Mild hypothermia (2°C to 3°C below normal) significantly protects the brain from damage after ischemia. After global forebrain ischemia, mild hypothermia protects selectively vulnerable neurons from delayed damage, although this protection might be short-lived. Several investigators have shown that hypothermia after focal ischemia reduces infarction. In contrast, mild hyperthermia aggravates ischemic neuronal damage after global forebrain ischemia and increases infarction after focal ischemia. Although the importance of brain temperature in minimizing or exacerbating brain damage after ischemia is well recognized, the mechanisms underlying this sensitivity to temperature remain poorly understood.

Foremost among the processes that might explain the temperature sensitivity of brain to ischemia are energy metabolism and functions requiring high energy use, such as ion transport. Ischemia is well known to be accompanied within minutes by sudden, large shifts in the concentrations of most extracellular ion species (anoxic depolarization [AD]), which suggests ionic equilibration across cellular membranes. In focal ischemia, these changes are limited to regions of severely limited blood flow. However, in regions that surround the ischemic core, transient ionic disturbances occur that closely resemble cortical spreading depression (SD). The ionic changes associated with focal ischemia are important because they may contribute to brain infarction.

Few reports exist on the effects of temperature on brain ion homeostasis after ischemia. However, a consensus seems to exist that hypothermia does not prevent AD associated with either global or focal ischemia, although studies have reported that the onset of AD may be delayed. Data also have been reported that indicate that mild hypothermia reduces the number of SD-like depolarizations associated with focal ischemia. The frequency of SD-like depolarizations has been associated with the degree of damage after focal ischemia.

Although considerable interest has been generated in the ionic changes that occur during focal ischemia, little attention has been paid to disturbances associated with reperfusion. Most earlier investigations, for example, have shown that extracellular potassium ion activity ([K⁺]o) recovers to or near...
preischemic levels upon reperfusion, which suggests normalization of potassium ion homeostasis. However, we have recently shown that focal ischemia is accompanied by early secondary elevation of \([\mathrm{K}^+]_o\), which is dependent on brain temperature but not blood flow. The goals of the present study were (1) to further investigate restoration of cortical potassium ion homeostasis after transient focal ischemia; (2) to investigate whether ischemia-induced alterations in cortical potassium ion homeostasis are accompanied changes in cortical excitability; and (3) to determine whether mild hypothermia protects against any ionic and excitability disturbances provoked by focal ischemia.

**Materials and Methods**

**Animal Preparation and Distal Middle Cerebral Artery Occlusion**

All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and were approved by the animal care committee of the University of Miami. Fasted male Sprague Dawley rats (n=20; weight, 280 to 340 g) were initially anesthetized with 3% halothane in 30% oxygen/70% nitrous oxide. The animals were intubated, placed on positive-pressure ventilation with 1% halothane in 30% oxygen/70% nitrous oxide, and immobilized with 0.75 mg/kg pancuronium bromide. Polyethylene catheters were placed in the femoral arteries for monitoring blood pressure, sampling arterial blood for blood gas analysis, and supplementing pancuronium when necessary. Oxygen content of the inspired gas mixture, ventilatory rate, and stroke volume were adjusted to maintain blood gas values and pH in the normal range.

Each rat was placed in a head-restraining device, the skin overlying the skull was reflected, and 3 burr holes were made in the right side of the skull. One hole in the zygomatic bone exposed the inferior cerebral vein. The second hole was 2 mm in diameter and was placed 6 to 7 mm lateral and 1 mm posterior to bregma (ischemic core region). The third hole was 2 mm in diameter and was placed 1 mm lateral and 1 mm anterior to lambda (penumbra). The dura overlying the cortex in each region was gently removed by microdissection. A small hook was placed under the distal MCA under microscopic control and was held in a micromanipulator. Retraction of the hook completely occluded the distal MCA. Brain temperature was monitored with a 33-gauge thermocouple inserted between the skull and the dura. Brain temperature was maintained throughout ischemia and for 1 hour of reperfusion at 35°C to 36°C (normothermia) or 31.5°C to 32°C (mild hypothermia) with a lamp connected to a temperature-regulated electrical relay.

The distal MCA was occluded for 1 hour by gentle retraction of the hook, and ischemia was confirmed by sudden elevation of \([\mathrm{K}^+]_o\) to >50 mmol/L in the core region. The sudden elevation of \([\mathrm{K}^+]_o\) indicated that cerebral blood flow was reduced to less than the ischemic threshold and thus was used to confirm that the severity of ischemia was similar in all animals. In initial experiments, elevation of \([\mathrm{K}^+]_o\) after distal MCA occlusion (MCAO) was often incomplete or transient, which indicated incomplete ischemia or reflow from collateral circulation. To ensure completeness of ischemia in all animals, blood pressure was lowered by withdrawal of arterial blood into a heparinized syringe so that \([\mathrm{K}^+]_o\) remained elevated to >50 mmol/L for the entire 1-hour period. Reperfusion was accomplished by releasing occlusion of the MCA and by returning blood to the femoral artery to raise blood pressure. A subpopulation of animals (n=5) was allowed to recover after MCAO and was reanesthetized 24 hours later for subsequent analysis of cortical \([\mathrm{K}^+]_o\).

**Measurements of \([\mathrm{K}^+]_o\)**

\([\mathrm{K}^+]_o\) was measured with double-barreled microelectrodes as described earlier. The microelectrodes were inserted into the cortex to a depth of approximately 500 μm from the surface through burr holes positioned over the ischemic core and ischemic penumbra (see above). All \([\mathrm{K}^+]_o\)-sensitive microelectrodes were calibrated at room temperature. No effect of temperature was observed on calibrations over a range of 37°C to 27°C. Cortical excitability was determined by applying constant current pulses directly to the cortex in the vicinity of the \([\mathrm{K}^+]_o\), recording electrodes. We chose this method instead of stimulation of an afferent pathway to avoid possible complications as a result of postischemic inhibition of synaptic transmission. Bipolar stimulating electrodes (1-mm tip separation) were placed on either side of the \([\mathrm{K}^+]_o\)-sensitive microelectrode so that electric current could be passed through the cortex in which \([\mathrm{K}^+]_o\) measurements were made. Increased \([\mathrm{K}^+]_o\) was provoked by applying 2-second trains (20 Hz) of constant current pulses (0.5-millisecond duration) directly to the cortical surface. Identical current intensities were used before MCAO, 1 hour after reperfusion, and again 24 hours after reperfusion.

**Measurement of Cortical Infarct Area**

Because \([\mathrm{K}^+]_o\) measurements were conducted in cortex, cortical surface infarct area rather than infarct volume was estimated. After completion of \([\mathrm{K}^+]_o\) measurements 24 hours after brain reperfusion, rats were euthanatized and their brains were removed and immersed in a 2% solution of 2,3,5-triphenyltetrazolium hydrochloride (TTC) in normal saline at 37°C for 1 hour. After staining, the brains were fixed in 10% phosphate-buffered formalin for photography and digital analysis of infarct area. Infarction was estimated from the cortical surface by removing the cortex from subcortical structures, sectioning it at the frontal and occipital poles, and laying it flat on a cover slip. This procedure was used to eliminate cortical curvature (similar to a Mercator map projection) for facilitation of estimates of surface infarct area. Digital images of the cortical surface were recorded, and both the total cortical area and the area of infarction (unstained region) were measured by digital morphometry. Infarct area was calculated as a percentage of the total cortical area.

**Statistical Analysis**

Although measurements of \([\mathrm{K}^+]_o\) were continuous, for statistical purposes, values were sampled before ischemia, at the end of 1 hour of MCAO, 1 hour after reperfusion, and again at 24 hours after reperfusion. Values are expressed as the mean±SE of the mean and were compared by ANOVA for repeated measures. The method of orthogonal contrasts was used for post hoc comparisons of individual means. Student’s *t* test was used when only a single comparison among groups was warranted (eg, comparison of latency to depolarization during ischemia).

**Results**

**Blood Chemistry**

Before the onset of ischemia (and brain hypothermia), arterial Po, Pco, and pH averaged 111.6±15.3 mm Hg, 37.4±4.5 mm Hg, and 7.46±0.05, respectively, in normothermic animals. In animals designated for mild hypothermia, arterial Po2, Pco2, and pH averaged 126.5±13.7 mm Hg, 39.2±4.4 mm Hg, and 7.44±0.05, respectively. More importantly for the present study, arterial Po2, Pco2, and pH averaged 115.2±15.4 mm Hg, 38.5±4.1 mm Hg, and 7.39±0.06, respectively, in normothermic animals during reperfusion. In hypothermic animals, arterial Po2, Pco2, and pH averaged 129.1±19.5 mm Hg, 37.1±2.7 mm Hg, and 7.42±0.04, respectively, during reperfusion after focal ische-
mia. No statistical differences in blood gases and pH were observed between normothermic and hypothermic animals during reperfusion after focal ischemia.

Effect of MCAO on $[K^+]_o$ in Core and Penumbra of Normothermic and Hypothermic Animals

In both normothermic and hypothermic animals, distal MCAO resulted in elevation of resting (unstimulated) $[K^+]_o$ in core and penumbral regions of cortex. Examples of these changes in cortical $[K^+]_o$ are shown in Figure 1. Values recorded before ischemia, 1 hour after onset of MCAO, 1 hour after reperfusion, and 24 hours after reperfusion are shown in Figures 2A (core) and B (penumbra). During MCAO, $[K^+]_o$ in the core of both normothermic and hypothermic animals was significantly elevated from preischemic levels ($F=78.8; P<0.0001$), and no significant differences were observed between the 2 groups. In normothermic animals, core $[K^+]_o$ during MCAO averaged 60.3±3.48 mmol/L (mean±SD), whereas in hypothermic animals, it averaged 50.6±4.9 mmol/L. Moreover, mild hypothermia did not prevent or significantly delay the onset of $[K^+]_o$ elevation in cortical ischemic core. In normothermic animals, the latency to $[K^+]_o$ elevation after MCAO averaged 48.6±7.2 seconds, whereas in hypothermic animals, the latency was 57.6±7.3 seconds ($t=0.83; P=0.2$).

One hour after reperfusion, baseline $[K^+]_o$ recovered to 4.5±1.4 and 3.1±0.8 mmol/L in normothermic and hypothermic animals, respectively. Twenty-four hours after reperfusion, baseline $[K^+]_o$ was 5.7±2.2 mmol/L in normothermic animals and 3.05±0.6 mmol/L in hypothermic animals. During reperfusion, $[K^+]_o$ in the core of normothermic animals remained significantly elevated above preischemic levels both at 1 hour ($F=6.8, P<0.05$) and 24 hours ($F=9.3, P<0.01$). In hypothermic animals, no significant elevation of $[K^+]_o$ was found following reperfusion after MCAO either at 1 or 24 hours.

In penumbra, resting $[K^+]_o$ (excluding SD-like events) was not significantly elevated above preischemic levels during MCAO or at any time after reperfusion. However, in penumbra, transient depolarizing waves were observed during which $[K^+]_o$ was elevated to near 50 mmol/L (see Figure 1). These transient events resembled cortical SD associated with MCAO, which has been described by others.20,29,30 The frequency and duration of the SD-like events was highly variable and ranged in frequency from 1 to 5 per 1-hour ischemic episode and in duration from 30 seconds to approximately 10 minutes. SD-like events were not observed following reperfusion after MCAO. No significant difference was found in the frequency of SD-like events, which in penumbra averaged 2.17±1.0 in normothermic animals and 2.89±1.2 in hypothermic animals. No apparent effect of hypothermia was found on the duration of SD-like events, although great variability in the waveform of these events (duration range, 1 to 5 minutes) precluded meaningful analysis.

Effect of Mild Hypothermia on $[K^+]_o$ After Direct Cortical Stimulation

Before MCAO, direct cortical stimulation (DCS) resulted in transient elevation of $[K^+]_o$ in both regions destined to become ischemic core and in regions destined to become penumbra (Figure 3). In normothermic animals, DCS failed to elevate $[K^+]_o$ during MCAO in ischemic core as expected (data not shown) but also failed to elevate $[K^+]_o$ 1 hour after reperfusion, when baseline $[K^+]_o$ had returned to near preischemic levels (Figure 3A, top trace). Elevation of $[K^+]_o$ after DCS in penumbra was possible but was attenuated (Figure

![Figure 1. Simultaneous measurements of $[K^+]_o$ in ischemic core and penumbra during 1 hour of transient distal MCAO.](image)

![Figure 2. Average (mean±SEM) values of $[K^+]_o$ recorded in the core (A) and penumbra (B) before MCAO at the end of 1 hour of MCAO, 1 hour after reperfusion, and 24 hours after reperfusion (**$P<0.01$; *$P<0.05$ vs preischemic values; values in parentheses indicate number of animals per group).](image)
Mild hypothermia protected cerebral cortex against loss of excitability (indicated by response of $[K^+]_o$ to DCS) in both core (Figure 3B, top trace) and penumbra (Figure 3B, bottom trace) 1 hour following reperfusion after MCAO.

The average changes in $[K^+]_o$ after DCS ($\Delta[K^+]_o$) recorded in normothermic and hypothermic animals following reperfusion after MCAO are shown in Figure 4. Mild hypothermia significantly protected against loss of excitability after MCAO ($F=9.83$, $P=0.004$). In the ischemic core region of normothermic animals, MCAO resulted in attenuation of $\Delta[K^+]_o$ to 16.2±6.3% of preischemic values 1 hour after reperfusion. In hypothermic animals, $\Delta[K^+]_o$ in the core region recovered to 57.2±15.8% of preischemic values.

Mild hypothermia also tended to improve recovery of $\Delta[K^+]_o$ in penumbra, although the degree of improvement was not as dramatic because of the lack of initial MCAO-induced impairment compared with the core region. In penumbra, $\Delta[K^+]_o$ in normothermic animals recovered to 39.1±13.7% of preischemic values 1 hour after reperfusion after MCAO. In hypothermic animals, $\Delta[K^+]_o$ in penumbra improved to 70.1±11.3% of preischemic values.

The response of cortical $[K^+]_o$ to DCS 24 hours following reperfusion after MCAO also was tested in a limited number of animals. As expected, no response of $[K^+]_o$ to DCS of the core region was seen in 5 of 5 normothermic animals. In hypothermic animals, only 2 of 5 animals failed to respond to DCS in the core 24 hours after MCAO. In the penumbra, 2 of 4 normothermic animals responded positively to DCS, whereas 6 of 6 hypothermic animals responded positively. The high variability among animals 24 hours after MCAO precluded meaningful statistical analysis, especially in the ischemic core region. However, some protection due to hypothermia still did appear to be evident at this time.

**Effect of Mild Hypothermia on Cortical Infarction After MCAO**

One hour of distal MCAO in normothermic animals consistently resulted in cortical infarction (measured by TTC staining) 24 hours after reperfusion. Mild hypothermia profoundly protected against cortical infarction. Average estimates of surface infarction area in normothermic and hypothermic animals are shown Figure 5. The average area of infarction in normothermic animals was 16.6±3.9% of the total cortical surface, whereas in hypothermic animals, the average infarction area was reduced to 1.4±2.0% of the cortical surface.

**Discussion**

In the present study, we compared the recovery of cortical potassium ion homeostasis after 1 hour of transient distal MCAO in normothermic and mildly hypothermic animals. In addition to recovery of resting $[K^+]_o$, we evaluated release of $K^+$ in response to DCS as an indication of cortical excitabil-
ity. In normothermic animals, reperfusion after 1 hour of transient MCAO resulted in incomplete restoration of [K\(^+\)], failure of [K\(^+\)], to increase with DCS, and widespread infarction 24 hours later. Mild hypothermia prevented the changes in K\(^+\) homeostasis and the ensuing infarction. The data indicate that ionic disturbances are evident very early following reperfusion after focal ischemia and that these early changes are temperature dependent.

The protective effect of mild hypothermia against cerebral infarction after focal ischemia is well documented. Early investigations sought to determine whether intraschemic hypothermic protection resulted from preservation of energy metabolism. Although the rate of decline of high-energy phosphates such as ATP and phosphocreatine was slowed by mild hypothermia, ischemic levels of these compounds nonetheless were observed within minutes. The suggestion that mild brain hypothermia does not provide protection by significantly slowing rates of ATP utilization was indirectly supported by measurements of ionic changes such as [K\(^+\)]. The sudden elevation of [K\(^+\)], seen during ischemia has been related to the decline in ATP levels associated with ischemia. Although hypothermia has been reported to delay the onset of [K\(^+\)], elevation after ischemia, the delay is only a few minutes and not likely to contribute significantly to the damage that ensues during focal ischemia, which might persist for >1 hour. Results from the present study further support the notion that hypothermic protection does not result from delaying the onset of energy failure and loss of ion homeostasis. First, the onset of sudden depolarization was not significantly delayed by mild hypothermia. Second, in the present study, the duration of focal ischemia was determined from the onset of depolarization so that normothermic and hypothermic animals remained in the depolarized state for exactly 1 hour. Thus, data from the present study indicate that mild hypothermia during brain ischemia does not provide protection by reducing energy use and subsequently by delaying neuronal depolarization.

A related issue that concerns mild hypothermia and infarct size after focal cerebral ischemia is the role of transient SD-like depolarizations. SD-like events have been shown to occur in penumbra and nonischemic regions of cerebral cortex after focal cerebral ischemia. Moreover, the frequency of these events has been related to infarct volume. We observed no significant decrease in the frequency of SD-like elevations of [K\(^+\)], in penumbra of hypothermic animals compared with normothermic animals. This appears to be in contrast with the observations of Chen et al, who reported that hypothermia decreased the number of DC deflections recorded after MCAO by intraluminal suture insertion. However, their study did not make clear whether the DC deflections were recorded in the ischemic core or in the penumbra. If the recordings were in the ischemic core, then repeated DC deflections might have indicated incomplete ischemia rather than SD-like events, because the latter should not be observed in completely depolarized tissue (ie, ischemic core). In the present study, the ischemic core remained depolarized throughout 1 hour of ischemia by lowering arterial blood pressure. In the absence of hypotension, we often observed incomplete or spontaneous reversibly elevation of [K\(^+\)], during MCAO. However, on the basis of our data, we conclude that hypothermia did not reduce infarction by limiting the number of SD-like peri-infarct depolarizations.

Our data are more consistent with the hypothesis that hypothermia protects against infarction by preventing secondary loss of ion homeostasis associated with reperfusion. Loss of ion homeostasis was indicated both by elevation of resting [K\(^+\)], and by the failure of DCS to elicite elevation of [K\(^+\)]. Gido et al also reported a secondary rise in [K\(^+\)], following reperfusion after MCAO, but in their study, the delayed elevation occurred 6 hours after the onset of reperfusion. Secondary elevation of [K\(^+\)], might reflect reperfusion-induced deterioration of brain energy state related to secondary failure of mitochondrial function.

The failure of [K\(^+\)], to elevate after DCS likely did not result solely from elevation of resting [K\(^+\)], which was only a few millimoles per liter greater than normal levels. Failure of [K\(^+\)], to respond to DCS suggests incomplete neuronal repolarization to the point of blocking action potential discharges. However, the fact that [K\(^+\)], recovers during reperfusion indicates that some restoration of transmembrane potential has occurred. Other investigators have shown that total brain potassium ion levels decrease after focal brain ischemia, which indicates a decrease in [K\(^+\)], which would further magnify neuronal depolarization by reducing the transmembrane potassium ion gradient. However, it is also possible that ischemia or reperfusion may directly alter properties of ion transport molecules, voltage-dependent ion channels in neurons, or release of excitatory neurotransmitters such as glutamate. For example, oxyradical formation, which increases after brain ischemia in a temperature-dependent manner, has been reported to inhibit neuronal Na\(^+\),K\(^+\)-ATPase.

In conclusion, we have shown in the present study changes in cortical potassium ion homeostasis and excitability that occur as early as 1 hour following reperfusion after transient focal cerebral ischemia. These ionic disturbances were ameliorated by mild brain hypothermia, as was subsequent cortical infarction measured 24 hours later. An important question not resolved by the present study is whether early changes in ion homeostasis following reperfusion after focal ischemia contribute to or cause eventual tissue infarction. It is also important to determine the window of protection provided by mild hypothermia for preservation of ion homeostasis and excitability.

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Mild brain hypothermia is known to be neuroprotective and to limit the extent of brain injury in experimental models of focal cerebral ischemia. Conversely, mild hyperthermia increases injury in the same experimental models. The mechanisms that account for the protective effect of hypothermia during ischemia are not well defined, however.

Ischemia is associated with many molecular, biochemical, and cellular changes that may contribute to brain injury. These changes include alterations to the normally tight regulation of ion homeostasis that is present in brain. For example, ischemia produces large changes in extracellular levels of potassium ion. The present study examined the hypothesis that hypothermia (to about 32°C) would protect the brain from deterioration of ion homeostasis, loss of excitability, and injury after focal ischemia with reperfusion. The results of the study suggest that after transient focal ischemia, early changes in potassium ion homeostasis occur with loss of neuronal excitability and that these changes are attenuated by hypothermia.

Thus, this study provides additional insight into effects of hypothermia during cerebral ischemia and indicates that the loss of ion homeostasis following reperfusion after ischemia is temperature dependent. The precise mechanisms that mediate the effect are unclear and not defined by these experiments. Possible mechanisms by which hypothermia might affect ionic changes and brain injury after ischemia include alterations in release of neurotransmitters, reduced production or increased degradation of reactive oxygen species, or changes in function or expression of ion channels and ion transport mechanisms.

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Mild Hypothermia Improves Recovery of Cortical Extracellular Potassium Ion Activity and Excitability After Middle Cerebral Artery Occlusion in the Rat
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