Cortical Evoked Potential and Extracellular K\(^+\) and H\(^+\) at Critical Levels of Brain Ischemia

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SUMMARY As shown previously, the electrical function of the brain is critically dependent on cerebral blood flow in the sense that reduction above an ischemic threshold of approximately 15 ml/100 gm per minute (approximately 35% of control) in the baboon leads to complete failure of the somatosensory evoked response. This study tests the hypothesis that electrical failure in ischemia may be directly associated with a massive release of intracellular K\(^+\) or with a critical degree of extracellular acidosis. By microelectrode techniques, measurements of blood flow, extracellular activity of K\(^+\) and H\(^+\) as well as evoked potential were made in the baboon neocortex. Reductions in blood flow were obtained by occlusion of the middle cerebral artery and depression beyond the ischemic threshold of electrical function achieved by a reduction of systemic blood pressure which, in the ischemic zones, changed local cerebral blood flow proportionally.

The aim of this study therefore was to test the association between electrical function and extracellular activities of K\(^+\) and H\(^+\) (K\(_e\) and pH\(_e\)) at the ischemic threshold of electrical failure.

To achieve this, rCBF was lowered by middle cerebral artery occlusion to approximately the ischemic threshold for the somatosensory evoked response, and then manipulated about the threshold by varying systemic blood pressure, while K\(_e\) and pH\(_e\) were continuously recorded using extracellular microelectrodes.

Methods

Animals, Preparations, EP and Hydrogen Electrodes

Four adult baboons (Papio cynocephalus) of either sex, weighing 10 to 16 kg, were used in this study. The techniques of anesthesia and continuous monitoring of basal functions have been described in detail previously.

After tranquilization with intramuscular phencyclidine (Sernylan), anesthesia was induced with a sleep dose of thiopentone and maintained with alpha-chloralose (60 mg per kilogram i.v.). The animals were paralyzed with gallamine triethiodide (1 mg per kilogram i.v.) and respiration maintained with a Starling pump (C. F. Palmer). Systemic blood pressure, arterial pH, Pco\(_2\) and Po\(_2\), and end-tidal CO\(_2\) were monitored. The exposure of the middle cerebral artery through the orbit and the exposure of the middle cerebral artery was clipped under direct vision close to its origin, with a small Scoville clip. Normal levels of arterial Pco\(_2\) between 38 and 43 mm Hg were maintained by varying the stroke volume of the Palmer pump.
pH and K⁺ Electrodes

Continuous measurements of $K_e$ and $pH_e$ were obtained from two double-barreled K⁺ microelectrodes and one miniaturized pH electrode. Tip diameters were 1 to 2 μm for the pH electrode. The electrodes were inserted to a depth of approximately 500 μm in cortical gray matter through a fine slit cut in the pia arachnoid. This was done before the paraffin pool was established. To obtain a corresponding set of measurements of $K_e$, $pH_e$, rCBF and EP, one K⁺ electrode and the pH electrode were positioned within a distance of 3 to 6 mm from the EP electrode, also containing the hydrogen electrode No. 1. The other K⁺ electrode was placed close to hydrogen electrode No. 5 in experiments Nos. 1 and 2, and to hydrogen electrode No. 3 in experiments Nos. 3 and 4. In this way paired measurements of $K_e$, $pH_e$, rCBF and EP were obtained. In the course of these experiments we had some evidence that withdrawal of an electrode into paraffin appeared to damage K⁺ sensitivity in certain instances, possibly by interference with the liquid K⁺ ion exchanger in the electrode tip. We were particularly careful, therefore, to ensure that the electrodes once introduced remained in the brain and, because of this interference, we also felt it unsatisfactory to recalibrate K⁺ electrodes after the experiment.

Drift and Recording Techniques

Since the usual way of recording drift during the experiment by recalibration was not at hand for the K⁺ electrodes, the absolute terminal values for $K_e$ should be taken with caution. Rapid changes in reference electrode potential were not observed but could easily have been recognized as identical potential changes in all three electrodes, since the two reference barrels were connected and served as common reference for the two K⁺ electrodes and the pH electrode. Amplifier drift was controlled by recording the zero (short-circuited amplifier inputs) (fig. 1). The three electrodes were sequentially scanned by a low current scanner (Keithley 702/7028) and the signals recorded by a differential high input impedance amplifier (Keithley 604) and a Rikadenki pen recorder.

Precautions Concerning Brain Surface Movements

Swelling or shrinking of the brain with subsequent movements of the brain surface is unavoidable. Regular movements occur with respiration, and surface movements of several millimeters may occur during CO₂ inhalation, after MCA occlusion and following exsanguination. To keep the K⁺ electrodes in the tissue, the connection to the K⁺ barrel was made by a copper wire (0.3 mm in diameter) which was coiled to serve as a spring in very light compression. To prevent the electrodes from sinking into white matter a small drop of epoxy cement was placed approximately 0.5 mm from the tip. These simple precautions permitted movements of the K⁺ electrodes with the brain surface. The pH electrode was left hanging in the soft connection wire after insertion. No artifacts due to the brain movements synchronous with respiration were noted on the electrode signals.

Electrode Calibration

The K⁺ electrodes were calibrated at room temperature in solutions containing 3, 5 and 50 mmol per liter KCl and 147, 145 and 100 mmol per liter NaCl, respectively. Between 5 and 50 mmol per liter a slope of 50.6 (range 48.5 to 52 mV) was obtained. Calibrations at room temperature were adjusted to 37°C according to the Nernst equation by multiplying the calibration potentials by

$$\frac{310}{273 + \text{room temperature}}.$$ 

Below 5 mmol per liter KCl sensitivity declined. Electrode resistance was approximately 10⁶Ω. The internal solution consisted of 100 mmol per liter KCl and the junction potentials were within a range of +10 to −1 mV.

The pH electrode was calibrated at room temperature in phosphate buffers in which pH measured at 37°C was 6.48 and 7.22, respectively. Sensitivity was 55.4 mV/pH. Electrode resistance was 10⁶ to 10⁸Ω. Calibrations were done using the reference barrel of the K⁺ electrodes, later serving as reference during the experiment to avoid a change in junction potential. The internal solution was 0.1 N HCl.

Results

Response to CO₂ Inhalation

To test the pH electrode CO₂ was added at the respirator inlet. This was performed in experiments Nos. 1 and 2. The electrode responded within one to two minutes. At steady-state arterial PCO₂ values from 34 to 60 and from 37 to 54 mm Hg, $pH_e$ changed from 7.33 to 7.07 and from 7.21 to 7.07, respectively. No changes in $K_e$ occurred.

Initial and Terminal Recordings of $K_e$ and $pH_e$

$K_e$ and $pH_e$ recorded immediately after insertion, just before MCA occlusion, and after terminating the ex-
TABLE 1  Initial and Terminal Measurements of rCBF (ml/100 gm/min), Arterial Pco2 (mm Hg), Ke (mmol/l) and pHe

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Insertion</th>
<th>Before MCA occlusion</th>
<th>&quot;Drift&quot;</th>
<th>Terminal measurements</th>
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<td>rCBF</td>
<td>Ke</td>
<td>pHe</td>
<td>rCBF</td>
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<td>29 3.7 7.46</td>
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<td>3 10 6.42</td>
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<tr>
<td>3</td>
<td>70 36</td>
<td>70 57 5.5</td>
<td>19 19 285</td>
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<tr>
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<td>30 50</td>
<td>30 3.0 7.36</td>
<td>44 44 318</td>
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<tr>
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<td></td>
<td>42 42 318</td>
<td></td>
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<tr>
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<td>50 50 318</td>
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<tr>
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<td>Pco2</td>
<td>40 40</td>
<td></td>
<td>40 40 216</td>
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</table>

"Drift" refers to observed changes in electrode potential in mV from time of insertion to MCA occlusion. Observation time and total length of experiments are given in minutes. The terminal Ke values were still increasing at time of disconnection.

Experiments by inhalation of N2 (in experiment No. 1: air), are given in table 1. The changes in Ke and pH during the time of observation between insertion and MCA occlusion were within 0 to 13 mV (table 1). Changes in Ke and pH subsequent to insertion are likely to occur from cell damage, bleeding and arterial Pco2 changes. This leaves only a proportion of the "drift" listed in table 1 to electrode drift. No identical changes in all electrode signals indicating drift in junction potential were observed.

The terminal recordings of Ke and pH are given in table 1. By recording the anoxic release of cellular K+ at the end of the experiment, the K+ electrode was proved to respond
TABLE 2  Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Control</th>
<th>Occlusion</th>
<th>Control</th>
<th>Occlusion</th>
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<th>pH_e</th>
<th>rCBF</th>
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<tr>
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<td>6.90</td>
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<tr>
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<td>3.6</td>
<td>—</td>
<td>—</td>
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</table>

Corresponding values of EP (% of control), K_e (mmol/l), pH_e and rCBF (ml/100 gm per minute), at minimum for EP immediately after MCA occlusion (see also figs. 2 and 3).

adequately. Thus, the electrode tip buried in the tissue was protected from paraffin contamination. The anoxic K⁺ release occurred gradually and a steady-state was not obtained even after 20 minutes of N₂ inhalation (18, 24 and 27 minutes, respectively).

MCA Occlusion

In all four experiments, MCA occlusion caused an immediate drop in rCBF and a subsequent moderate elevation of K_e, an extracellular acidosis and a decline in EP amplitude. The changes in K_e, pH_e and EP following MCA occlusion were similar in time and occurred more rapidly in the experiments with the largest initial drops in rCBF (experiments Nos. 1 and 2 compared to Nos. 3 and 4, figs. 2 and 3). Corresponding values of EP, K_e and pH_e and of rCBF following occlusion are given in table 2. It is seen from table 2 that abolition of EP is not necessarily associated with the biggest increase in K_e nor with the most severe extracellular acidosis. But, consistent with previous studies, abolition of EP is associated with the lowest rCBF values. Thus it appeared that no critical value of K_e nor pH_e could be associated with onset of electrical failure.

Effect of Blood Pressure on rCBF After MCA Occlusion

A good correlation (r = 0.93) was found between the relative changes in MABP and rCBF, as shown in figure 4. This indicates a passive vascular bed in the ischemic zones. The regression line has a slope of 1.23 and the intercept of 19.5 ± 2.11 MABP% (5% confidence limits) is not significantly different from zero.

Effect of Blood Pressure on EP, K_e and pH_e After MCA Occlusion

By varying systemic blood pressure and hence rCBF after MCA occlusion, the interrelations between rCBF, EP, K_e and pH_e at critical low levels of rCBF were studied further.

Recovery by Increase in MABP

By increasing MABP (by infusion of aramine or noradrenalin) a complete recovery of EP and a partial recovery of K_e and pH_e were obtained in experiments Nos. 3 and 4 (table 3, fig. 2). The recovery of EP, K_e and pH_e was closely related in time to the changes in MABP. In experiments Nos. 1 and 2, a partial recovery in K_e and pH_e but no recovery of EP were obtained by an increase in MABP. However, in experiment No. 2, EP, K_e and pH_e were normalized by release of the MCA occlusion accompanied by a large increase in rCBF (table 3, fig. 2). That normalization of pH_e and K_e is obtained in this situation is in accordance with the study by Heuser et al. Again, no critical levels of K_e and pH_e for electrical failure or recovery could be identified since comparisons between experiments as well as within the individual experiments show that a good recovery in EP is not necessarily associated with the lowest K_e values or with a less severe extracellular acidosis (table 3, fig. 3).

Massive Cellular Release of K⁺ by a Decrease in MABP

Reductions in MABP by exsanguination led to abolition of EP, to higher values of K_e and to more severe acidosis (table 3, figs. 2 and 3). Again, the changes were closely related in time to MABP. At MABP levels as low as 59, 60 or 70 mm Hg in the three animals so studied, a massive release of intracellular K⁺ noted as a steep rise in K_e occurred. The closest corresponding rCBF values were obtained at MABP of 57, 55 or 45 mm Hg, respectively (table 3), i.e., later in the exsanguination procedure when MABP and hence rCBF were even further reduced. The rCBF values

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so obtained were 11 > 5 > 2 ml/100 gm per minute and (after adjustment according to fig. 4) 18 > 6 > 2 ml/100 gm per minute (median with 5% confidence limits). The adjusted reductions in rCBF required to enter the state of K+ release from the state of electrical failure were 17 > 10.5 > 2 ml/100 gm per minute (median with 5% confidence limits). This indicates the ischemic threshold of K+ release.

Discussion

Reliability of Methods

The pH electrode used in this study measures extracellular pH (pH_e) in neocortex and does not merely reflect plasma pH, since blood-brain barrier integrity toward H+ and HCO3 ions is not interrupted by electrode insertion. Further, the time response is more than adequate since a step increase in arterial Pco2 is followed by a change in electrode signal within five seconds. With these previous observations and with the present finding of an adequate response of about 15 ml/100 gm per minute.3

Mechanism of the Complete Electrical Failure in Ischemia

On the basis of several studies, clinical as well as experimental, we will consider the critical dependence of cortical electrical function (EEG, EP or single neuron discharges) on rCBF as a physiological fact.4 In the present ischemic model, the EP is totally abolished at rCBF values of about 15 ml/100 gm per minute.4

Recently, Marshall et al.1 have discussed the various hypotheses possibly explaining the electrical failure in ischemia. These have been primarily depletion of high energy phosphates, synaptic depolarization by release of intracellular K+, severe lactacidosis and impaired neurotransmitter metabolism.

The results of both Branston et al.3 and Marshall et al.1 suggest the hypothesis of a synaptic depolarization subse-
quent to release of cellular K+.

However, the results obtained in this study demonstrate that only the possibility of highly localized release of K+ at synaptic sites and therefore perhaps not seen by the electrode cannot be definitely excluded. Only slight elevations in Ke were found at levels of ischemia producing complete electrical failure. These elevations in Ke must be considered too small to produce inexcitability, because full electrical activity is seen at Ke values as high as 10 to 12 mmol per liter during epileptic discharges and following electrical stimulation. Thus, the potassium hypothesis (association of massive K+ release with electrical failure in ischemia) cannot be supported by the present study. Further evidence against the potassium hypothesis has been provided by previous studies. In asphyxiated rats, EEG becomes isoelectric one to two minutes before the massive K+ release. Furthermore, recovery from the terminal state of hypoglycemia by intravenous glucose leads to a rapid normalization in Ke, but to a much slower recovery in EEG, and in a study on cats by Heuser et al. (1975) it was found that in the recovery from total ischemia by reperfusion, Ke may normalize while EEG stays isoelectric. The conclusion of these results is that the state of complete electrical failure need not be associated with a high Ke value; in fact Ke may stay normal or normalize at onset of isoelectricity. This has recently been observed in rats with epileptic seizures and hypotensive ischemia. On the other hand, a massive release of intracellular K+ is always associated with electrical failure, and in the phenomenon of spreading depression the potassium hypothesis may fully explain the local transient isoelectricity.

A causal relationship between tissue lactacidosis and complete electrical failure is discounted also by the present study. It is clearly demonstrated that neither abolition of EP nor recovery of EP could be associated with a critical level of tissue extracellular acidosis. Neither could the study of Heuser et al. relate EEG recovery after total ischemia to a critical pHe value. The close relationship in time between changes in pHe and EP is striking (figs. 2 and 3), but does not necessarily indicate a causal interrelationship. Rather, the changes in pHe, as well as in EP are concomitants of the general ischemic metabolic pattern, the pHe drop being related to lactic acid production and the EP abolition possibly to intermediates acting as neurotransmitters. The possible role of the reduction in glutamate and the rise in GABA for electrical function has been emphasized by several investigators. Release of K+ and the Existence of Two Ischemic Thresholds

The massive release of intracellular K+ recognized as a steep rise in Ke did not occur at the threshold of electrical failure of about 15 ml/100 gm per minute. K+ release first occurred only when systemic blood pressure and hence rCBF was further reduced by exsanguination to a level of about 6 ml/100 gm per minute. We have thus demonstrated that the ischemic threshold for K+ release is below that for complete electrical failure.

No data seem yet at hand which strictly relate the K+ release to the energy metabolism. We have not assigned the term "failure of the ionic pump" to the phenomenon of K+ release, since other mechanisms such as an increase in ionic permeability of the cell membranes might well be of importance.

Our conclusions are illustrated in figure 5. We have restricted the use of the term ischemia to a reduction in local blood flow of a degree leading to impaired tissue function, while oligemia is taken to mean a reduction in local blood flow which leaves functions unaltered. When electrical activity ceases, a further reduction of about 50% in rCBF is required to move the functional state of the tissue from isoelectricity to K+ release. In figure 5 we have indicated not only the absolute rCBF values describing the present experimental condition, but we have also given a percentage rCBF scale. This is done to indicate that the concept of ischemic thresholds of various functions as shown in figure 5 should not be restricted to the present acute preparation which must unavoidably involve some depression of metabolic rate. We assume that in ischemia the functional inactivations occur subsequent to a failure of oxygen supply and utilization. Even if oxygen utilization is extremely high as in epilepsy, or extremely low as in hypothermia or barbiturate anesthesia, the concept of clearly separate ischemic thresholds of various functions may still apply as a general pattern independent of experimental conditions. Evidence for this has recently been provided by the demonstration of clearly separated thresholds of complete electrical failure and of K+ release in rats with generalized epileptic discharges and systemic hypotension. The actual threshold values in ischemia may thus cover a large range dependent on cerebral metabolic rate.

Possible reversibility of the functional inactivation in ischemia is the key problem of interest here. Neither the state of complete electrical failure nor the state of K+ release of severe extracellular acidosis per se indicates irreversible tissue damage since recovery of EP and normalization of Ke and pHe can be obtained simply by increasing local blood flow above ischemic levels. In experiment No. 2, the occlusion was released and an almost complete recovery in EP and a normalization in pHe occurred, while Ke was about normal all the time (fig. 2). In experiment No. 1, rCBF was increased by an increase in MABP but stayed below the ischemic threshold of electrical function, with recovery only of Ke and pHe. That a completely normal Ke can be regained after the massive K+ release has occurred is not shown in the present study.

**FIGURE 5.** Ischemic thresholds for electrical failure and for release of cellular K+. For further details see text.
this study, but is known from the study of Heuser et al., as well as from other studies from our group.

According to figure 4, it is likely that in patients with clinical stroke, blood flow in the ischemic zone varies in parallel with systemic blood pressure. A clinical implication of a drop in blood pressure in the stroke patient is seen in figure 5, which suggests the conversion of zones with spared electrical function into a state of electrical failure, and the conversion of zones with electrical failure into a state in which a massive cellular $K^+$ release with subsequent increase in $K_+$ may occur. That such extensions of the areas of impaired brain function, produced by hypotension, may be reversed with subsequent clinical improvement simply by the reestablishment of normotension is not unknown in clinical work. The zones of complete electrical failure and $K^+$ release lie in the ischemic penumbra, with functional inactivation but not yet cell death.

Acknowledgment
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