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Mild hypothermia prevents the occurrence of cytotoxic brain edema in rats

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Abstract. Hypothermia maintains the impermeability of the blood-brain barrier to proteins and, therefore, presumably the development of vasogenic brain edema after brain ischemia. We intended to determine whether mild hypothermia would have a protective effect against cytotoxic brain edema, the early stage of ischemic brain edema. Two groups of Wistar rats (37°C and 35°C body temperature) were subjected to 6 h of moderate decrease of cerebral blood flow (CBF) by means of permanent bilateral carotid artery ligation, and compared to a third group of unaffected animals. Carotid artery ligation induced a local cerebral blood flow (LCBF) reduction to 50-80% of baseline values. LCBF in the frontal cortex was restored to a higher level in hypothermic animals than in normothermic ones ($P<0.05$). In normothermic animals, an increase of brain water content was detected in the frontoparietal and occipital cortex as well as in the hippocampus ($P<0.05$), but only in one region of the frontoparietal cortex in hypothermic animals. The impermeability of the blood-brain barrier to proteins was shown by the absence of staining with Evans blue as an indicator of vasogenic brain edema. We conclude that mild hypothermia offers protection against the development of cytotoxic brain edema.

Key words: CBF, cerebral blood flow, blood-brain barrier, forebrain ischemia, cytotoxic brain edema, brain water content, hypothermia, rat

INTRODUCTION

The extent of neuronal damage due to global brain ischemia is essentially influenced by brain temperature (for a review see Dietrich 1992). It has been shown that a moderate reduction of brain temperature by about 2-5°C has a protective effect on histopathological damage (Chopp et al. 1991, Chen et al. 1992a, b, Morikawa et al. 1992) and behavioral deficits (Green et al. 1992). One of the mechanisms discussed, regarding the protective effects of hypothermia is the maintenance of the impermeability of the blood-brain barrier to proteins and with it the prevention of the development of vasogenic brain edema, both after severe ischemia (Dempsey et al. 1987, Dietrich 1992) and traumatic brain injury (Jiang et al. 1992). This is of special pathogenetic and clinical interest as brain edema is an important factor in secondary brain damage. However, to our knowledge there is no evidence that hypothermia protects against cytotoxic brain edema, which is an early phase of ischemic brain edema.

During cytotoxic brain edema, the impermeability of the blood-brain barrier to proteins has not yet been disturbed (Gotoh et al. 1985, Hatashita and Hoff 1990). The increase in brain water content is caused by the energy deficiency of the neurons. The resulting dysfunction of the Na^+-K^+-ATPase in the neurons leads to an intracellular rise of Na^+ ions and water (Yang et al. 1992). This rise is increased by the activation of the Na^+-H^+-antiport due to the intracellular accumulation of H^+ by glycolysis. At the same time the extracellular rise of K^+ together with an intracellular Ca^{2+} increase leads to a local depolarization of synapses and a release of excitatory amino acids. The excitatory amino acids can induce an intracellular brain edema by opening the glutamatergic Na^+ channels (Baethmann et al. 1980, Kempski et al. 1982). The developing ion gradient between intracellular and extracellular space causes a Na^+ shift from the capillaries into the ischemic regions without a disturbance of the blood-brain barrier function of protecting the brain against an extravasation of proteins and without an increase in extracellullar Na^+ concentration (Gotoh et al. 1985, Hatashita and Hoff 1990, Ishimaru 1993, Menzies and Betz 1993). This Na^+ shift is rather caused by an increase in the Na^+-K^+-ATPase activity of the endothelial cells which is presumably due to the increased extracellular K^+ concentration (Schielke et al. 1991). Also, a further increase in brain osmolality is due to lactate accumulation and the development of "idiogenic" osmotic active substances. These are presumably cations and metabolites of the ATP hydrolysis (Hatashita et al. 1988).

In order to evaluate the protective effect of hypothermia concerning the development of cytotoxic brain edema due to brain blood flow reduction, we used permanent bilateral carotid artery ligation (PBCAL) in rats as a model for moderate decrease of global cerebral blood flow (Eklöf and Siesjö 1973, Harris and Symon 1984). Regional brain water content was determined by gravimetry. Moreover, the impermeability of the blood-brain barrier to proteins was shown by the absence of brain tissue staining with Evans blue as an indicator of vasogenic brain edema.

METHODS

Surgical procedure

The care and use of the animals reported on in this study were approved by the local authorities. Wistar rats were anesthetized with 1.5% halothane in a mixture of 70% N_2O and 30% O_2. Thirty minutes before and during decrease of CBF, anesthesia was maintained using 0.5% halothane in the inspired gas. A catheter was inserted into the abdominal aorta via the left femoral artery for blood sampling and recording arterial blood pressure. Two Laser probes were placed on the dura mater above the right hemisphere of the frontal (primarily motor) and occipital (visual) cortex (3 mm lateral to the midline suture, 1 mm in front and 4 mm behind the coronal suture respectively) and fixed with dental cement. Using a midline incision in the ventral neck, carotid arteries were occluded permanently by both an arterial clip and a loop of thread. Rectal temperature in all animals was kept at 37.0 ± 0.5°C during preparation and at 37.0 ± 0.5°C or 35.0 ± 0.5°C during the experiment by means of a self-controlled heating lamp and pad.

Experimental protocol

Two groups of adult male Wistar rats (Uje:wist, normothermic rats with a 37°C rectal temperature, $n = 6$; hypothermic rats with a 35°C rectal temperature, $n = 6$) were subjected to 6 h of moderate CBF reduction by permanent bilateral common carotid artery ligation. A third group of unaffected rats with a 37°C rectal temperature ($n = 8$) was not subjected to carotid artery ligation. Local cerebral blood flow (LCBF) was measured continuously by a 2-channel Laser Doppler flowmeter (MBF 2D,

Moor Instruments, England) together with electrocardiogram (ECG), and arterial blood pressure. In addition, blood gases and glucose were determined hourly. The LCBF values were expressed as percentages of the baseline values. Animals received 2 ml of 2% Evans blue (Fluka, Buchs, Switzerland) intravenously 45 min before sacrificing in order to test the functioning of the blood-brain barrier in relation to proteins and to distinguish the vasogenic from the cytotoxic brain edema. Six hours after onset of the CBF reduction animals were killed by an overdose of halothane. The brains were removed quickly from the skull and cut into four coronar slices. The slices were temporarily stored in kerosene in order to prevent an evaporative loss of water. The surface of the slices was checked carefully with a magnifiying glass for blue stainings as a sign of extravasation of Evans blue.

Brain water estimation

Brain tissue samples of about 5 mm^3 were taken from the coronar slices for brain water estimation by gravimetry. Samples were taken from each hemisphere from the frontoparietal cortex (primary motor cortex) at AP levels 2.5 and 0 according to Paxinos and Watsons atlas (1986), from the occipital cortex (visual cortex) at AP -3 and -6, from the prepiriform cortex at AP 2.5 and 0, from the piriform cortex at AP -3, from the entorhinal cortex at AP -6, and from the hippocampus (anterior and posterior horn).

The samples were placed on a gravimetric column of brombenzene and kerosene of known density according to the method of Marmarou et al. (1978). The specific gravity of the mixture in this column decreased linearly with the height of the cylinder. The columns were calibrated using a standard curve created by anhydrous standard potassium sulfate solutions with specific gravities of 1.025, 1.03, 1.035, 1.04, 1.045, 1.05, and 1.055. The brain water content was calculated according to the equations of Nelson et al. (1971). The estimation of the specific gravity corresponding to these equations required the determination of the specific gravity and dry weight of brain tissue in unaffected animals, for which 9 additional animals were used.

Statistical analysis

Differences in LCBF, mean arterial blood pressure (MABP), heart rate, blood gases, and specific weight values between normo-, hypothermic and unaffected animals were analyzed by the Mann-Whitney U-test. The Wilcoxon test was used to prove differences in MABP and heart rate before and during the decrease of CBF. P values of <0.05 were considered to be significant.

RESULTS

PBCAL caused a short term LCBF reduction to 40-50% of baseline values followed by an increase to 50-80% of baseline values (Fig. 1). In the frontal cortex, LCBF was restored to a higher level in hypothermic animals than in normothermic ones between 10 min and 2 h after the onset of PBCAL ($P<0.05$, Fig. 1 above). There were no significant differences in blood gas values and MABP between the normothermic and hypothermic animals before and during CBF reduction (Table I). MABP

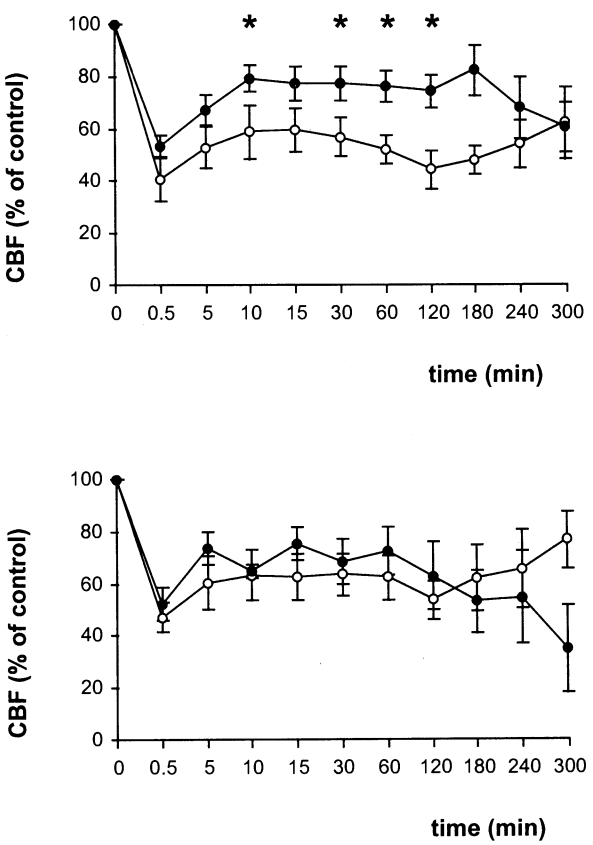

Fig. 1. Effects of temperature on frontal (above) and occipital LCBF (below) after permanent bilateral carotid artery ligation (normothermic animals: open circles; hypothermic animals: filled circles; $n = 6$ in each group; mean ± SEM; * $P<0.05$, * indicates comparison between normothermic and hypothermic animals.)

TABLE I

Physiological variables during baseline conditions (control), and 5 h after onset of carotid artery ligation

	control	5 hours of carotid artery ligation
arterial blood pressure (mm Hg)		
normothermic group	109 ± 14	123 ± 10
hypothermic group	116 ± 6	128 ± 8
heart rate (min^{-1})		
normothermic group	344 ± 15	411 ± 21
hypothermic group	316 ± 32	356 ± 31
arterial pO_2 (mm Hg)		
normothermic group	108 ± 8	102 ± 10
hypothermic group	104 ± 6	99 ± 2
arterial pCO_2 (mm Hg)		
normothermic group	39 ± 5	38 ± 5
hypothermic group	32 ± 2	32 ± 3
arterial pH		
normothermic group	7.42 ± 0.04	7.35 ± 0.05
hypothermic group	7.43 ± 0.10	7.37 ± 0.04

(Values are means ± SEM; comparison between normothermic and hypothermic animals revealed no significant differences.)

increased transiently for 2 h after the onset of PBCAL from 109 ± 14 up to 136 ± 13 mmHg (mean ± SEM) in the normothermic group and from 116 ± 6 up to 133 ± 6 mmHg in the hypothermic group (P <0.05). The control values of the heart rate did not differ between both groups either (Table I), however, heart rate increased slowly in the normothermic animals but not in the hypothermic ones from 344 ± 15 min^{-1} up to 411 ± 21 min^{-1} 5 h after onset of PBCAL (P <0.05). Heart rate was significantly lower in the hypothermic animals than in the normothermic ones 2 h (364.0 ± 26 *versus* 412 ± 17 min^{-1}, mean ± SEM), 3 h (337 ± 31 *versus* 425 ± 15 min^{-1}) and 4 h (335 ± 23 *versus* 414 ± 18 min^{-1}) after the onset of PBCAL (P<0.05).

No brain slice showed any blue staining by Evans blue, meaning the blood-brain barrier for proteins was not disturbed even 6 h after the onset of PBCAL. We found no significant differences in the brain water content between the corresponding regions of both hemispheres by gravimetry, and therefore the specific weight values of those regions were averaged. In spite of the absence of evidence of vasogenic brain edema, the brain water content of the normothermic animals increased by up to 1.2 % in both frontoparietal cortical regions investigated and in the occipital cortex at AP level -2 as well as in the anterior horn of the hippocampus, indicating the development of cytotoxic brain edema (P <0.05, Fig. 2). In the hypothermic group, an increase in brain water content occurred in only one region of the frontoparietal cortex (P<0.05, Fig. 2). In the posterior horn of the hippocampus, the brain water content of the normothermic animals was significantly higher (0.47%, P <0.05, Fig. 2) than in the hypothermic ones.

DISCUSSION

Six hours after the onset of moderate CBF reduction a brain edema was found mainly in the frontoparietal cortex and hippocampus. Mild reduction of the body temperature by 2°C during permanent bilateral common carotid artery ligation in rats resulted in a diminished reduction of LCBF in the frontal cortex and prevented the development of brain edema. This edema was presumably a cytotoxic one because the staining with Evans blue showed no extravasation of albumines which would have been indicative of a vasogenic brain edema. The development of vasogenic brain edema strongly correlates with the extravasation of albumin (Kuroiwa et al. 1985, Fredriksson et al. 1987), and Evans blue binds almost completely with albumin (Freedman and Johnson 1969). The decrease of cerebral blood flow in our study has presumably disturbed the ion homeostasis of the neurons. It has been shown that a LCBF reduction to 50-60% caused initial functional disturbances in neurons such as a reduction in electrical functions (Hossmann and Schuir 1980) and in protein synthesis (Mies et al. 1991). Although the notion of the strict division of the brain edema into a cytotoxic and vasogenic type has been criticized (Joo 1987), our experiments show the possibility of developing a brain edema without disrupting the blood-brain barrier to proteins.

Though we did not measure the brain temperature because we wanted to avoid traumatic injury to the brain, a 2°C reduction of brain temperature can be assumed. Brain temperature correlated closely with the rectal tem-

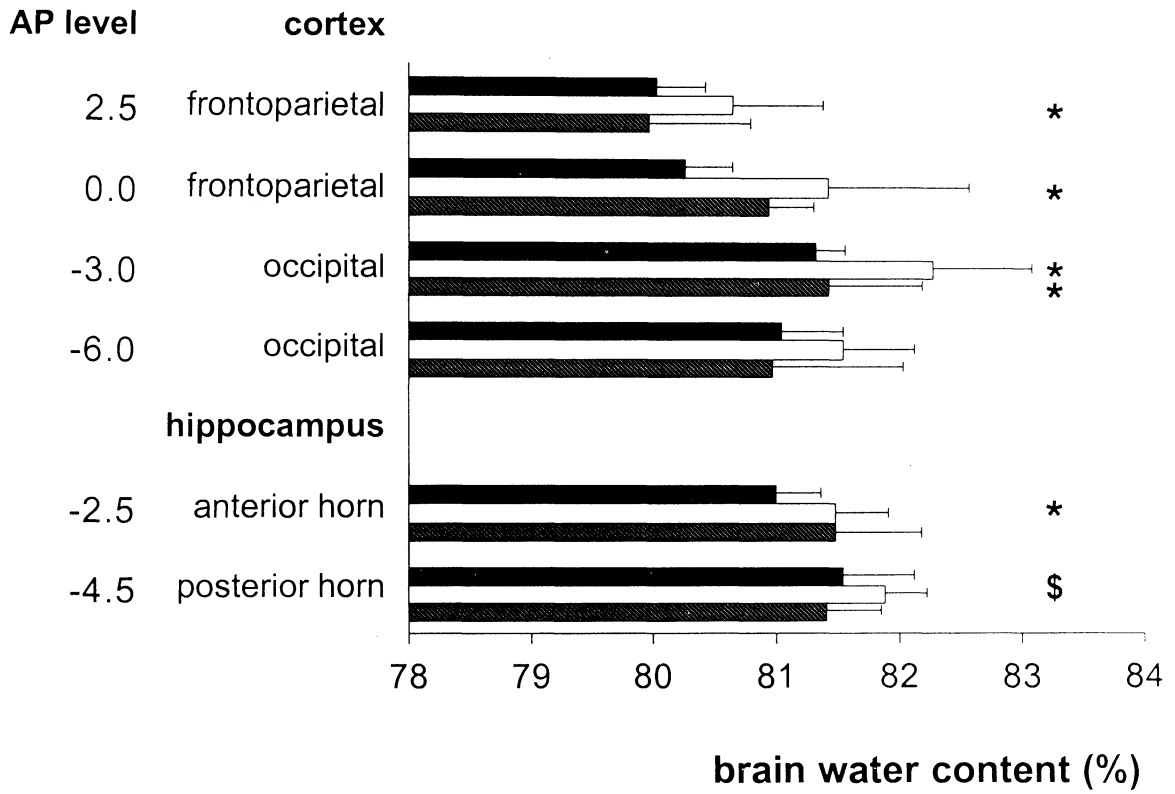

Fig. 2. Comparison of brain water content in unaffected animals (filled bars, $n = 8$), normothermic animals after 6 h of PBCAL (open bars, $n = 6$), and hypothermic animals after 6 h of PBCAL (hatched bars, $n = 6$). (Mean ± SEM are given; *, $ $P < 0.05$, * indicates comparison between unaffected animals and animals underwent 6 hours of PBCAL; $ indicates comparison between normothermic and hypothermic animals). Note the protective effects of hypothermia against an increase in brain water content in the parietal cortex and hippocampus.

perature (Yamashita et al. 1991) when the temperature probe was inserted into the rectum to at least 6 cm in order to measure the core temperature exactly (Lomax 1966, Miyazawa and Hossmann 1992).

Up to now, the protective effects of moderate hypothermia against the development of cytotoxic brain edema have never been taken into account among the effects of hypothermia. The preservation of LCBF could contribute to this effect. The frontal Laser probe was placed close to the areas of frontoparietal cortex where the cytotoxic brain edema was shown. However, the Laser probe did not influence the development of brain edema in this region because the probe was placed above the right hemisphere and cytotoxic brain edema could be proven to exist in the corresponding regions of both hemispheres. Furthermore, no edema was shown in the area surrounding the occipital Laser probe (occipital cortex sample taken at AP level -6). Here, no protective effect of mild hypothermia on LCBF could be proven. This lack of a protective effect of hypothermia in the occipital cortex can not be explained by our experimental approach. Generally, the changes of LCBF during hypothermia seem to be variable and depend on the degree of hypothermia, model of ischemia, and animal species (Kuluz et al. 1993). Thus, some authors found brain temperature to have no effect on LCBF in rats at temperatures between 39°C and 30°C during global (Busto et al. 1989) and focal (Morikawa et al. 1992) ischemia. Avoiding the systemic effects of hypothermia by selective brain cooling, Lo and Steinberg (1992) found no changes of LCBF at 33°C in focal ischemia in rabbits, however, they did find a decrease of LCBF at 30°C. Using a model of selective brain cooling as well, Kuluz et al. (1993) showed an increase of LCBF in nonischemic rats to about 200% at a cortical temperature of 33.4°C. Likewise, a systemic effect of hypothermia on CBF can not

be concluded from our experiments because a significant difference in MABP was not found between normo- and hypothermic animals. Heart rate was even significantly lower in the animals with 35°C rectal temperature during the period of CBF reduction. We assume that the increase of LCBF during mild hypothermia was mediated by a reduced cerebrovascular resistance. The metabolic control of LCBF seemed not to be affected, or only to a minor degree, by the mild hypothermia used. Busto et al. (1989) showed that the same mild decrease in brain temperature of 2°C during an ischemic insult did not affect the degree of high-energy phosphate depletion and lactate accumulation.

Other effects of hypothermia could play an additional role in the protection of the brain against a cytotoxic brain edema. It was shown that hypothermia has stabilizing effects on the ion balance of neuronal cell membranes resulting in a reduction in the number of ischemic depolarizations (Chen et al. 1993). These depolarizations occurred after LCBF reduction to 57% of control levels (Iijima et al. 1992), a threshold that was temporarily reached in our experiments. However, in these experiments DC signals were not measured. In addition, hypothermia diminishes the ischemically induced release of excitatory amino acids (Busto et al. 1989), probably via the inhibition of ischemic depolarisations (Ueda et al. 1992). An increase in glutamate release occurred at a LCBF threshold of about 40-48% of baseline values (Shimada et al. 1989, Takagi et al. 1993), which was partially observed in our study.

In conclusion, a mild hypothermia of 2°C prevents the development of cytotoxic brain edema which is the early stage of ischemic brain edema in rats in the first 6 h of moderate global CBF reduction. This is partly caused by an improvement of LCBF.

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ABBREVIATIONS

LCBF - local cerebral blood flow
PBCAL - permanent bilateral carotid artery ligation
MABP - mean arterial blood pressure

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