Preservation of Brain Temperature During Ischemia in Rats

Hiroaki Minamisawa, MD, Pekka Mellergård, MD, Maj-Lis Smith, PhD, Finn Bengtsson, MD, PhD, Sten Theander, BSci, Fredrik Boris-Möller, MD, and Bo K. Siesjö, MD, PhD

Our objectives were to study the loss of heat from ischemic brain and to devise a method of maintaining brain temperature. Reversible forebrain ischemia was induced by carotid clamping and exsanguination in 30 anesthetized and artificially ventilated rats. Rectal, skull, and brain temperatures were measured, confirming previous findings that brain temperature falls by 4–5° C during 15 minutes of ischemia unless measures are taken to maintain head temperature by external heating. Temperature gradients developed within the ischemic brain, superficial tissues being cooler than deep ones. These temperature gradients were reversed when skull temperature was maintained at core body (rectal) temperature by external heating. With rectal and skull temperatures maintained at 38°, 37°, 35°, or 33° C, brain temperatures nonetheless decreased by approximately 1° C during ischemia. This decrease in brain temperature could be prevented by placing the rat in a Plexiglas box with circulating air at temperatures close to that of the body core and a relative humidity of approximately 100%. We also found that, unless special precautions are taken, a temperature gradient develops between the brain and body core during recirculation. (Stroke 1990;21:758–764)

Brain tissues in many mammals maintain a temperature slightly greater than that of the arterial blood circulating within the tissue.1–3 In terms of heat exchange, therefore, brain tissue normally serves as the source and blood as the sink. However, the temperature of blood supplying brain tissue may be lower than the body core temperature, especially in animals that use the mechanism of countercurrent heat exchange between an arterial rete and the venous sinuses draining the mucous membranes of the nose and mouth.4–7 Furthermore, brain temperature is not uniform. For example, superficial tissues with their supplying arterial vessels lose heat to their surroundings by conduction, radiation, evaporation, and convection. Since evaporation occurs mainly from mucous membranes in, for example, the nasal and oral cavities, even deep tissues may lose heat to their surroundings. As a result of heat loss, there is often a temperature gradient between the deep and superficial tissues.8–12

Heat loss is a function of the efficacy of insulation by bone, muscles, skin, and hair,13,14 a function of the temperature and movement of ambient air,15–17 and a function of the humidity of the ambient air.18,19 Brain temperature also depends on regional blood flow and the temperature of the nasal mucosa.20–24 Normally, the surroundings are cooler and have a lower vapor pressure than body tissues, favoring heat loss. Since it retards or interrupts the arterial blood supply, ischemia upsets temperature regulation. In centrally located brain tissues temperature may rise, at least transiently, reflecting the failure of arterial blood to remove metabolic heat.3 However, superficial tissues cool, and in small mammals such as rats this cooling can be fast and can rapidly cause centrally located brain tissues to cool also. Thus, forebrain ischemia lasting 15–20 minutes is accompanied by a striatal temperature decrease of 4–5° C.25

Previous as well as more recent results25–31 have demonstrated that even small changes in brain temperature (1–3° C) significantly alter ischemic brain damage or the cerebral metabolic response to ischemia. This makes it imperative that experiments on ischemia are conducted with brain temperature under tight control. In fact, unrecognized differences in tissue temperature may explain many of the discrepant results reported in the literature.
Our study had two objectives. First, we wished to explore the factors that determine heat loss from ischemic brain and to assess temperature gradients within the brain. Second, we wanted to devise a method, preferably one not too complicated, for maintaining brain temperature during ischemia at any level.

Materials and Methods

We used 30 male Wistar rats (Møllegaard's Breeding Center, Copenhagen, Denmark) weighing 300–350 g. The rats were fasted the night before the experiments but were allowed free access to tap water. Anesthesia was induced with 3.5% isoflurane (Abbott Laboratories Ltd., Kent, England), and the rats were then tracheotomized and connected to a Starling-type ventilator delivering 1.5% isoflurane and 30% N2O in O2. Catheters were inserted into the tail artery and vein of each rat to allow blood pressure recording, blood sampling, and infusions. The common carotid arteries were isolated via a neck incision, and a silicone catheter was advanced into the inferior caval vein via the right jugular vein to allow later withdrawal of blood. A pair of needle electrodes were inserted into the temporal muscles for electroencephalographic (EEG) recording.

Following these procedures each rat received 50 IU heparin; 30 minutes passed before induction of reversible forebrain ischemia. The ventilator was adjusted to maintain PaO2 and PaCO2 at 90–115 and 35–40 mm Hg, respectively; arterial pH was thus kept at approximately 7.4. Mean arterial blood pressure was maintained at 100–120 mm Hg. Blood glucose concentration was measured. During the experiments the rats were immobilized with 0.3–0.6 mg i.v. d-tubocurarine.

We used copper-constantan thermocouples to measure temperature. Each thermocouple was calibrated in a temperature-controlled water bath to <0.1°C error at 37°C before each experiment. Rectal and esophageal temperatures were measured with a type A-RM 4 thermocouple (Ellab, Copenhagen, Denmark) inserted 5 cm into the rectum or into the esophagus to a position directly behind the heart. Arterial temperature was measured using a thermocouple constructed at the electronic workshop of Lund University Hospital and placed in the abdominal aortic artery via a cannula in the femoral artery. Skull temperature was measured with a type A-K3 thermocouple (Ellab) placed subcutaneously on the bregma. Brain temperature was measured using a type A-K19 needle thermocouple with an outer Teflon guide (Ellab). This thermocouple had thin plastic coating all but the distal 0.15 mm of the metals. When in place, the Teflon guide covered all but the distal 2 mm of the thermocouple. The diameters of the plastic-coated thermocouple and the Teflon guide were 0.2 and 0.8 mm, respectively. To check the sensitivity of this thermocouple to changes in the surrounding temperature, we stereotactically guided the thermocouple within a very strictly temperature-controlled water bath covered with a Teflon membrane at the same room temperature as the rat experiments. As expected, the distal 0.15 mm of the thermocouple was the actual temperature-measuring site, but the temperature readings were influenced slightly by the temperature around the 2 mm plastic-coated part of the thermocouple. However, the Teflon guide gave very good insulation so changes in temperature around the guide did not influence the temperature readings, the temperature resolution being 0.1°C.

For measuring the temperature gradient, a burr hole 2.5 mm in diameter was drilled with its center 5 mm lateral to the bregma for measuring caudoputaminal temperature. A second burr hole was made 4.5 mm lateral and 4.5 mm posterior to the bregma for measuring thalamic temperature. The dura was carefully lifted and punctured with a thin needle. The thermocouple was then stereotactically inserted into the left caudoputamen or thalamus to a depth of 5 mm from the cortex. The skin above the burr hole was sutured before the beginning of temperature measurements.

The temperature of each rat was maintained close to the desired level by either the use of a 55-W heating lamp placed above its head and a heated operating table or by the use of a Plexiglas box constructed for the experiments. The box measured 60x40x50 cm (length x width x height) and had no bottom so that it could be placed around the operating table. The box had small windows that could be opened, permitting all manipulations of the rat to take place without removing the box. It was equipped with a heating fan and a small water bath, a thermocouple at the level of the experimental animal, and a device for measuring humidity. The box also contained insulated inlets and outlets for cables and respiratory tubings.

The model of ischemia has been described in detail.32 Isoflurane was discontinued, and 1 minute later both common carotid arteries were clamped, followed by exsanguination to a blood pressure of 50 mm Hg. Cerebral ischemia was confirmed by cessation of EEG activity. At the end of the ischemic period, recirculation was instituted as the carotid clamps were removed and the blood was rein infused. When blood pressure was restored, 0.5 ml of 0.6 M sodium bicarbonate was injected intravenously to counteract systemic acidosis.

Rectal, skull, and caudoputaminal temperatures were measured in all rats. In one rat the rectal, esophageal, and abdominal aortic temperatures were also measured simultaneously. The rats were divided into seven groups. One rat was subjected to 20 minutes and the other 29 to 15 minutes of ischemia. Experiments in the first five groups (n=4 in each) were performed in room air (temperature 20–22°C, relative humidity 10–20%). In one group no attempt was made to control the temperature of the rat's head; in one of the four rats (subjected to 20 minutes of ischemia) the temperature at different depths in the brain were measured by moving the stereotacti-
FIGURE 1. Graph of mean±SEM rectal (△), caudoputaminal (○), and skull (●) temperatures during and following 15 minutes of reversible forebrain ischemia in four rats in which rectal temperature was maintained at 37°C while head was allowed to cool spontaneously.

FIGURE 2. Graph of mean±SEM rectal (△), caudoputaminal (○), and skull (●) temperatures during and following 15 minutes of reversible forebrain ischemia in rats. Rectal and skull temperatures were controlled by external heating to 38°C (top, n=4), 37°C (middle, n=4), or 35°C (bottom, n=4) during ischemia. At start of recirculation, heating was discontinued.

The rectal, esophageal, and abdominal aortic temperatures were measured simultaneously guided thermocouple through the parenchyma in 1-mm steps. In the other four groups rectal and skull temperatures were kept at 33°C, 35°C, 37°C, or 38°C during ischemia using the heating lamp and the heated operating table. Heating was discontinued during recirculation. In two of the 16 rats (one at 33°C and the other at 37°C) brain temperature gradients were measured as described above. Experiments in the two remaining groups took place in the Plexiglas box at a relative humidity of either 10–20% (n=5) or approximately 100% (n=5). In both groups, the box was opened for 5 minutes at the start of recirculation but closed at all other times. At the lower humidity, ambient air temperature was kept at 37°C, and the head of two rats and the body of one were wrapped in aluminum foil; in the two remaining rats the inspired air was heated to 37°C. At the higher relative humidity, ambient air was kept at 35°C in one rat, at 37°C in three rats, and at 39°C in one rat.

Values presented are mean±SEM. Due to the nature of the experiments, no other statistical calculations were made.

Results

Blood pressure, PaO₂, PaCO₂, arterial pH, and blood glucose concentration were kept within normal physiologic limits in all rats before and after ischemia. No differences in the variables were observed among the groups.

In the rat in which rectal, esophageal, and abdominal aortic temperatures were measured simultaneously, rectal temperature adequately reflected the central body core temperature. The maximum difference between the rectal and esophageal/abdominal aortic temperatures was 0.3°C.

In one group, spontaneous changes in brain temperature during and immediately after ischemia were measured. The results are shown in Figure 1. Immediately following induction of ischemia, skull and caudoputaminal temperatures began to fall, and they continued to fall in parallel during the entire ischemic period. These results confirm those of Busto et al., who noted an equally extensive reduction in brain temperature during ischemia. Skull temperature was 0.5°–1.0°C lower than caudoputaminal temperature. After 15 minutes of ischemia, skull temperature had fallen to <32°C and caudoputaminal temperature to approximately 32°C. When recirculation was instituted rectal temperature fell transiently, probably...
Because the reinfused blood had cooled. At the beginning of reperfusion, skull and caudoputaminal temperatures rose very rapidly. After 10 minutes of recirculation, caudoputaminal temperature approached its preischemic level while skull temperature remained approximately 1°C below its preischemic value.

In the next four groups, both rectal and skull temperatures were kept constant during ischemia. Results for three of the groups are displayed in Figure 2. Brain temperature, as measured in the caudoputamen, fell by approximately 1°C during ischemia. This decrease in brain temperature was similar regardless of whether rectal and skull temperatures were kept at 33°C (data not shown), 35°C, 37°C, or 38°C. Skull temperature fell very rapidly during the first 5 minutes of recirculation and continued to fall more slowly during the entire observation period, to 2–3°C below rectal temperature. Caudoputaminal temperature, on the other hand, increased rapidly to the rectal temperature immediately upon recirculation. In the group heated to 35°C, caudoputaminal temperature remained close to rectal temperature during recirculation. In the groups heated to 37°C or 38°C, after 10–15 minutes of recirculation caudoputaminal temperature again fell to 1–1.5°C below rectal temperature.

In the rat in which the skull was allowed to cool spontaneously during 20 minutes of ischemia, caudoputaminal temperature reached 32.3°C after 9 minutes of ischemia. The thermocouple was then withdrawn from the caudoputamen in 1-mm steps. This procedure disclosed a temperature gradient within the brain, the neocortex being 0.9°C cooler than the caudoputamen (Figure 3). The thermocouple was then inserted through the second burr hole into the thalamus, and
after 18 minutes of ischemia the temperature gradient was confirmed by the same procedure.

This same procedure was also performed in two rats in which skull temperature was controlled during ischemia. In these rats a temperature gradient of approximately 0.5° C was seen. When the head was heated, however, the temperature gradient was reversed compared with when the head was not heated, the deep structures being cooler than the brain surface (Figure 4). During recirculation, when heating was discontinued, the temperature gradient was again reversed, with the thalamus being up to 0.25° C warmer than the neocortex (Figure 4).

In two groups experiments were performed with the rats in the Plexiglas box. Keeping the air around the rat at 37° C with normal (approximately 10–20%) relative humidity did not prevent the decrease in brain temperature previously described. This decrease could not be prevented by wrapping the rat's head or body in aluminum foil or by heating the inspired air to 37° C. However, when the relative humidity was increased to approximately 100% it was possible to keep rectal, skull, and caudoputaminal temperatures at the desired level without any decrease in brain temperature (Figure 5). This was shown with ambient air heated to 35°, 37°, and 39° C.

Discussion

Our major findings are as follows. First, if no measures are taken to maintain the temperature of the skull during ischemia, brain temperature falls rapidly, with relatively small intracerebral gradients. Second, when the head is heated by a lamp so that the subcutaneous skull temperatures are maintained at 38°, 37°, 35°, or 33° C, at identical body core temperatures intracerebral temperatures nonetheless decrease by approximately 1° C. Third, if the brain is recirculated and skull heating is discontinued, the temperature gradient between the brain and body core changes with time. During recirculation this gradient first decreases, then increases. These changes probably reflect postischemic changes in blood flow, encompassing an initial hyperemia and a later hypoperfusion. Fourth, even when the body is placed in a box with circulating air at a temperature close to that of the brain, brain tissue loses heat during ischemia. In all probability, the decrease in brain temperature is due to evaporative heat losses since it is abolished by increasing the relative humidity to 100%.

Two factors favor heat loss from the brain. First, since the skin (which normally represents the outer temperature zone) is cooler than deep organs, there is a gradient favoring conductive heat loss to the surroundings.16,33,34 Second, even when the temperature of the skin is the same as that of the surroundings, heat is lost by evaporation. Evaporation may occur from all skin surfaces; however, it is more marked from the mucous membranes of the nasal and oral cavities due to their wet condition and their large surface area.18–20

To our knowledge, the first authors to recognize that the brains of ischemic animals cool were Hirsch et al,35 who reported that brain temperature could fall to 33° C during ischemia and that the rate of decline increased when the skull was exposed (duration of ischemia unknown). These authors devised two methods for preventing the decrease in brain
temperature: external heating of the head or enclosing the animal in a glass box, the air of which was heated to within 1–2°C of body temperature. They reported that, with the latter method, temperature differences between rectum and brain could be kept to ±0.15°C.22 However, they did not report increasing differences with longer ischemic periods, nor is the humidity of the air in their box known.

Heat loss from ischemic brain is facilitated by the reduction in blood flow to extracerebral cranial tissues that occurs in all models of forebrain and global ischemia. Predictably, when ischemia is focal, as when a major intracerebral blood vessel is obstructed or when intracranial pressure increases, the maintained circulation to extracerebral tissues reduces the heat lost from the ischemic tissue.

For many years and before we published a model of recovery from forebrain ischemia, it has been our practice to prevent heat losses from ischemic brain by placing a lamp some distance from the brain. Previously, we used a standard placement of the lamp, one that maintained intracerebral temperatures at approximately 37°C, but during the last few years we have used a thermistor placed on the skull to guide placement of the lamp. We now report that even with these precautions, intracerebral temperatures fall by approximately 1°C during 15 minutes of ischemia. In fact, even when the entire animal was placed inside a box maintained at body temperature, brain temperature fell. Since this decrease could be prevented by increasing the relative humidity of the warm air, our results prove that evaporative heat losses contribute to brain cooling during ischemia. In addition, our results demonstrate that deep-to-superficial temperature gradients during spontaneous cooling of the head could, at least in part, mask the true differences in susceptibility to damage of different neuronal populations. For example, hypothermia is cerebroprotective, and since the neocortex has the lowest temperature in this model of ischemia, the vulnerability of neocortical cells to ischemia may have been underestimated compared with deeper structures. We will report experimental results supporting this contention elsewhere.

Although local heating of the head may seem to be a simple and adequate method of maintaining brain temperature at any level, two problems can be identified. First, as has been discussed, intracerebral temperatures are not exactly those of the body core or a subcutaneous site on the skull. Second, it seems likely that local heating can cause an error opposite that arising from uncontrolled cooling, that is, brain damage that is worse on the surface than deep within the tissue. As discussed above, this issue can be studied using animals placed in a heated box at the desired temperature and a high relative humidity.

Previous as well as more recent results indicate that posts ischemic hypothermia may ameliorate brain damage due to trauma or transient ischemia. This possibility, coupled with the knowledge that temperature differences of only 1–2°C significantly alter the density of ischemic damage, justifies exploration of factors determining postischemic brain temperatures. Our present results clearly show that although brain temperature increases immediately after recirculation is initiated (presumably because of reactive hyperemia), the difference between brain and blood temperatures subsequently increases. We tentatively interpret this to be the result of reduced blood flow, allowing blood in the superficial arteries of the brain to cool before entering the tissue. Very likely, evaporative heat losses from mucous membranes contribute to postischemic brain cooling.

Clearly, in view of the possibility that postischemic brain temperatures modulate the final damage incurred, it seems highly justified that factors determining brain temperature during and after ischemia be better defined.

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References


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