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Brain Extracellular Ion Composition and EEG Activity Following 10 Minutes Ischemia in Normo- and Hyperglycemic Rats

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SUMMARY Hyperglycemia severely impairs the outcome from cerebral ischemia. In order to sort out whether impaired brain ion homeostasis contributes extracellular $[K^+]_o$, $[Ca^{++}]_o$, and $[H^+]_o$ concentrations, of brain cortex, as well as the EEG, were monitored during and after 10 minutes of complete cerebral ischemia in normo- and hyperglycemic rats. In both groups, the EEG-activity disappeared in 10-20 seconds of ischemia, at a time when $[K^+]_o$, $[Ca^{++}]_o$, and $[H^+]_o$ started to increase. After about 1.5 min, $[K^+]_o$ showed an abrupt increase and $[Ca^{++}]_o$ a steep decrease in the normoglycemic group. In the hyperglycemic group the same event took place after about 3 min of ischemia. pH_o decreased to 6.6 and 6.1 in the normoglycemic and hyperglycemic group, respectively. Following the ischemic episode $[K^+]_o$ reached pre-ischemic level after 4 min, $[Ca^{++}]_o$ after 13 min, and $[H^+]_o$ after 30 min in both groups. Recovery of the EEG, however, was clearly different in the 2 groups. EEG-activity reappeared later in the hyperglycemic group and showed after one hour a pattern of burst-suppression activity while the normoglycemic group showed asynchronous activity resembling the control pattern.

It is concluded that high glucose content in brain prior to ischemia — and hence lower brain pH during ischemia — does not interfere with the return of normal extracellular ion composition after cerebral ischemia, whereas the return and pattern of EEG activity is severely affected.

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IT HAS BEEN SHOWN that glucose administration prior to cerebral ischemia seriously aggravates the clinical outcome of rats.¹⁸ Also, the return of EEG activity after ischemia in hyperglycemic rats is significantly delayed. The reason for the detrimental effect of the high glucose level in blood, and hence in brain, has remained unknown, but since brain glucose during ischemia is converted to lactic acid, it was suggested that the augmented lactacidosis in the hyperglycemic rats was the culprit.¹⁴ How the lactacidosis affects brain function in the post-ischemic period and what cellular mechanism it eventually will damage is presently unknown.

To find out whether the delayed EEG appearance is caused by a delayed normalization of ion gradients across cell membranes in brain, we measured $[K^+]_o$

and $[Ca^{++}]_o$ in brain cortex during and after ischemia. We also determined the severity of brain acidosis by simultaneous measurement of extracellular pH during ischemia and ability of the brain to normalize pH after ischemia.

Material and Method

Preparation of Animals

The experiments were performed on male Wistar rats, weighing approximately 350 g. All rats were fasted overnight.

The rats were initially anesthetized with ether and intubated with a steel cannula. Following relaxation with suxamethonium, they were mechanically ventilated with 1% halothane and 35% O_2 in air. Polyethylene catheters were placed in the femoral artery and vein. The ventilation was adjusted to maintain arterial P_{CO_2} between 32-40 mm Hg. Rectal temperature was kept at 37°C by a heating lamp. One group of rats received an i.p. injection of 50% solution

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of glucose (3.5 g kg^{-1}) 40–60 minutes before ischemia. Plasma glucose was measured by a glucose oxidase method.¹

Craniotomy. A trephine opening was performed in the left parietal bone and the dura pierced without damaging the underlying pial vessels. Through the slit in the dura, ion-selective microelectrodes were lowered 0.5 mm below the cortical surface by means of a micromanipulator. EEG was recorded from 2 gold-coated screws, inserted bilaterally in the parietal bones 2 mm posterior to the bregma and 2 mm from the midline, against a reference inserted subcutaneously in the tail. The EEG activity was monitored until one hour after the ischemic episode.

Cerebral Ischemia. Ten minutes of cerebral ischemia was achieved by the method described previously.¹⁶ Three minutes prior to ischemia, approximately 50 IU kg^{-1} heparin was given intrarterially and halothane administration was discontinued. Arterial blood was slowly removed into a heparinized syringe until mean blood pressure equalled 50 mm Hg. Cerebral ischemia was achieved by compression of neck vessels with a pneumatic cuff, inflated to a pressure of 1 atm. After 10 minutes, the pressure was released and blood reinfused intrarterially within 15–30 seconds. Approximately $5 \mu\text{g}$ adrenaline was added to the last ml of blood in order to increase mean arterial blood pressure above 100 mm Hg.

Ion-selective Microelectrodes

Manufacture and use of K^+ -sensitive microelectrodes has been described elsewhere.³ In brief, they were made from double-barreled glass micropipettes where the tip of one barrel contained K^+ -exchanger (Corning 417377) and the reference barrel 150 mM KCl. In case of Ca^{++} -electrodes, one tip contained the Ca^{++} -exchanger¹⁶ (a generous gift from professor W. Simon, Zürich, Switzerland). pH electrodes consisted of a single glass pipette with a protruding pH-sensitive glass tip (Hincke-type electrode). The length of the protruding tip was 10–30 μm and the diameter 5 μm .

The response time of the K^+ and Ca^{++} electrode was fast ($< 1 \text{ sec}$), whereas the pH electrode response time was about 5 sec. The measurements with the pH-electrodes were either performed with an attached reference glass electrode or by using the reference electrode of the Ca^{++} electrode as common reference. The electrodes were mounted in a manifold (Narishige) and the tips approached within 50 μm . Thus, in 4 normoglycemic and 4 hyperglycemic rats, pH and Ca^{++} were measured concomitantly, whereas the measurements with K^+ electrodes were performed on other animals — 5 from each group.

The electrodes were connected via an Ag/AgCl connection to high-impedance differential amplifiers with driven shield inputs (input resistance $> 10^{14} \Omega$). The common ground, composed of a glass tube filled with 1 M KCl solidified in agar and placed in a skin flap in the head, was connected to the low impedance input. The extracellular potential, V_e , of the brain cortex was

TABLE 1 Mean arterial blood pressure, blood gases, and arterial pH in normo- and hyperglycemic rats before and after 10 minutes of complete cerebral ischemia. All values are expressed as mean \pm SD in mm Hg except pH. Asterisks denote values significantly different from normoglycemic rats. Number of animals in brackets

		Normoglycemic group (9)	Hyperglycemic group (9)
before	pH	7.38 \pm 0.07	7.36 \pm 0.07
ischemia	PCO ₂	38.7 \pm 4.1	36.0 \pm 5.2
	PO ₂	148 \pm 40	148 \pm 33
	MABP	> 100	> 100
5 min	pH	7.20 \pm 0.05	7.12 \pm 0.05*
after	PCO ₂	41.3 \pm 5.7	37.2 \pm 4.1
ischemia	PO ₂	172 \pm 29	188 \pm 42
	MABP	110 \pm 9	120 \pm 10
30 min	pH	7.27 \pm 0.08	7.14 \pm 0.05*
after	PCO ₂	43.2 \pm 7.1	40.2 \pm 3.8
ischemia	PO ₂	143 \pm 42	206 \pm 66
	MABP	120 \pm 10	140 \pm 15
60 min	pH	7.35 \pm 0.07	7.21 \pm 0.03*
after	PCO ₂	38.3 \pm 8.1	40.8 \pm 3.2
ischemia	PO ₂	156 \pm 55	203 \pm 97
	MABP	110 \pm 5	115 \pm 10

measured by the reference electrode against the common ground.

All results are expressed as means \pm SD. Statistical analyses used Student's *t*-test.

Results

The physiological variables of the rats are shown in table 1.

[K⁺]_e Changes

Figure 1 shows the change of $[\text{K}^+]_e$ in the parietal cortex of a normoglycemic and hyperglycemic rat exposed to 10 minutes of cerebral ischemia. The increase of $[\text{K}^+]_e$ following the onset of ischemia can be described by a slow rate of rise (phase I) followed by a rapid increase (phase II). The duration of phase I was twice as long in the hyperglycemic rats ($206 \pm 4 \text{ s}$) as in normoglycemic rats ($97 \pm 24 \text{ s}$), $p < 0.005$, in accordance with Hansen.⁴ Following the rapid rate of rise, a subsequent slow rise (phase III) elevated the $[\text{K}^+]_e$ to about 80 mM in both groups (table 2).

After termination of ischemia, the $[\text{K}^+]_e$ displayed a fall which in both groups took place in 2 phases: Phase A where $[\text{K}^+]_e$ fell slowly from 80 mM to approximately 50 mM during 2–3 min, and phase B where $[\text{K}^+]_e$ declined to pre-ischemic level in approximately 1 min. The duration of phase A in the normo- and hyperglycemic groups was $147 \pm 24 \text{ s}$ and 175 ± 17 , respectively. Phase B lasted $66 \pm 26 \text{ s}$ in the normoglycemic groups and $78 \pm 44 \text{ s}$ in the hyperglycemic rats.

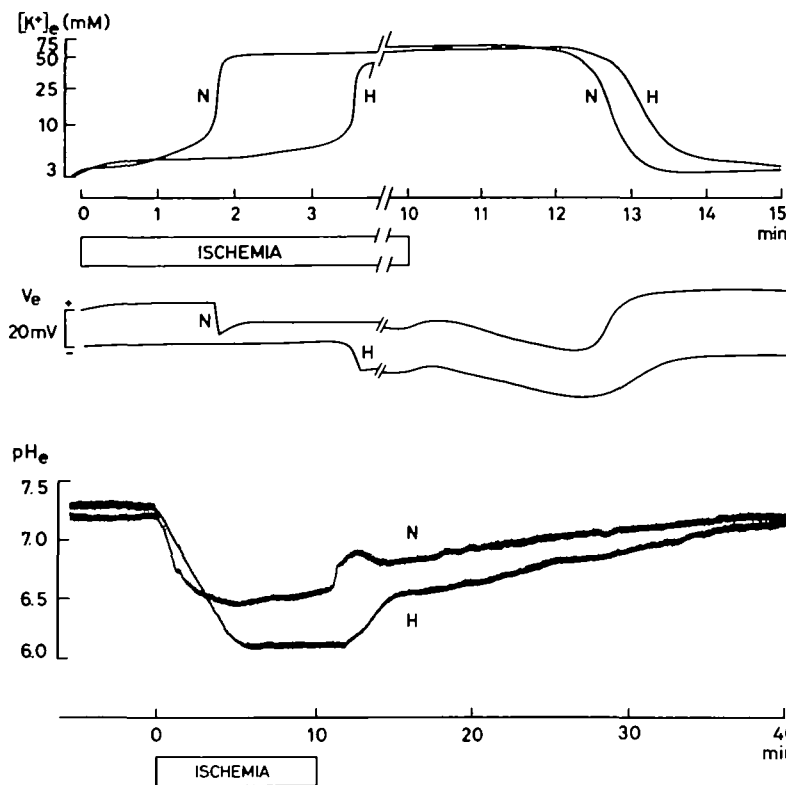


FIGURE 1. Upper panel: Extracellular potassium concentration, $[K^+]_e$, in parietal cortex (0.5 mm beneath the surface) before, during and after 10 minutes of complete cerebral ischemia in a normoglycemic rat (N) and a hyperglycemic rat (H). Lower panel: Electrical potential, V_e , in extracellular space of parietal cortex measured simultaneously with $[K^+]_e$.

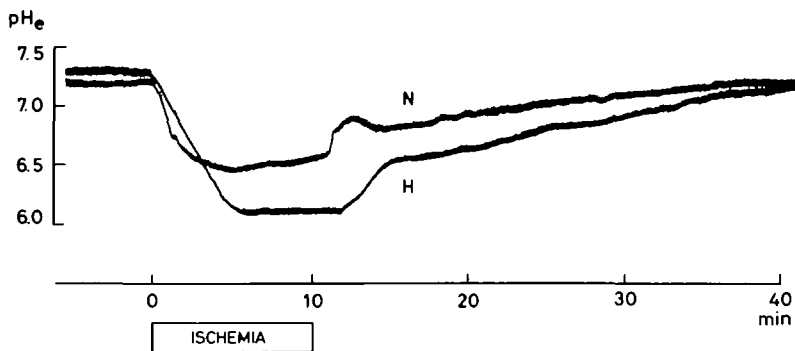


FIGURE 2. Extracellular pH in parietal cortex (0.5 mm beneath the surface) before, during and after 10 minutes of complete cerebral ischemia in a normoglycemic (N) and a hyperglycemic rat (H).

Changes of V_e . Figure 1 shows the change of the extracellular brain potential, V_e , (so-called DC-potential) during and after ischemia in the normo- and hyperglycemic rat. It is evident that the change of $[K^+]_e$ is reflected in the change of V_e . During the rapid rise of $[K^+]_e$ (phase II), there is a rapid negative deflection of V_e (anoxic depolarization¹²). V_e remains negative until termination of ischemia. Then, after a small positive and a small negative change during phase A, a large positive change is observed, coinciding with the rapid $[K^+]_e$ fall (phase B). In the following, phase A will denote the time interval after termination of ischemia until the start of the steep positive change of V_e . The time interval during the steep positive change is called phase B.

TABLE 2 Concentration of K^+ , H^+ , and Ca^{++} in the cortical extracellular space before and during 10 minutes of complete cerebral ischemia in normo- and hyperglycemic rats. All values are expressed as mean \pm SD. Asterisks denote values significantly different from normoglycemic rats. Number of animals in brackets

		Normoglycemic group	Hyperglycemic group
before	plasma (mM)	7.6 ± 2.0 (9)	$28.0 \pm 3.7^{***}$ (9)
ischemia	glucose		
	$[K^+]_e$ (mM)	2.8 ± 0.2 (5)	2.6 ± 0.8 (5)
	pH _e	7.22 ± 0.11 (4)	7.14 ± 0.13 (4)
	$[Ca^{++}]_e$ (mM)	1.2 ± 0.1 (4)	1.3 ± 0.1 (4)
during	$[K^+]_e$ (mM)	80 ± 10 (5)	74 ± 10 (5)
ischemia	pH _e	6.55 ± 0.20 (4)	$6.13 \pm 0.06^{**}$ (4)
	$[Ca^{++}]_e$ (mM)	0.13 ± 0.03 (4)	0.14 ± 0.05 (4)

pH Changes

Figure 2 shows pH_e in the parietal cortex of a normoglycemic and hyperglycemic rat during and after the ischemic challenge. In the normoglycemic group, there was an immediate fall of pH_e after the onset of ischemia. After approximately 2 min, coinciding with the start of phase II, pH_e reached a plateau value of about 6.5. Following the ischemic period, pH_e stayed unchanged during phase A — lasting approximately 2 min — and was then rapidly increased during the next 2 min. The start of this phase coincided with the start of phase B. Hereafter, a slow phase of pH_e increase followed, and, after about 20 min, the pre-ischemic level was reached.

In the hyperglycemic rats, the decline of pH_e after the onset of ischemia was parallel to that of the normoglycemic rats but lasted longer and reached a much lower value (table 2). Also, in this group the lowest pH_e value was attained at time of steep $[K^+]_e$ increase. pH_e stayed unchanged 2 min after termination of ischemia and showed, as in the normoglycemic rats, a fast increase lasting 2 min. Lastly, a slow phase of pH_e increase was seen during the 20 min (table 2), until the pre-ischemic pH_e value was reached. Thus, pH_e after 5 min in the normoglycemic was 6.84 ± 0.17 and 6.52 ± 0.1 in the hyperglycemic rats, and after 15 min was 7.07 ± 0.06 and 6.82 ± 0.08 , respectively.

$[Ca^{++}]_e$ Changes

Figure 3 shows an example of the change of $[Ca^{++}]_e$ during and after ischemia in the 2 groups. The onset of ischemia was followed by a small but significant in-

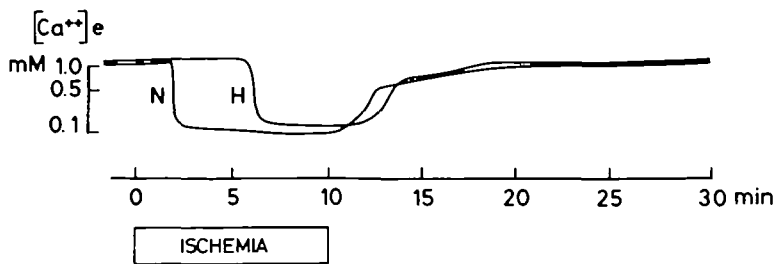


FIGURE 3. Extracellular calcium concentration, $[Ca^{++}]_e$, in parietal cortex (0.5 mm beneath the surface) before, during and after 10 minutes of complete cerebral ischemia in a normoglycemic (N) and in a hyperglycemic rat (H).

crease of $[Ca^{++}]_e$ in both groups — elevating $[Ca^{++}]_e$ by 20%. At phase II, $[Ca^{++}]_e$ fell rapidly, followed by a slower rate of decrease, to about 10% of normal level (table 2). After termination of ischemia, $[Ca^{++}]_e$ began to increase slowly during phase A. At phase B, a rapid rise doubled the concentration, followed by a slower rate of rise, raising $[Ca^{++}]_e$ to pre-ischemic levels in 10 min (table 3).

EEG Changes

No gross differences were detected among the 2 groups during the control period (fig. 4). After start of the ischemia, the EEG became isoelectric within 10–20 seconds in both groups. The EEG was isoelectric following termination of ischemia during a significantly shorter period in the normal glucose group (15 ± 2 min) than in the high glucose rats (33 ± 10 min). The first sign of EEG activity consisted of a spike activity separated by isoelectrical periods. In both groups, the isoelectric periods became shorter with time. After one hour, the EEG activity of the hyperglycemic group still showed a burst suppression

TABLE 3 Time to reach pre-ischemic concentrations of extracellular $[K^+]_e$, $[Ca^{++}]_e$ and pH_e in brain cortex of normo- and hyperglycemic rat after 10 minutes of complete cerebral ischemia. Number of animals in brackets

	Recovery time (min)	
	normoglycemic group	hyperglycemic group
$[K^+]_e$	3.6 ± 0.52 (5)	4.1 ± 0.6 (5)
pH_e	24.2 ± 3.5 (4)	29.2 ± 6.3 (4)
$[Ca^{++}]_e$	13.3 ± 2.6 (4)	13.2 ± 3.4 (4)

pattern while in the normoglycemic group the EEG activity was significantly improved and resembled the pattern of control EEG activity (fig. 4).

Discussion

This study has shown that the ability to normalize extracellular ion concentrations in the brain after a period of ischemia is unaffected by hyperglycemia, in contrast to the recovery of EEG activity.

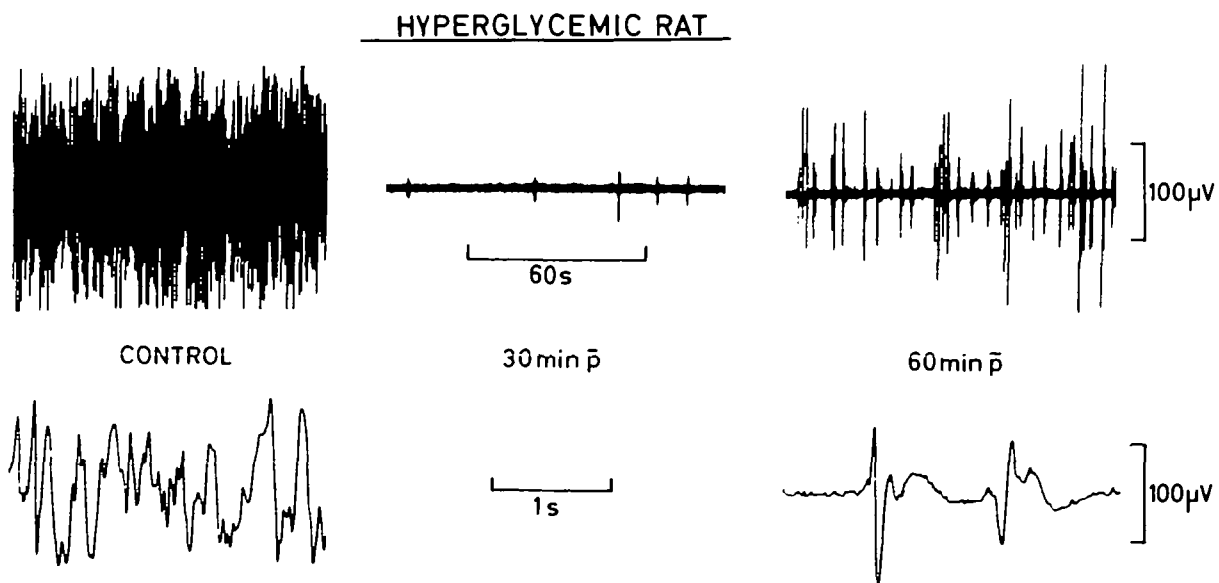


FIGURE 4. EEG activity before and after 10 minutes of complete cerebral ischemia in a normoglycemic and a hyperglycemic rat. To the left is shown EEG activity during control conditions. In the middle is shown the first appearance of electrical activity postischemia (\bar{p}) and to the right 60 min postischemia. Note earlier appearance of EEG activity in the normoglycemic rat and better recovery after 60 min.

Changes During Ischemia

The fact that the glucose content of the brain was increased in hyperglycemic rats¹³ provided an increased capability for anaerobic regeneration of ATP during ischemia,^{5, 13} keeping $[K^+]_o$ and $[Ca^{++}]_o$ near normal levels for a longer period than in the normoglycemic rats. $[H^+]_o$, however, began to increase at the start of ischemia in both groups. The rates of increase were similar in the two groups, but in the glucose-treated rats $[H^+]_o$ continued to increase for a longer time and reached a significantly higher level. In both groups, the increase stopped at the time of anoxic depolarization (steep $[K^+]_o$ increase), suggesting termination of anaerobic glycolysis and indicating that the rapid change of $[K^+]_o$ and $[Ca^{++}]_o$ was initiated by ion pump failure. The $[H^+]_o$ level was constant hereafter for the rest of the ischemic period, showing that the ischemic insult was indeed complete.¹⁵ Other changes of the extracellular ion composition during ischemia involve a decrease of $[Na^+]_o$ and $[Cl^-]_o$.^{5, 10}

Changes after Ischemia

The normalization of $[K^+]_o$, $[Ca^{++}]_o$, and $[H^+]_o$ after ischemia depends mainly upon cellular mechanisms and not upon clearance via the blood stream, as capillary permeability to ions is very low,⁷ and as ischemia does not increase the permeability of the blood-brain barrier.^{11, 17} Thus, the fall of $[K^+]_o$ is due to inward pumping activity of the membrane-bound Na-K-ATPase and the $[Ca^{++}]_o$ increase depends on an ATP-dependent Ca^{++} extrusion, whereas the fall of $[H^+]_o$ depends on the rate of combustion of accumulated lactate.¹³

We have previously shown, using the same experimental set-up, that the rapid decline of $[K^+]_o$ after ischemia is preceded by a cortical flow increase, suggesting that the fall of $[K^+]_o$ is due to renewed substrate delivery and subsequent cellular pumping.⁶ The Na⁺-K⁺ activated ATPase must be strongly activated during ischemia, since $[K^+]_o$ is high and the intracellular $[Na^+]_i$ is high.⁵ When sufficient ATP has been regenerated after the termination of ischemia, the K⁺ lost to the extracellular space is pumped into the cells and the Na⁺ out of the cells. The extracellular space (ECS), which is halved during ischemia,⁹ will approach the pre-ischemic level during the period of $[K^+]_o$ normalization. The rapid expansion of ECS could explain a part of the fall of $[H^+]_o$ during this period but not as a matter of course the increase of $[Ca^{++}]_o$. The most likely explanation for this is that the regenerated ATP also fuels an outward-directed Ca^{++} pump. It is not clear why $[Ca^{++}]_o$ only reached 30% of the pre-ischemic level at the end of phase B while $[K^+]_o$ at the same time is normalized. It may be that the resumption of mitochondrial function enables the mitochondria to accumulate Ca^{++} , and an outward-directed Ca^{++} pump could then be retarded by the ensuing low intracellular $[Ca^{++}]_i$. The prolonged duration for the normalization of $[H^+]_o$ in both groups implies a limited capacity for oxydation of the accumulated lactate. The observation that both

groups reached pre-ischemic levels within the same time, and that the hyperglycemic rats had a higher ischemic value of $[H^+]_o$, implies an increased rate of lactate removal, most likely by way of oxidation since blood-brain barrier transport is insignificant.^{2, 10}

It is a common statement that the low pH encountered during cerebral ischemia is the reason for cellular damage. It has been shown that ischemia in hyperglycemic rats does not affect the recovery of the energy metabolite level in brain,¹³ and we can now add that the capability of normalizing extracellular ion concentrations after ischemia is not affected by hyperglycemia, i.e., low pH in brain during ischemia; but the recovery of EEG activity was clearly inferior in this group of rats. The mechanism behind it remains to be identified.

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