Cerebral Blood Flows and Tissue Oxygen Levels Associated with Maintenance of the Somatosensory Evoked Potential and Cortical Neuronal Activity in Focal Ischemia

Philip E. Coyer, John J. Michele, James E. Lesnick, and Frederick A. Simeone

The middle cerebral artery was occluded in 18 cats to evaluate the physiological consequences of cerebral blood flow reductions on the somatosensory evoked potential, spontaneous neuronal activity, and oxygen availability in the ipsilateral and contralateral hemispheres. In the ipsilateral ectosylvian gyrus high-grade ischemia was produced as blood flow in the gray matter was reduced from $52.1 \pm 8.6$ (X ± SE) to $13.3 \pm 9.0$ ml/100 g/min and in the white matter from $33.8 \pm 5.6$ to $6.1 \pm 6.4$ ml/100 g/min. This significant reduction ($p<0.05$) was associated with abolition of the cortical component of the somatosensory evoked potentials. In all animals occlusion resulted in a predictable extended latency change and a variable amplitude response of the cortical component of the contralaterally recorded somatosensory evoked potentials. In 5 animals, oxygen availability was measured and spontaneous neuronal activity in the contralateral hemisphere was recorded. Volume expansion and hemodilution with either dextran or saline infusions elevated cerebral blood flow in the contralateral gray matter significantly ($p < 0.05$) compared with the control and clip values. Ipsilateral spontaneous activity stopped within 4–12 minutes of occlusion, while contralateral spike activity persisted at rates at least equal to those recorded immediately following occlusion. (Stroke 1987;18:77-84)

PHYSIOLOGICAL monitoring of the events following middle cerebral artery (MCA) occlusion in cats has been used to describe ischemic and nonischemic brain areas.\textsuperscript{1-3} Measurements of cerebral blood flow (CBF) and the metabolic rate for glucose (CMR-glu) vary over the ischemic and nonischemic brain regions as determined by the nature of the blood supply to these territories.\textsuperscript{1} On restoration of blood flow through the MCA, measurements of CBF and CMR-glu are less likely to predict the animal's neurological outcome than are biochemical determinations of such factors as adenosine triphosphate (ATP) and the somatosensory evoked potential (SEP) during reperfusion.\textsuperscript{3,5}

Electrophysiological measurements of extracellular $K^+$, the EEG, and the SEP can be used to characterize an area of near-threshold CBF for electrical failure. This borderline ischemic zone is the penumbra.\textsuperscript{4} Within this region, extracellular $K^+$ accumulates as CBF is reduced below 15 ml/100 g/min\textsuperscript{7} and the SEP undergoes changes in its latency and amplitude\textsuperscript{7-10} while the EEG is only slightly diminished in amplitude.\textsuperscript{7}

In models of focal ischemia (MCA occlusion), the ischemic penumbra of the cat brain includes the suprasylvian and marginal gyri\textsuperscript{7,11} and the ischemic core area involves the anterior edge of the ectosylvian gyrus.\textsuperscript{12} This area generates a secondary cortical representation of the SEP,\textsuperscript{13,14} as we have shown by electrocauterization.\textsuperscript{12} This configuration is sensitive to high-grade ischemia following MCA occlusion. In our attempts to elevate subthreshold CBF in the ipsilateral ectosylvian gyrus with saline or dextran infusions, we also monitored the contralateral CBF and observed changes in the SEP as in other studies of cerebral metabolism and the evoked potential.\textsuperscript{11,13,16} The purpose of these experiments was to describe the relations between the cortical oxygen availability and spontaneous neuronal activity and the amplitudes and latencies of the cortical component of the SEP. A preliminary report of this work appears in abstract form.\textsuperscript{47}

Materials and Methods

Eighteen adult cats of either sex ranging from 2.5 to 4.0 kg were used in these studies. Physiological monitoring of CBF, spontaneous neuronal activity, and oxygen availability were obtained from 10 of these animals. These electrophysiological determinations were made in the ipsilateral and contralateral hemispheres in 2 groups of 5 animals each. CBF data was collected for the other 8 animals for comparison of hemodilution and volume expansion treatments.

Surgical Preparation and Protocol. Animals were premedicated with atropine (0.03 mg/kg) and ketamine (35 mg/kg) through intramuscular injection. Standard surgical technique was used to catheterize a femoral artery for blood pressure monitoring and routine sampling of the arterial blood gases. All other...
preliminary procedures for physiological monitoring have been described by us in another paper.\textsuperscript{12}

MCA exposure was performed in 18 cats using a retro-orbital approach.\textsuperscript{18} Animals were then placed in a stereotactic frame, and a $1 \times 2$-cm craniectomy was made over each hemisphere. The dura was opened widely for placement of CBF and oxygen electrodes. Data was collected for control, MCA clip, and treatment conditions. Volume expansion and hemodilution were achieved with either 1 or 2 saline or dextran infusions (10–20 ml/kg) over a period of 2 hours to attain an approximately 30% reduction in hematocrit. Throughout the experiments, the animals' blood gases were monitored with an Instrumentation Laboratory Blood Gas Analyzer (Model 213). The $P_{O_2}$ values measured at $37^\circ C$ were well above 95 torr, and the pH was 7.35–7.45. The respiration rate and tidal volume were adjusted to maintain $P_{CO_2}$ at 35 ± 5 torr. Mean arterial pressures were 120–140 mm Hg, and the animals' temperatures as measured rectally were maintained at $37 \pm 2^\circ C$ with a heating blanket.

Somatosensory evoked potential monitoring and CBF measurements. SEP's were determined as described previously.\textsuperscript{12} CBF determinations were made in white and gray matter using the hydrogen clearance technique.\textsuperscript{19} CBF electrodes were stereotactically implanted into the gray and white matters according to the method of Lesnick et al.\textsuperscript{19} In several animals, a postmortem examination of the brain showed the pathways of electrodes in gray and white matters. SEP's were recorded from both hemispheres by placing bone screw electrodes across the somatosensory cortex and stimulating the median nerve to elicit a paw twitch. One member of the bipolar array was placed 1 cm lateral to the midline and approximately 5 mm anterior to the coronal suture while the reference was placed into the frontal sinus. This procedure was carried out on both sides of the cranium. SEP's were recorded and the signals were averaged through a delay circuit arising from the stimulator. Signals were averaged by analog-to-digital conversion of 256 successive sweeps of the EEG. The waveform resulting from this procedure was displayed on an Apple II Plus computer programmed to act as a digital oscilloscope through the use of a cursor. The reference wave was the stimulus artifact that occurs with a 4-msec delay after triggering the signal averager through the stimulator. A 50-μV, 100-Hz square pulse of known offset voltage applied to the probes of the EEG amplifiers allowed reading of the polarity and amplitudes of all waves lying within the evoked potential. Amplitudes and latencies were read to the nearest whole microvolt and to tenths of a millisecond, respectively. The evoked potentials of 5 additional animals were monitored over the duration of a typical experiment to determine whether there were systematic changes in the cortical wave latency with time.

Oxygen availability and spontaneous unit activity determinations. Platinum oxygen-sensing microelectrodes were purchased from an individual supplier. A current-to-voltage amplifier with adjustable feedback compensation was used for polarographic oxygen determinations in brain tissue. To monitor multiple unit action potentials, the output of these microelectrodes was capacity-coupled, a modification developed by others.\textsuperscript{20,21} The DC output of the microelectrodes reflected the polarographic oxygen current, which registered through a current-to-voltage converter as a voltage under polarization at $-650$ mV. The oxygen microelectrode was the cathode, and the silver–silver chloride reference inserted into the temporalis muscle was the anode. Action potentials resulting from the AC output were displayed on a Tektronix D10 dual beam oscilloscope to measure the time for peak deflection and were recorded on magnetic tape. At the termination of an experiment, multiunit action potentials were converted to pulses using a spike detector. These pulses were displayed as frequency–time histograms on an Apple II Plus computer using a program supplied by Biomedical Design Products (Cherry Hill, N.J.). Spike frequencies were recorded for 1-minute epochs for at least 10 minutes of control, 8–40 minutes of MCA clipping, and 2 hours of volume expansion and hemodilution during MCA occlusion.

Data analysis. Data were obtained for the rate of spontaneous neuronal activity, the latency and amplitude of the cortical component of the SEP, the oxygen availability, and the CBF of 18 cats. All values are expressed as mean ± SD. The spontaneous rate of neuron spiking is expressed as Hz, and the latencies and amplitudes of the cortical component of the SEP as percent of control. Oxygen availability is expressed as percent of the maximum observed tissue $P_{O_2}$, from calibration of the voltage output of the oxygen microelectrode to known concentrations of oxygen. Unpaired $t$ tests were used to compare % oxygen availability in the ipsilateral and contralateral hemispheres (Table 1). Standard error was calculated for the percent of control CBF based on 2 samples (Figure 2). Paired $t$ tests were used to compare gray and white matter CBF after MCA clipping and treatments with control values.

Results

Eighteen cats underwent left MCA occlusion as described above. Spontaneously occurring extracellular activity (population spikes) and oxygen availability were monitored in the ectosylvian gyri in 10 animals, 5

Table 1. Percent Tissue Oxygen availabilities in the Contralateral and Ipsilateral Cerebral Hemispheres Under Control, MCA Occlusion, and Treatment Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percent of control tissue oxygen availability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contralateral hemisphere ($n = 5$)</td>
</tr>
<tr>
<td>Control</td>
<td>100%</td>
</tr>
<tr>
<td>MCA occlusion</td>
<td>$80.1 \pm 22.5$</td>
</tr>
<tr>
<td>Treatment</td>
<td>$95.0 \pm 35.6$</td>
</tr>
</tbody>
</table>

Mean ± SD calculated from 5 cats for each hemisphere.

*Significantly less than the contralateral hemisphere percent oxygen availability; results of unpaired $t$ test.
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RIGHT HEMISPHERE

12.3 msec.

cbf α 29/100 gm/min

50 μV

UNIT ACTIVITY

1 min.

100 μV

PO2 (TORR)

15 sec.

5 msec.

cbf α 0

LEFT HEMISPHERE

12.3 msec.

COUNTS

50 μV

0 10 20 30 40 50

EPOCHS

FIGURE 1. Data collection method for white matter CBF (right and left hemispheres with MCA clip on the left MCA). Flows are reported for white matter electrodes only, and hydrogen clearance curves were reproduced from the originals. No gray matter curves are shown, but CBF was reduced from 52 to 10 ml/100 gm/min on the average. SEP's generated through contralateral median nerve stimulation appear above records of hydrogen clearance curves. Records of spontaneously occurring extracellular action potentials (population spikes) and oxygen availabilities from the ectosylvian gyrus appear on the left. Histogram analysis of neuronal spikes for 1 animal before and after MCA clip was placed ipsilaterally to the recording electrode appears in the lower right. Note the reduction in spike rate over the period of MCA occlusion following 10 minutes of control measurement. Records of population spikes were retouched for clarity, but the EEG trace was not.

with recordings from the hemisphere ipsilateral to the MCA clip, and 5 from the contralateral hemisphere. For technical reasons, only 6 or 7 of these animals (Figures 4 and 5) had concurrent measurements of CBF, oxygen availability, and SEP. Examples of the SEP waveforms, hydrogen clearance flow curves, spontaneous activity, EEG, and oxygen availability recordings are shown in Figure 1.

EFFECT OF MCA OCCLUSION ON CBF AND SEP. Consistent with our previous results, clipping of the left MCA in 18 cats produced a high degree of ischemia in the ipsilateral hemisphere. Significant reductions (p<0.05) in CBF from 52.1 ± 8.6 in the gray and 33.8 ± 5.6 in the white matter to 13.3 ± 9.0 and 6.1 ± 6.4 ml/100 g/min respectively were observed. No significant elevations above these values were achieved following hemodilution with either dextran (n = 9) or saline (n = 9). There was no difference between the saline and dextran treatments (p<0.05).

The control CBF values on the contralateral side were 52.3 ± 7.6 and 31.3 ± 6.1 ml/100 g/min for gray and white matter respectively. Gray matter blood flow was elevated significantly above control and clip values through hemodilution and volume expansion to 60.6 ± 8.1 ml/100 g/min. The clip gray matter blood flow was 47.6 ± 9.5 ml/100 g/min. White matter blood flow in the contralateral side was not significantly raised or lowered during MCA clipping or treatment in comparison to the control value (Figure 2).

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As we have reported previously, the reduction in CBF in the ipsilateral hemisphere was associated with abolition of the cortical component of the SEP in that hemisphere. Occlusion of the MCA also resulted in reduced amplitude and extended latency of the cortical component of the contralateral SEP. In the 5 control animals whose SEP's were monitored over time, no systematic change in the cortical wave latency was observed. The change in the SEP configuration for 1 animal during control and following MCA occlusion and treatment is shown in Figure 3. Latencies were predictably lengthened following contralateral MCA occlusion, but the amplitude response was variable and subject to change immediately after MCA occlusion (Figure 3, trace B) and about one-half hour following application of the clip (Figure 4, closed triangle for #89). Changes in the latencies of the cortical component were less subject to variability with regard to time than the amplitude responses. Alteration in the SEP cortical wave coincided with no change in gray matter blood flow and a decrease in oxygen availability in 5 of 7 animals (58, 63, 72, 88, and 89; Figure 4). Latencies from the onset of stimulation to the occurrence of the cortical component of the SEP were prolonged by at least 5% (p<0.05; paired t test), which is consistent
FIGURE 2. Bar graph representation of the ipsilateral (A) and contralateral (B) CBF during control, MCA occlusion, and treatment procedures. Note the significant decrease in gray and white matter blood flows in the hemisphere ipsilateral to the MCA clip indicated by the asterisks above open (white matter) and shaded (gray matter) bars. In the contralateral hemisphere there was a significant elevation of gray matter blood flow following hemodilution and volume expansion (treatment) indicated by the asterisk above the shaded bar. All CBF values are expressed as a percent ± SEM, vertical bars for 2 samples of the control (100%) white and gray matter CBF (NS = not significant, p < 0.05).

with earlier findings. After treatment the amplitudes of all animals' cortical components increased above control conditions, except for animal 88. This is evident in Figure 5, in which most of the solid triangles appear above the line corresponding to 100%. Latencies of the cortical component of the SEP also returned to control as blood flow in the contralateral gray matter was significantly raised (p < 0.05) above control and clip values, as shown in Figure 2B. In 2 animals (88 and 58), the oxygen availability remained unchanged. Animal 72 died before the treatment procedure, and consequently no data appears for it in Figure 5.

Effect of MCA Occlusion on Spontaneous Neuronal Activity and Oxygen Availability. Extracellular neuronal activity and tissue oxygen availability were monitored in 10 animals. Data were collected from the ipsilateral hemisphere in 5 animals and from the contralateral hemisphere in 5 animals. The pre- and postclip action potential frequencies are summarized in Figure 6. Each point represents the mean ± SD for a particular animal. Postocclusion neuronal firing in the contralateral hemisphere was depressed as indicated by the 4 points close to the abscissa. Preocclusion rates for the contralateral hemisphere registered 2–10 Hz while the postocclusion rates approached 1 Hz. Figure 6 also shows the results of action potential generation for the ipsilateral hemisphere following MCA occlusion. Although the rates were variable in the ipsilateral hemisphere, there was no tendency toward depression as in the contralateral hemisphere. In Figure 7, the posttreatment rate of action potential generation was nearly identical with postocclusion rate in the contra-

data summarizing oxygen availability in both hemispheres are found in Table 1. Each animal serves as its own control, and percent oxygen availability is the mean of the 5 animals in each group. There were no significant differences between the oxygen availabilities for postocclusion vs. posttreatment conditions within either hemisphere (p > 0.05; paired t test). In addition, no significant difference in oxygen availabil-

FIGURE 3. Sequential changes in configuration of the cortical component of the SEP recorded from the contralateral hemisphere of Cat 89 under control (A), MCA clip (B), and treatment (C and D) conditions. These SEP waveforms were redrawn from the computer tracing. Amplitudes and latencies were measured as described in "Materials and Methods" and were not subject to analysis by hand. Note the amplitude reduction and latency extension of the cortical component following MCA occlusion (B). These changes in configuration of the SEP were reversed with volume expansion and hemodilution as CBF in the gray matter was elevated from a clip value of 45 to a treatment one of 60 ml/100 g/min. Responses of the contralaterally recorded SEP's for other animals appear in Figures 4 and 5.
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Figure 4. Percent of control SEP cortical wave latency and amplitude and oxygen availability in the contralateral ectosylvian gyrus following MCA occlusion for animals 45, 58, 61, 63, 72, 88, and 89. Following MCA clipping, oxygen availability in 5 of 7 animals dropped below 100%. Amplitude responses (▲) were variable. The latency (●) of the cortical component for each animal was significantly extended by at least 5% (p<0.05, paired t test). A triangle in a circle, appearing at the intersection of the dotted lines, represents a normalized latency and amplitude value of 100%, the control for each animal.

Discussion

Three main conclusions can be made from our studies on the effects of MCA clipping on CBF and electrical activities recorded from the ectosylvian gyrus of the cat brain (both ipsilateral and contralateral hemispheres). First, in the model described by others, whole-hemisphere CBF measurements document high grade ischemia in the ectosylvian gyrus for the time during which we monitored it (about 2–3 hours). This finding also agrees with data on serial whole-brain CBF determinations in the marginal, suprasylvian, and ectosylvian gyri, and static measurements of CMR-glu during MCA occlusion. Second, MCA occlusion produced no significant changes in contralateral hemisphere gray and white matter blood flows. Volume expansion and hemodilution provided a means of significantly elevating CBF in the gray matter above control and clip values. There was a return of the latencies of the cortical component of the SEP in the contralateral hemisphere to control durations (Figures 3 and 5) associated with this increase in CBF. No systematic changes in cortical wave latencies or CBF were detected in the 5 control animals which experienced neither MCA occlusion nor volume expansion and hemodilution. Third, spontaneous neuronal activity was variable in both hemispheres, but unlike in the ipsilateral hemisphere where the spike rate was almost completely abolished in 4 of 5 animals within 4–12 minutes of MCA occlusion, neuronal activity in the contralateral hemisphere persisted although at lower...
rates in most cases (Figure 7; open circles). The long term changes observed in neuronal activity and oxygen availability of the contralateral hemisphere agree with that caused by a depression and subsequent return of the cortical component of the SEP as blood flow in the gray matter was augmented. Variations in the SEP and spontaneous neuronal generation responses of both hemispheres will be discussed later.

**CBF Reduction and Abolition of the Cortical Component of the SEP.** In the ipsilateral ectosylvian gyrus after MCA occlusion, failure to maintain the control rate of spontaneous activity agreed with abolition of the cortical component of the SEP and was associated with a decrease in oxygen availability. Cessation of spontaneous activity with ischemia has been reported by other investigators.222324

**CBF Elevation and Discrete Changes in the SEP.** Gray matter blood flows in the contralateral hemisphere responded to volume expansion and hemodilution following MCA clipping. An increase in the latencies and a decrease in the amplitudes of the cortical components of the SEP's occurred in the contralateral hemisphere (Figures 3–5). Expansion of blood volume resulted in significant elevations in the gray matter blood flow (Figure 2). As discussed in “Results,” there was no significant improvement in oxygen availability in the contralateral hemisphere between the time of MCA clipping and treatment. Significant differences in the oxygen availabilities of the ipsilateral and contralateral hemispheres were noted following treatment (see Table 1 and “Results”).

**Relation Between Spontaneous Neuronal Activities, Configuration of the SEP, and Tissue Oxygen Availability in Both Hemispheres.** Although the levels of spontaneous activity and tissue oxygen availability were variable within and between animals, SEP cortical wave latencies were much more uniform as seen in Figures 4 and 5. In only 2 cases was there enhancement of SEP cortical wave amplitudes associated with increases in the action potential frequency and oxygen availability in the contralateral hemisphere. In animals with no return of the cortical wave in the ipsilateral hemisphere, action potentials and oxygen availabilities were severely diminished (Figures 6 and 7, closed circles; Table 1).

In comparing the levels of spontaneous neuronal activity in both hemispheres with changes in the configuration of the SEP, continuation of the cortical component of the SEP and return of its amplitude and latency to control levels during volume expansion agree with the observed maintenance of neuronal activities in the contralateral hemisphere. In the ipsilateral hemisphere, neuronal activity persisted after MCA occlusion, but it was much depressed compared...
with that in the contralateral side. In 3 cases there were transient increases in the rate of action potential generation in the ipsilateral hemisphere (closed circles above the line of identity in Figure 6). One may ask how to explain this observation with respect to the lack of the SEP cortical component in the ipsilateral hemisphere after MCA occlusion. Perhaps these results can be linked to failure of the ipsilateral white matter to conduct the thalamically generated impulses to the cortex causing loss of the cortical component, while the gray matter (site of spontaneous action potential generation) reacts to hypoxia (Table 1). In the contralateral hemisphere, there is an immediate increase in the latency of the cortical component of the SEP along with a reduction in the amplitude of the cortical wave (Figure 3). Subsequent maintenance of action potential generation in this hemisphere after MCA occlusion and during treatment agree with the observed changes in the configuration of the SEP. No significant reductions in oxygen availability, which could trigger transient increases in rates of action potential generation linked to hypoxia, were observed. Maintenance of oxygen availability in the contralateral hemisphere may explain the coupling of action potential frequency in this side and the apparent uncoupling of action potential frequencies in the ipsilateral side. After hemorrhage and volume expansion, there was a significant reduction in oxygen availability in the ipsilateral hemisphere compared with the contralateral one (Table 1) and a depression in the action potential frequency from the transient increases noted following MCA occlusion (Figures 6 and 7, closed circles).

In conclusion: The SEP has a CBF threshold for maintenance of the cortical component's normal amplitude and latency. Changes in the amplitude and latency of the SEP in response to increases in CBF and Po2 after temporary occlusion and release of the MCA have been observed in baboons. Electrical failure occurs at CBF of 6–15 ml/100 g/min. This range is more predictable for maintenance of the SEP's normal integrity than for occurrence of spontaneous neuronal activity (this paper). Our results suggest that neuronal activity persists as long as there is oxygen to support cellular metabolism and agree with what others have predicted from a theoretical calculation of the cortical oxygen uptake, given flow and cortical arterial–venous differences. The continuation of spontaneous activity, which we observed even in the hemisphere ipsilateral to the MCA clip, occurred in most instances for short durations, and demonstrates different thresholds to reduced CBF of the mass electrical response (SEP) and individual neuronal activities. In studies of MCA occlusion, the observed changes in neuronal activity relative to the SEP configuration are represented in both hemispheres to varying degrees. Moreover, the observed uncoupling of electrical activities in the ischemic hemisphere (transient increases in action potential generation during subsequent loss of the SEP) may reflect the resumption of normal metabolism in the cortical area (site of action potential generation) where CBF is depressed. The uncoupling of blood flow and metabolism has been observed in other models of stroke and may be concomitant with brain ischemia.

With regard to the variable amplitude responses of the SEP in the contralateral hemisphere, in patients with unilateral vascular lesions the affected hemisphere may give rise to higher amplitudes of the evoked response than the unaffected hemisphere. In our animal experiments, the latency increase in the evoked response was more predictable than the amplitude changes in the unaffected hemisphere after MCA occlusion. Perhaps these disparate findings of amplitude and latency changes in clinical and experimental settings point out the need for further evaluation of the SEP in monitoring cerebrovascular insults.

Acknowledgment

The authors wish to thank Richard M. Martin, Ph.D., of the Department of Neurology, the University of Alabama in Birmingham Medical Center, Birmingham, Alabama, for supplying the oxygen microelectrodes used in this study.

References


**Key Words**  • cerebral blood flow (CBF)  • somatosensory evoked potential (SEP)  • focal ischemia  • neuronal activity  • tissue oxygen availability  • hydrogen clearance
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*Stroke*. 1987;18:77-84
doi: 10.1161/01.STR.18.1.77

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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