Cation Activities in Reversible Ischemia of the Cat Brain

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SUMMARY In normothermic anesthetized cats cerebral blood flow was interrupted completely for one hour by arterial clamping and induced hypotension. The effect of ischemia on the ionic gradients of the cerebral cortex was assayed by determining total cortical electrolytes and by recording the activities of extracellular potassium ([K⁺]) and subarachnoid sodium ions ([Na⁺]) with ion-sensitive electrodes. During ischemia [K⁺], increased from 3.3 ± 0.3 to 56 ± 5.4 mEq per liter (means ±SE) and [Na⁺], decreased from 133 ± 3.8 to 53 ± 5.8 mEq per liter. When the brains were recirculated with blood after one hour’s ischemia, [K⁺], and [Na⁺], gradually returned to normal within 45 minutes. The calculated intracellular uptake of sodium during ischemia amounted to 139 mEq per kilogram dry weight, whereas the intracellular release of potassium was only 64 mEq per kilogram. The increase in intracellular cation was accompanied by a movement of water from the extracellular into the intracellular compartment, causing a reversible shrinkage of the extracellular space from 18.9 to 8.5 vol %.

The changes in ionic gradients were related to the development and resolution of ischemic brain swelling, and to the electrophysiological events during and after ischemia.

Methods

Thirty cats of both sexes, weighing 2.5 to 3.5 kg, were used. The animals were anesthetized with a single injection of pentobarbital (30 mg per kilogram of Nembutal® intraperitoneally), immobilized with gallamine triethiodide (Flaxedil®) and mechanically ventilated with room air. The ventilation was adjusted to yield a Paco₂ of 28 to 32 mm Hg, and a Pao₂ of more than 100 mm Hg. Body temperature was kept constant at 36.8°C.

Cerebral ischemia of one hour’s duration was produced by intrathoracic clamping of the innominate, subclavian and internal mammary arteries, combined with induced hypotension in order to prevent collateral blood flow to the brain. Completeness of ischemia was ascertained by microscopic observation of the pial vasculature and by the absence of ¹³³Xe clearance, which was injected into the brain at the beginning of ischemia. One hour after the onset of ischemia and immediately before removal of the vessel clamps, blood pressure was raised with norfenefrin (Novadral®) to above 180 mm Hg resulting in recirculation of the brain. Changes in the acid-base status of the blood were rapidly corrected by adjusting the respiration and by controlled infusion of buffers.

The functional impact of ischemia and recovery upon recirculation was monitored by electrophysiological recording of the electrocorticogram and of various evoked responses, as has been described before. Serum electrolytes were measured by flame photometry in blood samples drawn from the femoral artery before and at the end of ischemia, and after 30 and 60 minutes of recirculation.

Extracellular ion activity of the brain was recorded continuously in the following way. Potassium activity ([K⁺]ₑ) was measured with double-barrelled microelectrodes, with a tip diameter of about 2 μ, inserted 1-mm deep into the sensorimotor cortex. The electrodes were prepared according to the methods described by Lux and Neher. The potassium-
sensitive barrel was filled with Corning Potassium Exchanger Resin No. 477317, and the reference barrel with 0.9% NaCl. The potential difference between the two barrels was amplified with a high impedance differential DC amplifier.

The activity of sodium ions was measured in the subarachnoid fluid with a combination macroelectrode (Orion, Model 96-11) which was brought into contact with the surface of the suprasylvian gyrus. The electrode potentials were recorded with a high impedance electrometer (Orion, Model 701). Interference with cortical DC potentials was prevented by grounding the animal via the reference element of the combination electrode.

Methods for the measurement of the extracellular fluid volume (vol_e) and the cortical content of water (H_2O_Cx) and electrolytes (K_Cx and Na_Cx) have been reported previously. The intracellular portion of cortical electrolytes was calculated in the following manner: (1) Extracellular water (H_2O_e) was derived from total H_2O_Cx and vol_e by:

\[ H_2O_e (l/kg d.w.) = \frac{H_2O_Cx (l/kg d.w.) \times vol_e(\%)}{H_2O_e (ml/100 gm w.w.)} \]

(2) Extracellular electrolyte content (E_{el}) was expressed as a product of extracellular electrolyte activity ([E_{el}]) and H_2O_e:

\[ E_{el}(mEq/kg d.w.) = [E_{el}] (mEq/l) \times H_2O_e (l/kg d.w.) \]

(3) Intracellular electrolyte (E_{il}) was calculated by subtracting the extracellular from the total cortical content:

\[ E_{il}(mEq/kg d.w.) = E_{il} (mEq/kg d.w.) - E_{el} (mEq/kg d.w.) \]

**Results**

**Electrophysiological Observations**

A detailed analysis of the electrophysiological events has been given before. In short, interruption of the cerebral blood flow caused an almost immediate suppression of the electrocorticogram, followed by suppression of synaptic and electrical excitability of the neurons within six minutes. When the brains were recirculated with blood after one hour's ischemia, electrical excitability began to recover after 8 to 15 minutes, synaptic excitability after 30 to 60 minutes, and low-frequency spontaneous electrocortical activity after one to two hours (fig. 1). In a few animals electrophysiological recovery was absent because posts ischemic blood recirculation could not be fully restored after ischemia. The results obtained in these animals were excluded from the main experimental group and will be described separately below.

**Extracellular Potassium Activity**

Successful recording of extracellular potassium activity ([K^+]_e) was obtained in eight experiments. Before ischemia, [K^+]_e was 3.3 ± 0.3 mEq per liter (means ± SE, eight animals). This value was identical with that of serum (3.3 ± 0.11 mEq per liter). When blood flow to the brain was interrupted, after a free interval of about one minute, [K^+]_e initially began to rise at a fast rate and later at a slow rate until it reached a mean value of 56 ± 5.4 mEq per liter after 60 minutes of ischemia (fig. 1). In nine animals water and total electrolyte content of the cortex were measured using tissue extraction techniques. During ischemia the cortical content of potassium decreased from 498.9 ± 12.5 to 458.7 ± 23.9 mEq per kilogram of dry weight, and cortical water increased from 4.291 ± 0.02 to 4.682 ± 0.013 liters per kilogram of dry weight. Extracellular space, during the same time, shrunk from 18.9 to 8.5 vol % (see below). From these data and [K^+]_e, intracellular potassium content could be evaluated. According to this calculation the average intracellular potassium decreased from 495 to 431 mEq per kilogram of dry weight during 60 minutes of ischemia. When the brains were recirculated with blood after ischemia, [K^+]_e, rapidly decreased (fig. 2). After 10 minutes of recirculation, [K^+]_e, returned to 8.5 ± 1.1 mEq per liter and after 40 minutes it was no longer significantly different from control levels. The water content of the cortex after

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**FIGURE 1.** Recording of the electrocorticogram (ECOG), extracellular potassium activity ([K^+]_e), systemic arterial blood pressure (SAP), and the pyramidal response (PR) during and after one hour's complete ischemia of the cat brain. The PR was evoked by electrical stimulation of the motor cortex at times which are indicated by A to F in the polygraph. The onset of ischemia is indicated by an upward arrow, the beginning of recirculation by a downward arrow.
one hour’s recirculation was 4.555 ± 0.34 liters per kilogram of dry weight, potassium content was 512 ± 37 mEq per kilogram of dry weight, and extracellular space 17.5 vol %. Average intracellular potassium thus amounted to 508 mEq per kilogram of dry weight, which demonstrates that the ischemic release of intracellular potassium was fully reversible.

The potassium concentration of the serum increased during ischemia from 3.3 ± 0.11 to 4.2 ± 0.36 mEq per liter, but returned to or below normal within 30 minutes after recirculation. The serum changes were minor in comparison to those of the brain, and could not be related to brain ischemia because the animals received infusions of different solutions for stabilization of blood pressure and equilibration of acid-base balance.

Extracellular potassium activity correlated well with electrical excitability of neurons (fig. 1). Electrical stimulation of the motor cortex evoked a response in the pyramidal tract which disappeared during ischemia when $[K^+]_e$ increased to about 16 mEq per liter, and the response reappeared upon recirculation when $[K^+]_e$ decreased below this level. The electrocorticogram, on the other hand, became isoelectric before potassium had significantly changed and began to recover long after potassium had returned to the control level.

Subarachnoid Sodium Activity

Subarachnoid sodium activity ($[Na^+]_s$) prior to ischemia was 133 ± 3.8 mEq per liter (means ± SE, 14 animals). This value was distinctly lower than the sodium concentration of the blood serum which was 153 ± 1.5 mEq per liter. During ischemia $[Na^+]_s$, shifted in the opposite direction of extracellular potassium activity and decreased to 53 ± 5.8 mEq per liter after 60 minutes (fig. 3). The time course of this shift was similar to that of potassium with the only difference being that the latency was about two minutes and the initial slope was less steep. Recirculation of the brain after one hour’s ischemia caused a rapid increase in $[Na^+]_s$, control levels being reached after 40 to 60 minutes.

Sodium content of cerebral cortex prior to ischemia was 240.7 ± 29.3 mEq per kilogram of dry weight. Sodium increased during ischemia to 272.2 ± 14.32 mEq per kilogram and returned to 251.6 ± 29.7 mEq per kilogram after one hour’s recirculation. The calculated intracellular sodium content consequently increased by 139 mEq, from 108 to 247 mEq per kilogram of dry weight during ischemia, and returned to 123 mEq per kilogram of dry weight after one hour’s recirculation.

The sodium concentration of the serum decreased during and after ischemia from 153 to about 140 mEq per liter. Because of low blood sodium, the concentration gradient between extracellular fluid and blood returned to normal after 20 minutes, i.e., before extracellular sodium activity had reached its control level.

Extracellular space

In an earlier investigation changes in the volume of the extracellular space during 30 to 90 minutes of reversible ischemia were calculated from cerebral impedance using the Maxwell equation. In one animal in the present series of experiments such a measurement was repeated for determining extracellular volume changes during ischemia of one hour’s duration (fig. 4). The results obtained were consistent with the earlier investigation. During ischemia extracellular space shrank from 18.9 to 8.5 vol % and expanded to 17.5 vol % within one hour of recirculation. The time course of variations in extracellular volume was similar to that of extracellular cation changes. The latency for the beginning shrinkage was two minutes, and a plateau was reached after about 30 minutes. This finding supports the earlier conclusion that changes in extracellular volume are closely related to electrolyte shifts during ischemia. ¹ ² ³ ⁴ ⁵ ⁶ ⁷ ⁸
Animals Without Electrophysiological Recovery

In five of 30 animals, electrophysiological recovery was absent. The reason for the failure of recovery was inadequate reoxygenization, which has been described in detail previously. Ion shifts during ischemia were similar to those of the main experimental group, but different and unforeseeable results were obtained upon recirculation. In some animals ion activities remained close to the ischemic values, in others a partial equilibration with serum occurred, but in none did ion activity return to normal. This indicates that in contrast to the animals with recovery of electrophysiological function, ion homeostasis was not reestablished after ischemia.

Discussion

Measurements of electrolyte concentration in the living tissue with ion-sensitive electrodes are limited in accuracy because of the low sensitivity of electrodes at high concentrations, the possible interference with steady potentials and other ions, and the fact that only dissociated ions (ion activities) are measured. Interference with steady potentials are almost canceled out by using combination electrodes but the other problems are inherent to this technique. On the other hand, the advantage of ion-sensitive electrodes is the unique opportunity to measure ion activities continuously in tissue fluids which before now could not be adequately sampled for chemical analysis.

Using this technique an extracellular potassium activity ([K+]e) of 3.3 mEq per liter was measured in the somatomotor cortex of cat prior to ischemia. This value is consistent with earlier recordings and differs only slightly from total potassium concentration of cisternal or subarachnoid cerebrospinal fluid which has been assessed by flame photometry. Subarachnoid sodium activity ([Na+]e), on the other hand, was only 133 ± 3.8 mEq per liter. This is about 20 mEq per liter less than the sodium concentration in cisterna magna fluid.

This difference may be due to a relatively low concentration of active sodium ions, but a systematic error due to the above-mentioned technical properties of the electrode cannot be excluded.

During ischemia a considerable shift in the cation activities of the extracellular fluid occurred. The increase of [K+]e from 3.3 to 56 mEq per liter was in the same direction but not of the same magnitude as previously described in hypoxia and cardiac arrest. During cardiac arrest, [K+]e increased to about 100 mEq per liter within 10 minutes. This is almost twice the level which we have observed in our model of cerebral ischemia. In hypoxia, on the other hand, the maximum increase in [K+]e varied only between 5 mEq per liter and 40 mEq per liter. The lower [K+]e during hypoxia can be easily explained by the shorter duration of oxygen deprivation and the sink action of the circulating blood. The much higher [K+]e during cardiac arrest, however, is at variance with our results. In a parallel investigation (Hossmann et al., in preparation), total potassium content of the cortex after one hour's ischemia was 83.3 mEq per kilogram of wet weight. This is the maximum value that [K+]e would reach when all of the potassium is dissociated and when full equilibration between intracellular and extracellular compartments exists. Since this is not likely to occur, the peak value must be below this level which, in fact, was the case in the present investigation.

A recording of extracellular sodium activity during oxygen deprivation has not been reported previously. In the present investigation sodium activity of the subarachnoid fluid ([Na+]e) decreased from 133 to 53 mEq per liter during 60 minutes of ischemia. Since the subarachnoid fluid was contaminated by cerebrospinal fluid, extracellular sodium activity in the depth of the cortex might have been even lower. The calculated intracellular sodium uptake of 139 mEq per kilogram of dry weight during 60 minutes of ischemia therefore might be an underestimation of the true value. Sodium uptake thus considerably exceeded extracellular potassium release which was only 64 mEq per kilogram, causing a net increase in intracellular cation of about 75 mEq per kilogram. This finding is in agreement with the earlier histochemical demonstration of a shift of extracellular sodium into the dendrites. It also supports Van Harreveld's conclusion that during ischemia sodium chloride is transported into the cells and that this transport is accompanied by an uptake of water from the extracellular space in order to maintain osmotic equilibrium.

The ischemic changes in extracellular sodium and potassium activities created concentration gradients between blood and brain, which were rapidly equilibrated upon recirculation. Schematically, this process may be divided into two phases (table 1). During the early recirculation phase a passive equilibration along the concentration gradients between extracellular space and blood occurs. Because of the higher concentration gradient of sodium, uptake of sodium exceeds the loss of potassium which, in turn,

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**Table 1** Schematic Representation of Electrolyte and Fluid Shifts During and After Complete Ischemia of the Brain

<table>
<thead>
<tr>
<th>Ischemia</th>
<th>Intracellular compartment</th>
<th>Extracellular compartment</th>
<th>Blood compartment</th>
<th>Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K⁺</td>
<td>Na⁺</td>
<td>H₂O</td>
<td>Increase in [K⁺]e, Decrease in [Na⁺]e, Narrowing of ECS</td>
</tr>
<tr>
<td>Recirculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 1</td>
<td>K⁺ →</td>
<td>Na⁺ →</td>
<td>H₂O</td>
<td>Decrease in [K⁺]e, Increase in [Na⁺]e, Expansion of ECS, Brain swelling</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Na⁺ ← K⁺</td>
<td>Na⁺ ←</td>
<td>H₂O</td>
<td>Normalization of [K⁺]e, Normalization of [Na⁺]e, Resolution of brain swelling</td>
</tr>
</tbody>
</table>

results in an increase in brain water. This process is further enhanced by the equilibration of an osmotic pressure gradient which develops during ischemia and also participates in postischemic brain swelling. During the later recirculation phase the passive equilibration of ions between blood and extracellular space is counterbalanced by an active redistribution of ions and water after reactivation of the sodium-potassium exchange pump. The initiation of this phase, which eventually leads to the resolution of ischemic brain swelling, depends on the recovery of the energy-producing metabolism and is possible only when blood recirculation is not impaired. This explains why, in earlier experiments, brain swelling was reversible only in such cases in which blood flow after ischemia was not obstructed.6

One of the purposes of the present investigation was the correlation of electrophysiological function with extracellular ion activities. During ischemia electrical excitability of cortical neurons disappeared when extracellular potassium increased to about 16 mEq per liter and after ischemia it returned when potassium had fallen below the same level. The electrocorticogram, on the other hand, became isoelectric before any ion shifts could be detected. This is in contrast to the observations of Kirchner et al.,29 who reported a close coincidence between EEG suppression and the increase in $[K^+]_e$. Our findings, therefore, do not lend support to the hypothesis that changes in $[K^+]_e$ are responsible for the “shut-down” reaction of the EEG during anoxic stress.2

The role of extracellular cation levels for the recovery of the electrocorticogram after ischemia also remains unclear. In none of the animals without electrophysiological recovery were cations normal, but in those with recovery of the electrocorticogram, extracellular cations normalized long before the EEG returned. This suggests that reestablishment of ion homeostasis is a prerequisite but not the limiting factor for the restoration of cortical electrogensis after prolonged ischemia of the brain.

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References


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