to ischemia (Levine and Sohn, 1969; Payan and Conrad, 1977; Matsuyama et al., 1983; Yao et al., 1991; Futrell et al., 1991; Yao et al., 1993; Davis et al., 1995; Sutherland et al., 1996), and they may also tolerate hypothermia better than old animals (Adolph, 1951; Maguire and Merendino, 1955).

Intraischemic Hypothermia

A substantial and diverse body of literature has shown that hypothermia during an ischemic episode markedly decreases ischemic injury (Dietrich, 1992; Ginsberg et al., 1992; Maher and Hachinski, 1993; Dietrich et al., 1996). From early experiments (Bigelow et al., 1950; Pontius et al., 1954; McMurrey et al., 1956; Rosomoff, 1956, 1957) to the recent findings in rodent global (Busto et al., 1987; Boris-Möller et al., 1989; Minamisawa et al., 1990a,b; Green et al., 1992; Dietrich et al., 1993; Nurse and Corbett, 1994) and focal ischemia models (Onesti et al., 1991; Baker et al., 1992; Chen et al., 1992; Kader et al., 1992; Morikawa et al., 1992; Moyer et al., 1992; Ridenour et al., 1992; Xue et al., 1992; Goto et al., 1993; Aronowski et al., 1994; Karibe et al., 1994a, b, 1995; Meden et al., 1994), the evidence is overwhelming. In parallel to these experimental observations, moderate to deep hypothermia is used during the repair of heart defects in children, which require complete or near-complete circulatory arrest (Bigelow et al., 1950; Lewis et al., 1954; Tharion et al., 1982). Similarly, it is common for

neurosurgeons to allow body temperature to decrease to about 35°C during craniotomy (Baker et al., 1994).

Importantly, intraischemic cooling provides lasting neuroprotection. Green and colleagues (1992) compared mild hypothermic (30°C) to normothermic (37°C) global ischemia in rats, and found long-term (2-mo) histological (CA1 and striatum) and functional protection (spatial learning) in the cooled group. Similarly, Nurse and Corbett (1994) found a lasting (3-week) reduction in hippocampal CA1 injury from mild intraischemic hypothermia (31.4°C) in the gerbil global ischemia model. There was also concomitant behavioral (habituation) and electrophysiological (CA1 field potentials) protection. Surprisingly, a similar long-term evaluation of intraischemic hypothermia has not been done in any experimental model of focal ischemia.

Postischemic Hypothermia

Early Literature

Although intraischemic hypothermia is remarkably neuroprotective, there is a need for postischemic intervention, since most patients who suffer a stroke do not reach treatment until hours after the onset of symptoms. Postischemic cooling was clinically applied many years ago. Williams and Spencer (1958) applied mild hypothermia (32–34°C) for 24–72 h in four cardiac arrest patients immediately following establishment of cardiac rhythm. Although there were no controls, they noted that similar victims rarely survived. In fact, three had no residual neurological abnormality at a 1–2 mo follow-up, whereas the other had a moderate visual defect. Similarly, Benson et al. (1959) applied lengthy (3 h to 8 d) hypothermia (30–32°C) to 12 cardiac arrest patients after a 1–3 h delay. Six of the 12 survived compared to only 1 of 7 untreated patients. This difference, however, was not statistically significant ($p = 0.14$, Fisher's exact test). In addition, the degree of ischemia was difficult to quantify, since it was based on neurological status immediately after arrest. The mean age in hypothermic (31 yr) and untreated patients (55 yr) also differed. Both studies, although suggestive, did not prove that postischemic hypothermia was beneficial.

The first experimental study was performed by Zimmerman and Spencer (1959), who subjected 26 dogs to 10 min of normothermic aortic occlusion. Following this, 14 dogs were cooled to ≈32°C for 18–36 h, whereas 12 were not treated. Only 25% of untreated dogs survived compared with 57% in the hypothermic group, a difference that was not statistically significant ($p = 0.1$, Fisher's exact test). However, the trend towards improved survival combined with a better neurological recovery rate in treated (79%) vs untreated dogs (17%) suggested a beneficial effect. Similar data were produced by Wolfe (1960). He subjected dogs to 5 min of ventricular fibrillation followed by slow cooling to 31°C, which was maintained for 24 h. No untreated dog survived. Hypothermia resulted in a prolonged life in seven dogs, of which three survived.

Mullan et al. (1961) failed to reduce mortality from 6 min of asphyxia-induced cardiac arrest in the dog. The hypothermic group was lowered to 28–30°C and allowed to spontaneously rewarm over 6–12 h. Six of 10 treated dogs died, whereas 7 of 10 normothermic dogs died. As current studies suggest (*see* Recent Studies section), this failure may have been owing to an insufficient duration of hypothermia. However, negative results were also reported in the cat, monkey, and dog by Michenfelder and colleagues (Michenfelder and Milde, 1977; Steen et al., 1979, 1980), who used extended moderate hypothermia (29°C) under repeated diazepam and pancuronium management. The first study examined a 48-h interval of hypothermia induced 30 min following onset of permanent MCAO in five Java monkeys. Hypothermia resulted in 100% mortality compared with 33% dead in a historical control, ischemic group (Michenfelder et al., 1976). The second study similarly found a consistently lethal effect of 48 h of hypothermia in MCA-occluded monkeys. Also, this period of

hypothermia in MCA-occluded cats resulted in excessive mortality (95%) compared with no treatment (20%). Hemodilution or alterations in $PaCO_2$ did not improve outcome with hypothermia. In the last study, a 24-h hypothermic period alone was found to be detrimental in the normal dog. The harmful effects of hypothermia were usually uncovered during or soon after rewarming. For example, the normal decline in CBF, cardiac output, and whole-body oxygen consumption during hypothermia failed to recover during rewarming (Steen et al., 1979, 1980).

MacPhee et al. (1958) also found that dogs subjected to 72 h of hypothermia (26–29°C under anesthetic) failed to recover fully with rewarming. Blair et al. (1956) cooled dogs to 30°C for 1 h by immersion in ice water followed by rapid rewarming in a warm bath (42–45°C). Five of 15 dogs died between 1 and 12 h following rewarming. At that time, left ventricular work and blood pressure remained subnormal, whereas oxygen consumption exceed baseline, and peripheral resistance remained elevated. Since cardiac output did not match oxygen demand, the authors believed there was inadequate circulation. Shivering, which was controlled during hypothermia, was not suppressed during rewarming, possibly accounting for the observed increased oxygen demand.

Fay (1958) also found significant human mortality (11.2%) in his treatment of various conditions, predominantly cancer. He utilized 169 bouts of hypothermia in 126 patients in which temperature was kept below 33°C for up to 7 d. Most of the deaths were during rewarming or within 24 h of rewarming.

Sedzimir (1959) and Hendrick (1959) used generalized refrigeration for head injury, mostly in children. In one case, the total period of hypothermia (31–32°C) was 35 d (Hendrick, 1959). Drake and Jory (1962) subjected patients with critical head injury to up to 10 d of cooling (>28°C). Lazorthes and Campan (1957) also used hypothermia for the treatment of severe head injuries. Although in all head injury studies it was concluded that hypothermia was of some benefit, each study was limited by the small population evaluated and the lack of appropriate control groups.

A survey of the literature does not exactly reveal why protracted, mild hypothermia was abandoned. Perhaps it was the lack of convincing clinical and experimental data, management problems, and reports of deleterious effects with moderate hypothermia in the cat, monkey, and dog. Furthermore, there may have been negative findings in humans that were not published. Perhaps, hypothermia was also supplanted by barbiturates, which were initially thought to provide better neuroprotection.

Recent Studies with Brief Cooling— The Resurrection of Postischemic Hypothermia

After about a decade of neglect, interest in brief, mild, postischemic hypothermia was rekindled with several reports of neuroprotection in rodent ischemia models (Table 1). First, Busto and colleagues (1989a) reduced hippocampal CA1 injury with 3 h of immediate, but not 30-min delayed postischemic hypothermia (30°C). Rats were subjected to 10 min of 2VO ischemia and allowed to survive for 3 d. Similarly, Boris-Möller et al. (1989) reduced CA1, neocortical, and striatal neuronal injury (7-d survival) with ≈2 h of hypothermia (27°C), which was initiated soon after 20 min of 2VO. However, results were variable, and a statistical analysis on their four-point grading scale was not given. Subsequently, Buchan and Pulsinelli (1990) subjected gerbils to 5 min of ischemia immediately followed by 8 h of mild hypothermia (34.5°C). This attenuated CA1 neuronal injury at a 5-d survival. Similarly, Coimbra and Cavalheiro (1990) significantly reduced CA1 injury (7-d survival) with 5 h of immediate postischemic (5 min) hypothermia (29°C) in the gerbil. Chopp et al. (1991) also found significant CA1 preservation (7-d survival) against 8, but not 12 min of 2VO ischemia in rats with 2 h of immediate postischemic cooling (34°C). Carroll and Beek (1992) reduced CA1 loss from 5 min of ischemia in gerbils at 4 d of survival by immediate or 1-h, but not 3-h, delayed hypothermia

Table 1
Recent Studies in Rodent Global Ischemia Models with Brief Postischemic Hypothermia[a]

Model	Delay, h	Degree, °C	Duration, h	Survival, d	Outcome	Ref.
2VO 10 min	0, 0.5	30	3	3	Reduced CA1 injury in 0, but not 30-min delay group	Busto et al., 1989a
2VO 20 min	None	27	2	7	Reduced CA1, striatal, and cortical injury (variable)	Boris-Möller et al., 1989
Gerbil 5 min	None	34.5	8	5	Reduced CA1 loss	Burchan and Pulsinelli, 1990
Gerbil 5 min	None	29	5	7	Reduced CA1 damage	Coimbra and Cavalheiro, 1990
2VO 8 and 12 min	None	34	2	7	CA1 protection against 8, but not 12 min of ischemia	Chopp et al., 1991
Gerbil 5 min	1, 3	28–32	2–6	4	Greater protection with longer hypothermia, but no protection with 3-h delay	Carroll and Beek, 1992
2VO 10 min	2	33	5	7	Reduced CA1 injury	Coimbra and Wieloch, 1992
2VO 10 min	2–8	33	0.5–5	7	CA1 protection depended on delay and duration of hypothermia	Coimbra and Wieloch, 1994
2VO 20 min	None	30	3	60	Partial reduction in spatial learning impairment with no lessening of the habituation impairment	Green et al., 1995

[a]Reports that find CA1 protection. The delay time is to the start of hypothermia induction. Note that in all histology studies, the survival time was 7 d or less. Similarly, brief (≤ 4 d) survival times were used in studies of postcardiac arrest hypothermia in the dog (Leonov et al., 1990a,b; Stertz et al., 1991; Weinrauch et al., 1992; Kuboyama et al., 1993; Safar et al., 1996).

(28–32°C, 2–6 h). Finally, Coimbra and Wieloch (1992, 1994) found significant CA1 preservation at 7 d in rats (10 min of 2VO) given 5 h (≈33°C) of delayed (up to 12 h) postischemic cooling. These authors also showed that a 3.5-h cooling period was much less effective than 5 h, and 30 min was completely ineffective when started at 2 h postischemia. Damage in the striatum was also reduced, but only when hypothermia was begun at 2 h postischemia. Although the optimal hypothermic therapy and the real therapeutic potential are not evident from these studies, it is clear that longer hypothermia is superior, and protection depends on the insult severity.

In the young adult dog, various levels and durations of postcardiac arrest hypothermia were studied by Safar and colleagues (Leonov et al., 1990a,b; Sterz et al., 1991; Weinrauch et al., 1992; Kuboyana et al., 1993; Safar et al., 1996). Sterz et al. (1991) subjected dogs to 10 min of ventricular fibrillation followed by external cardiopulmonary resuscitation (CPR). Some dogs were cooled to 34°C (1 h + 1 h to rewarm) starting with CPR, whereas in others, cooling began at the end of CPR. Functional outcome (i.e., graded as dead, coma, severe or moderate disability, and normal) was improved, and histopathological damage (various areas graded

Table 2
Recent Studies with Brief Postischemic Hypothermia in Rodent Global Ischemia Models[a]

Model	Delay, h	Degree, °C	Duration, h	Survival, d	Outcome	Ref.
Gerbil 5 min	None	23,33	2	7	No protection in CA1	Welsh and Harris, 1991
2VO 12 min	None	30	2	7	No protection in CA1	Chen et al., 1992
Gerbil 5 min	None	29–31	20 min	7	No protection in CA1	Iwai et al., 1993
2VO 10 min	None	33	3	3, 7, 60	Declining CA1 protection with no permanent benefit	Dietrich et al., 1993
Gerbil 5 min	None	29–31	20 min	7	No protection in CA1	Hara et al., 1995
2VO 10 min	2	33	7	7, 60	Declining protection in CA1	Coimbra et al., 1996

[a]Reports that fail to find benefit or where CA1 protection is only temporary.

on five-point scale) was significantly decreased by hypothermia (3-d survival). Moreover, their best therapy (Safar et al., 1996) was a combination of a 12-h period of mild postischemic cooling (34°C under anesthetic), which was begun 10 min following arrest (11 min), and augmentation of cerebral blood flow with hemodilution and elevated blood pressure. Total histopathological damage was reduced, and functional outcome was improved up to a 4-d survival. Hippocampal injury was decreased, but the protective effects, specifically in CA1, were not presented. These data are in contrast to Michenfelder's studies (Michenfelder and Milde, 1977; Steen et al., 1979, 1980) in the cat, dog, and monkey. This is perhaps owing to a greater and more prolonged temperature drop (29°C) in those earlier experiments. It is known that side effects of hypothermia (e.g., cardiac arrhythmias) become more prevalent when the temperature drops below 30°C (Schubert, 1995).

Reports that fail to find protection have appeared (Table 2). Welsh and Harris (1991) did not attenuate CA1 loss at 7 d with up to 2 h of immediate hypothermia (33 and 23°C) against 5 min of global ischemia in the gerbil. Chen et al. (1992a) similarly did not find protection (7-d survival) against 12 min of 2VO ischemia with 2 h of immediate postischemic hypothermia (30°C). A 20-min period of immediate postischemic cooling (29–31°C) also did not reduce CA1 injury (7-d survival) against a 5-min insult in gerbils (Iwai et al., 1993; Hara et al., 1995). Although these negative findings are likely explained by an insufficient duration of hypothermia for the severity of ischemia (Chopp et al., 1991; Carroll and Beek, 1992; Coimbra and Wieloch, 1996), a study by Dietrich and colleagues (1993) casts serious doubt on the long-term benefit of brief postischemic hypothermia. They subjected rats to 10 min of 2VO ischemia followed by 3 h of immediate postischemic cooling (30°C). CA1 protection was found with a 3-d survival as previously noted (Busto et al., 1989a). However, this benefit significantly declined by 7 d and was completely absent with a 2-mo survival. Since the above positive reports had sacrifice times of 7 d or less, it is not clear if brief postischemic cooling can provide prolonged CA1 protection. In fact, Coimbra et al. (1996) recently failed to find lasting histological benefit in CA1 with 7 h of postischemic hypothermia initiated 2 h following 10 min of 2VO ischemia. A 3-h period of immediate postischemic hypothermia (30°C) did, however, partially attenuate the ischemia-induced impairment in spatial learning as

Table 3
Recent Global Ischemia Studies With Prolonged Postischemic Hypothermia[a]

Model	Delay, h	Degree, °C	Duration, h	Survival, d	Outcome	Ref.
Gerbil 3 and 5 min	1	32	12,24	10,30	Persistent protection in CA1 with 12-h hypothermia against 3 min of ischemia and with 24-h hypothermia against 5 min of ischemia, habituation impairments were reduced	Colbourne and Corbett, 1994
Gerbil 5 min	1,4	32,34	24	10–180	Persistent protection in CA1 with reduced habituation and working memory impairments; protection depended on degreee and intervention delay	Colbourne and Corbett, 1995
Gerbil 3 min	2	35	24	4,10	CA1 protection diminished between 4 and 10 d	Nurse and Corbett, 1996
Gerbil 5 min	1	32	24	30	Persistent CA1 protection in aged (18-mo) gerbils	Corbett et al., 1996b
Gerbil 5 min	1	32	24	356	Three gerbils with permanent CA1 protection	This article

[a]Note that there is both long-term histological (CA1) and functional protection.

measured in a water maze test starting 8 wk postischemia (Green et al., 1995). There was no protection in a single open-field test session.

Striatal injury following 2VO ischemia in the rat, however, appears to be persistently (2 mo) reduced with brief (≤7 h) postischemic hypothermic periods (Dietrich et al., 1995; Coimbra et al., 1996). It must be noted that quantification of striatal injury is more difficult at longer survival times owing to shrinkage (Coimbra et al., 1996). Thus, the precise extent of neuroprotection in the striatum is unknown. Perhaps, quantitative behavioral assessment may overcome such shortcomings with histopathology and provide a true estimate of benefit.

Recent Studies with Long-Duration Hypothermia

It was thought that lengthy, but mild hypothermia would provide better and more persistent benefit in CA1 than that seen with brief hypothermia, while avoiding side effects more often associated with greater reductions in temperature. Accordingly, several studies were undertaken to examine systematically factors (e.g., duration of cooling) that were thought to affect neuroprotection (Table 3). In these studies, gerbils were exposed to normothermic forebrain ischemia under brief halothane anesthesia with brain temperature measured during ischemia and afterward in awake, freely moving gerbils (Colbourne et al., 1993b). Postischemic hypothermia was manually produced in the awake animal by a combination of cold water spray and a fan. Notably, pilot data have found excessive mortality when mild hypothermia was induced with prolonged halothane (1%) anesthesia in gerbils or repeated pentobarbital injections in rats. Similarly, Hansen (1954) found that hypothermia (15–17°C) was safer when produced in rats without anesthesia than under pentobarbital anesthesia (40 mg/kg). Although these "exposure techniques" are safe and uncomplicated by anesthetics, the method of

inducing hypothermia in humans is quite different (e.g., the need for an anesthetic and the control of shivering).

In the first study (Colbourne and Corbett, 1994) (Experiment 1), young adult female gerbils were subjected to either 3 or 5 min of normothermic ischemia followed by, in some, 12 h of hypothermia (32°C) starting at 1 h postocclusion. Against the severe 5-min insult, hypothermia resulted in a moderate, but declining amount of CA1 protection at a 10- and 30-d survival. Conversely, hypothermia provided approx 90% protection of CA1 against 3 min of ischemia at both 10- and 30-d survival. Hypothermia also attenuated the early (≤10 d postischemia) ischemia-induced open-field habituation impairments. Thus, for the first time, postischemic hypothermia was shown to provide sustained histological and functional protection. Like the study by Chopp et al. (1991), CA1 protection clearly depended on the duration of ischemia.

Since the amount and permanence of CA1 protection were believed to depend on the duration of hypothermia, a second experiment (Colbourne and Corbett, 1994) (Experiment 2) assessed a 24-h cooling period, which was initiated 1 h following 5 min of ischemia. CA1 protection at a 30-d survival was near perfect and much better than with the 12-h cooling period. Thus, the previous failure to find persistent benefit against 5 min of ischemia with brief hypothermia (12 h) was owing to an insufficient duration of hypothermia for the severity of ischemia.

To clarify further the effects of postischemic hypothermia, a study was conducted to determine whether 24 h of hypothermia (32°C) initiated at 1 h following ischemia (5 min) would reduce hippocampal CA1 loss at a 6-mo survival. Long-term functional outcome (T-maze—working memory, and open field—habituation) was also measured up to 6 mo. The effects of 34°C hypothermia (1-h delay) and 32°C cooling (4-h delay) were also determined to characterize better the effects of degree and intervention delay on hypothermic neuroprotection (Colbourne and Corbett, 1995).

Postischemic hypothermia (32°C, 1-h delay, 24-h length) provided substantial CA1 protection at 6 mo (≈70%). However, there was less benefit than at the 1-mo survival (≈94%). This was significant in medial CA1, whereas middle and lateral sectors were well protected. Thus, although some CA1 death may continue for months following postischemic hypothermia, the highly significant protection, especially in middle and lateral and more posterior CA1, at 6 mo proved that delayed postischemic hypothermia can provide persistent neuroprotection. Supporting this was a marked and chronic reduction in ischemia-induced open-field and T-maze learning impairments throughout the 6 mo following ischemia. In similar treated gerbils (N = 3, 1-h delay, 24 h at 32°C) allowed to survive for 1 yr following 5 min of normothermic ischemia, there was marked protection in the CA1 zone (pilot data of Colbourne, Sutherland, and Auer). Almost always, this insult results in complete CA1 destruction (Colbourne and Corbett, 1994, 1995). This (*see* Fig. 1) and the 6-mo data (Colbourne and Corbett, 1995) prove conclusively that postischemic hypothermia can convey permanent CA1 neuroprotection if the duration and degree of hypothermia are sufficient.

Although the 4-h delayed treatment (32°C) also provided significant protection at a 6-mo survival, this was significantly less than initiating hypothermia at 1 h. Thus, although there is a need for quick intervention following ischemia, there is a therapeutic window of at least 4 h. Recent data (Colbourne, Sutherland, and Auer, in progress) in the gerbil (5-min ischemia) indicate that extended hypothermia (32/34°C each for a day) provides greater protection in CA1, since persistent protection (2 mo) may be achieved with intervention delays at least as long as 12 h. Thus, 2 d of hypothermia appear to extend the therapeutic window compared with 1 d of cooling.

Cooling to 34°C was also beneficial at a 30-d survival, but <32°C at a 30-d survival (Colbourne and Corbett, 1995). Similarly, Nurse and Corbett (1996) found that a milder drop in temperature (≈1.5°C lower than

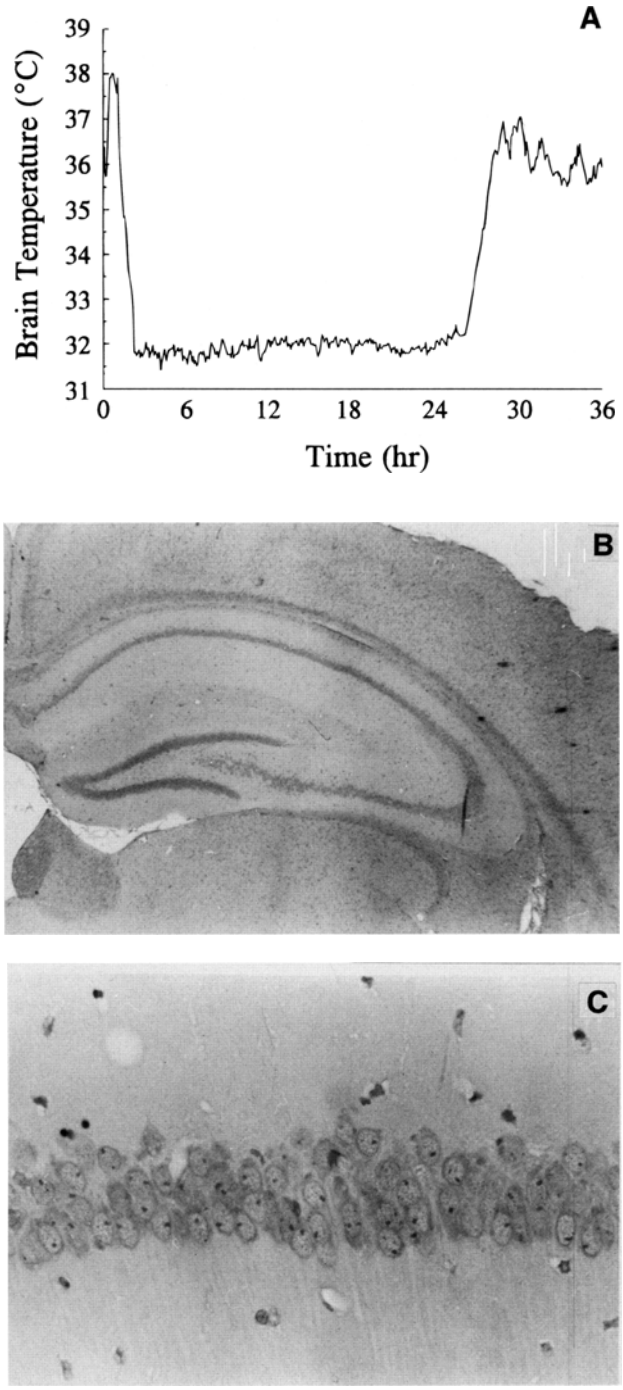

Fig. 1. Ischemia (5-min duration) was followed by 24 h of unanesthetized mild hypothermia (32°C) induced at a rate of 1.0°C/15 min starting 1 h postischemia with rewarming at a rate of 1.0°C/30 min **(A)**. Data are averaged every 5 min from the start of occlusion (0 h). Gerbils ($N = 3$) survived for 1 yr. CA1 protection **(B** and **C)** was consistently robust in the three gerbils and similar to that found at a 6-mo survival (Colbourne and Corbett, 1995). Untreated, this ischemic period consistently destroys >95% of CA1 neurons within a few days following ischemia (Colbourne et al., 1993b; Colbourne and Corbett, 1994, 1995).

untreated group for 26 h) induced 2 h following 3 min of ischemia in the gerbil was protective in CA1. However, in this case, CA1 counts significantly declined between 4 and 10 d (of survival) indicating that protection was only transient. Cooling to 32°C is clearly superior.

The separate effects of intraischemic (32°C) and postischemic hypothermia (1-h delay, 24 h at 32°C) were examined in middle-aged (18-mo) female gerbils. Both persistently reduced CA1 injury at a 30-d survival against 5 min of ischemia (Corbett et al., 1996b). Postischemic hypothermia was also well tolerated with no mortality, similar to the studies by Colbourne and Corbett (1994, 1995).

Finally, Shuaib et al. (1992) reduced injury in an astrocyte culture model of anoxia with 12 h, but not 30 min, of postinsult hypothermia (32°C). A study in mixed cortical cell culture (Bruno et al., 1994) similarly demonstrated significant protection with a 24-h hypothermic period (30°C) when initiated during combined oxygen/glucose deprivation. Hypothermia also reduced injury to high NMDA and glutamate exposure.

Based on the above in vivo global ischemia studies (Colbourne and Corbett, 1994, 1995; Corbett et al., 1996b; this article), it is apparent that protracted postischemic hypothermia can provide permanent CA1 protection in the gerbil. However, it is possible that postischemic hypothermia may convey a different degree of neuroprotection in other species, such as the rat. In fact, some investigators who feel that gerbils are more easily protected than rats believe that postischemic hypothermia is only transiently neuroprotective in the rat, as studies by Dietrich and collaborators (1993) and Coimbra et al. (1996) found with brief hypothermia. The assumption that gerbils are generally more easily protected than rats is groundless, considering that a 12-h duration of hypothermia was not persistently neuroprotective in gerbils subjected to 5 min of normothermic ischemia (Colbourne and Corbett, 1994). In fact, a 24-h or longer period of mild hypothermia was needed. When Coimbra et al. (1996) combined delayed, brief hypothermia with an extended antipyretic treatment, they did find lasting CA1 protection (2 mo) in the rat 2VO model (discussed in section on hyperthermia). Since greater drops in temperature (e.g., 32 vs 34°C) provide better protection (Colbourne and Corbett, 1994), we expect significantly better and chronic CA1 protection in the rat and other species when the cooling period is sufficient (degree and duration) for the severity of ischemia. Thus, although hypothermia studies in other species are certainly warranted, the basic findings should be similar when one carefully considers the many important factors that influence outcome (e.g., severity of insult, delay to treatment, and so forth).

Postischemic Hypothermia After Focal Ischemia

Although the previous in vivo experiments establish protracted, mild postischemic hypothermia as a true neuroprotectant in CA1 against global ischemia, the efficacy against focal ischemia is more clinically relevant. Fortunately, there are data suggesting that postischemic cooling is effective against temporary focal ischemia in the rat. Zhang, R. L., et al. (1993) and Zhang Z. G., et al. (1993) found that a 3-h hypothermic period (30°C) induced immediately or at 1 h postischemia reduced injury after 2 h of MCAO in the rat (1-wk survival). Markarian et al. (1996) studied various durations (1–4 h) of immediate postischemic (3 h MCAO) hypothermia (32–33°C) on 3-d infarct outcome. The 3- and 4-h durations were maximally neuroprotective. In addition, they studied a 3-h duration with 0-, 15-, 30-, and 45-min postischemic delays. Protection was found with up to a 30-min delay. Yanamoto and colleagues (1996) recently found that hypothermia beginning 30 min prior to the end of a 3-h MCAO and maintained for 24 h reduced infarct size at 24 and 48 h. Cooling (≈4°C drop from ischemic animals with range of 32–36°C) was maintained by placing the rats in a 4°C cold room. Brief (1-h) hypothermia was ineffective. There are as yet no published data on the long-term behavioral and histological effects of

postischemic hypothermia in a focal ischemia model in aged animals. Given the possibility of a slow progression of ischemic injury either following brief global (Li and Buchan, 1995a) or focal ischemia (Du et al., 1996), or after brief postischemic hypothermia (Dietrich et al., 1993) such an experiment is warranted.

Sirimanne et al. (1996) subjected 21-d-old rats to unilateral carotid artery ligation with hypoxia. Rats were then subjected to normothermia, 6 or 72 h of immediate mild hypothermia (≈2°C drop), and 6-h delayed hypothermia (66-h duration). With 3-d survival, the 72-h period of immediate cooling reduced both cortical and striatal injury, but not hippocampal damage. Cortical damage was also attenuated with a 21-d survival. The brief 6-h period and the 6-h delayed treatments were ineffective. Perhaps this explains the findings of Yager et al. (1993) who failed to reduce hypoxic/ischemic injury in the 7-d old rat pup with 3 h of postischemic hypothermia (32 and 34°C). Thus, as in the adult rodent, lengthy hypothermia may be necessary for cerebral protection in the neonate.

Intra- and Postischemic Hyperthermia

Hyperthermia during ischemia worsens outcome. Dietrich and colleagues (1990) compared 37 with 39°C ischemia (20 min of 4VO) in rats that survived for 1 or 3 d. They found that hyperthermia not only increased injury and mortality, but accelerated the appearance of ischemic injury and resulted in more frequent infarction. Other reports confirm the detrimental effects of hyperthermia during both global (Minamisawa et al., 1990a; Mitani and Kataoka, 1991; Sutherland et al., 1992) and focal ischemia (Chen et al., 1991; Xue et al., 1992; Meden et al., 1994).

In the rat, MCAO can result in prolonged postischemic hyperthermia (Zhao et al., 1994) presumably owing to hypothalamic damage. Global ischemia in the rat (Coimbra et al., 1996) and gerbil (Kuroiwa et al., 1990; Neill et al., 1990; Colbourne et al., 1993b; Colbourne and Corbett, 1994, 1995) also frequently results in postischemic hyperthermia. It is likely that such extended hyperthermia aggravates injury.

In rat permanent focal ischemia model, Chen et al. (1991) demonstrated that an elevation of brain temperature to 40°C for 1 h postischemia significantly increased infarct volume with a 4-d survival. The role of hyperthermia following global ischemia is less clear. A report by Kuroiwa et al. (1990) suggested that prevention of hyperthermia for 85 min after ischemia (5 min) in the gerbil was substantially protective in CA1 (7 d). However, another study found that gerbils with normal (not hyperthermic) brain temperature for 85 min after ischemia still had severe CA1 damage (Colbourne et al., 1993a). Data from Welsh and Harris (1991) also indicated that this immediate hyperthermia was not critical for CA1 injury in the gerbil (5-min ischemia). Additionally, variations in postischemic hyperthermia over the first 24 h did not predict CA1 loss in untreated ischemic (5-min) gerbils (Colbourne and Corbett, 1996).

Prevention of hyperthermia, however, may have some benefit against a mild insult or when combined with another neuroprotectant. For instance, Coimbra et al. (1996) found lasting benefit from a combination of early hypothermia (33°C for 7 h; 2-h delay) and prevention of hyperthermia for over 3 d in the rat 2VO model, whereas either therapy alone was less effective. It is not known if this combination therapy (brief hypothermia + prevention of hyperthermia) is as effective as protracted cooling alone.

Twenty years ago, Hindfelt (1976) concluded from a retrospective analysis of stroke patients that mild fever (i.e., 37.5–38.0°C) was associated with poorer functional outcome. Przelomski et al. (1986) in a prospective study also found that fever was more common in stroke patients with a large infarction than in those with small or moderate-sized infarcts. Furthermore, fever occurred most often on the first or second day after the stroke. A detailed and controlled study (Reith et al., 1996), utilizing patients admitted to the hospital within 6 h

of stroke, found lower mortality and better outcome in patients who were mildly hypothermic on admission. In contrast, those patients who were mildly hyperthermic had a worse behavioral outcome and a higher mortality rate. In another study (Azzimondi et al., 1995), 43% of stroke patients had fever within the first 7 d of hospitalization, and fever of at least 37.9°C was associated with poor prognosis at 30 d. It is important to note, however, that these correlational data do not prove that fever increases morbidity and mortality, since it is possible that a more severe stroke results in more frequent infection and fever, or results in damage to thermoregulatory centers. For example, 5 min of global ischemia in the gerbil resulted in longer-term hyperthermia than a 3-min ischemic insult (Colbourne and Corbett, 1994). Therefore, although it is quite likely that hyperthermia aggravates injury, there are as yet no conclusive data in humans.

Pharmacological Neuroprotection

We will not attempt to review the vast literature on drug-induced neuroprotection, since there are a several recent reviews on this topic (Buchan, 1990; Buchan et al., 1993; Koreshetz and Moskowitz, 1996). In the past 5–8 yr, numerous agents have been reported to ameliorate ischemic injury in animal models. These compounds include glutamate receptor antagonists, voltage-gated channel blockers, GABA and adenosine analogs, free radical scavengers, anticoagulants, and thrombolytic agents. A number of these compounds are now in clinical trials (Koroshetz and Moskowitz, 1996), and although it is hoped that some will prove effective, there are reasons for pessimism. For example, the dramatic reduction in ischemic neuronal injury provided by MK-801 (Gill et al., 1987, 1988) was shown to be largely owing to drug/anesthetic-induced hypothermia (Buchan and Pulsinelli, 1990; Corbett et al., 1990). Since these early studies with MK-801, additional negative findings have been reported (Nellgård et al., 1991; Nellgård and Wieloch, 1992; Sheardown et al., 1993), the few positive studies note a greatly diminished degree of protection (Gill and Woodruff, 1990) sometimes with questionable histological assessment (Gill and Woodruff, 1990; Hayward et al., 1993).

Hypothermic effects continue to confound pharmacological studies largely because most investigators have not yet realized that postischemic hypothermia, even if delayed by many hours (e.g., 12 h), can convey some neuroprotection. For example, the selective AMPA antagonist NBQX induces a mild hypothermia (1–1.5°C) that develops gradually and persists for several days (Nurse and Corbett, 1996). When this hypothermic effect of NBQX is eliminated, so too is the protection. The detection of this subtle temperature effect was made possible by continuous monitoring of brain temperature in freely moving animals (Colbourne et al., 1993b). We have observed that the considerable stress associated with taking rectal temperature measurements is sufficient to elevate brain temperature temporarily into the normal range as others have noted (Briese and Cabanac, 1991). Many compounds with reported neuroprotective actions have not undergone similar scrutiny. Another serious shortcoming of drug studies is that the survival time has been very short, usually ranging from 2–7 d after the insult. Thus, it is likely that many of these drug treatments, like brief duration (e.g., 3-h) postischemic hypothermia, only delay rather than prevent cell death. Indeed, in the gerbil model of global ischemia, the degree of protection obtained with NBQX is impressive with 4-d survival. However, protection declines substantially by 10 d (Nurse and Corbett, 1996). In the rat, robust CA1 preservation has been noted at 7 d with NBQX and SNX-111, an Ω-conopeptide that blocks N-type Ca^{2+} channels (Nellgård et al., 1991; Buchan et al., 1991; Nellgård and Wieloch, 1992; Diemer, et al., 1992; Sheardown et al., 1993). However, the protection has dissipated completely when survival has been extended to 1 mo (Li and Buchan, 1995b). A similar decline with increasing survival time (i.e., 3 vs 28 d) has also been observed in focal ischemia where the NMDA antagonist MK-801 seemed merely to postpone infarct evolution (Valtysson et al., 1994).

Many other treatments, including barbiturates (Corbett et al., 1996a), (unpublished data), diazepam (Schwartz et al., 1994, 1995), 7-nitroindazole (Horn and Bloom, 1996; unpublished data), and even hypoglycemia (Strauch et al., 1969; Freinkel et al., 1973), that have been found to be neuroprotective can also induce significant hypothermia. The list of compounds that affect temperature is in fact extensive (Clark, 1979; Clark and Clark, 1980a,b, 1981). Unfortunately, meticulous intra- and postischemic temperature control, as is now possible (Colbourne et al., 1996), has generally not been done in these anti-ischemia drug evaluations.

Thus, many drugs that offer promise for treating stroke may fail because hypothermic effects in humans are likely to be negligible. To date, there are no drugs in clinical trials that offer the degree of protection over extended survival times (i.e., 6 mo and longer) as that provided by long- duration hypothermia (Colbourne and Corbett, 1994, 1995) (Fig. 1).

Mechanisms of Hypothermic Protection

At the outset, it is important to recognize that few studies have investigated potential mechanisms contributing to postischemic hypothermic neuroprotection. This is because postischemic cooling has only been recently shown to provide lasting benefit (Colbourne and Corbett, 1994, 1995; Sirimanne et al., 1996) (Fig. 1). As a result, findings from experiments using intraischemic hypothermia may not generalize to the situation of postischemic hypothermia, particularly when it is initiated several hours after ischemia.

Glutamate Toxicity

Excessive release of glutamate during ischemia (Benveniste et al., 1984; Globus et al., 1991) is widely regarded as an important factor in the pathogenesis of ischemic neuronal injury (Rothman and Olney, 1986; Choi, 1990). Glutamate levels rise abruptly and peak during ischemia, slowly declining to preischemic levels within 10–20 min of reperfusion (Benveniste et al., 1984; Globus et al., 1991; Mitani and Kataoka, 1991). The release of glutamate and other transmitters (e.g., glycine) is very sensitive to temperature (Busto et al., 1989b; Neill et al., 1990; Baker, 1991; Baker et al., 1995; Nakashima and Todd, 1996; Winfree et al., 1996). For example, Mitani and Kataoka (1991) have shown graded increases in glutamate release when gerbils were occluded at temperatures ranging from 31–39°C. Since postischemic hypothermia is often initiated one or more hours after occlusion (Xue et al., 1992; Zhang R.L., et al., 1993; Coimbra and Wieloch, 1994; Colbourne and Corbett; 1994, 1995), a time when glutamate levels have normalized, it might seem that an attenuation of glutamate release then would be of little benefit. However, intracellular recordings from slices taken several days after global ischemia suggest that vulnerable CA1 neurons may be irreversibly depolarized by low-frequency input fiber stimulation (Tsubokawa et al., 1992). Other studies have shown a dramatic and potentially lethal rise of Ca^{2+} in CA1 dendrites following afferent stimulation (Andiné et al., 1988, 1992; Silver and Erecinska, 1992). In addition, some (Suzuki et al., 1983; Schiene et al., 1996), but not all (Buzsáki et al., 1989; Mitani et al.,1990; Imon et al., 1991), studies find postischemic neuronal hyperactivity. Regardless of that controversy, there are certainly cases of hyperactivity, such as generalized seizures, especially common in the rat ischemia models. Overall, these events suggest that beginning early in the postischemic period, neurons are in a particularly vulnerable state, so much so that even normal and especially overactive synaptic drive can trigger cell death. If this is the case, then reducing glutamate levels and inhibiting neuronal activity for relatively long periods of time (e.g., 12–48 h) may allow recovery processes within compromised cells to proceed to the point where they can survive. In addition, although microdialysis studies have shown that glutamate levels return to baseline within minutes of reperfusion, it may be that there are late surges

of glutamate release (Andiné et al., 1991) that have gone undetected, since in most studies, the monitoring time is only a few hours into the postischemic period (Beveniste et al., 1984; Mitani and Kataoka, 1991; Globus et al., 1991).

Cerebral Metabolism

It is generally accepted that at least one, but not the sole mechanism by which mild hypothermia protects the brain from an ischemic challenge is by reducing the metabolic rate (Rosomoff and Holaday, 1954; Stone et al., 1956; Michenfelder and Thaye, 1970; Hägerdal et al., 1975; Astrup et al.,1981; Michenfelder and Milde 1991, 1992; Baldwin et al., 1991; Hindman et al., 1995). Accordingly, hypothermia reduces the rate at which energy stores are depleted, the time to electrical silence, and helps to preserve tissue pH (Michenfelder and Thaye, 1970; Astrup et al., 1980; Chopp et al., 1989; Welsh et al., 1990). Moreover, cellular metabolism and pH normalize more rapidly on perfusion in animals rendered hypothermic during ischemia (Globus et al., 1991; Sutherland et al., 1992). In a rodent study using postischemic hypothermia (30°C) initiated immediately after 12 min of forebrain ischemia (2VO), there was a slight reduction in postischemic pH and an elevated concentration of Mg^{2+} as measured by ^{31}P NMR spectroscopy. However, neither of these changes translated into histological savings (Chen et al., 1992) probably because the duration of postischemic hypothermia was only 2 h. Others have also found increased serum Mg^{2+} levels in nonischemic animals subjected to hypothermia (Platner and Hosko, 1953). Furthermore, it is possible that a greater and more prolonged elevation in Mg^{2+}, such as with protracted cooling, may act to blunt injury by virtue of magnesium's ability to block the NMDA channel noncompetitively (Nowak et al., 1984) and to cause vasodilation (Nadler et al., 1987).

Brain ischemia, either focal or global, leads to a state of inappropriate metabolism. That is, in global ischemia, there is a brief period of hyperemia followed by a more prolonged period of hypoperfusion (Harrison et al., 1975; Pulsinelli et al., 1982). Thus, when metabolic demand is very high, the substrate is in limited supply (Lind et al., 1975; Ginsberg, 1996). In the penumbral regions, metabolic demand also becomes very high, a situation that may create an imbalance owing to the hypoperfused condition of the tissue (Obrenovitch, 1995). A major cause of this mismatch between metabolic rate and supply is likely owing, at least in focal ischemia, to waves of spreading depression (Hossmann, 1994; Obrenovitch, 1995). Although repeated surges of K^+, which give rise to spreading depression, are by themselves noninjurious, they could be lethal to neurons under a state of reduced blood flow (Hansen 1990). Spreading depression requires glutamate synaptic transmission, since it can be abolished by NMDA antagonists, such as MK-801 (Lauritzen and Hansen, 1992; Iijima et al., 1992). Interestingly, MK-801 has been reported to increase CBF after both transient and permanent focal ischemia (Buchan et al., 1992). It is tempting to speculate that the protective effects of NMDA antagonists in focal ischemia are the result of the combined action of limiting spreading depression and increasing blood flow to penumbral regions, although hypothermia must also be considered in these cases since spreading depression is temperature-sensitive (Chen et al., 1993; Takaoka et al., 1996). Mild hypothermia (30–34°C) may convey protection by virtue of improving the ratio between metabolic demand and substrate supply. This could be the result of mild hypothermia producing a smaller reduction in CBF than the reduction in oxygen consumption (Croughwell et al., 1992). In addition to hypothermia reducing CBF intraischemically, recent data indicate that rats subjected to a combination of intra- and postischemic hypothermia (30°C) for 6 h showed a more rapid return of CBF on reperfusion of the MCA than did a normothermic group. This increase in blood flow was associated with a better histological outcome (Jiang et al., 1994). A similar attenuation of postischemic hypoperfusion has been found with intraischemic hypothermia in the gerbil

global ischemia model (Widmann et al., 1993) and in the rat MCAO model (Karibe et al., 1994a). Thus, better reflow in the postischemic period may contribute significantly to the beneficial actions of mild hypothermia.

Thoresen et al. (1995) subjected piglets to bilateral carotid artery occlusion with hypoxemia. After resuscitation, the temperature of some animals was lowered to 35°C for 12 h. Hypothermia significantly attenuated the delayed energy failure over the following 3 d, which was found in the untreated group by using ^{31}P magnetic resonance spectroscopy.

Assuming that a decrease in $CMRO_2$ is beneficial, then the studies where hypothermia was maintained in awake animals (Colbourne and Corbett, 1994, 1995; Yanamoto et al., 1996) may have underestimated the neuroprotective potential of hypothermia, since shivering (no anesthesia) would have greatly increased $CMRO_2$ (Dill and Forbes, 1941; Penrod, 1949; Bigelow et al., 1950; Spurr et al., 1954; Stone et al., 1956) at least until the animals stopped shivering perhaps because of declining glucose levels, which inhibit shivering (Dworking and Finney, 1927). Lowered glucose levels, owing to hypothermia-induced shivering, following ischemia may also reduce injury. For example, insulin-induced hypoglycemia has been found to reduce focal injury (Hamilton et al., 1995). Unfortunately, hypoglycemia-induced hypothermia (Dworking and Finney, 1927; Strauch et al., 1969) was not ruled out.

Calcium and Intracellular Signaling

An overload of intracellular calcium has frequently been suggested as being a pivotal event in ischemic cell death (Choi, 1988; Siesjö and Bengtsson, 1989). In addition to activating lipases and proteases, which ultimately can culminate in neuronal destruction, calcium regulates a number of kinases that participate in phosphorylation. Among other things, these kinases affect important cellular functions, such as membrane excitability, transmitter release, mobilization of synaptic vesicles, and cytoskeletal dynamics (Churn et al., 1992; Wieloch et al., 1993). Alteration of protein kinase C (PKC) and Calcium/calmodulin-dependent protein kinase II (CaM-kinase II) has both been implicated in ischemic injury (Cardell et al., 1990; Churn et al., 1992). Ischemia inhibits (by translocation) both of these enzymes (Cardell et al., 1990; Churn et al., 1990; Cardell and Wieloch, 1993), an effect that can be attenuated by intraischemic hypothermia (Churn et al., 1990; Busto et al., 1994). It is also noteworthy that mild hyperthermia, which worsens ischemic injury, potentiates the reduction in PKC and CaM-kinase II activity (Churn et al., 1990; Busto et al., 1994). Recent experiments in mice using gene knockout techniques to reduce CaM-kinase II activity have shown a doubling of focal ischemic injury compared to their wild littermates (Waxham et al., 1996). Hippocampal slices taken from CaM-kinase II knockout mice yield more robust post-tetanic potentiation, a finding that suggests CaM-kinase II regulates glutamate release (Chapman et al., 1995). It is not known whether postischemic hypothermia would restore the activity of these kinases. In one study, postischemic hypothermia failed to prevent the alteration of PKC (Cardell et al., 1991). However, the duration of postischemic hypothermia was not specified, and the only brain area examined for PKC activity was the striatum, a region that typically undergoes irreversible injury in advance of the hippocampus (Pulsinelli et al., 1982; Kirino, 1982).

Protein Synthesis

Prolonged inhibition of protein synthesis has been suggested as a possible cause of ischemic cell death (Bodsch et al., 1985; Thilmann et al., 1986; Araki et al., 1990; Widmann et al., 1991). Protein synthesis as assessed by ^{14}C leucine autoradiography declines to low levels during both global and focal ischemia (Widmann et al., 1991, 1993). On reflow, there is recovery of protein synthesis in nonvulnerable brain areas (e.g., dentate, CA3) and eventually a partial recovery in vulnerable regions, such as CA1 (Widmann et al., 1991, 1993). The recovery in CA1 is undoubtedly confounded by the

presence of increasing gliosis with longer survival times (Widmann et al., 1991; Frank et al., 1993). Importantly, intraischemic hypothermia does not prevent the initial protein synthesis inhibition, but does promote recovery (Widmann et al., 1993) to near-normal levels. Similar effects on protein synthesis are observed following postischemic treatment with barbiturates or the selective glutamate AMPA receptor antagonist, NBQX (Bonnekoh et al., 1992; Frank et al., 1993). It is quite possible that the recovery of protein synthesis observed with these drugs is because of their hypothermic actions. Importantly, immunoreactivity for ubiquitin, an intracellular protein essential in the removal of damaged proteins (Finley and Varshavsky, 1985), is lost following global ischemia (Yamashita et al., 1991; Widmann et al., 1993) whereas intraischemic hypothermia promotes the recovery of ubiquitin synthesis (Yamashita et al., 1991). Thus, a prolonged period of postischemic hypothermia may help to restore normal protein synthesis and degradation, and thereby maintain neuronal viability.

Free Radicals

Free radicals have also been implicated in the ischemic injury process on reperfusion (Phillis and Clough-Helfman, 1990; Clemens et al., 1993; Sutherland et al., 1993; Chan, 1996). Indeed, the conditions of the reperfused brain are ideal for the formation of several oxygen radicals, including the superoxide, hydroxyl, and nitric oxide types. Normally, these toxic ion species are scavenged by protective enzymes, such as superoxide dismutase and glutathione (Coyle and Puttfarcken, 1993; Chan 1996). Although direct measurement of free radicals is not possible because of their short half-life, there is evidence linking hypothermia with a reduction in free radical formation. Karibe et al. (1994c) subjected rats to 3 h of middle cerebral artery occlusion under conditions of intraischemic normothermia (37°C) or hypothermia (33°C). After 3 h of reperfusion, the normothermic group had greater reductions of ascorbate and glutathione in the cortex. Thus, hypothermia blunted the use of endogenous antioxidants, indicating less free radical activity in that group. More directly, Globus and colleagues (1995) trapped hydroxyl radicals with salicylate, and measured the stable adducts (DHBA) during hypothermic (30°C), normothermic (37°C), and hyperthermic (39°C) global ischemia in the rat. Hypothermia blunted the decline in DHBA during and following (up to 1 h) ischemia (less hydroxyl radicals) compared with normothermic animals, whereas hyperthermic ischemia greatly increased the concentration of DHBA. Kil et al. (1996) found similar results in a more recent study. Intraischemic hypothermia (33°C) also reduced the ischemia-induced increase in nitrite, cGMP, and NOS activity during MCAO (Kader et al., 1994). Thus, one beneficial action of mild intraischemic hypothermia may be through a reduction in NO formation. NO can lead to the formation of hydroxyl radicals by combining with a superoxide anion (peroxynitrite), which then breaks down into hydroxyl radicals. Finally, the effects of postischemic hypothermia on lipid peroxidation were studied by Baiping et al. (1994). They subjected dogs to cardiac arrest with resuscitation in which some dogs were cooled to 30–32°C for 2 h beginning 10 min after resuscitation. Two hours of hypothermic reperfusion resulted in significantly less malondialdehyde, with greater concentrations of glutathione, superoxide dismutase, and glutathione peroxidase, indicating less lipid peroxidation in the hypothermic group. These results could be expected on the basis that hypothermia decreases the demand for oxygen during ischemia and in the reperfusion period. A reduction in $[Ca^{2+}]_i$, by hypothermia could decrease mitochondrial oxidative stress and, therefore, diminish radical production. Similarly, hypothermia may blunt the Ca^{2+}-stimulated conversion of xanthine dehydrogenase to xanthine oxidase, resulting in less O_2^- production. A reduction in $CMRO_2$ would reduce enzymatic reactions that generate free radicals, whereas a reduction in lactic acidosis would decrease the catalytic facilitation of

iron-catalyzed free radical formation (Smith and Hall, 1996).

Other Potential Protective Actions

Disruption of the blood–brain barrier could lead to exacerbation of neuronal injury by permitting toxic blood-borne substances to gain access to the brain (Dietrich et al., 1990). Breakdown of the blood–brain barrier following ischemia has been shown to be sensitive to temperature changes during ischemia, with hypothermia preventing and mild hyperthermia exacerbating breakdown (Dietrich et al., 1990; Karibe et al., 1994a).

It now recognized that inflammatory cytokines, such as IL-1β and TNF-α (Liu et al., 1993; Goss et al., 1995), are increased by focal ischemia, which in turn may trigger an increase in various cellular adhesion molecules. Antagonists of these cytokines and adhesion molecules have been found to reduce the degree of focal ischemic injury arising from MCAO (Relton and Rothwell, 1992; Chopp et al., 1994) and in a neonatal hypoxic/ischemic model (Martin et al., 1994). Hypothermia applied for 4 h following traumatic brain injury in the rat attenuated the usual rise in IL-1β (Goss et al., 1995). Hypothermia may provide protection via similar mechanisms in ischemia.

Hypothermia has been found to reduce edema and intracranial pressure (ICP) (Rosomoff and Gilbert, 1955; Rosomoff, 1959; Laskowski et al., 1960; Shapiro et al., 1974; Baldwin et al., 1991; Pomeranz et al., 1993). Accordingly, protracted mild hypothermia has been repeatedly used in the treatment of severe traumatic brain injury in humans (Fay, 1945; Lazorthes and Campan, 1957; Sedzimir, 1959; Hendrick, 1959; Drake and Jory, 1962; Dempsey et al., 1989; Marion et al., 1993; Shiozaki et al., 1993). Such injuries are often associated with decreased CBF and elevated ICP, which puts the patient at particular risk for ischemic brain injury. Protracted postischemic cooling may reduce the delayed postischemic hypoperfusion following cerebral ischemia, while at the same time reducing the metabolic needs of the tissue.

Recent findings have indicated that some ischemic cell death may result from an apoptotic process (MacManus et al., 1993; 1994). However, this view remains controversial, because necrosis and apoptosis exhibit several overlapping biochemical and morphological features (Charriaut-Marlangue et al., 1996). Regardless, it may be that prolonged hypothermia interrupts the apoptotic cascade by preventing a threshold level of cellular injury (via other mechanisms discussed) that ordinarily activates gene pathways responsible for inducing apoptosis. Hypothermia may also directly interfere with apoptotic genes, which consequently results in less neuronal injury. Furthermore, the mechanisms contributing to the very slow CA1 death following brief (Dietrich et al., 1993; Coimbra et al., 1996) or longer postischemic (global) hypothermic periods (Colbourne and Corbett, 1994, 1995) may also involve apoptosis. Unfortunately, no studies have critically examined these possibilities.

In summary, it is not possible to point to a single mechanism that underlies the robust neuroprotection provided by long-duration postischemic hypothermia. Indeed, the remarkable benefit provided by mild hypothermia is likely owing to a multitude of actions, which on the whole make it the ultimate neuroprotective cocktail. This may explain why the effectiveness of pharmacotherapy pales in comparison to that attainable with mild hypothermia, since drug treatments have tended to target a single receptor subtype (e.g., NMDA receptors) or ion channel (e.g., calcium L-channels). What is clear, however, is that the mechanisms by which hypothermia conveys its protection continue throughout the maturation period and not just in the first few hours of recirculation. Choi (1990) defined this time as the amplification and expression periods.

Conclusions

There is an impressive amount of animal data showing neuroprotection with mild postischemic hypothermia, whereas ischemic

injury is exacerbated by hyperthermia. Furthermore, unlike many putative neuroprotective drugs, hypothermia has been found to provide lasting histological and functional benefit in several models of global and focal ischemia. It is clear that hypothermia should be initiated as quickly as possible following the onset of ischemia with, ideally, no more than a 2–3 h delay. Cooling to 32°C is best, since it can provide significant protection, while avoiding systemic side effects often associated with moderate and severe hypothermia (Schubert, 1995). In patients with increased risk (e.g., coronary artery disease), milder hypothermic levels (34°C) or at least prevention of fever may be employed. Although it is known that prolonged hypothermia is better than brief cooling (a few hours), the optimal duration has not been defined and is likely to depend on many factors, such as the type (e.g., focal vs global) and severity (e.g., temporary vs permanent) of the insult. It appears from studies in the dog (Safar et al., 1996), gerbil (Colbourne and Corbett, 1994, 1995; Colbourne et al., in progress), and rat (Sirimanne et al., 1996; Yanamoto et al., 1996; Coimbra et al., 1996) that extended hypothermia of at least 12 h and perhaps several days is warranted. The optimal rates of cooling and rewarming have yet to be determined. It is likely, however, that a gradual or stepwise rewarming phase would be best, since it may avoid difficulties observed by several investigators (MacPhee et al., 1958; Fay, 1958; Michenfelder and Milde, 1997; Steen et al., 1979, 1980).

Interest in the use of hypothermia for severe traumatic brain injury has resurfaced in animal studies (Clifton et al., 1991; Dietrich et al., 1994; Bramlett et al., 1995) and in clinical reports by Clifton and colleagues (Clifton et al., 1992; Clifton, 1995), Marion et al. (1993), and Shiozaki et al. (1993). Shiozaki et al. reduced temperature to ≈34°C for 2 d by cooling blankets and continuous infusion of barbiturates to eliminate shivering. Complications during rewarming occurred in two patients, of which one died. Nonetheless, hypothermia resulted in a significant reduction in ICP and significantly better survival. Marion et al. reduced temperature to 32–33°C and maintained it at that level for 24 h prior to rewarming over a 12-h period. Hypothermia significantly reduced CBF, ICP, and $CMRO_2$ during cooling. There was no difference in the incidence of complications. However, only a trend toward improved outcome was found in the hypothermic group. Clifton and colleagues (National Acute Brain Injury Study) are conducting a phase III study using surface cooling to 32°C for 48 h with slow rewarming in the treatment of severe traumatic brain injury given that the phase II study found the therapy safe (Clifton et al., 1994; Clifton, 1995).

Systemic side effects (see Schubert, 1995 for a recent review) seen in some earlier studies in humans and animal models of ischemia are much less common in recent studies in humans with mild hypothermia. This is at least partially owing to technical improvements. Furthermore, the use of mild vs moderate or severe hypothermia results in lower morbidity and mortality, while still providing benefit. Also, slow rates of rewarming and attention to various potential hazards have likely improved this therapy. The use of selective brain cooling should also minimize side effects (Cabanac, 1993). Although all stroke victims will not be suitable for hypothermic therapy, it is possible to treat fever consistently. A controlled clinical trial of hypothermia in acute stroke is certainly warranted. Further cellular and molecular studies into the mechanisms of this remarkable neuroprotectant will hopefully lead to more selective and effective therapy for patients who suffer stroke or traumatic brain injury.

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