Experimental Focal Ischemia in Cats: Changes in Multimodality Evoked Potentials as Related to Local Cerebral Blood Flow and Ischemic Brain Edema

K. Kataoka, R. Graf, G. Rosner, and W.-D. Heiss

Somatosensory and auditory evoked cortical potentials (SEP's and AEP's), regional cerebral blood flow, regional brain water content, and alteration of the blood–brain barrier were investigated in 3 cortical areas during permanent and 1- and 2-hour transient occlusion of the left middle cerebral artery and after restoration of blood flow in cats. During occlusion, blood flow in the auditory cortex was severely suppressed. In the fore limb projection area of the somatosensory cortex, blood flow was moderately reduced while it was nearly unaffected in the hind limb projection area. Despite different degrees of ischemia in the 3 cortical areas, all evoked responses were completely abolished within 10 minutes after occlusion. During permanent occlusion, the pattern of blood flow reduction persisted, and all evoked potentials stayed abolished. Recirculation after occlusion restored blood flow rapidly. AEP's recovered poorly after both 1 and 2 hours of ischemia. SEP's regained normal amplitudes soon after recirculation in the group with 1-hour occlusion. After 2 hours of ischemia, the recovery of SEP's was variable but better than that of the AEP's. Remarkable water accumulation was observed in the auditory cortex of all 3 groups and was accompanied in the 2-hour ischemia group by a disruption of the blood–brain barrier. In the 2-hour group, water accumulation was also found in the subcortical white matter radiation, whereas significant changes in regional water content were not observed in the somatosensory areas. The present study indicates that abolition of SEP's during middle cerebral artery occlusion in cats is caused by lesions in the afferent pathway leading to cortical deafferentation rather than by cortical ischemia. Restoration of blood supply results in a reactivation of cortical functions in the somatosensory area even after 2 hours of ischemia. The good prognosis of cortical deafferentation may be limited by concurrently developing water accumulation in the subcortical white matter, as documented in the group with 2 hours of transient ischemia. (Stroke 1987;18:188–194).

IN FOCAL ischemia the center, with low residual blood flow, is surrounded by areas with disturbed perfusion of gradually diminishing severity. With respect to this gradient of regional blood flow, other parameters like ion homeostasis, electrical activity, histology, and water content have been investigated in the different ischemic zones.1–5

Studying afferent neuronal functions after occlusion of the left middle cerebral artery (MCA) in cats,6 we found complete abolition of evoked responses despite a gradient of regional cerebral blood flow (rCBF) disturbance among the different areas, ranging from severe suppression to unimpaired circulation. In an area with intact perfusion (hind limb projection area of the primary somatosensory cortex), the spontaneous ECoG and spontaneous single unit activity persisted during ischemia while sensory evoked responses ceased. The functional integrity of this area was also documented by the persistence of transcellularly evoked electrical activity after abolition of sensory potentials. The loss of afferent functions in SH has been interpreted as a deafferentation caused by subcortical ischemia.6

In the mentioned study, restoration of blood flow after a brief ischemic period of 15 minutes resulted in an immediate recovery of afferent functions in areas with only moderately reduced or unimpaired rCBF compared with a slow recovery of evoked responses in areas with severe suppression of blood flow.6 We were therefore interested in the prognosis of afferent functions (multimodality evoked potentials, MEP's) after prolonged transient and permanent ischemia. Local blood flow and 3 kinds of sensory evoked potentials (auditory, AEP; front limb somatosensory, SEP; and hind limb somatosensory, SEPH) were investigated in the corresponding primary cortical areas during 4-hour (permanent) and during and after 1- and 2-hour transient MCA occlusion in cats. Because of the possible deteriorative effects of brain edema development during prolonged ischemia and after revascularization,7 regional brain water content was additionally determined; for examination of the blood–brain barrier (BBB), the permeability for Evans blue was evaluated in the 3 cortical areas.

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Materials and Methods

The experiments were carried out on 25 male cats weighing between 2.0 and 3.7 kg. After i.m. injection of ketamine hydrochloride (25 mg/kg), the left femoral artery and vein were cannulated. Anesthesia was maintained by i.v. injection of α-chloralose (60 mg/kg). After tracheostomy and immobilization with pancuronium bromide (0.5 mg/kg), the animals were artificially ventilated with a mixture of 25–30% oxygen and 70–75% nitrogen. During surgery, nitrogen was replaced by nitrous oxide for analgesia; additionally, supplemental injections of ketamine hydrochloride and topical applications of a lidocaine solution (0.5%) were used. Rectal temperature was kept constant at 37–38°C by means of a temperature-controlled pad. The mean arterial blood pressure was kept between 90 and 130 mm Hg. Arterial blood gas parameters were maintained within normal range (P0₂, 100–120 mm Hg; Pco₂, 27–33 mm Hg). For the measurement of rCBF and cortical evoked responses, 3 platinum-iridium electrodes (etched wires with a diameter of 150 μm, glass-coated to within 1 mm of the tip) were implanted and fixed with dental cement into the left middle ectosylvian gyrus (primary auditory cortex, A), the lateral part of the left posterior sigmoid gyrus (fore limb projection area within the primary somatosensory cortex, SF) and the medial part of the left posterior syngmoid gyrus (hind limb projection area within the primary somatosensory cortex, SH). A common reference electrode was placed in the neck muscle. Under microscopic control, the left MCA was exposed just above the left optic nerve using the translaminar route. Control measurements of rCBF and evoked potentials were performed more than 2 hours after α-chloralose injection. The MCA was occluded near the intracranial carotid bifurcation using a Yasargil miniature aneurysm clip. Animals were chosen for further examination when the left cortical AEP's stayed completely abolished during the first hour after occlusion; the described type of ischemia was denoted as critical focal ischemia. The experimental animals were separated into 4 groups. In 6 animals, the occlusion was permanent, and they were observed 4 hours after the insult. Thirteen other animals were divided into 2 groups, one group with a 1-hour occlusion followed by a 3-hour period of recirculation (n = 6), and another group with a 2-hour occlusion followed by a 2-hour period of recirculation (n = 7). The last group (n = 6) served as controls for brain water content determinations.

rCBF and Cortical Evoked Potentials

Regional CBF was measured by means of the hydrogen clearance technique. The 2 initial minutes of the clearance curves were analyzed and the blood flow values were calculated according to the formula

\[
\text{rCBF (ml/100 g/min)} = 69.3 \times T_{1/2}
\]

The cortical AEP elicited by right ear click stimulation (intensity, supramaximal; duration, 0.1 msec; frequency, 2 Hz) was recorded with the electrodes placed in A. The responses evoked by electrical stimulation of the right median nerve and the right tibial nerve (intensity, supramaximal; duration, 0.1 msec; frequency, 2 Hz) were recorded with the electrodes placed in SF and SH, respectively. Each kind of stimulus was delivered separately to avoid interaction with responses to the other stimuli. After amplification and appropriate filtering (high pass, 1 Hz; low pass, 5,000 Hz), the cortical MEP's were recorded on magnetic tape and averaged off-line (100 sweeps) with a laboratory computer (Minc, Digital Equipment).

Regional Brain Water Content

Thirty minutes before termination of the experiments, a 2% Evans blue solution (2.5 ml/kg) was injected i.v. to determine alterations of the BBB. After inducing cardiac arrest by means of an i.v. injection of saturated potassium hydrochloride solution, the cranium was immediately removed and brain tissue samples (80–150 mg) were obtained and stored in preweighed plastic test tubes. The samples were dried for 24 hours at 95°C. The brain tissue water content was evaluated as percent of total weight. Control measurements were obtained from 6 sham-operated cats.

Results

All kinds of cortical responses evoked by the different stimulus modalities were altered in peak latency and in peak-to-peak amplitude by chloralose compared with halothane anesthesia. In Figure 1, typical examples of the different responses from various cortical areas are shown. In 5 of the experimental animals, poststimulus latencies and peak-to-peak differences were evaluated before and 1 hour after chloralose injection (Table 1). The uniform shape of the chloralose-influenced potentials allowed us to easily discriminate primary cortical response amplitudes by evaluating peak-to-peak differences between the positive (P) and negative (N) peaks of the different potentials.

Before occlusion, the mean amplitude of the primary cortical AEP was 440 ± 235 μV (mean ± SD of all investigated animals). The two primary cortical SEP's amounted to 980 ± 440 μV in SF (SEPf) and 490 ± 220 μV in SH (SEPH). The mean values of rCBF prior to occlusion were 68 ± 21, 82 ± 24, and 61 ± 15 ml/100 g/min (mean ± SD of all investigated animals) in A, SF, and SH, respectively.

Effects on rCBF and MEP's

Changes of evoked responses and rCBF during MCA occlusion of different duration and during recirculation are summarized in Figure 2. In A, rCBF was markedly reduced during occlusion. A mild CBF reduction to mean values between 30 and 40 ml/100 g/min was observed in SF, while cortical blood flow was insignificantly affected in SH. Removal of the clips restored the blood flow to the ischemic region. Postischemic hyperperfusion was observed in A and SF. Hyperperfusion reached higher values in the 1-hour occlusion group. Despite the different blood flow
disturbances in the 3 examined areas, all evoked responses were homogeneously suppressed during occlusion. Depending on the cortical area and the duration of occlusion, however, differences in recovery of the evoked responses were found.

In the 1-hour occlusion group, AEP's recovered only poorly, whereas SEPF's recovered well, and SEPH's were completely restored. In the 2-hour group, 90 minutes after recirculation the recovery of both SEPH's was significantly (*p < 0.01, t test) smaller than in the 1-hour group. However, the SEP's showed significantly (*p < 0.001) better recovery than the AEP.

Effects on BBB and Regional Brain Water Content

In both control and permanent-occlusion animals, no extravasation of Evans blue was found. In the 1-hour occlusion group, only 1 of 6 animals showed remarkable staining in the left A. On the other hand, in 5 of the 7 animals of the 2-hour ischemia group, extravasation of Evans blue occurred in A and, in 1 of these, the staining extended to SF.

The water content in the left A was significantly increased in all experimental groups compared with controls, the 1-hour ischemia group being less affected than the other two groups (Table 2). Moreover, the water content of cortical areas with BBB disruption amounted to 84.4 ± 1.0% (mean ± SD), which is significantly (*p < 0.01, t test) higher than that of the left A (82.4 ± 1.4%) in the permanent-occlusion group. Of the other investigated regions, significant increases in water content were observed only in the left white matter radiation in the 2-hour occlusion group (*p < 0.05). The comparison between residual rCBF and brain water content showed that an increase in water content occurs only below a critical level (< 15 ml/100 g/min) (Figure 3). From the diagram of the relation between brain water content and evoked potentials, it is evident (Figure 4) that evoked responses exhibited a good recovery as long as water content remained in the normal range. Only when water content was raised above 81% were evoked potentials severely suppressed after reperfusion.

Table 1. Chloralose Influence on Poststimulus Peak Latencies and Peak-to-Peak Amplitudes of MEP's in 5 Cats

<table>
<thead>
<tr>
<th>Area</th>
<th>Anesthetic</th>
<th>Poststimulus peak latencies (msec)</th>
<th>Peak-to-peak amplitudes (μV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P1</td>
<td>N1</td>
</tr>
<tr>
<td>SH</td>
<td>Halothane</td>
<td>11.2±0.5</td>
<td>16.7±1.5</td>
</tr>
<tr>
<td></td>
<td>Chloralose</td>
<td>11.2±0.7</td>
<td>19.9±1.4*</td>
</tr>
<tr>
<td>SF</td>
<td>Halothane</td>
<td>7.1±0.6</td>
<td>11.0±1.1</td>
</tr>
<tr>
<td></td>
<td>Chloralose</td>
<td>7.5±0.5</td>
<td>13.2±0.8*</td>
</tr>
<tr>
<td>A</td>
<td>Halothane</td>
<td>7.6±0.8</td>
<td>13.9±1.4</td>
</tr>
<tr>
<td></td>
<td>Chloralose</td>
<td>8.9±0.9</td>
<td>16.3±1.1*</td>
</tr>
</tbody>
</table>

Abbreviations as in Figure 1; values are mean ± SD.

*Significantly different from mean values obtained under halothane anesthesia (*p < 0.01, t test).
**Figure 2.** rCBF and MEP amplitudes during MCA occlusion of different duration (stippled) and after recirculation. Mean values (± SD) from all animals belonging to the different experimental groups are shown: 1-hour occlusion group, n = 6; 2-hour occlusion group, n = 7; permanent occlusion group, n = 6. Abbreviations as in Figure 1.

**Table 2.** Regional Brain Water Content of Various Brain Regions in 3 Different Experimental Groups 4 Hours after MCA Occlusion and of Sham-Operated Controls

<table>
<thead>
<tr>
<th>Brain water content (% total wt)</th>
<th>A</th>
<th>SF</th>
<th>SH</th>
<th>Thalamus</th>
<th>White matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sham-operated</td>
<td>79.5 ± 0.6</td>
<td>79.3 ± 0.7</td>
<td>79.3 ± 0.8</td>
<td>77.0 ± 0.9</td>
<td>67.5 ± 1.0</td>
</tr>
<tr>
<td>Permanent occlusion (4 hours)</td>
<td>83.4 ± 1.4*</td>
<td>79.6 ± 1.3</td>
<td>79.6 ± 0.6</td>
<td>77.7 ± 1.4</td>
<td>69.0 ± 1.8</td>
</tr>
<tr>
<td>Transient occlusion (1 hour)</td>
<td>81.4 ± 1.3†</td>
<td>79.8 ± 0.8</td>
<td>79.9 ± 0.5</td>
<td>76.7 ± 0.7</td>
<td>68.2 ± 1.0</td>
</tr>
<tr>
<td>Transient occlusion (2 hours)</td>
<td>83.7 ± 1.5‡</td>
<td>80.4 ± 1.9</td>
<td>79.8 ± 0.8</td>
<td>76.7 ± 1.0</td>
<td>69.9 ± 2.1†</td>
</tr>
</tbody>
</table>

Abbreviations as in Figure 1; values are mean ± SD.
*†‡Significantly different from controls at p < 0.01, p < 0.05, and p < 0.001, respectively.
Discussion

Methodological Considerations

A direct correlation of local CBF and electrical neuronal activity can be achieved by using the same platinum electrode. The hydrogen clearance curve is simply obtained by appropriate filtering.\(^1\)\(^-\)\(^3\) Depth recordings result in a polarity change of cortical evoked responses compared with surface recordings.\(^4\) Considering the MEP's, a more uniform shape with a two- to fourfold increase in amplitude was achieved in all responses 1 hour after injection of a-chloralose.\(^5\) N\(_1\), has been interpreted to be of primary cortical origin.\(^6\)\(^-\)\(^7\) In another series of experiments, we had measured single unit activity in the described cortical areas at latencies in the time range of N\(_1\).\(^5\) Therefore, the difference between P\(_1\) and N\(_1\), was taken in this study to be the amplitude of the primary cortical sensory response. Latency shifts were not calculated because of the high variability after the onset of ischemia, which has also been described by other authors.\(^8\)

The experiments with total suppression of the AEP, accompanied by a low residual CBF and an increase in water content in A, were comparable with the critical ischemia group described by Hossmann and Schuier.\(^2\)\(^,\)\(^3\) Only experiments with this type of ischemia were used for further analysis; experiments with spontaneous recovery of the AEP and a high residual CBF in A were eliminated. With respect to the MCA occlusion model in cats, its high variability due to individual differences in the development of collateral circulation has been described.\(^1\)\(^,\)\(^2\)\(^,\)\(^9\)\(^-\)\(^1\)\(^0\)

Effects of Permanent Ischemia on rCBF and MEP's

Experimental MCA occlusion causes neurological symptoms that derive from direct cortical and/or subcortical ischemic damage.\(^2\)\(^1\)\(^-\)\(^2\)\(^3\) The abolition of AEP's in A found in the present study was caused by the severe suppression of rCBF in A. In contrast, the abolition of the two SEP's can be explained only by other than direct cortical effects because no (in SH) or only mild (in SF) alterations of rCBF were found. Evidence for the hypothesis of subcortical deafferentation causing the abolition of SEP's was provided by the fact that spontaneous neuronal activity remained in the somatosensory areas, and that it was even possible to evoke responses in one of the areas (SH) transcortically by stimulation of the contralateral hemisphere.\(^6\) Preliminary experiments have additionally shown that SEP's in the thalamic somatosensory relay nucleus (the ven-
central posterolateral nucleus) remained unchanged, while cortical responses ceased.

The present study showed a persisting gradation of residual rCBF after occlusion of the MCA over periods of up to 4 hours. Despite this gradation, no spontaneous recovery of sensory evoked cortical potentials was observed during the whole period. The water content measurements supported the hypothesis of cortical and/or subcortical damage causing cortical deactivation. In the group with permanent ischemia, the highest water accumulation was found in the ischemic center (A) followed by that in the subcortical white matter radiation. In contrast, very little or no increase in water content was found in SH and in the thalamus.

Effects of Blood Flow Restoration

It was documented that restoration of blood flow to an area with severe 1- and 2-hour ischemia, like A, failed to reestablish integrated neuroaxial functions. In global ischemia, Carter and colleagues24,25 have shown that at CBF<10 ml/100 g/min, the time limits for recovery of direct cortical responses were in the range of 14–50 minutes. Histological infarction was found after 2 hours of transient focal ischemia, with a residual CBF of approximately 10–15 ml/100 g/min.20,26,27

It has been shown, however, that even after 2 hours of transient ischemia, a recovery of SEP’s is possible. This better prognosis might be related to a higher tolerance of the subcortical white matter to ischemia. A higher resistance of white matter compared with gray matter structures was also described for the development of pathologic lesions27 and for recovery of neurologic symptoms26 in the monkey MCA occlusion model.

Clinical revascularization in acute ischemic stroke might be effective within 5–6 hours after the insult, despite variable results with regard to survival and functional recovery.28-31 Our finding of a relatively good prognosis for cortical deactivation caused by deafferentation may explain this variable effectiveness.

With regard to brain edema formation, restoration of CBF after 2 hours of ischemia resulted in a remarkable water accumulation in the ischemic center (A) accompanied by a disruption of the BBB. Schuier and Hossmann32 have shown significant increases in cortical water content in cats after 2 hours of critical ischemia produced by MCA occlusion. Once water accumulation has started, restoration of CBF to the ischemic cortex causes deterioration of edema with disruption of the BBB as observed in other previous reports.20,32 Ischemic cerebral edema leads to a more serious state when it expands rapidly and causes transtentorial herniation. In a considerable number of ischemic stroke patients, such an ischemic brain edema causes clinical deterioration or death.33 With regard to the surgical treatment of ischemic patients, the restoration of CBF to the ischemic brain may result in a deterioration of edema as well as in neurofunctional recovery within the peripheral zones of the ischemic focus as shown in our study.

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**KEY WORDS** • brain water content • cat • cerebral blood flow • deafferentation • focal cerebral ischemia • multi-modality evoked potential
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