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CEREBRAL EXTRACELLULAR POTASSIUM CONCENTRATION CHANGE AND CEREBRAL IMPEDANCE CHANGE IN SHORT-TERM ISCHEMIA IN GERBIL

BY

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ABSTRACT

Extracellular potassium and calcium ion concentration, field potential (Fp), local EEG and cortical impedance were measured continuously in the cortex and hippocampus of the pentobarbital anesthetized gerbils subjected to 5-minute ischemia produced by bilateral carotid occlusion. Extracellular potassium ($[K^+]_e$) and extracellular calcium ($[Ca^{++}]_e$) showed a triphasic change during ischemia followed by a biphasic change after recirculation. The EEG became flat within about 20 seconds. There was a sharp increase in the $[K^+]_e$ and a sharp decrease in the $[Ca^{++}]_e$, which coincided with the sharp increase in the Fp around one and a half minutes. The impedance increased simultaneously with a large amplitude change of EEG. The $[Ca^{++}]_e$ continued to decrease for another 1–2 minutes before returning to the normal values after recirculation whereas the $[K^+]_e$ began to decrease immediately. The extracellular space (ECS) did not change until depolarization. The rise of the $[K^+]_e$ and $[Ca^{++}]_e$ in the pre-depolarization phase was not explained by the shrinkage of the ECS. The possible explanation for the change of the $[Ca^{++}]_e$ in ischemia was discussed.

Key words: Cerebral ischemia, extracellular ion concentration, cerebral impedance

INTRODUCTION

The maintenance of the ionic gradient between the inside of the cell and the extracellular fluid is essential for all mammalian cells. A recent study [1] has indicated that the normal mammalian cell membrane leakage of the Na-K ions and their replacement by the active transport consumes 40–60% of the total energy expenditure in the brain. Using the brain slice preparation, it is estimated that 25–40% of the metabolic rate was dependent on the cation flux [2]. Under physiological conditions, most results indicate that an outward movement of 3 Na^+ and an inward movement of 2 K^+ for each molecule of ATP consumed. Assuming a clearance rate of 1 mM/sec of K^+, the potassium clearance would require 15–30% of the cortical ATP utilization [2].

In cerebral ischemia, the blood cannot convey oxygen and glucose which are the main energy sources of the brain. Consequently, ATP generation is limited to the metabolism of the endogenous substrates under an anaerobic condition. Although the anaerobic glycolytic rate can increase, the brain can supply no more than 30% of its normal energy requirements [3]. Therefore, the deple-

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tion of ATP is presumed to lead to the dysfunction of the Na^+-K^+ ATPase [3]. Disturbance of the membrane permeability is the membrane permeability is the first step toward cell derangement in cerebral ischemia.

Recently, calcium has been identified as the trigger to the cellular damage [4, 5], in addition to its important role under physiological conditions. Following the development of ion-selective electrodes [6], the extracellular ion changes in ischemia have been investigated and elucidated, along with the other physiological phenomena. A massive efflux of potassium [7–12] and influx of calcium [3, 13–16] in cerebral ischemia have been reported.

The author developed the triple-barrel electrodes by which two ion activities could be measured at the same point simultaneously and continuously. The electrical impedance of the brain tissue is considered as an index of the volume of the extracellular space. In this study, we tried to elucidate the change of the extracellular calcium concentration ($[Ca^{++}]_e$) during and after 5-minutes cerebral ischemia in the gerbils in relation to the change of the extracellular potassium concentration ($[K^+]_e$). The relationship between the change of the extacellular space and the ionic change in ischemia is also to be clarified.

Materials and Methods

I. Materials

Adult Mongolian gerbils (60–80 g) were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). Through a midline skin incision, both carotid arteries were exposed and freed carefully from the surrounding structure and a 4–0 silk thread was looped around each artery. The body temperature was measured rectally and kept at 37°C by a heated blanket. The head was fixed in a stereotaxic instrument. A small bony opening was made by a microsurgical drill at 2.0–2.5 mm posterior and 1.8–2.0 mm lateral to the bregma. The dura overlying the cortex was pierced with a 30G hypodermic needle without damaging the underlying pia and the cortex to avoid the damage to the tips of the electrodes.

II. Microelectrodes

The ion-selective electrodes used in this study are shown schematically in Fig. 1. Three glass tubes, glued together, were pulled in a microelectrode puller (Micropipette puller MI, Industrial Science Associates, Inc.) in the conventional way. The tip was then broken back to make the total tip diameter approximately 10–20 μm. The two ion-selective barrels were siliconized with 15% hexamethyldisilane (Fisher), while air was continuously blown through the reference barrel to keep it clear. The electrode was then baked in an oven for 30 minutes to one hour at 220°C. The ion-selective resins used were Corning 477317 for potassium and WPI IE-202 for calcium. The reference barrel was filled with 154 mM NaCl. The electrodes were connected via the Ag-AgCl

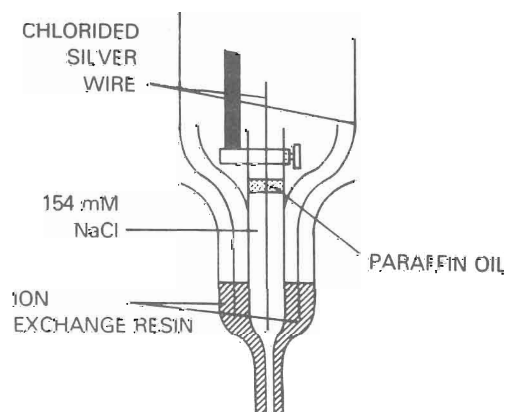

Fig. 1. Triple-barrel electrode. Outer two barrels are filled with ion-exchange resins. The center barrel is filled with 154 mM NaCl.

leads to the differential amplifiers which were mounted on a micromanipulator arm just above the electrodes. The amplified output was displayed on a multichannel recorder (Gould Recorder 2800). The electrodes and the head stage preamplifier were lowered by the hydraulic microdrive (David Kopf Instrument). There was no crosstalk between the signals from the ion-selective barrels. Calibration of the electrodes was performed before and after each experiment. The calibration solutions were 3, 5, 10, 50 and 100 mM KCl and 0.02, 0.1, 0.2, 1 and 2 mM $CaCl_2$ made up in 154 mM, respectively. The animals were grounded through the ear bars. From the reference electrode, the field potential (Fp) (DC potential) and EEG were recorded also on a multichannel recorder.

III. Impedance Measurement

To measure the cortical impedance in the present study, we used a four-electrode array patterned on that described originally by Ranck [17].

The platinum-iridium alloy electrodes were insulated with Epoxy adhesive except for the 30–50 μm of the tip of the electrode which was kept bare and platinized. The electrodes were spaced 250 μm apart so that the entire array width was less than one mm. We used monophasic or biphasic square waves of 1 or 5 μA and a duration of 1 ms. The frequency of current application used was 30 per minute. The voltage difference between inner electrodes was displayed on the oscilloscope for inspection and was also electronically stretched so that it could be displayed on a multichannel chart recorder. From the recording electrodes, EEG was monitored simultaneously.

IV. Ischemic Procedures

The author set the tip of the ion-selective electrodes at 0.7 mm below the surface when we measured the ion activity in the cerebral cortex and at 1.35 mm when we measured it in the hippocampus. The bare tips of the impedance electrodes were placed at the depth of 0.7 mm in the cortex in a different series of gerbils.

After inserting the electrodes, paraffin oil was applied to the surface of the brain to prevent drying of the exposed brain. The animal was observed until the values ($[K^+]_e$, $[Ca^{++}]_e$, or the impedance) were stabilized for 20 or 30 minutes. Then the sutures around the two common carotid arteries were pulled by 10-gram weight to occlude the circulation. Five minutes later the suture was cut and removed to restore the circulation.

RESULTS

I. Ionic Changes

Fig. 2 shows the course of the ionic changes in the cortex and hippocampus in the gerbils exposed to five minutes of cerebral ischemia. The course of the ionic changes was almost the same as in the cortex and hippocampus. When the circulation was interrupted, the local EEG became flat within about 20 seconds while the $[K^+]_e$ increased slowly from 4.2 mM to 9.0 mM (Table 1). During this period the $[Ca^{++}]_e$ showed a small increase from 1.28 mM to 1.47 mM. The Fp showed a very slight negative deflection (pre-depolarization phase) (Fig. 3). Later the Fp showed a large negative deflection (anoxic depolarizationn) (18) at the same time when the $[K^+]_e$ began a steep increase and the $[Ca^{++}]_e$ showed a decrease. The $[K^+]_e$ increased to 54 mM and the $[Ca^{++}]_e$ decreased to 0.88 mM in the cortex. Finally the $[K^+]_e$ rose gradually and the $[Ca^{++}]_e$ also decreased somewhat further until the occlusion was terminated (post-depolarization phase). When the animals were recirculated, the

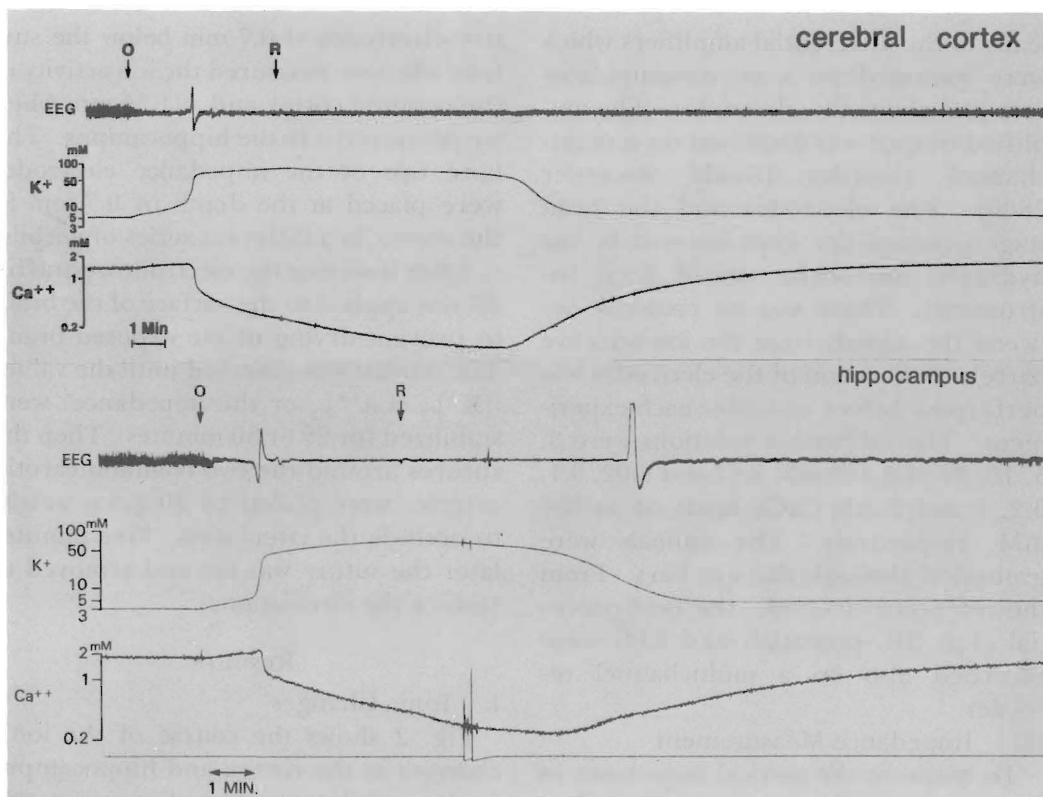

Fig. 2. EEG, $[K^+]_e$ and $[Ca^{++}]_e$ change in the cortex and hippocampus in the gerbils during and following the 5-minute ischemia.

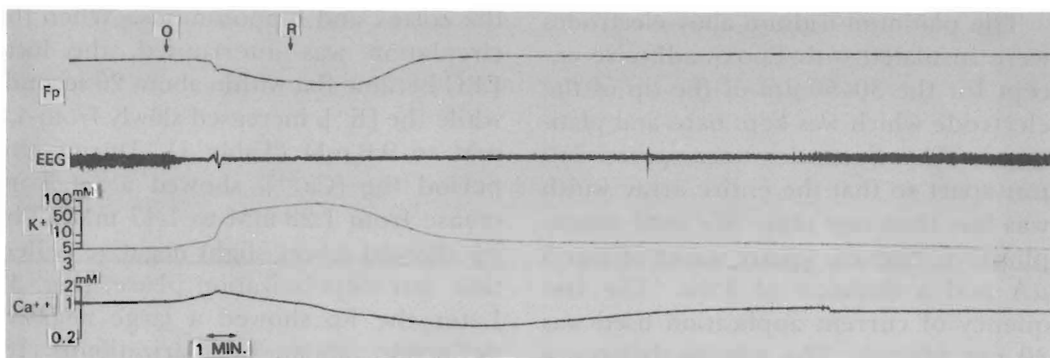

Fig. 3. Fp, EEG, $[K^+]_e$ and $[Ca^{++}]_e$ are recorded in the same experiment.

$[K^+]_e$ started to decrease slowly but the $[Ca^{++}]_e$ steadily decreased and the Fp continued to go down (pre-repolarization phase). Then the $[K^+]_e$ decreased rather rapidly whereas the $[Ca^{++}]_e$ started to increase when the Fp went up to the base line (repolarization phase). The $[K^+]_e$ returned to the base line 9.7 minutes after recirculation in the cortex whereas the $[Ca^{++}]_e$ was normalized in 23.4 minutes. The EEG showed some activity after the $[K^+]_e$ was normalized in some cases.

Table 1. The values of $[K^+]_e$ and $[Ca^{++}]_e$ in the cortex and hippocampus in the gerbils exposed to the 5-minutes ischemic insult. Values are mean±SEM. It also shows how long it takes for the cells to depolarize and for $[K^+]_e$ and $[Ca^{++}]_e$ to be normalized

(mM)	Baseline	At start of depolarization phase	At end of depolarization phase	At 5 minutes	At start of repolarization phase	At end of repolarization phase
CORTEX N=5						
$[K^+]_e$	4.2 ±0.6	9.0 ±1.6	54.3 ±5.3	75.3 ±5.2	49.6 ±5.8	4.2 ±0.5
$[Ca^{++}]_e$	1.28±0.11	1.47±0.12	0.88±0.09	0.42±0.11	0.32±0.07	1.28±0.14
HIPPOCAMPUS N=5						
$[K^+]_e$	3.4 ±0.2	5.7 ±0.2	41.2 ±7.1	78.8 ±0.11	44.4 ±5.7	3.8 ±0.4
$[Ca^{++}]_e$	1.37 4?0.15	1.69±0.16	1.26±0.24	0.70±0.11	0.40±0.07	1.36±0.17

(sec)	Anoxic depolarization	Normalization of $[K^+]_e$ after recirculation	Normalization of $[Ca^{++}]_e$ after recirculation
CORTEX	109±18	580±53	1403±185
HIPPOCAMPUS	95±14	498±20	1196±164

Fig. 4. EEG and impedance change in the cortex during and following the 5-minute cerebral ischemia.

II. Impedance Change

In these experiments, the author recorded the impedance and the local depth EEG via the sensing electrodes inthe cortex. In the EEG, depolarization could usually be detected but repolarization, a slower process, could not always be detected (Fig. 4). When the common carotid arteries were occluded, the impedance did not change even afer the EEG disappeared. The impedance went up to 182% rapidly around 1.2 minutes, then remained high. After the recirculation the impedance did not change for a while, then decreased to the previous level. The duration of normalization was 6.7 minutes (Table 2).

The extracellular space (ECS) was calculated by the Maxwell equation, using the impedance values.

$$\frac{\rho}{2} = \frac{1-\gamma_1/\gamma}{2+\gamma_1/\gamma}$$

where ρ equals the volume fraction occupied by the cells; γ_1 and γ equal the specific impedance of the extracellular fluid in the brain tissue and of the brain tissue, respectively. By definition, $1-\rho$ is the volume fraction of the extracellular compartment [18, 19]. Therefore, $1-\rho$ stands for ECS. According to Fenstermacher [18], the impedance of the cerebrospinal fluid at 37°C is 55 ohm cm. We used this value for the calculation of the ECS.

The ECS in the control phase was 24.2%. By five-minute ischemia, the ECS decreased to 14.0%, then returned

Table 2. The impedance values and calculated extracellular space (ECS). It also shows when anoxic depolarization happens after occlusion and normalization (repolarization) after recirculation

(N=5)	baseline	at 5-minutes
Impedance	100%	182±9%
ECS	24.2±1.4%	14.0±0.6%
Time		
anoxic depolarization	70± 4 sec	
repolarization	403±31 sec	

to the previous value after normalization. After the five-minute ischemia, the ECS decreased to 58% of the control phase.

Discussion

Gerbils have been used to study the cerebral ischemia for nearly 20 years. Crockard [20] reported that the blood flow was less than 4 m*l*/100 g/min in bilateral common carotid occlusion of the gerbil. There have been some reports about the threshold value of the blood flow before the noticeable electrophysiological changes occur. EEG becomes flat when the blood flow falls to 16–20 m*l*/100 g/min [21] or becomes below 15 m*l*/100 g/min [22].

Evoked potentials begin to decrease at the flow values below 20 m*l*/100 g/min and are completely suppressed below 15 m*l*/100 g/min [23]. Extracellular potassium changes are expected when the blood flow falls below 8–11 m*l*/100 g/min [23, 24]. In the bilateral carotid occlusion in the gerbils, we could see the change of the extracellular potassium regularly. In this study, it can be said that the insult was sufficient to initiate the extracellular potassium change. In the pre-depolarization phase, as the $[K^+]_e$ rose almost doublefold, the $[Ca^{++}]_e$ increased by 14% ($p<0.05$) in the cortex. Similar results had been observed by Hansen [3] and Harris [14]. But they did not pay attention to the small increase of $[Ca^{++}]_e$ just before anoxic depolarization. Harris [14] supposed that there is a decrease in the size of the extracellular space with a relative increase of the extracellular calcium. But according to the impedance data obtained by the author and his group, the extracellular space did not change in the pre-depolarization phase. In the middle cerebral artery occlusion in the cats, the impedance increased at the same time when the $[K^+]_e$ rose steeply (unpublished data). Putting together the data mentioned above, we conclude that the $[Ca^{++}]_e$ increased a little but in a significant amount, not due to the shrinkage of the extracellular space but due to other reasons yet unknown and to be determined.

In the depolarization phase, the $[K^+]_e$ increased rapidly and also the $[Ca^{++}]_e$ decreased, whereas the Fp showed a negative deflection. In this phase, the membrane permeability was disrupted. It is said that the disrupted membrane permeability causes a redistribution of the ions to equalize the ionic concentration gradient across the membranes of the cellular elements [11, 14]. In this phase, the cells start to swell and the extracellular space decreases. In the post-depolarization phase there is a slow increase of $[K^+]_e$ and a slow decrease of $[Ca^{++}]_e$. After the disruption of the membrane permeability, the ions move toward equilibrium. In the pre-repolarization phase after recirculation, the $[K^+]_e$ started to decrease sometimes after a short lag. In contrast to the $[K^+]_e$, the $[Ca^{++}]_e$ still decreased until repolarization. The possible explanation is the following: When the brain is recirculated, glucose and oxygen are supplied. ATP is regenerated immediately and supplied for the restoration of the membrane function. The

intracellular concentration of calcium ($[Ca^{++}]_i$) may be regulated by several processes: 1) Na^+-Ca^{++} exchange at the plasma membrane, 2) calmodulin-regulated $(Mg^{++}+Ca^{++})$-ATPase at the plasma membrane, 3) active transport into the mitochondria, 4) $Ca^{++}-Na^+$ exchange by the mitochondria and 5) ATP-dependent binding by the endoplasmic reticulum [2]. Among them, the modulation of the mitochondrial calcium transport by Na^+ could have a physiological significance [2]. When recirculated, the $[Ca^{++}]_i$ is very high and so every process can work to reduce the $[Ca^{++}]_i$. But as the energy for Ca^{++} extrusion may be provided by the inward movement of Na^+ by the Na^+-Ca^{++} exchange mechanism, the $[Ca^{++}]_i$ cannot be extruded, because in this phase the massive influx of K^+ and the massive efflux of Na^+ occur. When ATP is provided, in order to reduce the $[Ca^{++}]_i$, mitocondria utilize most of the energy derived from the electron transport to the sequester Ca^{++} rather than to the phosphorylate ADP [25]. And also the energy-dependent binding by the endoplasmic reticulum is more sensitive than the mitochondria and so this system may work to sequester Ca^{++} in order to reduce the $[Ca^{++}]_i$. Once the membrane is repolarized, the Na^+-Ca^{++} exchange at the membrane is expected to extrude the Ca^{++} and the Ca^{++} once sequestered in the mitochondria and endoplasmic reticulum moves reversely. So gradually the $[Ca^{++}]_e$ recovers to the normal level.

Recently, calcium has been identified as the trigger to cause a cellular damage [4, 5, 14, 26]. Increased intracellular calcium concentration would stimulate the turnover of the phosphatidylinositol cycle, with serious consequence on the neurotransmitter metabolism during reperfusion and also stimulate the phospholipase activation with the release of free-fatty acids [4, 14]. Pathologically, sequestration of the calcium in the mitochondria has been reported [26, 27]. Sequestered calcium is supposed to deteriorate the mitochondrial function.

In this study the calcium change after recirculation is dissociated from the potassium recovery. There are supposed to be different mechanism to maintain the ionic homeostasis for K^+ and Ca^{++}, which also react differently to the ischemic insults. The change of $[Ca^{++}]_e$ after recirculation can suggest the turmoil of the intracellular calcium ion homeostasis which can damage the structure instead of $[K^+]_e$ normalization.

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