Failure of the Somatosensory Evoked Potential Following Middle Cerebral Artery Occlusion and High-Grade Ischemia in the Cat — Effects of Hemodilution

Philip E. Coyer, Ph.D.,* James E. Lesnick, M.D.,† John J. Michele, B.S.,* and Frederick A. Simeone, M.D.*

SUMMARY Acute focal ischemia was created in 10 cats by unilateral retro-orbital middle cerebral artery (MCA) occlusion. Regional cerebral blood flow (CBF) was determined utilizing the hydrogen clearance technique from electrode recordings within the gray matter and white matter of the ectosylvian gyrus of both hemispheres. The somatosensory evoked potential (SSEP) was obtained during contralateral median nerve stimulation. When the MCA was clipped the white and gray matter blood flows in the ipsilateral ectosylvian gyrus were reduced to $14.8 \pm 19.6\%$ and $19.3 \pm 23.7\%$ of control, and the cortical component of the SSEP was abolished. In the contralateral hemisphere an average increase of $3.5\%$ above the control latency and a $10\%$ mean depression in the amplitude of the cortical component of the SSEP was observed following occlusion. CBF in the contralateral hemisphere was unaffected by the MCA clip. Infusion of saline or dextran to lower the hematocrit by approximately $45\%$ did not significantly improve blood flow or restore the SSEP in the hemisphere ipsilateral to the MCA clip. However, significant increases in the contralateral hemisphere gray matter CBF occurred following hemodilution while the latency of the cortical component of the SSEP in this same hemisphere was significantly extended. Elevations in gray and white matter blood flows were achieved in the experimental hemisphere of 3 of 10 cats suggesting a wide range of variation in the collateral circulation.

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Materials and Methods

Ten adult cats of either sex ranging in weight from 2.5 to 4.0 kg were used in these studies.

Surgical Preparation

Following premedication with atropine (0.03 mg/kg) and ketamine (25–35 mg/kg) through intramuscular injection, standard surgical technique was used to catheterize the femoral artery for blood pressure monitoring and routine sampling of the arterial blood gases. An intravenous line was also introduced into the animals' forelimbs for administration of saline, dextran 40, or pancuronium bromide (0.05 mg/kg).

After the initial preparation, the animals were moved to an electrostatically-shielded laboratory where they were first intubated and placed on a respirator breathing a 3:1 nitrous oxide/oxygen mixture. Before muscle paralysis, bipolar stimulating electrodes were placed into the median nerve region of both forelimbs so that evoked potentials could be elicited in each hemisphere contralateral to the stimulation site. Placement of these electrodes was tested by bringing about a paw twitch with a capacity-coupled square wave of 0.1 msec duration, 12 V in strength, and 4 Hz in frequency.

Evoked Potential Monitoring

Bipolar electroencephalogram (EEG) recordings were accomplished by placement of screw electrodes (one on each side) 1 cm lateral to the midline and approximately 5 mm anterior to the coronal suture to which a Grass P5A EEG amplifier was connected using a reference electrode placed in the frontal sinus. The recording system was adjusted to a bandwidth of 3–3000 Hz. EEG averaging was performed over 256 responses and displayed on an Apple II Plus computer following analogue to digital conversion of 256 data points taken at 100 μsec intervals. Absolute latencies and amplitudes of the cortical components were computed on-line.

To localize the site of SSEP generation, one experiment was undertaken in which sequential sections of the ectosylvian gyrus were destroyed by cautery during evoked potential monitoring (See fig. 1C). The cortical component was ablated at the anterior edge of the ectosylvian gyrus. Anatomical locations of these sites have been described for cat and monkey using surface recordings of the cortical response during somesthetic stimulation in combination with tissue ablation experiments.13

Measurement of Cerebral Blood Flow

CBF was recorded utilizing the hydrogen clearance technique using platinum electrodes which were acutely placed into the gray and white matters of both hemispheres. The platinum electrodes (Frederick Haer #1788) having 1 mm exposed tip lengths with average tip diameters of 25 μm were routinely cleaned and replatinized to obtain greater sensitivity to hydrogen. A 1 × 2 cm craniectomy was made in both hemi-
spheres, and the platinum electrode probes, mounted on micromanipulators, were placed into the ecosylvian gyrus of both hemispheres following removal of the dura. A silver-silver chloride reference electrode was placed in the temporalis muscle, and the potentiographic current resulting from the application of a 250 mV source (active electrode positive) was converted to a voltage output by a current to voltage amplifier. Administration of 400 ml of hydrogen gas through an endotracheal tube (about 9% of the total nitrous oxide and oxygen mixture) lasted for two minutes. The hydrogen clearance curves were displayed on a video screen utilizing an analogue to digital converter and 256 sampling points made at 2 sec intervals over an 8½ min sampling period.

Curve Analysis
Flow values were obtained by biexponential analysis of the curves generated at each site. All results are expressed in ml/100 gm/min. White matter electrodes ordinarily recorded monoeponential curves while gray matter recordings were often but not always (especially during ischemia) biexponential. There is yet no physiological explanation for this phenomenon which has also been observed to be variable by others. Because of the mathematical error introduced in the analysis of very slow and prolonged clearance curves, differences in flows ranging between 0 and 5 were not considered to be significantly different from 0 and 10 respectively. Therefore, flow values lying within these two ranges were rounded up or down to 10 or 0 as appears in table 1. We do not feel that this procedure biases our results since our conclusions are based upon distinguishing flows above and below 15 ml/100 gm/min which the technique allows us to do.

Experimental Protocol
MCA occlusion was performed in 10 cats by a retroorbital approach. Following a reduction in blood flow with MCA occlusion, all animals were infused with either physiological saline (0.9%) or dextran 40 (Pharmacia; 10% w/v) in the amount of 20 ml/kg animal weight for 1 to 2 trials while the MCA clip remained in place. Over the course of each half hour following the infusion of the fluids, CBF and SSEP monitoring continued as the hematocrit was determined. Throughout the experiments, the animals’ blood gases were monitored with an Instrumentation Laboratory Blood Gas Analyzer. The normal PO2 values measured at 37°C were well above 95 Torr, and the pH fell within a range of 7.35–7.45. The respiratory volume was adjusted to maintain the PCO2 to within 35 ± 5 Torr. Mean arterial blood pressures were within the range of 130–160 mm Hg, and the animals’ temperatures as measured rectally were maintained at 37 ± 2°C with the aid of a heating blanket. At the end of the experiments, the brains were frozen in situ with liquid nitrogen for biochemical analysis. Methods and results of these investigations will be presented elsewhere.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Percentage of control CBF following MCA occlusion (% of control)</th>
<th>Percentage of control CBF following first hemodilution (% of control)</th>
<th>Percentage of control CBF following second hemodilution (% of control)</th>
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<td>9.9 ± 11.1</td>
<td>7.3 ± 11.5</td>
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The control hematocrit was 33.1 ± 7.9% (n = 10) of the cell volume. 23.7 ± 9.4% (n = 10) after hemodilution, and 18.3 ± 2.2% (n = 7) after the second hemodilution. Means and standard deviations (X ± SD) are expressed at the bottom of the table for all blood flows. Reductions in the white and gray matter CBF values following MCA occlusion are expressed as percentages (X ± SD%). Percentage of control CBF X ± SD 14.8 ± 19.6%, 19.3 ± 23.7%.

*Animals whose CBF's increased after hemodilution.
†One dextran 40 infusion.
‡One saline infusion.
Abolition of the cortical component occurred when the experimental conditions. In figure 1A the top trace latency of 12.5 msec from the onset of the stimulus. shows the cortical component (V) appearing with a latency of 13.9 msec. SSEP was observed. The blood flows recorded under control conditions and zero for both flows following application of the MCA clip. After clipping both flows were reduced to values of 49 and 16 ml/100 gm/min. Within 35 min of removing the clip, the flows stabilized at values of 44 and 16 ml/100 gm/min following removal of the clip. Within 35 min of removing the clip, the latency of the cortical component became shorter (12.9 msec) and returned to a value close to the pre-clip one of 13.7 msec. The flow data measured by the hydrogen clearance technique indicated that the control gray and white matter flows were 50 and 17 ml/100 gm/min respectively. After clipping both flows were reduced to zero, but they returned to values of 49 and 16 ml/100 gm/min following removal of the clip. Within 35 min of removing the clip, the latency of the cortical component became shorter (12.9 msec) and returned to a value close to the pre-clip one of 12.5 msec (not shown). There were no changes in the flow values recorded over this period of time. The results of a longer-duration clip are shown in figure 1B. The cortical component, which occurred with a latency of 13.9 msec in the top trace, was irreversibly lost following the MCA clip. After 2 hr, the clip was removed, and the SSEP was recorded intermittently over an additional 2 hr. No return in the cortical component of the SSEP was observed. The blood flows recorded under these conditions were 56 and 33 ml/100 gm/min for the gray and white matter respectively under control conditions and zero for both flows following application of the clip. After a brief period of hyperemia following removal of the clip, the flows stabilized at values of 44 and 26 ml/100 gm/min.

Data Analysis

Data were obtained from 10 cats (See tables 1 and 2). Means and standard deviations (X ± S.D.) of the blood flows (ml/100 gm/min) are reported. The cortical wave amplitudes and latencies are expressed as a percentage of control. Paired t-tests were performed on the mean percentage differences from control for latencies and amplitudes, which were measured in the contralateral hemisphere (non-clip side) under the following conditions: control vs clip, control vs infusion, and clip vs infusion. For the contralateral hemisphere, t-tests were also conducted among the means of gray and white matter flows following MCA occlusion and hemodilution. In assessing the effect of hemodilution on the blood flow, the percentage of control flow is expressed (tables 1 and 2).

Results

Effects of CBF Reduction in Gray and White Matter on the Cortical Components of the SSEP

In 2 animals the MCA clip was applied for 12 min and 2 hr respectively before removal. SSEP and CBF were monitored before application of the clip, while it was left in place to impede flow, and upon its removal. Figure 1 shows the configuration of SSEP's which were recorded from these cats under control and two experimental conditions. In figure 1A the top trace shows the cortical component (V) appearing with a latency of 12.5 msec from the onset of the stimulus. Abolition of the cortical component occurred when the clip was applied to the MCA as shown by the flat appearance in the middle trace. When the clip was removed after a period of 12 min (lower trace 3 in A), the cortical component appeared with a latency of 13.7 msec. The flow data measured by the hydrogen clearance technique indicated that the control gray and white matter flows were 50 and 17 ml/100 gm/min respectively. After clipping both flows were reduced to zero, but they returned to values of 49 and 16 ml/100 gm/min following removal of the clip. Within 35 min of removing the clip, the latency of the cortical component became shorter (12.9 msec) and returned to a value close to the pre-clip one of 12.5 msec (not shown). There were no changes in the flow values recorded over this period of time. The results of a longer-duration clip are shown in figure 1B. The cortical component, which occurred with a latency of 13.9 msec in the top trace, was irreversibly lost following the MCA clip. After 2 hr, the clip was removed, and the SSEP was recorded intermittently over an additional 2 hr. No return in the cortical component of the SSEP was observed. The blood flows recorded under these conditions were 56 and 33 ml/100 gm/min for the gray and white matter respectively under control conditions and zero for both flows following application of the clip. After a brief period of hyperemia following removal of the clip, the flows stabilized at values of 44 and 26 ml/100 gm/min.

Finally, in a third cat we monitored CBF and SSEP's to confirm the locus of SSEP cortical wave generation. SSEP's were examined before and after

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<td>37(205.5)</td>
<td>54(145.9)</td>
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<td>X ± SD</td>
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<td>36.6 ± 10.7</td>
<td>60.4 ± 13.4</td>
<td>44.3 ± 8.8</td>
<td>63.9 ± 13.1</td>
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Means and standard deviations (X ± SD) are expressed at the bottom of the table for all blood flows. Gray matter blood flow increased significantly from the occluded condition (p < 0.05, t-tests on the means). Control and experimental hematocrit values are the same as reported in Table 1.

*Significantly greater than post-clip flows; n.s. not significantly different from post-clip flows.
†One dextran 40 infusion.
‡One saline infusion.
Reduction in CBF and SSEP Values in the Experimental Hemisphere

Figure 3 is a bar graph representation of the gray and white matter flows and the percentage of control SSEP amplitudes and latencies for the experimental hemisphere following MCA occlusion. Control (pre-clip) white and gray matter flows were 32.0 ± 8.6 and 50.8 ± 11.3 ml/100 gm/min (X ± S.D., n = 10) respectively. We compared gray and white matter CBF in the experimental hemisphere following MCA occlusion. The cortical component of the SSEP following MCA occlusion was abolished in all animals. As indicated in table 1, a reduction in cerebral blood flow in both the gray and white matter of the ectosylvian gyrus occurred in all of the 10 cats following MCA occlusion. Flow was reduced to 14.8 ± 19.6% of control in the white matter and 19.3 ± 23.7% of control in the gray matter. Average normal white matter blood flow was 32.0 ± 8.6 ml/100 gm/min while the clip flow was 4.1 ± 5.3 ml/100 gm/min. In 6 of 10 cats the white matter flow was reduced to values which were indistinguishable from zero while in the remaining 4 the percentage of control flows varied from 30.5 to 47.6. Although the gray matter showed a lesser percentage reduction in flow values (solid and open bars respectively; flows in ml/100 gm/min) and the percentage of control SSEP amplitudes (A) and latencies (L) for the experimental (left) hemisphere under control, MCA clip, saline or dextran 40 infusion, and additional saline or dextran 40 infusion conditions. Note that the MCA occlusion resulted in a drastic reduction in white and gray matter flows and a concomitant abolition of the cortical wave of the SSEP. Hemodilution increased gray and white matter flows insignificantly (See text) and did not restore the cortical component of the SSEP.

Volume expansion and concomitant hemodilution were used in treating high-grade ischemia. Hemodilution with either dextran 40 or saline infusions raised the flow values to means (X ± S.D.) of 10.0 ± 13.8 and 17.6 ± 19.1 ml/100 gm/min for white and gray matter respectively (fig. 2; table 1, third column). Though these values suggest an increase, they were not significantly greater (p > 0.05) than the untreated, clip flow values which were measured immediately following application of the clip. Thus, it appears as if hemodilution through dextran or saline was ineffective in increasing CBF after MCA clipping in this preparation.

There was a large degree of variability in blood flows in both the gray and white matter after hemodilution treatment as indicated by the large standard deviation about the means in the preceding paragraph. In only 3 animals (#'s 40, 46, and 53, * in table 1) did both the gray and white CBF increase above that of the occluded condition. In the 7 remaining animals, there was no consistent change.

Changes in CBF and the SSEP in the Control Hemisphere

In figure 4 a bar graph indicates the results of gray and white matter flows and the percentage of control SSEP amplitude and latencies for the unclipped hemisphere before and after hemodilution. Control white
and gray matter blood flows were 31.5 ± 8.1 and 53.0 ± 12.5 ml/100 gm/min (X ± S.D., n = 10) respectively. Blood flows were not significantly affected by the MCA clip (p > 0.05), but they did increase after hemodilution. The changes in the amplitude of the cortical component of the evoked potential were variable, but there was a tendency for this wave to be depressed following the clipping and treatment procedures. The latency of the cortical component was extended even as the flows remained above preocclusion values. Significant increases in the cortical wave latency, i.e., greater than 100% of control, were observed when comparing the values recorded following the first hemodilution with the control (p < 0.05). However, there was not a significant difference between the control and normalized latencies at the time of MCA occlusion in the contralateral hemisphere (p > 0.05). Furthermore, percentage changes in the amplitudes were not significantly different from the control values of the cortical component recorded from the right (contralateral) hemisphere (p > 0.05). Gray matter flows in this hemisphere increased significantly (p < 0.05) from control and clip values to those measured after the second dextran or saline infusion (See table 2; 45.7 ± 14.2 vs 63.9 ± 13.1 ml/100 gm/min).

Discussion

Occlusion of the middle cerebral artery (MCA) produces high-grade cerebral ischemia in the cat. Loss of the SSEP cortical component occurred following significant reductions in the CBF. In numerous animals (6 out of 10) the white matter flow was reduced to immeasurably low levels. Therefore, the ischemic insult produced in the ectosylvian gyrus as a result of clipping the ipsilateral MCA contrasts with the more medial gyri which have been studied by others. In the level of flow reduction. The decrease in flow in the suprasylvian and marginal gyri are within the flow range characteristic of the ischemic penumbra. In the ectosylvian gyrus there was a large reduction in blood flow and a corresponding loss of electrical activity as measured by the components of the SSEP.

The large amount of variability in not only the percentage of control flows during ischemia but also the response to treatment may be attributed to a number of factors. First, one must consider that there is much variation in the ability of the collateral circulation to function in the cat during MCA occlusion. Inter-animal variation has been observed in the cat during MCA occlusion. Another consideration is the variability resulting from electrode placement from cat to cat. Hemodilution neither increased CBF in the experimental hemisphere nor elevated the mean arterial blood pressure significantly. Flow did not improve the critical level of 15 ml/100 gm/min, a level which supposedly corresponds to a failure of electrical activity. In our experiments the electrical activity of the brain was not restored as flows in the white matter did not exceed the critical level. Gray matter flows were also variable and were only slightly above the critical level following hemodilution and volume expansion. The preferential effect in returning flow to the gray matter following hemodilution may be due to the higher metabolic demand of this area compared to the white matter and also the richer vascular supply.

Two other interesting yet not fully understood observations deserve comment. Unidirectional changes in the configuration of the SSEP's of both hemispheres; i.e., loss of the cortical wave in the experimental hemisphere and depression of it in the control hemisphere, in light of the bidirectional flow changes may indicate trans-hemispheric communication. Contralateral reductions in blood flow have been reported in patients undergoing unilateral cerebral infarctions (diaschisis). In our experiments we observed a reduction in the amplitude and extended latency in the cortical component of the contralateral SSEP without reductions in CBF. This type of diaschisis may reflect neuronal communication to the opposite cerebral hemisphere, possibly through connections within the corpus callosum. This is merely speculative, and we are not sure how these changes in the SSEP wave evolved in the contralateral hemisphere following clipping and treatment. Since the animals' vital signs were carefully monitored, it is doubtful that these changes occurred spontaneously during the experiment.

Secondly, as we observed during destruction of the anterior edge of the ectosylvian gyrus through cautery, MCA clipping had the same effect on the SSEP in the sense that cortical wave abolition was permanent. Moreover, if the clip was removed after 2
hr (fig. 1B), the cortical wave failed to reappear even though blood flows were restored to control values. This series of observations seems to indicate that there is irreversible injury to the sites responsible for generating the cortical component of the SSEP both with destruction through cauterization and ischemia rendered through long durations of MCA clipping. The length of clip time over which damage ensues is within the 12 min to 2 hr range. In experiments in which the clip was removed within a short time (See fig. 1A), the cortical component returned with a longer latency than measured under control conditions. However, within 35 min the shorter, control latency was restored presumably as this length of time is necessary for recovery of the metabolic processes underlying normal nervous transmission of synaptic and action potentials. Presently, we are addressing the question of how long the MCA clip can be applied before there is irreversible change in both the CBF and SSEP.

References
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