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RESEARCH ARTICLE

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Influence of hypoglycemic coma on brain water and osmolality

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Abstract To study the effects of pronounced hypoglycemia on brain osmolality and brain edema formation, fasted rats were rendered hypoglycemic by injection of insulin, and subjected to 30 min of hypoglycemic coma. Recovery was accomplished by glucose administration. The change in water content in different brain regions was measured as a change in specific gravity after 30 min of hypoglycemic coma, or 30, 60, and 180 min after glucose administration. Plasma and brain tissue osmolality were measured in separate animals. The results show a significant decrease in specific gravity (increase in water content) in all structures measured (caudoputamen, neocortex, hippocampus, and cerebellum) at the end of the period of coma, as well as after 30 min and 60 min of recovery. At 180 min of recovery, brain water was normalized. The edema affected all structures to the same degree regardless of their vulnerability to hypoglycemic damage. Brain tissue osmolality showed a tendency to decrease with decreasing tissue glucose content. The decrease was significant ($P<0.01$) at 30 min of isoelectric coma. In the recovery phase, normal brain osmolality was restored within 30 min. Measurements of blood-brain barrier (BBB) permeability after 30 min of hypoglycemic coma showed no extravasation of Evan's blue, though a small but significant increase in the permeability for aminoisobutyric acid (AIB) in caudoputamen and in cerebellum was found. To analyze the importance of tissue acidosis for formation of edema, hypoglycemic animals were made acidotic by increasing the CO_2 concentration in inspired air to produce an arterial plasma pH of 6.8–6.9. In these animals the edema was of a similar degree to the normocapnic animals, and the permeability for AIB was normal. We conclude that osmolytic mechanisms are not the primary cause of the selective neuronal vulnerability in hypoglycemic coma. Furthermore, the BBB is largely intact during a hypoglycemic insult.

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Introduction

The main objective of the present study was to clarify whether hypoglycemic coma is accompanied by tissue edema and to obtain additional information on events which could contribute to shifts of water between blood and brain. Hypoglycemia and ischemia both give rise to brain damage, affecting selectively vulnerable neuronal populations. The localization of the lesions is similar, but not identical, suggesting differences in pathophysiology (Auer and Siesjö 1988). One important difference between ischemia and severe hypoglycemia is the absence of tissue acidosis in the latter situation (Bengtsson et al. 1990), due to the absence of anaerobic glycolysis, and to oxidation of anions of metabolic acids (Lewis et al. 1974a, b; Feise et al. 1976; Norberg and Siesjö 1976).

The decrease in glucose supply during hypoglycemic coma leads to energy failure with a reduction in the concentrations of ATP and other nucleoside triphosphates (Lewis et al. 1974b; Feise et al. 1976; Agardh et al. 1981; Chapman et al. 1981; Katsura et al. 1993). According to classic concepts, cell edema should result when ATP production fails and leak pathways for osmotically active ions are established (Macknight et al. 1994).

Hypoglycemic coma is accompanied by transamination with an accumulation of aspartate and by massive release of aspartate and glutamate (Lewis et al. 1974b, Agardh et al. 1981; Wieloch 1985; Sandberg et al. 1986). Since stimulation of receptors for excitatory amino acids activates a major conductance for Na^+ (and K^+), one would expect that hypoglycemia leads to edema and osmolytic damage to neurons, particularly in the selectively vulnerable areas (Rothman and Olney 1987). In addition, the cerebral blood flow during hypoglycemic coma

is increased, providing opportunities for edema fluid to enter the brain (Abdul-Rahman et al. 1980; Siesjö and Deshpande 1987).

Results from previous studies on brain edema during hypoglycemia are not internally consistent, probably because different species were used, and different degrees of hypoglycemia were studied. Most studies have, however, found experimental evidence of brain edema and electrolyte changes when the hypoglycemic situation was severe enough to produce electroencephalographic (EEG) silence and depolarization of cell membranes (Arieff et al. 1974; Thurston et al. 1976, 1977; Siesjö and Deshpande 1987). The direction of the ionic movements have caused controversy; Na^+ and K^+ have been suggested to: (1) move into the brain tissue from blood (Arieff et al. 1974), (2) show no changes (Thurston et al. 1976, 1977), or (3) change in opposite directions (Siesjö and Deshpande 1987). Accumulation of Na^+ and K^+ would promote the increase in brain osmolality, and such an increase was also reported by Arieff and collaborators (Arieff et al. 1974). However, in a study from this laboratory (Siesjö and Deshpande 1987), the increase in Na^+ was only moderately larger than the loss off K^+, and the change in Cl^- content was small.

Increase of tissue osmolality is considered to contribute to edema formation (Arieff et al. 1974; Hossman and Takagi 1976). However, the behavior of cells in anisosmotic media is often different from that predicted for a perfect osmometer, and caution is required in extrapolating from in vitro responses of isolated cells to the responses of tissues in vivo (Macknight 1988). Tomita, in a simulated "cell" model, demonstrated fluid shifts even against an osmotic gradient (Tomita and Gotoh 1992).

In this study plasma and tissue osmolality were followed throughout the hypoglycemic insult and the results were analyzed in relation to the formation of edema. Due to the absence of tissue acidosis, the hypoglycemic tissue could be expected to have a reduced capacity to form osmotically active metabolites. The capacity of hypoglycemic brains to form these metabolites was tested by exposing them to global ischemia.

The formation of brain edema, assessed as changes in specific gravity in brain slices, after 30 min of insulin-induced hypoglycemic coma was studied at the end of the coma period and after 30, 60, and 180 min of recovery. To study the coupling, if any, between edema and selective neuronal vulnerability, samples were taken from three vulnerable regions, *viz*. the hippocampus, neocortex, and caudoputamen, and from a resistant one, the cerebellum (Agardh and Siesjö 1981; Auer et al. 1984b; Kleihues et al. 1986).

Kristián et al. (1995) showed that addition of hypercapnia (to about 200 mm Hg) to the hypoglycemic insult aggravated the neuronal damage. To investigate whether enhanced edema may be involved in that process, tissue specific gravity was measured following 30 min of combined hypoglycemic coma and hypercapnia.

Formation of edema is closely connected to the permeability of the blood-brain barrier (BBB), but BBB damage in connection with hypoglycemic damage has previously not been extensively analyzed. In this study, the permeability of the BBB was investigated qualitatively with Evan's blue and quantitatively by measuring the rate of uptake of aminoisobutyric acid (AIB) into the tissue.

Materials and methods

Animals and operative techniques

All experiments, and all handling of the animals described in this study, were approved by the Ethical Committee for Laboratory Animal Experiments at the University of Lund. Male Wistar rats of an SPF-strain (Møllegaard's Breeding Centre, Copenhagen, Denmark) were used for this study. In total, 87 animals were examined for specific gravity or osmolality of the brain, devided into normal controls, insulin-treated controls given glucose to maintain normal glucose levels, and animals with different times of hypoglycemic coma either without recovery or, after 30 min of coma, with different times of recovery (30, 60, and 180 min). Two precomatose groups at a stage of hypoglycemia giving a slow-wave pattern in the electroencephalogram were also analyzed. The different groups and number of animals in each group are shown in Table 1. For the measurement of edema after hypoglycemic coma combined with acidosis, another 22 animals were utilized, 8 new controls and 14 animals subjected to hypoglycemic coma in combination with hypercapnia. Permeability of the BBB was measured by injection of Evan's blue in rats with 30 min of hypoglycemic coma, and by injection of AIB in normal anesthetised animals, in rats after 30 min of hypoglycemic coma, and in animals subjected to 30 min of ischemia combined with hypercap-

Table 1 Overview of the number of animals in the different experimental groups (*AIB* aminoisobutyric acid, *iso* isoelectric coma)

Analysis	Type of experiment													
	Nonisoelectric animals (*n*)			Isoelectric coma (*n*)			Min of recovery after 30-min coma (*n*)			20-Min decapitation ischemia (*n*)			Hypercapnic animals (*n*)	
	Control	Insulin control	Slow wave	1–2 min	30 min	60 min	30 min	60 min	180 min	Fasted controls	Slow wave	30-Min iso	Control	30-Min iso
Specific gravity	9	10	4	–	8	4	6	6	5	–	–	–	8	14
Osmolality	11	4	4	4	4	–	4	–	4	3	4	4	–	–
AIB	4	–	–	–	5	–	–	–	–	–	–	–	–	4
Evan's blue	–	–	–	–	3	–	–	–	–				–	–

nia. The influence on brain osmolality of a 20-min period of decapitation ischemia in hypoglycemic animals was measured in fasted animals, in the slow wave phase, and in animals after 30 min of isoelectric coma (Table 1).

Operative procedures and experimental protocols were as described previously (Auer et al. 1984a). In summary, to create hypoglycemic coma, the rats were injected *i.p.* with 2 IU·kg^{-1} of regular insulin 1 h before the operation (Actrapid; Novo Industri, Copenhagen, Denmark). At the start of operation, anesthesia was induced with 3.5% halothane in O_2–N_2O (30:70) and thereafter maintained on 1% halothane during the operation. The trachea was intubated and the lungs were mechanically ventilated. Catheters were placed in the tail artery for blood pressure recording and arterial blood sampling, and in a tail vein for drug and glucose administration. Needle electrodes were inserted bilaterally into the temporalis muscles for EEG recording. A central venous catheter was also inserted for rapid control of the blood pressure by exsanguination at the onset of isoelectric EEG activity.

Experimental procedures

After surgery the halothane concentration was reduced to 0.5%, and the animals were given an *i.v.* dose of 1 mg suxamethonium chloride (Celocurin; Vitrum, Stockholm, Sweden) for muscle relaxation, repeated as required. The animals were heparinized (50 IU) and $PaCO_2$ and PaO_2 were set at normal levels by proper adjustment of the ventilator and the inspired O_2. Plasma glucose levels were measured at short intervals, and arterial blood pressure and EEG activity were continuously recorded. Atropine (0.1 mg) was given *i.v.* just before the onset of isoelectricity. Systolic blood pressure was kept in the range 90–150 mm Hg by withdrawal of blood from the central venous catheter or, whenever needed, by reinfusion of blood. In the recovery experiments, glucose was infused *i.v.* at the end of the 30-min period of isoelectricity to restore normal plasma glucose levels. At the end of the experiment, the animals destined for specific gravity measurements were decapitated, and their brains were rapidly removed from the skull and immediately transferred to a humidified glove box for direct analysis. The brains of the animals destined for osmometry were frozen in situ with liquid nitrogen (Pontén et al. 1973), chiseled out from the skull in the frozen state, and stored at −80° C until analysis.

In the experiments for BBB studies, 1 ml of a 2% Evan's blue solution was injected *i.v.* 2 min before decapitation, or 15 μCi[^{14}C]AIB was injected *i.v.* 20 min before decapitation, *i.e.,* 10 min after onset of the coma period in the isoelectric groups.

Acidosis by hypercapnia was achieved by addition of carbon dioxide to the inspired anesthetic gases as soon as an isoelectric electroencephalogram was confirmed, and $PaCO_2$ was adjusted to 180–200 mm Hg, corresponding to a pH of 6.8–6.9.

Rats exposed to decapitation ischemia and intended for tissue osmolality measurements were made hypoglycemic as described above. When the slow-wave phase was attained or when an isoelectric electroencephalogram had lasted for 30 min, the animal was decapitated. The head was then put into a plastic bag which was placed in a water bath at 37° C for 19 min. The brains were then removed and frozen in 2-methylbutane chilled to −50° C, and subsequently stored at −80° C until analysis.

Specific gravity measurements

Specific gravity was measured immediately following decapitation. The brain was placed on an ice-cold plate in a humidity chamber (relative humidity more than 90%). Small samples (≈10 mg) were dissected bilaterally from caudoputamen, frontoparietal cortex, hippocampus, and cerebellar cortex. The tissue samples were introduced into a density gradient column and their position determined after 3 min.

The specific gravity columns were prepared from a Percoll solution (Pharmacia, Uppsala, Sweden) as described by Tengvar and collaborators (1983). The gradient was calibrated using glass spheres of known densities (Scientific Glass, Bloomfield, N.J., USA). Only gradients with a linear calibration curve were used.

Osmolality measurements

Osmolality was measured with the vapour pressure method described by Tornheim (1980). The frozen brains were transferred to a glove box with a temperature of −20 to −25° C. A cylindrical column of tissue with a diameter of 5 mm was taken unilaterally from the forebrain (Gisselsson et al. 1992). From this cylinder, representing mainly cortex and caudoputamen, slices of tissue with a thickness of 0.7–0.8 mm were cut and immediately transferred to the measuring chamber of the vapour pressure osmometer (Wescor 5500; Wescor, Logan, Utah, USA). Plasma samples were also analyzed. The osmometer was calibrated with standard solutions at the start, and after every third measurement.

AIB penetration

The integrity of the BBB to AIB was tested as follows. A bolus of 15 μCi [^{14}C]AIB diluted in 1.0 ml saline was injected *i.v.* Arterial plasma samples (20 μl) were repeatedly collected over a period of 20 min to allow integration of plasma activity. At the end of the experiment, the animals were decapitated, and their brains were removed and frozen in 2-methylbutane chilled to −50° C. Tissue samples, weighing 10–15 mg, were dissected out at −15° C from caudoputamen, parietal cortex, hippocampus, and cerebellum to allow assessment of tissue tracer activity.

The plasma-to-brain AIB transfer coefficient K (μl·g·min^{-1}) was calculated as described by Blasberg et al. (1983), with the help of the following equation:

$$K = \frac{C^*_{br}(T)}{\int_0^T C^*_{pl}(t)\mathrm{d}t}$$

in which C^*_{br} is the brain tissue concentration of radioactivity at 20 min, and C^*_{pl} is the plasma activity. The cerebral blood volume was set to 0.01 ml·g^{-1} of tissue, according to Ohta et al. (1992), to allow correction for AIB remaining in the vasculature.

Statistics

Statistical evaluation of the material was made with one-factor analysis of variance (ANOVA), followed by Dunnett's test for differences between control and experimental groups. Statistical differences between normal fasted control animals and insulin-treated, glucose-infused control animals and between controls and hypercapnic, hypoglycemic animals animals were tested with unpaired, two-tailed Student's *t*-test. A *P*-value of less than 0.05 was regarded as statistically significant.

Results

Physiological variables

Table 2 shows physiological variables for the animals in the specific gravity study. All animals had normal blood gases and pH throughout the experiment, and body temperature was close to 37° C. All other groups had similar values (not shown), with the exception of the group in which animals were made hypercapnic by inhalation of CO_2. These animals had a $PaCO_2$ of 180–200 mm Hg and an arterial pH of 6.8–6.9.

Table 2 Physiological parameters for rats engaged in the specific gravity measurements (BP=mean arterial blood pressure)

	Start of experiment								End of experiment							
	Temp (°C)		BP (mm Hg)		$PaCO_2$ (mm Hg)		PaO_2 (mm Hg)		pH		Temp (°C)		BP (mm Hg)		$PaCO_2$ (mm Hg)		PaO_2 (mm Hg)		pH	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Control	37.1	±0.2	134	±10	36.6	±1.9	112	±11	7.40	±0.03										
Insulin control	37.1	±0.3	134	±13	37.8	±2.3	114	±4	7.36	±0.04										
30 min isoelectr.	37.5	±0.5	138	±22	37.5	±2.3	101	±10	7.40	±0.04	37.1	±0.5	140	±20	36.9	±4.9	112	±11	7.38	±0.05
30 min iso+30 min rec	37.1	±0.7	132	±13	38.6	±2.8	102	±14	7.39	±0.06	36.9	±0.1	165	±11	37.4	±4.9	112	±7	7.32	±0.01
30 min iso+60 min rec	37.1	±0.4	133	±10	38.2	±1.4	111	±9	7.38	±0.03	37.0	±0.3	159	±15	35.9	±3.2	109	±8	7.36	±0.01
30 min iso+180 min rec	37.3	±0.8	130	±11	36.8	±3.6	110	±14	7.40	±0.04	37.1	±0.3	141	±15	38.4	±1.5	106	±10	7.41	±0.03

The mean arterial blood pressure (MABP) increase at the onset of isoelectric coma was counteracted by exsanguination from the central venous catheter, as were increases in MABP during the coma period. Withdrawn blood was reinfused as needed. Fasted controls had a plasma glucose level of about 8 $mmol \cdot l^{-1}$, whereas the insulin-treated controls were given glucose to maintain a glucose level of about 4 $mmol \cdot l^{-1}$. Blood glucose determinations in the experimental groups were made after 5 min and 25 min of isoelectricity, giving values consistently below 0.5 $mmol \cdot l^{-1}$. In the recovery groups, infusion of glucose was started after 30 min of isoelectric EEG activity, as described in previous publications (Auer et al. 1984b).

Brain specific gravity

The normal values for specific gravity in insulin-injected controls were: caudoputamen 1.0434±0.0009 $g \cdot cm^{-3}$, cortex 1.0438±0.0009 $g \cdot cm^{-3}$, hippocampus 1.0421±0.0008 $g \cdot cm^{-3}$, and cerebellum 1.0456±0.0008 $g \cdot cm^{-3}$ (mean± SD). These values were similar to those obtained in normal fasted control animals, so the two control groups were pooled.

The specific gravity decreased during hypoglycemic coma (Fig. 1). After 30 min of isoelectric EEG activity, the decrease in specific gravity was highly significant in neocortex, hippocampus, and cerebellum, while caudoputamen only showed a small, nonsignificant reduction in tissue density. Durin the 1st h of recovery, specific gravity remained significantly reduced in all structures examined. After 3 h the specific gravity had returned to near-normal levels.

We also examined four animals prior to the onset of isoelectricity in which the electroencephalogram showed a slow-wave pattern, and four animals after 60 min of hypoglycemic coma. Since these groups were small and since only caudoputamen and cortex were analyzed, the animals were not included in the statistical analysis. During the slow-wave phase, specific gravity in caudoputamen was 1.0433±0.0007 $g \cdot cm^{-3}$ and in cortex 1.0430±0.0008 $g \cdot cm^{-3}$. The values were very similar to control values. We conclude from these results that a decrease in specific gravity of the tissue does not occur until "coma" ensues.

After 60 min of hypoglycemic coma, the specific gravity in caudoputamen and cortex was 1.0423±0.0008 $g \cdot cm^{-3}$ and 1.0419±0.0010 $g \cdot cm^{-3}$, respectively. These should be compared with values of 1.0425±0.0008 $g \cdot cm^{-3}$ and 1.0419±0.0010 $g \cdot cm^{-3}$, respectively, after 30 min of hypoglycemia. The results suggest that no further fall in specific gravity occurred when the coma period was prolonged from 30 to 60 min. In a separate series, edema after 30 min of hypoglycemic coma combined with hypercapnia was measured. This part of the study was performed later than the previous part, and the new control values differed somewhat from the old ones. The decrease in specific gravity after 30 min of hypoglycemic coma was, however, similar to normocapnic

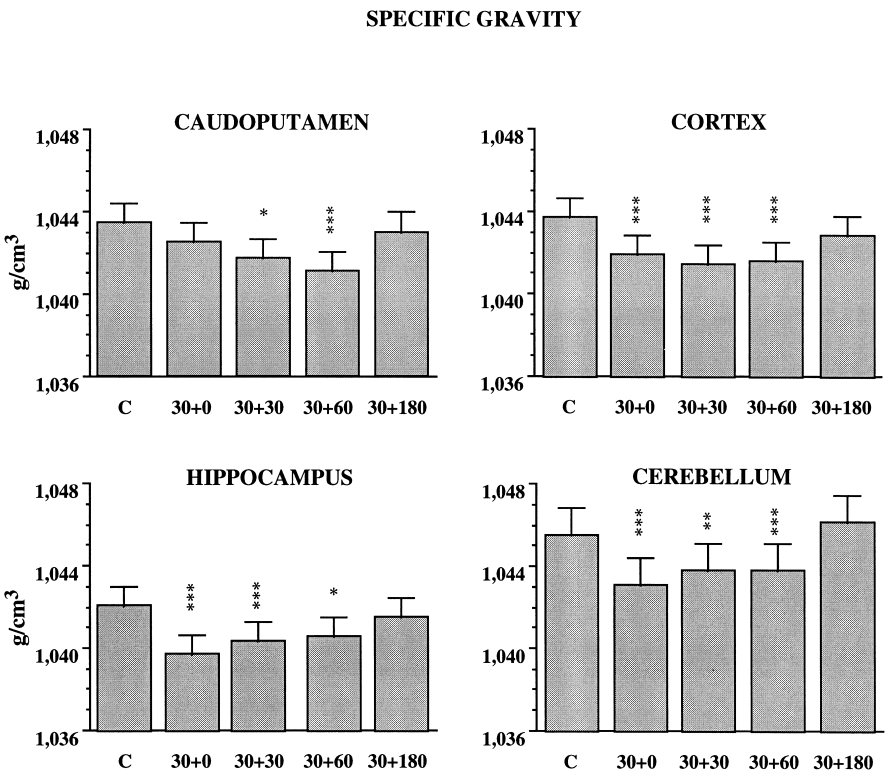

Fig. 1 Specific gravity in four different regions of the brain, in control animals (*C*), during hypoglycemic coma and in the recovery phase after 30 min of coma. * $P<0.05$; ** $P<0.01$; *** $P<0.001$ versus C

animals, *i.e.*, the edema did not increase in the hypercapnic animals.

Osmometry

Plasma

The normal plasma osmolality was found to be 287±5 mmol·kg^{-1}. For insulin-treated controls, it was 285±4 mmol·kg^{-1}. The osmolality of plasma (Fig. 2) showed a slight tendency to decrease with decreasing plasma glucose concentration, so that after 1 min and 30 min of hypoglycemic coma the osmolality was 280±3 mmol·kg^{-1} and 282±4 mmol·kg^{-1}, respectively. When glucose was administered in the recovery period, plasma osmolality increased to values 4–5 mmol·kg^{-1} higher than normal. None of these suggested changes in plasma osmolality was statistically significant.

Brain

Fasted, sham-operated rats had a brain osmolality of 305±6 mmol·kg^{-1} Rats made slightly hypoglycemic by insulin injection, followed by glucose infusion, which was adjusted to maintain a plasma glucose level of 4 mmol·l^{-1}, had a brain osmolality of 297±5 mmol·kg^{-1}, not significantly lower than the normal fasted controls. However, in the coma period tissue osmolality fell further, the decrease at 1 min and 30 min of isoelectricity reaching statistical significance ($P<0.05$), when compared with nontreated controls (see Fig. 2). During the first 30 min of recovery, brain osmolality increased to control values, and after 180 min of recovery we found an osmolality of 312±5 mmol·kg^{-1}, not significantly different from the control.

The osmotic gradient between brain and plasma

In normal control animals the osmotic difference between tissue and plasma was 17±6 mmol·kg^{-1}. In insulin-treated controls it was somewhat lower, 13±3 mmol·kg^{-1}. During the coma period the osmolality in tissue decreased more than in plasma, hence the mean value for the gradient numerically diminished, to a lowest value of 1±6 ($P<0.01$). In the recovery period the osmotic gradient gradually returned to control values (*cf.* Fig. 2).

Decapitation ischemia

Decapitation ischemia of 20 min duration in normoglycemic, fasted, animals led to an increase in brain osmolality from 307±4.5 mmol·kg^{-1} to 319±2 mmol·kg^{-1}. If the animal was in the slow-wave phase before the ischemic insult, the brain osmolality increased from 301±4 mmol·kg^{-1} to 309±11 mmol·kg^{-1}. After 30 min of hypoglycemic coma, brain osmolality was 283±8 mmol·kg^{-1}. A 20-min period of decapitation ischemia in such animals resulted in an elevation of tissue osmolality to 296±8

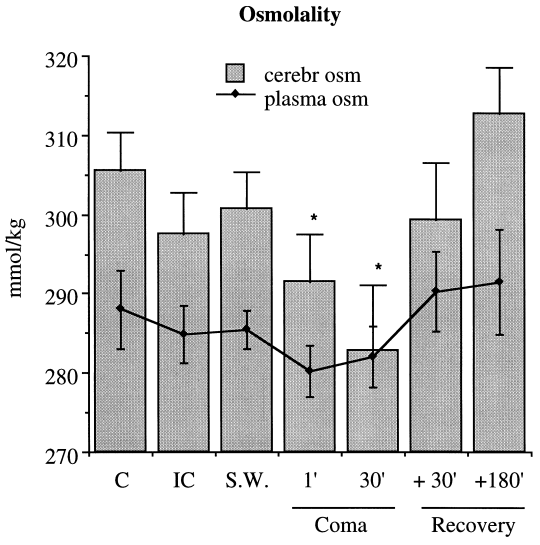

Fig. 2 Brain (*cerebr osm*) and plasma osmolality (*plasma osm*) in normal control animals (*C*), in insulin controls (*IC*), in hypoglycemic animals in the slow-wave phase (*S.W.*), during coma (1 min and 30 min), and in the recovery period (30 min and 180 min). * $P<0.05$ versus C

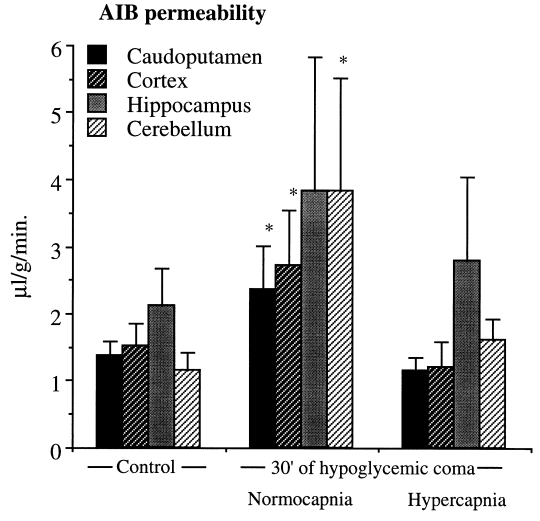

Fig. 3 Aminoisobutyric acid (*AIB*) permeability in different regions of the brain in control animals, and after 30 min of isoelectric coma in normo- and hypercapnic animals. * $P<0.05$ versus control value of the same structure

mmol·kg^{-1}. In other words, the ischemia-induced increase in osmolality was similar in animals with hypoglycemic coma and in controls.

BBB permeability during hypoglycemic coma

No sign of Evan's blue permeability was seen at 30 min of hypoglycemic coma. The permeability of AIB in control animals was: in caudoputamen 1.38±0.21 µl·g·min^{-1}, in parietal cortex 1.51±0.35 µl·g·min^{-1}, in hippocampus 2.11±0.56 µl·g·min^{-1}, and in cerebellum 1.15±0.26 µl·g·min^{-1}. After 30 min of hypoglycemic coma, we found a statistically significant ($P<0.05$) increase in caudoputamen, parietal cortex, and in cerebellum. The hypoglycemic value for hippocampus was unchanged (Fig. 3).

Acidosis by hypercapnia

If 30 min of hypoglycemic coma was combined with hypercapnia, to induce a blood pH of 6.8–6.9, the permeability for AIB remained at control levels in all the structures studied (Fig. 3).

Discussion

Edema

Tissue water content of hypoglycemic animals increased in all structures studied, but the edema did not progress with time (*cf.* Siesjö and Deshpande 1987) and was reabsorbed within 3 h after a hypoglycemic insult of 30 min duration.

Edema in normocapnic animals

The increase in tissue water content in this study was similar to what is seen in the early phase after forebrain ischemia in the same rat strain (Warner et al. 1987; Gisselsson et al. 1992). In ischemia, the cytotoxic component dominates (Ting et al. 1986; Todd et al. 1986; Seida et al. 1989). To clarify whether hypoglycemic edema had a vasogenic component, the integrity of the BBB was studied. Our results showed that 30 min of coma induced an increase in AIB influx into all structures except the hippocampus. It is noteworthy that one of these structures was the cerebellum, which is relatively resistant to hypoglycemic damage (Agardh and Siesjö 1981; Auer et al. 1984b; Kleihues et al. 1986). This means that the vasogenic edema observed correlated poorly to the occurrence of neuronal necrosis. It should also be pointed out that the increase in AIB permeability was small, suggesting a moderate vasogenic component of the edema. An electron-microscopic study of hypoglycemia has demonstrated mild capillary alterations, which may indicate changes in vascular permeability (Auer et al. 1985). The conclusion of a moderate BBB influence is supported by the absence of a detectable extravasation of Evan's blue. Probably, the increase in AIB permeability also reflects the vasodilatation and increase in cerebral blood flow during the hypoglycemic insult.

The changes in brain water content were assessed by measurements of specific gravity. This method is well established for determination of cytotoxic edema (Nelson et al. 1971; Marmarou et al. 1982; Tengvar et al. 1983), but requires that the specific gravity of tissue solids (sp. gr.$_s$) remains constant. However, when a vasogenic component contributes to the edema formation, sp. gr.$_s$ may change, and furthermore, hypoglycemic coma is associated with breakdown of lipids, proteins and nucleic acids

(Agardh and Siesjö 1981; Chapman et al. 1981), a situation in which sp. $gr._s$ may be expected to change. The direction of such changes is not predictable. sp. $gr._s$ was not measured, but, as the vasogenic component in our experiments was small, we regard the specific gravity method suitable for edema measurements in this study. The present results give qualitatively similar results to an earlier study from this laboratory, where wet minus dry weight was used to determine the edema after hypoglycemic coma (Siesjö and Deshpande 1987).

Acidosis by hypercapnia

The outcome after an *ischemic* insult is worse if an animal is hyperglycemic. A tentative explanation for this has been that a lower tissue pH leads to a more pronounced edema (Myers 1979; Hakim and Shoubridge 1989; Yura 1991; Morimoto et al. 1993; Katsura et al. 1994; Siesjö et al. 1996). However, when the hypoglycemic insult was combined with hypercapnia, the edema after 30-min coma was not worse than after a hypoglycemic insult alone, and a combination of hypercapnia and hypoglycemic coma did not increase the permeability of AIB.

Osmolality

Control animals

Brain tissue osmolality in control animals was 17±6 $mmol \cdot kg^{-1}$ higher than plasma osmolality in the same animals. The fact that the osmolality in brain is higher than that in plasma has been verified in many studies (Hossman and Takagi 1976; Bandaranayake et al. 1978; Tornheim 1980; Gisselsson et al. 1992). Many authors also found an osmolality of the cerebrospinal fluid that exceeds the plasma osmolality (Melton and Nattie 1983; De Pascuale et al. 1989; Wells 1990).

Although it is sometimes maintained that a difference in osmolality cannot exist over membranes which are permeable to water, this contention is probably too simplistic. A difference in osmolality between plasma and brain may be upheld by factors which are related to the active transport of ions across charged membranes (MacKnight and Leaf 1977) and by a relatively restricted water diffusion across membranes, including those constituting the BBB (Fenstermacher 1984; Fenstermacher and Rapoport 1984). However, we have to anticipate that in the control animals the water distribution over the BBB was at equilibrium, obviously representing a balance maintained by energy.

Hypoglycemic animals

Brain tissue osmolality decreased during hypoglycemia, while plasma osmolality remained essentially constant. The total decrease in tissue osmolality after 30 min of isoelectric EEG activity was about 20 $mmol \cdot kg^{-1}$. After restoration of plasma glucose to normal levels, the tissue osmolality returned to control values.

During coma, the shortage of glucose will directly reduce plasma osmolality. Simultaneously tissue concentrations of glucose and glycogen will decrease (Agardh et al. 1978). As a consequence we consider the direct effect of the decreased glucose concentration on the tissue-plasma osmolality gradient as moderate.

There are few investigations on the change of osmolality during hypoglycemic coma, but Arieff and collaborators (Arieff et al. 1974) found a substantial, but short-lasting, increase in brain tissue osmolality about 2 h after the *i.v.* administration of insulin to rabbits. The results from the two laboratories are thus contradictory, suggesting differences in experimental protocols and conditions. The study of Arieff and coworkers (Arieff et al. 1974) was made on awake, non-ventilated animals, and the insult was longer than that employed in studies from our laboratory. Thus their results could have been influenced by hypoventilation, hypotension, and seizures. Besides, the model used by these authors has not been extensively documented in terms of bioenergetic state, ion homeostasis, or histopathology.

Decapitation ischemia

Hypoglycemic rats were exposed to 20 min of anoxia. We found that animals subjected to 30 min of hypoglycemic coma showed the same increase in tissue osmolality after 20 min of decapitation ischemia as normoglycemic animals. Little is known about mechanisms that lead to an increased tissue osmolality in ischemia, an increase which is assumed to be responsible for postischemic edema (for results and further references, see Hossman and Takagi 1976; Gisselsson et al. 1992). We used the unique metabolic characteristics of hypoglycemic coma to obtain further information on this issue, since hypoglycemic coma leads to depletion of glucose and glycogen. Since little lactate (plus H^+) can be formed during ischemia, and since about three-quarters of the pool of nucleoside triphosphates is depleted, we expected the increase in osmolality during a superimposed ischemia to be small. Our results suggest that the increase in osmolality during ischemia is not related only to lactic acidosis or to hydrolysis of nucleoside triphosphates.

Objections could be raised toward the technique for measuring plasma or brain osmolalilty. The reliability of measurements of plasma osmolality is known to be biased by technical artifacts (Bohnen et al. 1992). On the other hand, our values for plasma osmolality are close to what are considered "normal values," and the SD is low. In tissue, postmortem artifacts may easily occur. Since small molecules such as ammonia or inorganic phosphate rapidly accumulate in brain within seconds after death and many methods tend to overestimate tissue osmolality (Katzman and Pappius 1973). We tried to avoid this type of alteration by freezing the brains in situ and by storing them at a temperature of −80° C. The values for tissue osmolality in this study, lies close to what has previously been found for whole brain in rats (Maffly and Leaf 1959; Bandaranayake et al. 1978).

The problem encountered in studying plasma and tissue is that the osmolalities in a solution and a gel phase are compared, and interpretations must be made with caution. Nonetheless, osmolality in brain tissue clearly decreased during hypoglycemic coma, while no significant differences in plasma osmolality were found.

Mechanisms of cell water control

The osmolality difference between plasma and blood remained stabile until the time of depolarization, including the 1st min of isoelectric coma. After 30 min of isoelectric EEG activity, the difference between brain and plasma osmolality was significantly decreased in relation to control, and the value approached zero. Tissue osmolality returned to normal after restitution of plasma glucose to control values. Despite the decreased osmolality gradient, edema occurred in all structures after 30 min of isoelectric coma and persisted in all structures during the first 30 min of recovery. Since the combined findings of edema and decreased tissue osmolality are contradictory, we will discuss possible mechanisms below.

The theory proposed by Macknight and Leaf (1977) predicts that the control of cell volume is exerted by the active extrusion of Na^+ from cells. This theory is more understandable if it is realised that extrusion of Na^+ occurs by $3Na^+/2K^+$ exchange and that efflux of one Na^+ must occur with a stoichiometrical amount of Cl^-. The operation of the Na^+/K^+ exchange means that Na^+ plus Cl^- is exported from cells, together with osmotically obliged water. Thus, active transport of Na^+ involves the "pumping" of water from intra- to extracellular fluids. Therefore, interruption of Na^+/K^+ transport should lead to cell swelling.

This theory has been extended by Tomita and Gotoh (1985, 1992). Using an in vitro system, these authors demonstrated that fluid shifts could occur in the absence of an osmotic gradient. In fact, they found that it could take place *against* such a gradient. They postulated that metabolic energy is used to create a large thermodynamic potential maintaining cells in the "contracted" state. This potential is discharged when cells loose their energy source or if their membranes become pathologically leaky to ions. As a result, cells swell. Evidently, their hypothesis fits well with our data which demonstrate that the osmotic difference between brain and plasma is disturbed during hypoglycemic coma, a condition that leads to energy failure and to dissipation of energy dependent ion gradients. It remains, however, to be demonstrated how the edema-associated water flux relates to the ionic changes in extra- and intracellular fluids. In vitro studies have shown that energy failure per se does not suffice to induce glial cell swelling (Kempski et al. 1987), and Ames and Nesbett (1983), working on isolated rabbit retinas, found that even a combination of anoxia and energy failure did not lead to irreversible cell swelling. In vivo, as Kempski emphasises, the circumstances are different, and membrane permeability to e.g., Na^+ is of importance, and a competent energy metabolism is essential to support cell volume regulation (Kempski et al. 1987).

Conclusion

Hypoglycemic coma was found to decrease tissue and brain osmolality and to influence the permeability of the BBB as shown by a moderate increase in uptake of amino-isobutyric acid, but not of Evan's blue. Hypoglycemia was also found to decrease specific gravity, indicative of brain edema formation is cerebral cortex, hippocampus, and cerebellum, and to some extent also in the caudoputamen. The edema occurred in spite of a decrease in tissue-plasma osmolality and also in structures not vulnerable to hypoglycemic damage. The magnitude was the same as previously found during 15 min of ischemia in normo- as well as in hyperglycemic animals (Gisselsson et al. 1992). All changes were reversible within 3 h following normalization of plasma glucose. Acidosis combined with hypoglycemic coma does not seem to further increase the edema or the permeability of the BBB.

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