Preischemic Hyperglycemia Leads to Delayed Postischemic Hyperthermia

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Background and Purpose Temperature alterations are known to influence the outcome of transient ischemia, even when instituted in the postischemic period. Since preischemic hyperglycemia aggravates ischemic brain damage, the question of whether hyperglycemic animals become hyperthermic arose. To explore this possibility, we measured body temperature telemetrically in normoglycemic and hyperglycemic rats subjected to 10 minutes of forebrain ischemia at a body (and brain) temperature of 37°C.

Methods Isoflurane-anesthetized animals were subjected to 10 minutes of forebrain ischemia under normoglycemic or hyperglycemic conditions. Temperature changes after ischemia were measured by means of a telemetric temperature coil.

Results In normoglycemic animals, temperature decreased to 35.6±1.1°C (mean±SD) during the first 4 hours of recovery, after which it gradually increased to normal values (38°C). Hyperglycemic animals behaved differently in that they remained normothermic for approximately 10 hours during recovery and later became hyperthermic, with core temperatures rising above 39°C. The rise in temperature was not due to the osmotic load of the glucose administered because infusion of mannitol, which gave a comparable increase in plasma osmolality, failed to cause delayed postischemic hyperthermia. Excessive hypercapnia during ischemia in normoglycemic animals, which produces cerebral acidosis of a magnitude similar to that of hyperglycemia and is known to aggravate ischemic lesions, likewise failed to induce hyperthermia. When postischemic seizures ensued in hyperglycemic subjects, temperature was 39.8±0.6°C. Animals with seizures invariably died. To evaluate the influence of postischemic hyperthermia on the outcome, an additional series of experiments was performed in which delayed hyperthermia was avoided by gentle cooling (n=6) or by acetaminophen administration (n=5). Although these procedures prevented delayed hyperthermia, they neither blocked seizure induction nor affected the fatal outcome. Postischemic seizures developed when the core temperatures of animals were 37.9±0.1°C and 37.8±0.2°C in the cooled and acetaminophen-treated groups, respectively.

Conclusions The results suggest that both delayed hyperthermia and delayed seizures in hyperglycemic animals are caused by the aggravated damage incurred by these animals during or immediately after the ischemic insult. (Stroke. 1994;25:1825-1829.)

Key Words • cerebral ischemia • hyperglycemia • seizures • temperature • rats

Transient ischemia leads to brain damage of two types. In the first, cellular necrosis affects neurons in selectively vulnerable areas. In the second type, damage affects all cell populations, yielding pannecrosis or infarction.

Preischemic hyperglycemia aggravates damage due to dense, transient ischemia in adult animals. Cardinal features of hyperglycemia-augmented damage are the selection of a proportion of neurons into necrosis, the rapid evolution of brain damage, the involvement of additional structures, such as the cingulate cortex and substantia nigra pars reticulata, and the development of postischemic seizures. The worsening of outcome is assumed to be related to accumulation of lactate plus H+ produced by the increased amount of available glucose. Another factor that aggravates brain damage due to transient ischemia is hyperthermia, which, like hyperglycemia, transforms

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selective neuronal vulnerability into infarction. The mechanisms are not necessarily identical, however; for example, hyperthermia has not been reported to trigger postischemic seizures. These results raise the question of whether hyperglycemic animals develop postischemic hyperthermia and whether such hyperthermia could explain the appearance of seizures and the aggravation of damage. To shed light on this problem, we recorded core temperature telemetrically in normoglycemic and hyperglycemic animals subjected to transient ischemia. Additional series were studied to assess the effect of an increase in plasma osmolality and of enhanced tissue acidosis in the absence of hyperglycemia. When the result revealed that only hyperglycemic animals developed delayed hyperthermia, experiments were performed in which the hyperthermic response was prevented by environmental cooling or administration of acetaminophen, and seizure incidence was recorded in these temperature-regulated animals.

Materials and Methods

The experiments were performed on adult male Wistar rats (weight, 290 to 370 g) of a specific-pathogen-free strain (Mallegaard's Breeding Center, Copenhagen, Denmark). The animals were housed in macrolon cages and exposed to a 12-hour day and night cycle and a room temperature of 22°C.
Before ischemia the animals fasted overnight but were allowed water ad libitum. As described earlier, the animals were anesthetized with isoflurane (3.5% isoflurane and 70% \( \text{N}_2\text{O} \) in \( \text{O}_2 \)), intubated, and connected to a respirator with the isoflurane concentration decreased to 1.5%. Central venous as well as tail venous and arterial catheters were inserted, and strings were placed around each common carotid artery. Skull and rectal temperatures were measured, and a telemetric temperature probe (receiver RA-1010, Mini-Mitter Co Inc) was placed into the peritoneal cavity. After the surgical procedures, 50 IU IV of heparin was administered. During a 30-minute preischemic period, hyperglycemia (plasma glucose, 20 to 25 mmol/L) was achieved by intravenous infusion of a 25% glucose solution. Normoglycemic animals were given a similar amount of saline solution. Ischemia was induced by bilateral common carotid artery clamping combined with central venous exsanguination to a blood pressure of 50 mm Hg. After 10 minutes of ischemia, brain circulation was restored by removal of the carotid clamps and reinfusion of shed blood. The isoflurane administration was discontinued, and animals were extubated after 20 to 40 minutes of recovery. During the experiment, the blood pressure was continuously recorded, and temperature, \( \text{PaO}_2 \), \( \text{PaCO}_2 \), and \( \text{pH} \) were controlled. Skull and rectal temperatures were carefully adjusted to 37°C throughout the steady-state period as well as during ischemia and the early recirculation phase (20 minutes).

After extubation the animal was moved to a cage, with continuous telemetric monitoring of the core temperature. The animal was observed for abnormal behavior and seizures during the recovery period. Room temperature was kept at 22°C during temperature recording. When we found that the temperature increased during the recovery period in hyperglycemic but not in normoglycemic animals, a second series of experiments was performed, comprising four groups of animals. In the first group, animals were placed in a cold environment at the time when temperature increase started, i.e., after 10 hours of recovery. Cooling of the air was achieved by packing ice around the cage. In the second group of animals, acetaminophen (Curadon, Astra-Tika) was administered to avoid the temperature increase. Acetaminophen was given orally by a gastric tube in two to four doses of 75 mg·kg\(^{-1}\) every 3 hours. The third group was ventilated with 50% \( \text{CO}_2 \) starting to 15 minutes before ischemia and continued throughout the period of ischemia (see Reference 21). The fourth group was given 15% mannitol intravenously to increase the plasma osmolality to values observed in hyperglycemic ischemia (total volume, 1.5 mL). There were thus six groups of animals observed for core temperature and neurological behavior, subjected to ischemia under conditions of normoglycemia (n=9), hyperglycemia (n=17), hyperglycemia plus cooling (n=6), hyperglycemia plus acetaminophen (n=5), hypercapnia (n=4), and hyperosmolality (n=4).

The experimental procedures conformed to the guidelines set by the American Physiological Society and were approved by the Ethical Committee for Laboratory Animal Experiments at the University of Lund.

Statistical evaluation of physiological variables was performed with ANOVA followed by Dunnett's test, and the time course of temperature changes among groups was statistically assessed with two-factor ANOVA followed by Scheffé's tests. A value of \( P<.05 \) was considered statistically significant.

**Results**

**Physiological Parameters**

No statistical differences were observed in blood pressure, temperature, \( \text{PaO}_2 \), \( \text{PaCO}_2 \), and \( \text{pH} \) between the normoglycemic and hyperglycemic groups or between any of the hyperglycemic groups before, during, or after ischemia until 20 to 40 minutes of recovery. Skull and rectal temperatures were kept at 37°C. Blood glucose concentrations differed as expected between groups. Predictably, the \( \text{CO}_2 \) tension was markedly increased in the group ventilated with \( \text{CO}_2 \), with \( \text{PaCO}_2 \) values close to 300 mm Hg. Plasma osmolality was similar in glucose- and mannitol-infused animals, ranging from 295 to 310 mmol/L.

**Core Temperature After Transient Brain Ischemia in Normoglycemic and Hyperglycemic Rats**

The results of this series are given in Fig 1. In the normoglycemic group (n=9), the temperature during the first 4 hours gradually decreased to a mean value of 35.6°C and thereafter slowly increased to and remained at approximately 38°C, the temperature of a normal awake rat (Fig 1A). Some variability was evident, since two rats showed hyperthermia (>39.0°C) after 15 hours of recovery. One of these developed seizures after 17.5 hours of recovery and died.

The hyperglycemic rats (Fig 1B) showed a different pattern. They remained normothermic during the early phase of recovery, but temperature increased spontaneously after approximately 10 hours of recirculation, to mean values exceeding 39°C (Fig 1B). Six of 17 hyperglycemic rats developed early postischemic seizures, and 5 of 6 died in close relation to these seizures (between 3 and 6 hours of recovery). The 6 rats that developed early seizures did not differ in temperature from the 11 animals that did not develop early postischemic seizures (Fig 1B). When the 5 rats that succumbed early were excluded, the hyperglycemic animals showed generalized convulsions at a mean recovery time of 18.2±1.7 hours and subsequently died (20.2±3.4 hours of recovery). The temperature of the dying animals fell, as seen in Fig 1B. The mean temperature when the first seizure was observed was 39.8±0.6°C. Interestingly, animals consis-
Hypercapnic and Mannitol-Infused Rats

To study whether the rise in temperature of hyperglycemic animals was related to the exaggeration of intracellular and extracellular acidosis, such exaggeration was reproduced by superimposed hypercapnia. As Fig 2A shows, hypercapnic animals remained moderately hypothermic for 16 to 20 hours and never developed hyperthermia. In agreement with data reported by Katsura et al., we found that hypercapnic animals did not develop postischemic seizures (observation period, 24 hours of recovery).

The results were similar in animals infused with mannitol to increase their plasma osmolality (Fig 2B). Thus, hyperosmolality per se cannot be held responsible for delayed hyperthermia.

Effect of Suppression of Hyperthermia

In the second series of animals, we attempted to counteract the temperature increase in the hyperglycemic animals by cooling the ambient air by packing ice around the cage or by oral administration of acetaminophen. Fig 3 illustrates that both air cooling and acetaminophen could completely suppress delayed hyperthermia. After 12 hours of recovery and thereafter, animals in the cooled or acetaminophen-treated groups showed significantly lower core temperatures than the untreated hyperglycemic groups. No rats showed early seizures.

Although hyperthermia could be avoided, the time point for the development of late postischemic seizures did not change, since seizures occurred after 19.3±3.3 hours in the cooled group and after 20.0±1.8 hours in the acetaminophen-treated group, starting when the animals showed a core temperature of 37.9±0.1°C and 37.8±0.2°C, respectively. In addition, although 3 of 11 cooled animals survived until 2 to 4 days of recovery, most animals died after 20 to 25 hours, and none survived for more than 4 days.

Discussion

The results of the present study demonstrate that animals rendered hyperglycemic before a transient ischemic insult develop a delayed increase in body temperature, which becomes manifest after approximately 10 hours of recirculation. This rise in temperature thus precedes the appearance of late postischemic seizures, which are observed after 18 to 26 hours of recovery.

However, the tendency for hyperglycemic animals to develop hyperthermia is evident already during the first 6 to 8 hours of recovery. Thus, normoglycemic rats demonstrate a transient decrease in body temperature during the early recirculation hours, hyperglycemic rats maintain body temperature 1.0°C to 2.0°C higher in that period. We will discuss the mechanisms involved in this difference in temperature response and the pathophysiological effects of the immediate and delayed rises in body temperature. This discussion is facilitated by our results in hypercapnic and mannitol-infused animals since they show that neither aggravation of tissue acidosis alone nor hyperosmolality can be held responsible for the hyperthermia.

Body temperature is controlled by thermoregulatory centers in the brain, mainly localized in the anterior (preoptic area) and posterior hypothalamus.
in the anterior hypothalamus are known to cause hyperthermia. Such lesions may explain why middle cerebral artery occlusion by an intraluminal filament frequently leads to hyperthermia. However, although middle cerebral artery occlusion by this technique may lead to hypothalamic lesions, ischemia. In hyperglycemic subjects is not known to cause such lesions. It would thus seem that more subtle alterations in the function of temperature-regulating centers are responsible for the hyperthermic effect. However, by reexamining histological material from a previous study, we found discrete lesions in hypothalamic nuclei in 15 of 36 animals with preischemic hyperglycemia.

Postischemic hyperthermia has previously been described in gerbils and in the absence of deliberately raised environmental temperature. In this species, temperatures increase to 38.5°C to 39°C within the first 2 to 3 hours of recovery. This early hyperthermia has been found to be associated with an aggravation of brain damage. However, in the present experiments on rats, normoglycemic animals showed a postischemic decrease in body temperature to minimal values of 35.5°C, although they were freely moving in an environment of normal temperature (22°C) and humidity. In contrast, hyperglycemic animals showed a rise in temperature toward 36.0°C, the normal body temperature of awake rats.

Very probably, the transient decrease in temperature in normoglycemic animals reflects a sustained effect of transient anesthesia plus hypotension. However, it remains to be explained why hyperglycemic animals behaved differently. The hyperthermic effect observed in these animals was probably not due to the increased osmolality since mannitol-infused animals failed to show a similar temperature response. Furthermore, it was probably not related to the additional decrease in extracellular and intracellular pH since hypercapnic/normoglycemic animals showed a temperature pattern different from that of hyperglycemic animals. It remains to be shown whether the decisive factor is hyperglycemia, i.e., the glucose molecule per se, perhaps acting in conjunction with the acidosis.

As previously reported, at least in the gerbil a rise in postischemic body temperature leads to aggravation of ischemic brain damage. In the present experiments body temperature differed by 1.0°C to 2.0°C between normoglycemic and hyperglycemic animals during the first 2 to 4 hours of recovery. It remains to be shown whether this difference in early recovery temperature could explain at least part of the difference in outcome.

Although it was tempting to assume that delayed hyperthermia is responsible for the postischemic seizures and for the aggravation of brain damage in hyperglycemic subjects, neither external cooling nor acetaminophen could prevent the occurrence of seizures or the fatal outcome. It seems more likely, therefore, that delayed hyperthermia is the result of more severe damage in hyperglycemic subjects rather than its cause. Tentatively, we can thus envision a scenario in which hyperglycemia aggravates damage during or immediately after ischemia, a damage that secondarily leads to increased temperature and to postischemic seizures.

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Intraischemic and postischemic hyperthermia have been shown in several ischemia models to aggravate brain injury. In adult animals, preischemic hyperglycemia also exacerbates ischemic damage. The results reported by Uchino and colleagues nicely demonstrate that hyperglycemic animals develop a delayed increase in body temperature after 10 hours of recirculation. Thus, the question of whether this delayed hyperthermic response is a cause or consequence of hyperglycemic tissue damage and seizure activity was investigated.

The present data indicate that in hyperglycemic rats in which delayed hyperthermia was avoided, seizure induction and fatal outcome were not prevented. Thus, early structural damage to thermoregulatory brain regions may have caused the late temperature elevations. Since preischemic hyperglycemia aggravates the early neuronal and microvascular consequences of ischemia, early tissue injury may lead to the generation and release of factors that could also affect body temperature.

The use of continuous telemetric monitoring of the core temperature provided new information regarding the natural response of body temperature to cerebral ischemic damage. In previous studies, spontaneous hyperthermic periods had been documented after middle cerebral artery occlusion in rats and after bilateral common carotid artery occlusion in gerbils. Based on the present findings, one might question whether the temporal response or the magnitude of the hyperthermic periods is similar between body and brain.

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