Spontaneous Cerebral Hypothermia Diminishes Focal Infarction in Rat Brain

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Background and Purpose: Brain temperature during ischemia is known to strongly influence the extent of cellular injury. The objectives of the present study were to determine the effect of severe focal ischemia on brain temperature and to assess the influence of those changes on focal infarction.

Methods: Severe focal ischemia was produced in rats using permanent occlusion of the distal middle cerebral artery combined with transient (60-minute) bilateral carotid artery occlusion. The temperature of the ischemic focus was measured with a small subdural probe. Three groups of rats were studied. In the first group, brain temperature was permitted to decline spontaneously to 32°C after occlusion. In the second group, brain temperature was maintained at 37.5°C during occlusion. In the third group, the brain temperature was maintained at 37.5°C for 40 minutes postocclusion before cooling. After recovery for 24 hours, the volume of infarction was measured in histological sections.

Results: In the absence of cranial heating, the brain temperature fell to 33°C by 10 minutes postocclusion, and infarct volume was 19±9 mm³ (mean±SEM; n=6). Maintaining brain temperature at 37.5°C increased the volume of infarction to 82±16 mm³ (n=7; p<0.001). Delayed cooling did not prevent the increase in infarct volume (75±16 mm³; n=6).

Conclusions: These results demonstrate that in the present model of transient focal ischemia, spontaneous cooling of the brain during ischemia diminishes the extent of focal infarction, relative to that observed when cerebral hypothermia is prevented or delayed for 40 minutes. (Stroke 1992;23:1812-1816)

KEY WORDS • cerebral arteries • cerebral ischemia • hypothermia • rats

In experimental models of global ischemia, the extent of cellular injury is strongly dependent on brain temperature.1-3 However, in models of focal ischemia, the influence of brain temperature on tissue injury has not yet been clarified. Early investigations indicated that the effects of focal ischemia were diminished by transient hypothermia4 but were exacerbated when the duration of hypothermia (29°C) was extended to 48 hours.5,6 With the development of models of focal ischemia in rat brain7-9 has come the opportunity to investigate the effects of hypothermia on focal injury more extensively.

Recently it was reported that transient cooling to 24°C decreased the volume of infarction caused by permanent occlusion of the middle cerebral artery (MCA) in rats.10 The efficacy of transient hypothermia in a model of permanent occlusion is interesting because it implies that early pathogenic events may be both transient and temperature dependent. However, preliminary reports from a number of laboratories, including our own, have failed to detect hypothermic protection in models of focal ischemia unless reperfusion is permitted.11-13 Further, it is not yet established whether moderate reductions in brain temperature exert a strong influence on focal injury, as reported for global ischemia. Finally, the rodent model of combined occlusion of the MCA and carotid arteries9 is likely to induce cerebral hypothermia during occlusion. Thus, the objectives of the present study were 1) to determine the effect of combined occlusion of the MCA and carotids on brain temperature and 2) to assess the influence of the measured temperature changes on tissue injury. The results of this study have been presented previously in preliminary form.11

Materials and Methods

Male Wistar rats weighing 350-550 g were given free access to food and water before surgery. The animals were anesthetized with pentobarbital (50 mg/kg i.p.), and O₂ was suffused over the nose of the spontaneously breathing animal through a loose-fitting nose cone. Before surgery a local anesthetic (0.5% solution of bupivacaine, Winthrop Pharmaceuticals, New York) was injected into the operative sites. A rectal temperature probe connected to a heat lamp was inserted to maintain core temperature at 37.5°C. The tail artery was catheterized for measurement of arterial pressure, Po₂, Pco₂, and pH.

Severe focal cerebral ischemia was produced using a modification of the model of combined MCA/carotid occlusion.8,14,15 In brief, a 2-3-cm linear incision was made in the midline of the neck, and the common carotid arteries were isolated and encircled loosely with silk thread. The rat was then turned on its side, and a
2–3-cm vertical incision was made 0.5 cm behind the right eye. The temporalis muscle was incised and retracted to expose the skull. A small burr hole (3–4 mm) was made anterior to the junction of the zygomatic arch and cranium. The dura was removed over the main trunk of the MCA. A stainless steel wire, 18 μm in diameter and bent at 90° to form a hook, was maneuvered using a micromanipulator under the MCA just above the rhinal fissure and vein but below the first arterial branch. The hook (carrying the MCA) was lifted 2–3 mm above the cortical surface and heated with a microcautery to coagulate and divide the raised Heifetz clips within 2 minutes of MCA occlusion.

At the onset of carotid occlusion, the rats were randomized into two groups, spontaneously hypothermic (n=6) and normothermic (n=7). In the spontaneously hypothermic group, brain and body temperatures were permitted to fall passively until the brain temperature reached 32°C. The brain temperature was regulated at 37.5°C in both groups of rats (Figure 1). In normothermic animals, temperature was permitted to fall spontaneously to 34.2°C because of administration of O2 through the nose cone. Hypothermic animals were mildly hypercapnic as rectal temperatures were raised gradually to 37.5°C using the overhead lamps, but CO2 levels were maintained at 38–40 mm Hg.

Microscopic examination revealed regions with pancellular necrosis, as evidenced by nuclear condensation and cytoplasmic acidophilia. The boundary between normal and necrotic tissue was well delineated, although acidophilic neurons were occasionally present in regions adjacent to the infarct. The areas of infarction were measured at six predetermined brain levels, 10, 8, 6, 4, 2, and 0 mm anterior to the zero vertical plane of de Groot. After tracing the region of infarction onto brain diagrams, areas of infarction were quantitated gravimetrically and integrated to obtain infarct volume. Differences of mean infarct volume between groups were tested using Scheffe’s criteria for multiple comparisons with a 95% confidence limit.

Results

Arterial variables did not differ significantly between the normothermic and spontaneously hypothermic rats (Table 1). Arterial Po2 was above the normal range because of administration of O2 through the nose cone. Hypothermic animals were mildly hypercapnic as rectal temperature was permitted to fall passively to 34.2°C during occlusion.

Immediately after occlusion of the MCA and carotid arteries, subdural temperature ranged from 35.0°C to 35.9°C in both groups of rats (Figure 1). In normothermic animals, temperature was permitted to fall passively to 37°C within 30 minutes of reperfusion, then rewarmed gradually to 37.5°C.

After survival for 24 hours, the rats were killed with a lethal injection of pentobarbital, and the brain was removed from the cranium and frozen in a bed of dry ice. The brain was sectioned at –11°C at a thickness of 20 μm, and sections were air-dried on glass slides. The dried sections were fixed in acetic acid/formaldehyde/methanol and stained with thionin and acid fuchsin. These stains are similar to hematoxylin and eosin, which have been used previously to detect infarction in frozen sections.

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FIGURE 2. Schematic representation showing distribution of infarction after focal ischemia in normothermic (37.5°C) and hypothermic (32.0°C) brain. Focal ischemia was produced using permanent occlusion of the distal middle cerebral artery combined with transient occlusion of both carotid arteries for 1 hour. In one group of rats (panel A, n=7), brain temperature was maintained at 37.5°C during occlusion and 30 minutes of reperfusion. In the hypothermic group (panel B, n=6), brain temperature was permitted to fall spontaneously to 32°C after occlusion and rewarmed during reperfusion. Area of infarction was measured in stained sections at several levels of brain after 24-hour recovery. Solid areas denote infarction in 100% of animals, hatched areas in >50% of animals.

mic animals, brain temperature was raised to 37.5°C by 10 minutes and maintained at this level for the duration of occlusion and for 30 minutes of reperfusion. In spontaneously hypothermic rats, brain temperature was allowed to fall to 32.1°C over the course of 20 minutes, maintained at 32°C for the duration of the occlusion period, and returned to 36.7°C during the initial 30 minutes of reperfusion.

The extent of neocortical infarction, measured after survival for 24 hours, was strongly influenced by the temperature of the brain during ischemia. Thus, in normothermic rats, infarction extended from the frontal pole to the level of the hippocampus in more than half of the animals (Figure 2A). However, the incidence and extent of infarction were markedly diminished in rats in which the brain was allowed to cool spontaneously to 32°C (Figure 2B). At several levels within the brain, the area of infarction was significantly smaller in hypothermic brain than in normothermic brain (Figure 3). Thus, the overall volume of infarction was reduced from 82±16 mm³ (mean±SEM; n=7) in the normothermic group to 19±9 mm³ (n=6; p<0.001) in the hypothermic group (Figure 4). In a subsequent group of rats, cerebral normothermia was maintained for 40 minutes postocclusion before permitting the temperature of the brain to fall to 32°C. The volume of infarction in this group of animals (75±16 mm³; n=6) was not significantly different from that in normothermic rats (Figure 4).

Discussion

The effect of focal cerebral ischemia on brain temperature has not been well characterized in rodent models of focal ischemia. In the present model, occlusion of the MCA and carotid arteries caused a rapid decrease in brain temperature when no attempt was made to maintain cerebral normothermia. Undoubtedly, the interruption of carotid blood flow contributed significantly to the drop in brain temperature. In addition, the small decrease in body temperature during passive cooling may have contributed to cerebral hypothermia. Nevertheless, brain temperature during isch-
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emia was consistently 2–3°C below body temperature in the absence of selective heating. Moreover, in a previous study, subdural temperature decreased to 34–35°C despite the regulation of rectal temperature at 37.5°C. These results indicate that a significant decline in brain temperature will occur in the present model of focal ischemia unless selective heating is used to maintain cerebral normothermia.

The influence of moderate changes in brain temperature on tissue injury in models of focal ischemia remains poorly defined. Reduction of temporalis muscle temperature to 24°C before and for 60 minutes after permanent occlusion of the proximal MCA was reported to reduce the volume of infarction to 71% of that in normothermic (36°C) animals. Thus, profound, transient hypothermia appears to offer protection even after permanent occlusion. The present model of combined occlusion and reperfusion of the carotid arteries presumably permits recirculation of the previously ischemic focus, although the level of reperfusion is not known. In previous studies, reperfusion of the carotid arteries was followed by reoxidation of NADH, regeneration of ATP and lowering of tissue lactate— all indicators of reperfusion. In addition, synthesis of mRNA for the 70-kd heat-shock protein has been demonstrated in the ischemic focus during reperfusion, again indicating reperfusion and regeneration of ATP. In the present study, hypothermia reduced the volume of infarction to 23% of that caused by 60 minutes of normothermic (37.5°C) ischemia. Thus, in the setting of focal ischemia and reperfusion, even moderate cooling had a marked influence on the extent of tissue damage. This protection is similar to that observed in models of transient global ischemia, in which small decreases in brain temperature have profound effects on ischemic injury. Presumably, recirculation permits the maximum benefit of intraischemic hypothermia.

The present results failed to detect a reduction of infarct volume when cerebral hypothermia was delayed for 40 minutes after the onset of ischemia. However, the lack of effect of delayed hypothermia should be considered provisional because the variance and limited size of each group would have prevented detection of a 50% reduction in infarct volume. Postischemic hypothermia has been reported to reduce ischemic injury in some preparations of global ischemia but not others. Thus, it would not be surprising if delayed hypothermia were to diminish the extent of infarction in models of focal ischemia. However, it may be necessary to reduce brain temperature below 32°C in order to achieve protection comparable to that attained by prompt cooling.

The mechanism by which hypothermia diminishes ischemic injury remains poorly understood. Even a minor reduction of brain temperature is known to cause significant preservation of energy metabolites, presumably because of a decrease in energy use. In a previous study, focal depletion of energy metabolites occurred by 60 minutes after combined occlusion of the distal MCA and both carotid arteries, despite a reduction of brain temperature to 34–35°C. Although metabolite levels were not measured in the present study, hypothermic protection after global ischemia has been reported without a change in the end-insult levels of tissue metabolites. However, the time course of high-energy phosphate depletion during ischemia is markedly retarded by hypothermia. Thus, hypothermia may shorten the duration of energy depletion during ischemia and thereby reduce ischemic injury. Such preservation of energy metabolites may be especially important in models of focal ischemia, in which incomplete loss of flow may enable many regions to benefit from a reduction in energy utilization. Undoubtedly, hypothermia may modulate other pathological factors, such as changes in extracellular glutamate and intracellular calcium. It is also possible that hypothermia may slow the process of infarction, so that by 24 hours the lesion is not fully developed. Whatever the mechanism(s) of protection, the present results indicate that moderate hypothermia has a strong influence on the extent of brain injury after transient focal ischemia. Although the model of ischemia used in the present study was not intended to accurately model human stroke, it is nevertheless a useful experimental preparation for identifying important factors in ischemic injury.

References

The well-known neuroprotective effects of whole body hypothermia have been prophylactically applied in human surgical interventions for at least three decades. Several investigators, whose work is mentioned in the accompanying article by Moyer et al, have shown that brain hypothermia (accompanying the ischemic injury) or hypothermia induced before the injury ameliorates the effects of global ischemia on rodent brains.

This communication by Moyer et al reports two important facts: 1) Simultaneous occlusion of one intracranial artery (the middle cerebral artery) and two carotid arteries rapidly lowers the brain temperature; this effect was partly attributed by Moyer et al to the direct effects of decreased blood flow. 2) Animals in which this brain hypothermia is not actively corrected develop smaller brain lesions than those observed in the appropriate control groups, as measured 24 hours after the ischemic insult (p < 0.001). Similar results were recently reported by Chen et al (1992) in experiments based on a rat model of single-artery occlusion and reperfusion: preischemic hypothermia protects the brain. In these experiments, the extent of neuronal, astrocytic, and cellular inflammatory responses was lessened by hypothermia when measured 96 hours after ischemia/reperfusion and compared with the corresponding normothermic experiments.

The next pertinent question may be whether brain hypothermia, induced 1–2 hours after a single-artery occlusion, offers any degree of protection. In this model of multiple vessel occlusion it did not seem to have a beneficial effect; however, as the authors appropriately mention, the sample size may have been inadequate to compensate for the variance. Equally important is the issue, mentioned by Moyer et al, that hypothermia may simply delay or slow down the maturation (ripening) effect typical of the lesion that an arterial occlusion sets in motion. Selman et al (1990) described a time-dependent metabolic worsening of the brain lesion induced by an arterial occlusion, and Garcia et al (1992) have reported a significant morphological growth of the resulting brain lesion; in normothermic animals, the growth of such lesion peaks approximately 72 hours after the artery is occluded. It could be that the development of a “mature” infarct is postponed several hours by hypothermia.
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