Mild hypothermia decreases the incidence of transient ADC reduction detected with diffusion MRI and expression of c-fos and hsp70 mRNA during acute focal ischemia in rats

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Abstract

The effects of mild hypothermia on the apparent diffusion coefficient of water (ADC) and expression of c-fos and hsp70 mRNA were examined during acute focal cerebral ischemia. Young adult rats were subjected to 60-min middle cerebral artery occlusion under either normothermia (37.5°C) or hypothermia (33°C). Diffusion-weighted echo-planar magnetic resonance imaging was used to monitor changes in ADC throughout the ischemic period. Perfusion MRI with dysprosium contrast was used at the end of the ischemic period to verify that the occlusion was successful. C-fos and hsp70 mRNA expression were examined with in situ hybridization at the end of the ischemic period. The results indicate that the size of the region that exhibited reduced ADC was smaller during hypothermia than during normothermia. Hypothermia also decreased the frequency of occurrence of transient ADC reductions, especially in dorsal aspects of cortex. Expression of both c-fos and hsp70 mRNA were markedly reduced by hypothermia. Transient ADC reduction and c-fos expression are associated with spreading depression, which is believed to contribute to lesion expansion during acute focal ischemia. The results suggest that part of the neuroprotective effect of hypothermia may be due to a reduced incidence of spreading depression.

Theme: Disorders of the nervous system

Topic: Ischemia

Keywords: Hypothermia; Diffusion MRI; Apparent diffusion coefficient; Focal cerebral ischemia; C-fos; Hsp70

1. Introduction

Hypothermia is highly effective in reducing injury during cerebral ischemia. Deep to moderate hypothermia (20 to 30°C) has been found to be useful clinically following head trauma and during surgical procedures that disrupt the cerebral circulation [23]. However, it is also associated with numerous adverse affects such as cardiac dysfunction [45,62]. Mild hypothermia (~33°C), while less protective than deep hypothermia [4,24], has gained considerable interest recently because it provides significant neuroprotection with far fewer complications [36]. The effect of mild hypothermia on clinical ischemic stroke has not yet been examined in large clinical trials [21,36].

In animal models of global ischemia, mild hypothermia
can reduce infarct size and the extent of ischemic neuronal damage in non-infarcted regions [8,11,15,17]. For focal ischemia, mild hypothermia can reduce the size of the necrotic region [32,35,50] and the extent of induction of apoptosis in non-necrotic regions [46]. It can also help to prevent neuronal and endothelial damage associated with reperfusion injury, if it is initiated during [28,35] or after ischemia [41]. Applying hypothermia during the acute phase of prolonged (3 to 4 day) permanent ischemia can reduce the rate of pathophysiological changes, but it generally does not reduce the final extent of injury [53].

Hypothermia has many effects on the underlying pathophysiological changes that occur during cerebral ischemia. It reduces cerebral metabolic rate (CMR) as evidenced by decreased glucose and oxygen consumption rates [4], which delays the onset of energy failure and terminal ischemic depolarization. Direct measurement of ATP levels by 31P NMR and invasive methods has confirmed that hypothermia slows the rate of energy depletion [42,63]. However, this does not completely account for its neuroprotective capacity since anesthetics that diminish CMR to the same extent as hypothermia do not provide the same degree of neuroprotection [52,61].

Hypothermia also reduces neurotransmitter release during ischemia, including glutamate [3,12,28,64], aspartate [7], glycine, gamma-aminobutyric acid [51], and dopamine [12]. Glutamate is a potent excitotoxin that can hasten ischemic depolarization in regions where energy depletion is incomplete [57]. In focal ischemia, hypothermia reduces intra-ischemic increases of glutamate in penumbral [64] but not core regions [28]. However, recent results suggest that the reduction in glutamate release does not fully account for the neuroprotective effect of hypothermia [66].

Diffusion-weighted imaging is sensitive to changes in the apparent diffusion coefficient of water (ADC). Reduced ADC during ischemia is believed to be, at least in part, the result of cellular edema, which occurs with ischemic depolarization [60]. Such depolarization is primarily caused by energy failure [6,13,20] or by excitotoxic injury from neurotransmitters [6]. Reduced ADC also occurs during spreading depression due to abnormal ion fluxes that produce temporary cellular edema [10,43]. Spreading depression is believed to be an important component of lesion size expansion during focal ischemia [27]. Additional phenomena that are not as yet completely understood may also contribute to reduced ADC during ischemia [25]. Diffusion-weighted imaging has been shown previously to be sensitive to reduced water diffusion during hypothermic ischemia [31].

Focal ischemia induces immediate early genes including c-fos [26,38] and stress genes including hsp70 [49] that may be important in protecting the brain from ischemic injury. We previously observed that during acute focal ischemia, c-fos expression was associated with brief transient ADC reduction [47], which was probably caused by transient cellular depolarization [10]. Hsp70 expression occurred in regions exhibiting both transient and permanent ADC reduction [47].

Hypothermia can affect postischemic expression of both c-fos and hsp70 mRNA following temporary occlusion. C-fos expression following temporary forebrain ischemia is hastened by moderate (30°C) hypothermia [33,39]. Cerebral hsp70 expression can be induced by 8-h sustained mild hypothermia without ischemia [18]. Expression following brief forebrain ischemia is reduced by moderate hypothermia [16,40]. The effect of hypothermia on c-fos and hsp70 expression during acute ischemia has not been reported.

In this work, the effect of intra-ischemic mild (33°C) hypothermia on both transient and persistent ADC reduction and expression of c-fos and hsp70 mRNA was examined. The hypotheses tested were that hypothermia would reduce the extent of persistent ADC reduction, the incidence of transient ADC reduction and c-fos mRNA expression.

2. Methods

2.1. Animal model

Male Sprague–Dawley rats weighing 270–325 g were used. They were given free access to food and water prior to use in experiments. Anesthesia was induced with 3% isoflurane in a closed chamber and subsequently maintained with 1.5% isoflurane (both in 70/30 N2/O2) through an intratracheal 16-gauge Teflon® tube. A rodent ventilator (Harvard Instruments, South Natick, MA) was used for mechanical ventilation; respiratory adjustments were made to maintain normal arterial blood gas levels. Just before imaging, pancuronium bromide (0.2 mg in 0.2 ml of 0.9% saline) was administered intraperitoneally to induce paralysis and thereby minimize motion artifacts.

Permanent focal ischemia was produced by occluding the MCA as described previously [34]. A 3-0 monofilament nylon suture with a heat-rounded tip was introduced into an internal carotid artery (ICA), through the stump of a transected external carotid artery (ECA). It was advanced 22 mm past the bifurcation of the common carotid artery (CCA) to block the origin of the MCA. The CCA proximal to the occluded MCA was left patent. The duration of the occlusion was 60 min.

Rats were randomly assigned to two groups, either normothermic, 37.5±1°C (n=7) or hypothermic, 33±1°C (n=11). Body temperature was monitored with a rectal thermistor encased in a grounded electrical shield. A thermostatically controlled warm water pad under the animal was used to maintain rectal temperature within the specified ranges. During imaging, the head was held in a polycarbonate stereotactic frame constructed in our laboratory. The frame helped to eliminate brain motion and reduce heat loss from the head of the rat. In this device, the
rectal temperature accurately represented the brain temperature [47]. Blood gas samples were taken from a femoral artery catheter before and during the ischemic period. Arterial blood pressure was monitored with the same catheter.

2.2. Magnetic resonance methods

Diffusion-weighted echo-planar images were acquired with an MBEST pulse sequence on a 2-Tesla Omega CSI system (Bruker Medical Systems, Fremont, CA), equipped with 20 gauss/cm self-shielded gradients. A 5.5-cm diameter low-pass birdcage coil was used for both pulse transmission and signal reception. Half sine diffusion-sensitizing gradients of up to 6 gauss/cm were used to produce $b$-values of up to 1710 s/mm$^2$. The diffusion gradients were applied parallel to the long axis of the brain. Other image acquisition parameters were: FOV=50 mm, slice thickness=2 mm, and acquisition matrix size = 128×128. The relaxation delay between images was 3.0 s. Images were acquired for a single coronal slice in each rat, centered at the level of bregma. A series of 8 to 11 images were used to construct each ADC map. To verify that the occlusion of the MCA was successful, perfusion imaging was performed with dysprosium DTPA–BMA contrast at the end of the 60-min ischemic period. Additional details of the imaging methods were described previously [47].

ADC maps were constructed with an IDL (Research Systems Inc., Boulder, CO) program written in our laboratory [47]. The program performed pixel-by-pixel least-squares regression to the line: $\ln(S/S_0) = -b(ADC)$, where $S$ is the diffusion-gradient attenuated pixel intensity, $S_0$ is the unattenuated pixel intensity, and $b$ is the attenuation factor [44]. Values of $b$ were calculated from the equation: $b = (2G\gamma/\pi^2)(\Delta - \delta/4)$, where $\gamma$ is the gyromagnetic ratio of the hydrogen nucleus, $G$ is the diffusion gradient magnitude, and $\delta$ and $\Delta$ are the duration and inter-pulse delay, respectively, for the diffusion gradients. The program was also used to compute ADC threshold maps and to interpolate the results to 256×256 resolution. A reduction of 20%, relative to the average value of the non-ischemic hemisphere, was assumed for distinguishing the ADC lesion from the rest of the brain.

A second IDL program was used to identify regions with transient reduction in ADC (TR-ADC). The program initially masked the region with persistently reduced ADC on all maps in each time series. Persistent reduction in ADC (PR-ADC) was determined by averaging the final three ADC maps and identifying the pixels with reductions of 20% or more. The program was then used to determine the size of the ADC lesions (as a function of time) within the five regions of interest (ROI — 1, 2, 3, 4, and 5) shown in Fig. 1. A region was considered positive for TR-ADC if at least 30% of the region was transiently included in the ADC lesion. Similarly, the same five regions of interest were used to analyze the c-fos auto-radiographs. A 30% area threshold was assumed for classifying a region as c-fos-positive.

2.3. In situ hybridization for c-fos and hsp70 mRNA

Frozen brains were cut with a cryostat (Hacker Instruments, Fairfield, NJ) into 20-μm-thick coronal sections. The sections were analyzed for expression of c-fos and hsp70 mRNA with modified versions [37] of methods described by Schalling [54]. The probe used to detect hsp70 was a synthetic oligonucleotide that corresponds to highly conserved amino acids (122–129) near the 5′-end of the human hsp70 coding sequence [29]. For c-fos, the sequence complementary to bases 142–186 was used [19]. Additional details were described previously [47].

2.4. Statistical analysis

A single-tailed $t$-test was used to determine differences in ADC lesion size between the two groups. Differences in the frequency of transient ADC reduction between the normothermic and hypothermic groups in the regions of interest of Fig. 1 and in c-fos-positive regions were determined with the Mann–Whitney Rank Sum Test. The Fisher Exact Test was used to determine if c-fos and hsp70 expression occurred more frequently during normothermia than hypothermia. The association of TR-ADC reduction with c-fos expression was evaluated with the Chi-Squared Test. All statistical calculations were performed with Sigma Stat 2.03 (SPSS, Chicago, IL). Values presented in Tables 1, 2 and 4 are mean±S.D.

3. Results

3.1. Physiological data

Blood pressure and blood gas results for the pre- and
The physiologic blood gas data during pre and intra-ischemic periods are summarized in Table 1. The blood pressure, pH, and P\textsubscript{O\textsubscript{2}} values are all in the normal physiological range. However, the P\textsubscript{CO\textsubscript{2}} levels before ischemia for both groups and during ischemia for the normothermic group were slightly low. The potential significance of this observation will be presented in the discussion section.

### 3.2. Normothermic group

Fig. 2 shows typical ADC maps and autoradiographs for rats in both the normothermic and hypothermic groups. Regions with ADC reductions greater than 20% of the mean value of the contralateral hemisphere (ADC lesion) are shown in color. For the normothermic rat in Fig. 2A, ischemia initially produced significant ADC reductions of 20–45% in the lateral basal ganglia. The region with reduced ADC spread during the following 57 min and eventually encompassed almost all of the MCA territory. The second ADC map in the figure represents the average of the final three ADC maps; the colored region for this averaged result was defined as the persistently ischemic region. On the third ADC map, the persistently ischemic region is shown in dark gray. By digitally superimposing this region on all of the ADC maps acquired during the 60-min ischemic period, regions exhibiting TR-ADC were readily identified. For example, the ADC maps from 42 to 45 min after the onset of ischemia (lower part of Fig. 2A) indicate that TR-ADC occurred in region 1 (defined in Fig. 1). The duration of ischemia (min:sec) is shown below each ADC map.

The complete time-course for the ADC lesion size for the rat in Fig. 2A (as a percentage of the ischemic hemisphere) is shown in Fig. 3A. The data indicate that much of the expansion of the ADC lesion occurred during the first 20 min of ischemia. Transient expansion of the ADC lesion into dorsal medial cortex occurred repeatedly. The time-course for the percentage of ROI-1 (see Fig. 1) included in the ADC lesion is shown in Fig. 3B. Three significant transient increases occurred with a duration of approximately 3 min. The maximum extent of the transient increases was slightly more than 50% of ROI-1.

Results very similar to those shown in Fig. 2A were observed in two other rats for the normothermic group, which also showed transient ADC reduction and c-fos expression in ROI-1. Smaller final ADC lesions were observed in the remaining four rats. Two rats had ADC lesions that encompassed all of the basal ganglia and a small part of cortex. For both of these rats, TR-ADC occurred repeatedly in much of cortex. The remaining two rats had persistent ADC reduction in only part of the basal ganglia; in these rats TR-ADC occurred over a large part of the MCA territory.

For the rat in Fig. 2A, c-fos mRNA was induced in the region where TR-ADC was observed. Hsp70 mRNA was weakly induced over a large part of the MCA distribution. It was induced to a much greater extent at the dorsomedial edge of the MCA territory in cortex, where TR-ADC was observed, but not in cingulate cortex.

C-fos expression was observed in all seven rats of the normothermic group, almost exclusively in regions with TR-ADC. Three rats exhibited c-fos in only medial frontal and cingulate cortex, similar to the results shown in Fig. 2A. The remaining four rats exhibited c-fos in a larger region of cortex, which included medial frontal and cingulate cortex. Two rats with c-fos expression throughout cortex also showed c-fos in the lateral basal ganglia. The time-course for ADC changes within the c-fos-positive region of two rats is shown in Figs. 4A and B. The ordinate of these graphs represents the percent of the
Fig. 2. ADC maps and autoradiographs for c-fos and hsp70 expression in rats subjected to 1 h permanent MCA occlusion. Typical normothermic results are shown in A. The color scale indicates the extent of ADC reduction relative to the mean value of the non-ischemic hemisphere. ADC reductions ranging from 20 to 25% are shown in red; each further 5% increment in ADC reduction is indicated with a different color. The second ADC map is the average of the final three ADC maps and the entire colored region was defined as the persistently ischemic region. This region is shown in dark gray in the third ADC map and in the time series of ADC maps in the bottom of A. Transient ADC reduction was observed in medial frontal cortex, where c-fos was also observed. Hsp70 was weakly expressed throughout the region with persistently reduced ADC and strongly expressed at the dorsomedial edge of this region in cortex. Many rats of the hypothermic group showed little transient ADC reduction (TR-ADC), c-fos or hsp70 expression (B). However, four rats in the hypothermic group showed a single incident of TR-ADC that was associated with c-fos expression (C). Small bands of hsp70 expression were observed for these rats.
Fig. 3. Representative ADC lesion size changes for a normothermic and a hypothermic rat. The top graphs represent the results for the entire hemisphere and the bottom graphs represent the results for ROI-1 (defined in Fig. 1).

Fig. 4. Representative changes in the size of the region with significant ADC reduction in c-fos-positive regions of two normothermic and two hypothermic rats.
c-fos-positive region that exhibited significant ADC reduction. The results in Fig. 4A are for the rat that was shown in Fig. 2A. The graph indicates that three significant peaks in ADC lesion size occurred in the c-fos-positive region. Four significant peaks were observed for the rat represented by Fig. 4B. Two peaks were observed for all of the other rats of the normothermic group except one, which had only one peak.

All seven rats of the normothermic group exhibited hsp70 mRNA expression. Expression was weak in central portions and strong along the periphery of the PR-ADC region. Hsp70 was generally limited to the MCA territory; almost no hsp70 was observed in medial frontal or cingulate cortex where TR-ADC occurred. Strong hsp70 expression in much of the MCA territory was observed in one rat. For this rat, the ADC lesion initially expanded very rapidly to cover a large part of the MCA territory, but subsequently contracted to encompass only about half of the basal ganglia.

3.3. Hypothermic group

ADC, c-fos and hsp70 results for a hypothermic rat are shown in Fig. 2B. The initial ADC lesion was very small. It expanded gradually to eventually include much of the basal ganglia and a small portion of cortex. The time-course for the size of the ADC lesion is shown in Fig. 3C. The five ADC maps at the bottom of Fig. 2B indicate that TR-ADC did not occur during early ischemia. For the normothermic group, TR-ADC was most commonly observed during early ischemia and in ROI-1. The time-course for ADC changes in ROI-1 is shown in Fig. 3D; no significant TR-ADC was observed in this or any other ROI. Also, no c-fos or hsp70 expression was detected. Six other rats of the hypothermic group exhibited no c-fos or hsp70 and almost no TR-ADC. Three of these rats had PR-ADC regions that involved all of the basal ganglia and part of cortex; one had a PR-ADC region that was limited to the basal ganglia. Three rats had PR-ADC regions that covered less than 10% of the ischemic hemisphere, despite exhibiting large perfusion deficits. This contrasts sharply with the findings for the normothermic group, where TR-ADC, c-fos and hsp70 were observed in all seven rats.

Four rats of the hypothermic group showed a single incident of TR-ADC at the beginning of the ischemic period. Results for one such rat are shown in Fig. 2C. No significant ADC lesion was observed in the first map. In subsequent maps (not shown), a small region with reduced ADC appeared in lateral basal ganglia and lateral cortex. As shown at the bottom of Fig. 2C, this region expanded abruptly to include most of the dorsal half of cortex, including cingulate cortex. It contracted during the next 5 min and eventually was limited to the basal ganglia at the end of the ischemic period. The complete time-course for the size of the region with reduced ADC is shown in Fig. 5B. TR-ADC did not recur in this or any hypothermic rat.

One normothermic rat also showed a single TR-ADC that was fairly large (Fig. 5A). C-fos was induced throughout the region that exhibited TR-ADC in Fig. 2C. Two typical time-courses for the ADC lesion size within the c-fos-positive region are shown in Figs. 4C and D. Transient ADC reductions in the hypothermic group were longer than they were in the normothermic group (Figs. 4A and B) and did not recur.

Moderate hsp70 mRNA expression was observed in a small band of lateral cortex for the rat in Fig. 2C. The faint hsp70 expression normally observed in the persistently ischemic region of normothermic rats was not observed in this or any other hypothermic rat. Three rats exhibited moderate hsp70 expression in a thin band along the dorsal periphery of the PR-ADC region. One rat exhibited strong hsp70 expression in a large part of cortex within the MCA distribution, where a single transient reduction in ADC occurred during the first 10 min of ischemia.

3.4. Statistical comparisons between the normothermic and hypothermic groups

Mean ADC lesion sizes for the normothermic and hypothermic groups following 15, 30, 45 and 60 min of ischemia are summarized in Table 2. The values for the normothermic group were significantly larger than the corresponding values for the hypothermic group at all four time-points.

Table 3 summarizes the association of TR-ADC with
Table 3
Association of c-fos mRNA expression and transient ADC reduction

<table>
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<th>Region</th>
<th>Normothermic (n=7)</th>
<th>Hypothermic (n=11)</th>
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*Regions used for this analysis are shown in the anatomical map in Fig. 1.

c-fos expression, based on the regions of interest shown in Fig. 1. For the normothermic group, all seven rats exhibited both c-fos and TR-ADC in region 1. In regions 2 and 3, the incidence of c-fos expression was lower, but nearly all c-fos-positive regions exhibited TR-ADC. Little c-fos or TR-ADC was observed in regions 4 and 5. Of the total 35 regions of interest for the normothermic group, only five exhibited c-fos without TR-ADC or TR-ADC without c-fos. Statistically, the association between c-fos and TR-ADC was highly significant (P<0.001). For the hypothermic group, the frequency of c-fos expression in regions 1, 2 and 3 was less than it was for the normothermic group (on a percentage basis). Of the 11 rats, c-fos was observed in only four rats for regions 1, 2 and 3. For the hypothermic group, only six of the 11 rats exhibited TR-ADC; the association between these two parameters was again highly significant (P<0.001).

Table 4 summarizes the frequency of TR-ADC in the regions of interest shown in Fig. 1. For region 1, the mean number of incidents of TR-ADC for the normothermic group was 1.9±0.6, which is significantly higher than the 0.5±0.7 for the hypothermic group (P<0.01). The frequency of TR-ADC for the normothermic group was also higher in regions 2 and 5, but the differences were not significant (P>0.05).

The frequency of transient ADC reduction within the c-fos-positive regions was much lower in the hypothermic group than in the normothermic group. For the c-fos-positive regions of the normothermic group, the average number of peaks in ADC lesion size was 2.3±1.0, which was significantly higher than the 1.0±0.0 for the hypothermic group (P<0.05). For the hypothermic rats, the duration of the single ADC lesion size peak was much longer than it was for the normothermic group, generally lasting for about 10 min.

All seven rats of the normothermic group exhibited moderate hsp70 expression in at least some part of the ischemic hemisphere. Only four of the 11 hypothermic rats exhibited any hsp70, which was significantly less than for the normothermic group (P<0.05).

4. Discussion

The most significant findings of this work were that during acute focal cerebral ischemia, mild hypothermia diminished the incidence of transient ADC reduction and c-fos mRNA expression. TR-ADC was observed much more frequently in dorsal cortex for the normothermic group than it was in the hypothermic group. Also, c-fos-positive regions of rats in the normothermic group had a higher number of significant peaks in ADC lesion size, 2.3±1.0, than the hypothermic group, 1.0±0.0. Both of these findings support the second hypothesis of this work, that hypothermia decreases the incidence of transient ADC reduction. C-fos expression was observed in all seven rats of the normothermic group. For the hypothermic group, a markedly reduced incidence of c-fos expression was observed (five of 11 rats), which is consistent with the third hypothesis. We also previously reported a very high incidence of c-fos expression during normothermic MCA occlusion [47]. Both TR-ADC [10,43] and c-fos expression [26] are believed to be associated with spreading depression. Thus, the results of this work suggest that mild hypothermia reduces the incidence of spreading depression during acute focal ischemia.

Hypothermia has been shown to reduce the incidence of transient recurring DC potential shifts measured with cortical electrodes during MCA occlusion [14]. Such shifts are believed to be caused by transient cellular depolarization that occurs during spreading depression. Moderate hypothermia reduced the average number of DC potential shifts from 3.75 (at 37°C) to 2.0 (at 30°C). These results are consistent with our observation that hypothermia...
attenuated changes believed to be associated with spreading depression. However, in a recent study done with electrode measurement of extracellular potassium in cortical penumbra, hypothermia did not affect the number of peaks in potassium concentration during acute focal ischemia [56]. Such peaks are believed to be caused by spreading depression. The findings of Sick et al. contrast with those of this work and the work of Chen et al. However, the potassium measurements of Sick et al. were only made at a single point in penumbra. Further studies, with electrodes at multiple penumbral cites, would be useful for clarifying this discrepancy.

Spreading depression during acute focal ischemia is initiated by high concentrations of interstitial excitatory amino acids and/or potassium [27]. During MCA occlusion in rats, measurements with dialysis probes have shown that mild hypothermia significantly reduces interstitial glutamate accumulation in cortical regions of the MCA territory. After approximately 1 h of MCA occlusion, interstitial glutamate levels in dorsolateral parietal cortex were 2.5–3 times higher at 37°C than they were at 33°C [3,64]. A more recent study has confirmed that mild hypothermia (32°C) markedly reduces cortical but not striatal glutamate levels [28]. Similar effects were reported for interstitial accumulation of glycine [28]. Thus, the reduced incidence of TR-ADC and c-fos expression observed in this work may have been caused by diminished levels of interstitial glutamate in cortical regions.

During MCA occlusion, mild hypothermia only slightly reduces steady state extracellular potassium levels in penumbral and core regions [55,56]. In addition, mild hypothermia does not inhibit the initiation of spreading depression by cortical application of potassium chloride [59]. These results suggest that the neuroprotective effect of hypothermia during focal ischemia is not mediated by changes in potassium-induced spreading depression.

Reduced incidence of spreading depression during ischemia could be an important component of the neuroprotective effect of hypothermia, since spreading depression is believed to increase the size of the necrotic region during focal ischemia [9,58]. Blood perfusion levels in a region depolarized by spreading depression increase in response to the increased energy demand necessary to restore ionic gradients [27]. Spreading depression in the absence of marked perfusion deficit does not produce permanent injury. However, in the partially ischemic region where blood flow is restricted, perfusion levels can not increase to meet the increased energy demand. This hastens energy failure, which allows calcium to cross the cell membrane. High levels of intracellular calcium activate enzymes that cause necrosis [57].

A number of studies with cortical electrodes have demonstrated that spreading depression contributes to lesion size growth during focal ischemia. Inhibition of spreading depression by the NMDA receptor antagonist MK-801 (dizocilpine maleate) [22,30] reduces the eventual infarct size. Conversely, increased incidence of spreading depression during MCA occlusion, caused by electrical stimulation, increases neuronal damage and infarct size [2].

The slowing of ADC lesion expansion by hypothermia is also consistent with the first hypothesis of this study and with observations described in a recent preliminary report [67]. In addition to reduced incidence of spreading depression, the slowed rate may have been caused directly by reduced rates of ATP depletion or excitotoxin release. Consistent with both of these mechanisms, DC potential measurements during acute ischemia have shown that hypothermia delays the onset of terminal ischemic depolarization [5].

In addition to being caused by spreading depression, some of the transient ADC reductions observed in this work may have been caused by transient ischemia. Following the initial blood flow reduction with occlusion, an ischemia-induced increase in collateral blood flow may have occurred. For example, the results in Figs. 5A and 5B may have been caused by this mechanism. This would most likely occur in rats with only a limited reduction in blood flow, during the earliest part of the ischemic period. Four hypothermic rats and one normothermic rat exhibited a single TR-ADC during early ischemia. Thus, hypothermia may have preserved energy metabolism sufficiently to allow improved recovery from the brief ischemia. However, not enough rats exhibited these patterns to statistically evaluate this hypothesis. We previously reported that blood flow reduction in our model of focal ischemia can be somewhat variable [48]. The variability is likely a consequence of leaving the CCA proximal to the occluded MCA patent. This model is used in our work because it is a better model for clinical stroke, where some residual blood flow in the ischemic region is usually present. MCA occlusion models with an intraluminal suture and CCA ligation may give more reproducible lesion sizes, but they may also be too severe to accurately represent the pathophysiological changes that occur clinically.

Whether caused by spreading depression or transient ischemia, an important finding of this work is that during both normothermic and hypothermic acute ischemia, c-fos expression is associated with TR-ADC. This observation is consistent with our previous findings for normothermic rats subjected to 30 or 60 min MCA occlusion [47].

Intra-ischemic hypothermia has been shown to hasten c-fos and fos-B protein expression following temporary global ischemia [39]. Gerbils subjected to 10-min bilateral common carotid artery (CCA) occlusion exhibited C-FOS protein in the CA2–CA4 regions of hippocampus within 1 h after moderate hypothermic (30°C) ischemia. This was significantly earlier than for normothermic control animals. Similarly, hippocampal c-fos mRNA expression during early reperfusion was hastened by mild hypothermia (33°C) following 15-min transient forebrain ischemia in rats [33]. The authors of both studies hypothesized that the
faster expression may have been the result of faster recovery of intracellular signaling with hypothermia. These results indicate that induction of c-fos by transient cerebral depolarization is not blocked by hypothermia. In our work, the reduced incidence of c-fos expression may have resulted from hypothermic attenuation of the stimulus for expression, i.e., spreading depression.

The effect of intra-ischemic hypothermia on HSP70 protein expression has been examined following transient forebrain ischemia [40]. Normothermic gerbils subjected to 10-min bilateral CCA occlusion exhibited HSP70 protein in hippocampus and neocortex 24 h after reperfusion. With moderate hypothermia during ischemia (30°C), almost no HSP70 was observed. Similarly, rats subjected to 8-min bilateral CCA and hypotension at 37°C showed strong HSP70 protein expression in hippocampus 48 h after ischemia, but very little HSP70 expression when the intra-ischemic temperature was reduced to 30°C [16]. These results, and the results of this study, indicate that the ischemic stimulus for hsp70 expression is diminished by hypothermia. Hsp70 induction is believed to be controlled by the formation of denatured proteins [1]. Hence, hypothermia may play an important role in reducing the denaturation of proteins during ischemia. The results of this work also suggest that the neuroprotective effect of hypothermia is not related to HSP70 protein expression, at least not during acute focal ischemia.

A limitation of this study is that transient ADC reduction was generally not observed in 100% of the c-fos-positive regions. Three possible reasons for this are: (1) the temporal resolution of the ADC maps was not sufficient to consistently capture complete waves of transient ADC reduction, (2) partial volume effects may have obscured some of the ADC changes, and (3) an ADC reduction threshold of 20% was too high to detect all occurrences of transient ADC reduction. Nevertheless, the results demonstrate a statistically significant association of hypothermia with reduced TR-ADC and c-fos expression.

The low Pco2 values during ischemia for the normothermic group may have resulted in slightly reduced cerebral blood flow levels, relative to the hypothermic group. However, because the degree of hypocapnia was very slight (~10 mmHg), the extent of reduction in perfusion was likely very small [65], especially since isoflurane was used as an anesthetic [68]. The reduced perfusion rates could potentially reduce the incidence of spreading depression in the normothermic group. However, we observed that the incidence of spreading depression was much higher for the normothermic group than it was for the hypothermic group. Thus, the slight hypocapnia probably did impact our primary conclusions.

In summary, the observations presented in this work indicate that mild hypothermia reduced the incidence of TR-ADC and c-fos expression. Both of these phenomena are believed to be associated with spreading depression. Therefore, part of the neuroprotective affect of hypothermia during focal ischemia may be due to a reduced incidence of spreading depression. Hypothermia also significantly reduced the ADC lesion size. Hsp70 expression was markedly diminished by hypothermia, indicating that the neuroprotective affect of hypothermia is not mediated by expression of this stress protein.

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References


