Relationship Between Metabolic Recovery and the EEG After Prolonged Ischemia of Cat Brain

R. Schmidt-Kastner, M.D., K.-A. Hossmann, M.D., Ph.D., and B. Grosse Ophoff, M.D.

SUMMARY In normothermic cats, cerebral blood flow was arrested for 1 hour followed by blood recirculation for 5-6 hours. Functional recovery was evaluated by qualitative and quantitative EEG analysis, and metabolic recovery by measuring metabolite and electrolyte levels in tissue samples taken from the cerebral cortex.

In 5 out of 12 animals EEG activity did not recover after ischemia (group I); in 3 animals, intermittent EEG activity (group II) and in 4 animals continuous EEG activity returned during the observation period (group III). In group I the energy state was severely disturbed and an increase of calcium was detected, in group II this disturbance was much less pronounced, and in group III changes in energy metabolism and ion concentration were absent with the only exception of lower ADP levels. During recovery, the total intensity of EEG correlated positively with ATP ($p < 0.01$) and inversely with lactate ($p < 0.05$), and the intensity of the delta band inversely with sodium content ($p < 0.05$).

The results obtained demonstrate that electrophysiological recovery after prolonged ischemia is closely correlated with the restoration of the energy state and of electrolyte homeostasis of the brain. The inverse relationship of EEG intensity with lactate and sodium are interpreted as evidence for the adverse effects of ongoing post-ischemic glycolysis, resulting in the activation of the H+/Na+ antiporter for the regulation of intracellular pH.

Restoration of high energy organic phosphates in brain tissue is generally accepted to be a reliable indicator of global metabolic recovery after a period of cerebral ischemia. Similarly, return of electrophysiological signals, such as somatically evoked potentials or EEG activity, is an early detector of functional recovery of the brain. Electrophysiological recordings and metabolic studies are frequently combined in the recovery of the brain tissue is generally accepted to be a reliable indicator of global metabolic recovery after a period of cerebral ischemia. Similarly, return of electrophysiological signals, such as somatically evoked potentials or EEG activity, is an early detector of functional recovery of the brain.

Materials and Methods

Induction of Ischemia

Cats of both sexes ($n = 18$; body weight 2.1 to 4.2 kg) were not fed overnight, tranquilized by intramuscular injection of ketamine (35 mg), and then anesthe-
mm Hg, blood gases were frequently analyzed and kept normal by appropriate adjustment of ventilation and, if necessary, by infusion of sodium bicarbonate. Controls (n = 5) were prepared in a similar way as experimental animals except for thoracotomy, and were maintained under halothane anesthesia.

EEG-recording
A craniotomy was performed over the frontal pole of the right hemisphere, and the electroencephalogram (EEG) was recorded with two silver ball electrodes placed a few mm apart on the intact dura over the posterior sigmoid gyrus (sensorimotor cortex). The signal was fed to a preamplifier (Type 122 Low-Level Preamplifier, Tektronix, Oregon, USA) connected to a chart recorder (Type RM Dynograph® Recorder, Beckman, Illinois, USA) and displayed on paper. Filter settings were 30 Hz for high and 0.8 Hz for low pass. Grading of EEG recovery was performed by visual inspection of the recordings, as described in the Results.

In animals recovering continuous EEG activity, Fourier frequency analysis was performed using a laboratory computer (PDP12, Digital Equipment, MA). EEG intensity was calculated by summing the square roots of Fourier coefficients covering the frequency range of 1–20 Hz. EEG recovery was expressed as a percentage of control intensity recorded in the same animal before ischemia. Intensity of the conventional EEG frequency bands was also calculated, and expressed as a percentage of control intensity recorded in the same laboratory computer (PDP12, Digital Equipment, MA). Fourier frequency analysis was performed using a laboratory computer (PDP12, Digital Equipment, MA).

Cerebral Blood Flow
Cerebral blood flow (CBF) was measured using the intra-arterial 133Xenon injection technique. A bolus of 1 mCi 133Xenon dissolved in 500 μl of Ringer’s solution was injected into the innominate artery via the subclavian artery catheter. Radioactivity was measured with an extracranial detector mounted over the right hemisphere. CBF was calculated from the initial two minutes of tracer clearance (two-minute flow index) according to Hutten and Brock.7 Completeness of ischemia was tested by injecting a bolus of 133Xenon immediately before vascular occlusion.

Tissue Sampling and Biochemical Analysis
At the end of 6 hours recirculation, the frontal craniotomy was enlarged under an operating microscope and the dura split. A frontal sample of cortex roughly corresponding to the EEG recording site was removed with a spatula, immediately freeze-clamped in liquid nitrogen with a maximum delay of 2–3 sec, and stored at −80°C until use. For enzymatic analysis, a small tissue sample was transferred to a glove box kept at −28°C, weighed and placed in pre-cooled tubes together with 0.3 N-HClO4 and 1 mM-EDTA, for immediate homogenization by sonication. After centrifugation, the supernatant fluid was collected and the pellet was re-extracted by the same procedure. The pooled supernate was neutralized with a solution of 1.5 N-KOH, 0.4 M-imidazole base and 0.3 M-KCl, and the KClO4 precipitate was removed by centrifugation. The final extracts were stored at −80°C until analyzed. ATP, ADP, AMP, phosphocreatine (PCr), glucose and lactate were measured by enzymatic fluorometric methods.8 The total adenylates (Σ Ad) was calculated as: Σ Ad = ATP + ADP + AMP, and the energy charge (E.C.) as E.C. = (ATP + 0.5 ADP)/Σ Ad.9 All values are given in μmol/g wet weight of brain tissue.

Brain Electrolytes
Tissue electrolyte content (Na+, K+, Mg2+, Ca2+) was measured in cortical samples taken from an area close to that used for biochemical analysis. After weighing, samples were transferred into 1 M-HNO3, sonified and digested for 24 hours. Electrolyte content was determined in the supernatant by atomic absorption spectrometry (Type 2280, Perkin Elmer, Überlingen, West Germany) and expressed as μeq/g w.w.

Statistical Analysis
To facilitate interpretation of the data, 3 groups were defined according to the quality of EEG recovery (see Results). Comparisons between controls and the three groups of EEG recovery was made with respect to metabolites, electrolyte content, and CBF. After appropriate transformation, the data were subjected to analysis of variance (ANOVA; p < 0.05) and tested with modified t-statistics using the Bonferroni method (p < 0.05).10 Quantitative EEG data were correlated with sodium, ATP and lactate content using Spearman’s Rank Test.11 Values are given as means ± SEM.

Results
General Physiological Parameters
All animals exhibited complete cerebral ischemia followed by rapid blood recirculation, as evidenced by the absence of 133Xenon clearance during 60 min vascular occlusion and fast washout of the tracer after removal of the clamps. One animal was excluded from further analysis because of severe post-ischemic hypotension. In the others general physiological parameters were in the normal range at the time of biochemical sampling: arterial pO2 was 147.2 ± 7.5 mm Hg, arterial pCO2 was 30.7 ± 1.5 mm Hg, pH 7.39 ± 0.01, and systolic blood pressure was above 80 mm Hg. Plasma glucose was 10.92 ± 1.80 nmol/ml in recirculated animals at the time of sacrifice; this value was not significantly different from controls (8.22 ± 0.53 μmol/ml).

EEG Recovery
Representative strip chart recordings of the EEG are shown in figure 1. Control EEG exhibited continuous background activity of about 100 μV in all animals, confirming the intactness of the preparation. Occasional slow wave complexes superimposed on back-
EEG control

EEG recovery

FIGURE 1. Electroencephalogram (EEG) of control and post-ischemic animals. Group I: no recovery of EEG, group II: non-continuous activity, group III: continuous EEG recovery.

ground activity can be attributed to the effect of ketamine anesthesia. Upon clamping the large intrathoracic vessels, EEG flattened within 15 sec and remained isoelectric throughout ischemia in all animals. During recirculation after 1 hour ischemia, greatly varying patterns of EEG recovery were observed. In order to facilitate comparison with metabolic parameters, 3 groups of animals were differentiated according to the degree of EEG recovery after 5-6 hours of recirculation. Group I animals did not present any signs of spontaneous cortical activity even at high amplification (n = 5). Group II animals exhibited intermittent slow polymorphic potentials with or without minor background activity (n = 3). Group III animals were characterized by continuous background activity — usually of lower frequency and amplitude than before ischemia — with some intermingled spikes (n = 4).

In all animals with recovery of EEG, quantification of background activity was carried out by fast Fourier transform. After 5-6 hours of recirculation, total EEG intensity ranged between 12 and 103% (m = 41%) of the pre-ischemic value, the delta band between 14 and 104% (m = 46%), the theta band between 11 and 145% (m = 50%) the alpha band between 9 and 163% (m = 50%) and the beta band between 11 and 79% (m = 28%).

Cerebral Blood Flow

In the control group under halothane/nitrous oxide anesthesia, cerebral blood flow (CBF) was 54.4 ± 10.9 ml/100g/min. After one hour ischemia and 4-6 hours recirculation, CBF was only 21.2 ± 2.74 ml/100g/min, indicating post-ischemic hypoperfusion. The differences between pre- and post-ischemic blood flow were significant for all grades of EEG recovery (ANOVA, modified t-test). As no statistical differences were detected between the three grades of EEG recovery, no further comparison of CBF to EEG and metabolic data was pursued.

Brain Metabolites

The results of the fluorometric analysis of tissue energy metabolites are given in Table 1. Freeze-clamped material from control brains maintained under halothane/nitrous oxide anesthesia exhibited slightly lower PCR levels and higher ADP levels than previously reported for cat cortical tissue frozen in situ under barbiturate or nitrous oxide anesthesia. This is explained by the short interval between sampling and freezing of the tissue which results in a decrease of PCR and an increase of ADP whereas the ATP and total adenylate do not change.

In animals with recovery of continuous EEG activity (group III), ADP was significantly lower than in controls. ATP, glucose and lactate, however, were not different from controls, and a non-significant overshoot of PCR and a significant overshoot of E.C. were detected. Animals with intermittent EEG activity (group II) recovered ATP, E.C., total adenylate, PCR and glucose levels, but lactate tended to be elevated. In animals without recovery of spontaneous activity (group I) ATP, ADP, total adenylate, and energy charge were significantly lower and lactate significantly higher than in the controls.

The most dramatic difference between the various grades of EEG recovery was the tissue content of lactate, more than 20 µmol/g w.w. being incompatible with EEG recovery. ADP of all recirculated animals was lower than in the controls, even in animals with continuous EEG activity. This is explained by the lower energy utilization rate of the post-ischemic brain.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Brain Tissue Metabolite Content in Control and Post-Ischemic Animals (group I–III), at 6 Hours Recirculation after 1 Hour of Global Brain Circulatory Arrest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n =  ATP</td>
</tr>
<tr>
<td>Controls 5</td>
<td>2.05 ± 0.06</td>
</tr>
<tr>
<td>Group I 5</td>
<td>0.90 ± 0.26*</td>
</tr>
<tr>
<td>Group II 3</td>
<td>1.88 ± 0.21</td>
</tr>
<tr>
<td>Group III 4</td>
<td>2.13 ± 0.20</td>
</tr>
</tbody>
</table>

* Post-ischemic animals were grouped according to EEG recovery: group I — no EEG recovery, group II — intermittent EEG activity, group III — continuous EEG activity.

Values are means ± SEM, expressed as µmol/g wet weight. Statistical analysis was performed using ANOVA and modified t-test with the Bonferroni method (*p < 0.05).
which renders the tissue less sensitive to catabolic changes during the freeze clamping procedure.

Brain Electrolytes

Results of the brain tissue content measurements of electrolytes are shown in table 2. Na⁺ did not reveal a significant difference in all post-ischemic groups (ANOVA: F = 1.015). Animals with EEG recovery tended to have slightly elevated Na⁺ despite normal K⁺ levels, but this difference did not reach statistical significance. The only consistent finding was a significant increase of calcium in the group of animals without EEG recovery (group I).

Correlation between EEG, Electrolytes and Energy Metabolism

In the animals with recovery of spontaneous EEG activity, total EEG intensity and the intensity of the conventional frequencies bands was correlated with the tissue content of ATP, sodium and lactate. There was a correlation of ATP with total EEG intensity (p < 0.01), with the intensity of the theta band (p < 0.05) and alpha band (p < 0.01) but not with the beta and delta band. Lactate did not correlate because one animal with low amplitude EEG exhibited low tissue lactate in the presence of subnormal plasma and brain glucose levels. When this animal was excluded, correlation between the increase in EEG and the decrease of lactate was also significant for total power, delta and theta activity (p < 0.05). The decrease of sodium was correlated with the increase of the delta activity (p < 0.05) but not with the activity in the other bands.

Discussion

The present series of experiments corroborates our earlier observations in cats and monkeys that restitution of energy metabolism, electrolyte homeostasis and EEG activity is possible after complete cerebrocirculatory arrest of one hour duration.1, 16-18 Extensive recovery of energy metabolism has also been shown in the rat brain subjected to up to 30 min of severe forebrain ischemia induced by either intracranial hypertension,19 or arterial occlusion;6 in the rabbit after 15 min of compression ischemia,2 and in the artificially perfused dog brain after 30 min of perfusion stop.20

It has been repeatedly stressed that recovery of energy-producing metabolism is a pre-requisite for the restitution of EEG activity. EEG reflects the spatial summation of postsynaptic potentials,21 and therefore requires the restitution of all biochemical processes associated with synaptic transmission. First of all, energy metabolism must provide ATP for driving the various ion exchange pumps necessary to maintain transmembrane ion concentration gradients.22 Next, most biochemical processes associated with synaptic activity are energy-dependent; in particular biosynthesis, release, re-uptake and degradation of neurotransmitters.22 Finally, synaptic activity results in release of macromolecules, such as acetylcholine esterase,24 that must be restored by active protein synthesis. The energy requirements for these processes are considerable. By flattening the EEG with high dose of pentobarbital, the percentage of cerebral oxygen consumption used for generation of EEG has been estimated to amount to about 30%.25 Reversal of Na⁺ and K⁺ leak fluxes consumes about 40% of the remaining oxygen needs.25 In comparison, the energy used for turnover of proteins and lipids is much smaller and amounts to less than 10% of ATP production.26 It is, therefore, not surprising that in all animals in which continuous EEG activity had recovered after ischemia (group III), high concentration of energy-rich phosphates were present.

We did not expect, however, that in animals without EEG recovery (group I) energy metabolism was consistently impaired. Several authors have pointed out that energy metabolism may recover although electrical function is irreversibly suppressed.27 Our study does not support this notion, but clearly indicates that after one hour of complete cerebro-circulatory arrest and 5–6 hours recirculation, energy metabolism is impaired when EEG does not recover. The limiting factor for recovery of EEG is ATP, as evidenced by the significant correlation with the intensity of EEG background activity. Post-ischemic ATP concentration depends on three factors: the rate of adenine nucleotide phosphorylation, the rate of ATP consumption, and the total adenine nucleotide content. During ischemia, ATP and ADP are reduced to AMP which is further degraded to adenosine and hypoxanthine.28, 29 After the beginning of recirculation, oxidative phosphorylation is rapidly resumed and adenylylate energy charge returns to near normal within 30 min,30 indicating that energy production and energy consumption are in equilibrium. However, in order to restore normal ATP levels, the pool of adenine nucleotides has to be replenished. During the early recirculation phase, this is achieved by rapid salvage of a substantial amount of purine

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>48.14 ± 3.43</td>
<td>97.56 ± 6.44</td>
<td>2.26 ± 0.31</td>
<td>11.47 ± 0.47</td>
</tr>
<tr>
<td>Group I</td>
<td>51.15 ± 7.88</td>
<td>74.89 ± 6.89</td>
<td>3.64 ± 0.42*</td>
<td>9.68 ± 0.45</td>
</tr>
<tr>
<td>Group II</td>
<td>62.02 ± 6.28</td>
<td>78.08 ± 10.7</td>
<td>2.53 ± 0.19</td>
<td>10.13 ± 0.66</td>
</tr>
<tr>
<td>Group III</td>
<td>56.38 ± 2.33</td>
<td>91.88 ± 3.61</td>
<td>2.28 ± 0.16</td>
<td>11.10 ± 0.69</td>
</tr>
</tbody>
</table>

Post-ischemic animals were grouped according to EEG recovery: group I — no EEG recovery, group II — intermittent EEG activity, group III — continuous EEG activity. Electrolytes were measured in cortical tissue samples and expressed as μeq/g w.w. (means ± SEM). Statistical difference was tested by ANOVA and modified t-statistics using the Bonferroni method (*p < 0.05).
nucleotides and free bases. Further increase of adenylate requires purine de novo synthesis which is a slow process and which, depending on the adenylate loss during ischemia, may take up to several days for completion. The degree of ATP restoration depends crucially on how much adenylate is salvaged after restitution of blood flow.

The comparison of the present investigation with previous studies suggests that adenylate salvage varies with the type of anesthesia used. In cats anesthetized by a single intraperitoneal injection of 30 mg/kg pentobarbital, total adenine nucleotides were reduced to 34% of normal during 60 min ischemia, and only 68% were recovered during recirculation by salvage pathways. ATP, therefore, returned to only 62% of control although energy charge was restored to within 7% of the pre-ischemic value. In the present study in which animals were anesthetized before ischemia with halothane/nitrous oxide, total adenylate returned to 85% after 5-6 hours of recirculation in animals with EEG recovery. Barbiturates seem to delay the post-ischemic restoration of adenylate, and they are apparently responsible for the fact that in the previous study, the quality of metabolic recovery was distinctly lower than in the present investigation.

The importance of phosphocreatine (PCr) for electrophysiological recovery is less clear. It has been demonstrated in numerous studies before, that low PCr levels are incompatible with return of EEG activity. On the other hand, Barzaghi et al. found an overshoot of PCr at 6 hours after 10 min bilateral carotid occlusion in the gerbil although EEG frequency analysis revealed EEG slowing. Overshooting PCr levels were also reported in rats after 30 min severe forebrain ischemia, in the rabbit after 15 min compression ischemia, and in the gerbil after 60 min unilateral carotid ligation. One of the reasons for this overshoot is transient post-ischemic alkalosis resulting in a shift of the pH-dependent creatin-kinase equilibrium toward PCr.

The present study confirms that PCr — in contrast to ATP — may overshoot in animals in which EEG recovered, but it never does so in animals without EEG activity. This fact raises interesting questions related to the energy equilibrium of synaptic activity. It was previously shown that PCr overshoot is a transient phenomenon which is followed by a decrease after longer survival times as EEG activity further improves. This implies that subnormal synaptic activity during the early phase of brain recovery may be directly linked to the overshoot of PCr. The reverse situation is seen in status epilepticus where PCr is reduced in face of maximally enhanced electrical activity. PCr is an immediate energy reserve of the brain. PCr overshoot indicates that the energy reserves are filled up, either by increased production or decreased utilization of energy-rich phosphates. As ATP levels are normal or decreased during this phase, reduced energy consumption due to the still reduced synaptic activity is more likely.

Another interesting aspect of this study is the relationship between tissue lactate content and EEG recovery. During 60 min ischemia, lactate rises up to 20 μm/g w.w. followed by a gradual decline after ischemia provided blood recirculation is not impaired. Adequate post-ischemic recirculation is also of importance for functional recovery, and it is not surprising that animals without EEG recovery exhibited greatly enhanced lactate levels. There was — although at a lower level — a correlation between lactate and EEG intensity in the animals with recovery. This relationship can be explained by two mechanisms both of which interfere with the electrical function of the brain. One is slightly inhomogeneous reperfusion resulting in micro-areas of low blood flow in which lactate remains elevated due to persisting anaerobic glycolysis. The other factor is partial uncoupling of oxidative phosphorylation, as evidenced by the previously described disturbance of state 3 respiration of mitochondria. In either case, an excess of protons is produced which have to be removed from the intracellular compartment in order to maintain normal pH. One of the mechanisms responsible for regulating intracellular pH is the H⁺/Na⁺ antiporter driven by the sodium gradient which in turn is built up by the ATP-dependent Na⁺/K⁺ exchange pump. This antiporter has been found in various types of cells, and it is reasonable to assume that it also exists in the brain. Activation of this antiporter could explain that tissue sodium may be increased after ischemia and that this increase correlated inversely in a similar way as lactate with the intensity of the EEG in the group with EEG recovery.

In this context, the role of the other electrolytes for EEG recovery should be briefly mentioned. In animals without recovery, the well-known accumulation of Ca²⁺ was observed. It is difficult to decide whether these changes are the reason for, or the consequence of, deranged energy state; but they are closely associated with each other and reflect the persistent injury inflicted upon the brain by the preceding ischemic impact.

In conclusion, the present investigation clearly demonstrates that restoration of EEG activity after prolonged ischemia is a reliable indicator of the post-ischemic restoration of energy metabolism and electrolyte homeostasis. Similarly, post-ischemic reactivation of energy metabolism results in functional recovery, provided animals are allowed to survive long enough. Previous reports on the dissociation between energy metabolism and functional recovery can be explained by the time lag between the two events. An elegant demonstration of this fact comes from studies combining in vivo 31 phosphorus nuclear magnetic resonance spectroscopy with EEG recording. After 30 min of forebrain ischemia in the rat, the ATP and PCr peaks recovered to normal at 30 min recirculation; whereas, the EEG took about 12 hours to normalize in the very same animal.

This further stresses the importance of sufficiently long recirculation times for evaluation of post-ischemic resuscitation potentials.
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