Possible control of intermittent cerebral ischemia by monitoring of direct-current potentials

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**Object.** Neurosurgically induced temporary occlusion of intracranial arteries carries the risk of cerebral ischemic damage. Because negative shifts in the cortical direct-current (DC) potential indicate tissue depolarization and, thus, critical ischemic stress, the authors hypothesized that recordings of these potentials could help to determine the optimal duration and frequency of induced intermittent focal ischemia to prevent brain injury. The investigators related the results of DC recordings both to simultaneously recorded decreases in extracellular Ca^{++} concentration ([Ca^{++}]_{o}), which reflect Ca^{++} entry into cells, and to histological outcome.

**Methods.** In cats anesthetized with halothane the effects of intermittent brief (10 minutes long, six times [6 × 10-min group]) and prolonged (20 minutes long, three times [3 × 20-min group]) episodes of middle cerebral artery occlusions were compared with those of a single continuous episode (1 × 60-min group). Laser Doppler flow probes and ion-selective microelectrodes were used to measure cerebral blood flow, DC potentials, and [Ca^{++}]_{o} in cortical tissues of ectosylvian gyri.

Negative shifts in DC potential were evaluated in the three groups during the entire 60-minute-long period of ischemia and were smallest in the 6 × 10-min group, larger in the 3 × 20-min group, and largest in the 1 × 60-min group. Accordingly, infarct volumes were smallest in the 6 × 10-min group, intermediate in the 3 × 20-min group, and largest in the 1 × 60-min group. Decreases in ischemic [Ca^{++}]_{o} were significantly greater in the 1 × 60-min group than in the two groups in which there were repetitive occlusions, and recovery of [Ca^{++}]_{o} after reperfusion normalized only in the 1 × 60-min group.

**Conclusions.** The DC potential may provide a reliable measure to optimize intermittent ischemia and to achieve minimal ischemic brain injury during temporary neurosurgical occlusion of cerebral arteries.

**KEY WORDS ** intermittent ischemia • aneurysm surgery • temporary focal ischemia • direct current potential • extracellular calcium concentration • reperfusion

Temporary occlusion of intracranial arteries is frequently used during aneurysm surgery to prevent intraoperative rupture, to control excessive bleeding from premature rupture, and to achieve clipping of aneurysms. Despite these advantages, it carries the risk of inducing ischemic sequelae, depending on the duration of the occlusion and the presence of collateral blood channels. Repeated occlusions of brief duration that are interrupted by reperfusion episodes have been thought to minimize ischemic damage, and authors of several experimental reports have supported this notion by showing that repeated episodes of focal ischemia cause less histological injury than a single episode. On the other hand, arguments have been raised that interrupted arterial clipping is not only technically more difficult than a single prolonged occlusion, but worsens the outcome of surgery, particularly in cases of reperfusion injury resulting from repeated recirculation of blood flow.

In this study, we used a model of intermittent focal ischemia in cats to ascertain whether an easily applicable method, real-time monitoring of the DC potential known to reflect ischemic tissue depolarization, provides an index for intraoperative control of ischemic injury, as determined by histological outcome. Simultaneous recordings of [Ca^{++}]_{o}, provided a means for comparing the results with one of the most important steps in the cascade of ischemic deterioration, that is, Ca^{++} entry into cells.

**Materials and Methods**

**Animal Preparation**

Fourteen adult cats of either sex, each weighing 2.5 to 4 kg were used. The study was approved by the local animal care committee and by the Regierungspräsident of Cologne. Ketamine hydrochloride (25 mg/kg administered intramuscularly) was used to induce general anesthesia. The left femoral artery and vein were catheterized to administer drugs and to measure arterial blood pressure, arterial blood gases, hematocrit, and plasma glucose concentration. Each cat underwent tracheostomy, was immobilized by an intravenous bolus of 0.2 mg/kg pancuronium bromide followed by a continuous infusion of 2 ml/kg/hr gallamine triethiodide, and under-

**Abbreviations used in this paper:** [Ca^{++}]_{o} = concentration of extracellular calcium; CBF = cerebral blood flow; DC = direct current; ECoG = electrocorticography; MCA = middle cerebral artery; rCBF = regional CBF.
went artificial ventilation with a 70%:30% NO2/O2 gas mixture containing 0.8 to 1.5% halothane. Arterial and expiratory gases were controlled within normal physiological ranges. Deep body and brain temperatures were independently maintained at 37˚C by using a heating blanket and heating lamps. The proximal left MCA was prepared transorbitally, and a device for repetitive remote occlusion was implanted. This device consists of an outer cannula, the tip of which forms a hook to be placed around the MCA, and an inner occluder that can be slid into the hook through the cannula. By pushing the inner occluder toward the silicone-coated wall of the hook, the MCA is compressed gently and firmly between the occluder and the hook wall, yielding total arterial occlusion; by pulling back the occluder, the MCA occlusion is easily relieved.

Each cat received a craniotomy 3 mm in diameter above the left ectosylvian gyrus, and located 8 mm anterior and 15 mm lateral from the gyrus, according to a stereotactic atlas. After a careful incision of the dura mater, a microelectrode to be used for recording DC potentials and [Ca2+]o levels was inserted 1-mm deep into the cortical tissue. For this purpose, double-barreled ion-selective microelectrodes with tip diameters of 1.5 to 2 μm were manufactured using theta glass with thick septa. The ion-selective barrel was filled with Ca2+ ionophore and 150 mM CaCl2, and the reference barrel was filled with 150 mM NaCl. Calibration at 37˚C was performed in 0.03-, 0.3-, and 3-mM solutions of CaCl2, with a constant background of 150 mM NaCl and 3 mM KCl. The reference barrel served also for DC potential and ECoG recordings. The DC potential was recorded against a calomel electrode placed at the nasal region to avoid electrode polarization.

Next to the electrode was a thermocouple used to measure regional brain temperature, and a laser Doppler probe with a tip diameter of 800 μm was used to measure rCBF on the cortical surface. Burr holes in the orbit and above the cortex were filled with Gelfoam containing cerebrospinal fluid and were closely covered with dental cement to avoid leakage of cerebrospinal fluid and to make the intracranial space a closed system.

Experimental Groups and Variables Measured

The various parameters (blood pressure, brain temperature, rCBF, ECoG activity, and DC potential) were continuously recorded using a personal computer–based data acquisition system. The animals were subjected to the following three experimental procedures: 1) a single 1-hour occlusion of the MCA (1 × 60-min group, four cats); 2) three 20-minute occlusions of the MCA interrupted by 5-minute intervals of reperfusion (3 × 20-min group, five cats); and 3) six 10-minute occlusions of the MCA interrupted by 5-minute intervals of reperfusion (6 × 10-min group, five cats). After 15 hours of reperfusion, the animals were perfused with 4% paraformaldehyde solution to fix their brains. The brains were removed and embedded in paraffin, and 10-μm-thick coronal sections of brain were cut every 2 mm and stained with hematoxylin and eosin. Areas of infarction were evaluated in a blinded fashion by using an image analyzer. Total infarct volumes were calculated by integrating the infarcted area of all brain slices (area of infarct measured in square millimeters multiplied by the 2-mm thickness).

The means of accumulated DC potential shifts recorded during all ischemic episodes and during the 60-minute recirculation following the last reperfusion, and the means of infarct volumes were compared among experimental groups by using analysis of variance and multiple post hoc comparisons.

Sources of Supplies and Equipment

The Ca2+ ionophore was obtained from Fluka (Neu-Ulm, Germany). The laser Doppler probe was manufactured by Moor Instruments (Axminster, UK). The personal computer–based data acquisition system was obtained from DASYLab (Münchengladbach, Germany). The imaging analyzer was obtained from Gesotec (Darmstadt, Germany). Statistica (StatSoft, Inc., Tulsa, OK) software was used in the statistical analysis of data.

Results

Regional CBF

In all three experimental groups, MCA occlusion reduced rCBF immediately, and the level of rCBF reduction...
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stayed almost constant during single ischemic episodes, as shown in single examples of original laser Doppler flowmetry traces in Fig. 1 upper. The mean values of rCBF reduction were 16.2 ± 5.6% of preischemic control values in the 1 × 60-min group, 13.6 ± 14.8% in the 3 × 20-min group, and 11.3 ± 3.7% in the 6 × 10-min group, and did not differ among the three groups. Reopening of the MCA in all sites was confirmed by recovery of rCBF to more than 90% of the preischemic control value, although the degree of recovery showed a certain variation among local recordings, ranging from delayed recovery with a certain period of hypoperfusion to a rapid response with hyperperfusion that was sometimes followed by hypoperfusion.

Electrocorticography Recordings

When evaluated according to amplitude, ECoG activity significantly decreased in all groups during individual ischemic episodes (1 × 60-min group, 21.4 ± 12% of preischemic control values; 3 × 20-min group, 20.6 ± 7.3%; and 6 × 10-min group, 29.6 ± 5.2% immediately after occlusion). One hour after the final reperfusion, recovery of ECoG activity was still incomplete in all groups, although the 6 × 10-min group exhibited by far the best recovery (1 × 60-min group, 29.4 ± 12.7% of preischemic control values; 3 × 20-min group, 39.2 ± 7.2%; and 6 × 10-min group, 62 ± 14.4%).

Traces of Cortical DC Potential

In all three experimental groups, original traces of the cortical DC potential (Fig. 1 center) consistently displayed negative shifts for single and for repetitive MCA occlusions. In the 1 × 60-min group, the negative shifts persisted until reperfusion. After reperfusion, recovery of the DC potential did not reach baseline levels throughout the observation period. In the 3 × 20-min group, the recovery of the DC potential following consecutive reperfusion worsened to some extent. In the 6 × 10-min group, the DC potential seemed to decrease less with consecutive occlusions and, in contrast with the other groups, recovery of the DC potential during consecutive reperfusion episodes was always complete.

An analysis of shifts in DC potential for all ischemic episodes in the various groups revealed significant differences: the mean negative shifts in the 1 × 60-min group were markedly greater than those in the 3 × 20-min group, and the mean negative shifts in the 6 × 10-min group were the smallest (Fig. 2 upper). Similarly, the recovery of the DC potential was significantly higher in the 6 × 10-min group than in the other two groups, in which there were longer periods of occlusion (Fig. 2 upper right). The 3 × 20-min group and 1 × 60-min group did not differ significantly with respect to recovery of the DC potential after recirculation.

Extracellular Ca\(^{++}\) Concentration

During occlusion, in all three experimental groups there was an initial brief phase of gradual increase in [Ca\(^{++}\)], followed by an abrupt steep drop corresponding to the sharp decrease in the DC potential (Fig 1 lower). In the 1 × 60-min group, the low level of [Ca\(^{++}\)], persisted and, after reperfusion, recovery of [Ca\(^{++}\)], remained incomplete during the observation period. In the 3 × 20-min group, recovery of [Ca\(^{++}\)], also was incomplete following each single occlusion. In the 6 × 10-min group, in contrast, decreases in [Ca\(^{++}\)], became smaller following successive arterial occlusions, and final recovery after the last occlusion was almost complete.

The shifts in [Ca\(^{++}\)], which were analyzed for all ischemic episodes in the three groups, revealed significant differences. During ischemia, the shifts were much greater in the 1 × 60-min group than in the 3 × 20-min and 6 × 10-min groups (Fig. 2 lower left). The latter two groups did not differ markedly. Recovery of [Ca\(^{++}\)], was significantly higher in the 6 × 10-min group than in the two groups in which there were longer periods of occlusion (Fig. 2 lower right).

Infarct Volume

Infarct volumes were by far largest in the nonrepetitive 1 × 60-min group. Compared with this group, infarct volumes were significantly reduced by 38% (p < 0.05) in the 3 × 20-min group and by 93% (p < 0.001) in the 6 × 10-min group (Fig. 3). Infarction in the 6 × 10-min group was not significant in the hemisphere ipsilateral to the occlusion, compared with the contralateral hemisphere, thus underlining the small degree of neuronal injury in this group subjected to repetitive short-term ischemia.

Discussion

Using a model of proximal MCA occlusion in cats, we have shown that six 10-minute episodes of ischemia in-
Depolarization is reached. In focal ischemia, the DC decreases in the DC potential before a final state of low minimization of brain damage. Monitoring this potential has been shown to reflect gradual ischemic disturbances in an integrative manner, differentiating not only between the ischemic core and penumbra, but also within the ischemic core between different grades of blood flow reduction and ischemic stress. In particular, the slope of the decline of the DC potential seems to be a good indicator for the degree of disturbance. This has also been documented in studies in which negative shifts in DC potentials have been compared with decreases in apparent diffusion coefficients for brain water assessed using diffusion-weighted magnetic resonance imaging, the latter occurring slightly earlier than the shift in the DC potential. Thus, it is not surprising that, in the present study, DC potential recordings provided good correlates of final pathological outcome after intermittent ischemia. The cumulative mean DC potential deflection for all ischemic episodes in the 6 × 10-min group was only half (47%) that of the 1 × 60-min group and approximately two thirds (58%) that of the 3 × 20-min group. The comparison between the 3 × 20-min group and the 6 × 10-min group revealed that complete recovery of the DC potential after successive ischemic episodes could only be achieved after short occlusions. Furthermore, it seemed as though, in the 6 × 10-min group, negative DC potential shifts were attenuated in consecutive occlusions (Fig. 1). One might speculate that this effect is indicative of a yet unknown mechanism of enhancing ischemic tolerance. Because negative shifts in the DC potential reflect anoxic tissue depolarization, they are paralleled by severe depletion of adenosine triphosphate. We assume that only in the 6 × 10-min group did this depletion remain subcritical, with the potential for a full recovery, whereas in other groups, the duration of ischemic episodes was too long. Additionally, the time left for recovery by intermittent reperfusion may have been too short in the case of the 3 × 20-min group, even though arguments have been raised against reperfusion episodes lasting longer than 2 to 5 minutes because of the risk of reperfusion injury.

Assessments of [Ca\(^{2+}\)]
revealed changes similar to those in the DC potential. Three phases of [Ca\(^{2+}\)]
perturbations in response to MCA occlusion were observed: a first small transient increase, followed by a steep decrease, and later a more moderate decrease. These three phases were a little more prominent than those of the DC potential deflections. Cumulative analysis of [Ca\(^{2+}\)], after reperfusion revealed less pronounced recovery compared with the DC potential. Even in the 6 × 10-min group, basal levels were not reached during the observation period, possibly reflecting a rather slow recovery process involved in the transmembrane regulation of this specific ion.

Further studies should be undertaken to elucidate an optimum balance between timing of intermittent occlusion and reperfusion. The DC potential provides a means for optimizing strategies in intermittent ischemia that allow minimization of brain damage. Monitoring this potential might also be performed in combination with more specific measures of underlying mechanisms of ischemic and reperfusion injury, such as determination of [Ca\(^{2+}\)], or other ion activities, and possibly assessments of transmitter imbalances made by using microdialysis combined with high-pressure liquid chromatography. In our view, the DC potential could even prove to be a tool for intra-

![Fig. 3. Bar graph showing infarct volumes recorded 15 hours after the final reperfusion in the three experimental groups. The means ± SDs are plotted, and significant differences between groups are indicated.](image-url)
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operative monitoring, if electrode techniques were to be advanced for this purpose.

Conclusions

The outcome of induced temporary focal ischemia can be considerably improved by interrupting arterial occlusion. The duration of single ischemic episodes is most decisive for the final outcome, with 10 to 20 minutes being the tolerable upper limit. The DC potential provides a reliable measure to monitor this process and, therefore, we suggest that it be used as a tool to optimize intermittent ischemia to achieve minimum neuronal injury.

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References


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