Functional Impairment Due to White Matter Ischemia After Middle Cerebral Artery Occlusion in Cats

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We recorded regional cerebral blood flow, somatosensory evoked potentials, and auditory evoked potentials in the thalamic relay nuclei (ventral posterior lateral nucleus and medial geniculate body) and in the somatosensory and auditory cortices during and after 1 hour of transient left middle cerebral artery occlusion in nine cats. Regional cerebral blood flow was also measured in the thalamocortical tracts of five of these cats. Additionally, the integrity of thalamocortical connections was tested by retrograde labeling of the thalamic nuclei with horseradish peroxidase in eight cats (three of which experienced no ischemia). Regional cerebral blood flow was severely reduced during middle cerebral artery occlusion in the left primary auditory cortex (8.5 ml/100 g/min) and in white matter pathways (6.4–7.6 ml/100 g/min). In contrast, regional cerebral blood flow did not change significantly in the somatosensory cortex or in either thalamic nucleus. Evoked potentials were abolished in both cortices but remained unchanged in the thalamic nuclei. Cortical somatosensory evoked potentials disappeared 5–8 minutes later than auditory evoked potentials. Recirculation after 1 hour of ischemia resulted in rapid and almost complete recovery (94%) of somatosensory evoked potentials and little recovery (18.4%) of auditory evoked potentials. We conclude that in the auditory pathway both cortical and fiber tract ischemia are (perhaps synergistically) responsible for dysfunction, while in the somatosensory cortex evoked potentials are abolished due to white matter ischemia. The delayed disappearance and better recovery of somatosensory than of auditory evoked potentials indicate that ischemic tolerance is higher in fiber tracts than in cortex. (Stroke 1990;21:923–928)

Numerous experimental studies have concentrated on the effect of ischemia on the functional and morphologic integrity of the cerebral cortex.1–6 In many investigations, evoked potentials (EPs) recorded from specific cortical areas have served as the paradigm of function, and disturbances of EPs were usually interpreted as indicating cortical disorder.1,3,6–9 In contrast, regional cerebral blood flow did not change significantly in the somatosensory cortex or in either thalamic nucleus. Evoked potentials were abolished in both cortices but remained unchanged in the thalamic nuclei. Cortical somatosensory evoked potentials disappeared 5–8 minutes later than auditory evoked potentials. Recirculation after 1 hour of ischemia resulted in rapid and almost complete recovery (94%) of somatosensory evoked potentials and little recovery (18.4%) of auditory evoked potentials. We conclude that in the auditory pathway both cortical and fiber tract ischemia are (perhaps synergistically) responsible for dysfunction, while in the somatosensory cortex evoked potentials are abolished due to white matter ischemia. The delayed disappearance and better recovery of somatosensory than of auditory evoked potentials indicate that ischemic tolerance is higher in fiber tracts than in cortex.

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Materials and Methods

We used 17 adult cats weighing 2.3-4.0 kg. After the injection of 25 mg/kg i.m. ketamine hydrochloride, the cats were tracheotomized, immobilized with 0.5 mg/kg pancuronium bromide, and ventilated with 30% O2/70% N2O. Anesthesia was maintained during surgery by adding 0.5%-1.5% halothane to the gas mixture. After the left femoral vein and artery were cannulated, the cats received a continuous intravenous infusion (2 ml/kg/hr) of 5 mg/kg/hr gallamine triethiodide to maintain paralysis. Arterial blood pressure and blood gases (Paco2, PacO2, and arterial pH) as well as body temperature were frequently monitored throughout the experiment and remained within the normal range for cats.

Using the hydrogen clearance technique, rCBF (as milliliters per 100 g brain per minute) was measured at different locations in the thalamocortical somatosensory and auditory pathways (Figure 1). Platinum electrodes were implanted as previously described. Cortical locations were the hind limb projection area of the primary somatosensory cortex and the left primary auditory cortex; thalamic sites were the ventral posterior lateral nucleus (VPL) and the medial geniculate body (GM). Thalamocortical tract electrodes were implanted at three sites (WM1-3) exhibiting horseradish peroxidase (HRP) staining in the tracing experiments. The clearance curves were analyzed by means of the initial slope method as rCBF = 69.3+T1/2.14

At the cortical and thalamic sites, the electrodes used for hydrogen clearance measurements also served for EP recordings after appropriate amplification and filtering. Somatosensory EPs were induced by tibial nerve electrical stimulation (0.1 msec duration, supramaximal intensity), and auditory EPs were elicited by right ear click stimulation (0.1 msec duration, supramaximal intensity). Cyclic delivery of the different stimuli with a period of 1 second was used to avoid interaction between responses to the two kinds of stimuli. The responses were stored on magnetic tape and averaged off-line (100 sweeps, MINC, Digital Equipment Corp.). To quantify amplitude, the difference between the first positive and the first large negative wave of cortical potentials and the maximal peak-to-peak value of thalamic potentials—the cortical and thalamic origin of which have been confirmed for somatosensory15'16 and auditory17'18 EPs—were analyzed.

For retrograde tracing and assessment of axonal flow of HRP in control and posts ischemic conditions, 0.5 μl wheatgerm-agglutinated HRP was injected into the somatosensory and auditory cortices in control cats and in ischemic cats 30 minutes after inducing ischemia. After the microinjection, the cats were kept for approximately 20 hours before transcardial perfusion with physiological saline followed by Karnovsky fixative. Using the tetramethyl benzidine (TMB) method described by Mesulam,40 40-μm cryostat cross-sections of the brain were stained.

Three cats served as controls and experienced no focal cerebral ischemia. For inducing ischemia in the remaining 14 cats, the left MCA was exposed via the transorbital route and reversibly occluded using a Yasargil miniature aneurysm clip. We studied only the cats exhibiting critical ischemia, defined by the complete and persistent inhibition of auditory EPs and severe ischemia in the auditory cortex as previously
TABLE 1. Regional Cerebral Blood Flow in Cortex, Thalamus, and Thalamocortical Pathways of Cats Before, During, and After 1 Hour of Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Location</th>
<th>Regional cerebral blood flow (mg/100 g/min)</th>
<th>Before</th>
<th>During</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Somatosensory</td>
<td></td>
<td></td>
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<tr>
<td>Auditory</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventral posterior lateral nucleus</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Medial geniculate body</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Thalamocortical</td>
<td></td>
<td></td>
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<tr>
<td>WM$_1$</td>
<td></td>
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<tr>
<td>WM$_2$</td>
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<tr>
<td>WM$_3$</td>
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</tbody>
</table>

Data are mean±SD. WM$_1$-$3$, locations in subcortical fiber tracts.

*p<0.05 different from before.

Results

In the first experiment, hydrogen clearance determinations of rCBF at different locations along the pathways (Table 1) revealed very low values in the auditory cortex and the thalamocortical tracts during MCA occlusion. After reperfusion, rCBF values significantly greater than baseline were obtained at WM$_1$-$3$. In the somatosensory cortex and the thalamus, rCBF did not change significantly.

MCA occlusion abolished somatosensory and auditory EPs recorded in the two cortices. In contrast to findings in the cortex, amplitudes of the thalamic EPs were slightly greater during ischemia (Table 2). In the somatosensory cortex, the EP decay was delayed (Figure 2). Almost-complete recovery of the EPs was obtained in the somatosensory but not in the auditory cortex during reperfusion (Table 2).

In the second experiment, microinjections of HRP into the somatosensory and auditory cortices of the three control cats revealed massive fiber tract labeling and staining in the VPL and GM (Figure 1, Table 3). In the five ischemic cats, rCBF and cortical EPs were recorded before HRP injection or during the initial 30 minutes of ischemia. EPs were again measured approximately 20 hours after the injection of HRP.

**TABLE 2. Amplitudes of Somatosensory and Auditory Evoked Potentials in Cortex and Thalamus of Cats Before, During, and After 1 Hour of Middle Cerebral Artery Occlusion**

<table>
<thead>
<tr>
<th>Location</th>
<th>Amplitude of evoked potentials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before (µV)</td>
</tr>
<tr>
<td>Cortex</td>
<td></td>
</tr>
<tr>
<td>Somatosensory</td>
<td>240.0±56.6</td>
</tr>
<tr>
<td>Auditory</td>
<td>634.0±127.0</td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
</tr>
<tr>
<td>Ventral posterior lateral nucleus</td>
<td>45.0±13.4</td>
</tr>
<tr>
<td>Medial geniculate body</td>
<td>38.4±4.2</td>
</tr>
</tbody>
</table>

Data are mean±SD of nine cats.

*p<0.01 different from before.
FIGURE 2. Somatosensory and auditory evoked potentials in cortical and diencephalic regions of somatosensory (SH and VPL) and auditory (A and GM) pathways before (control) and during middle cerebral artery occlusion. SH, hind limb projection area of primary somatosensory cortex; VPL, ventral posterior lateral nucleus; A, primary auditory cortex; GM, medial geniculate body.

(Table 3). The higher degree of labeling in the somatosensory relay nucleus corresponded to a better recovery of EPs in the somatosensory cortex than in the auditory cortex.

Discussion

The prerequisites of a study focusing on white matter ischemia and distinguishing functional impairment caused by blood flow disturbance in the cortex from that in the afferent pathway are methods to record simultaneously rCBF and electrophysiologic activity in various sections of a sensory system. This task can be fulfilled repeatedly only by using electrodes capable of recording local electrical AC phenomena (e.g., EPs and electroencephalogram) and DC changes as elicited by saturation and clearance of hydrogen in tissue used for rCBF determination. Despite the problems of quantifying rCBF using the hydrogen clearance method discussed repeatedly,22-24 perfusion of gray and white matter are easily differentiated,23 and regional changes during and after MCA occlusion are well documented. This technique was well suited for our present study since together with rCBF determinations, EPs could be recorded repeatedly at various levels of two afferent pathways, permitting the correlation of changes in rCBF and local functional impairment.

Our second experiment confirmed results obtained in previous tracing studies.25-26 Despite their qualitative nature, our results support the findings of impaired fiber tract integrity by proving that ischemia influences axonal flow of HRP. Because of the relatively slow rate of retrograde flow (50-120 mm/day),27 an incubation time after MCA reperfusion of approximately 20 hours was necessary. During this time, massive edema formation occurs in the cat model in response to blood-brain barrier disruption.21 Cryostat sections of these brains were difficult to process with the TMB method,20 which involves floating the sections during the staining procedure, resulting regularly in destruction of the sections. In the cats included in the study, it was possible to identify cellular labeling in the thalamic relay nuclei, which appeared to be better preserved than the cerebral cortex or the thalamocortical tract.

Our results show that afferent deactivation of the somatosensory cortex is caused by severe ischemia in the thalamocortical fiber tract and not by cortical or thalamic impairment. Therefore, special characteristics of this kind of ischemic disturbance, that is, delayed abolition of somatosensory EPs and good

<table>
<thead>
<tr>
<th>Cat</th>
<th>Somatosensory (% of baseline)</th>
<th>VPL staining</th>
<th>Auditory (% of baseline)</th>
<th>GM staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>No occlusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>++</td>
<td>85</td>
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<tr>
<td>11</td>
<td>95</td>
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<td>100</td>
<td>++</td>
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<tr>
<td>12</td>
<td>90</td>
<td>++</td>
<td>90</td>
<td>++</td>
</tr>
<tr>
<td>Occlusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>25</td>
<td>+</td>
<td>0</td>
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<td>15</td>
<td>40</td>
<td>+</td>
<td>0</td>
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<td>17</td>
<td>60</td>
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<tr>
<td>14</td>
<td>85</td>
<td>++</td>
<td>40</td>
<td>++</td>
</tr>
</tbody>
</table>

EP, evoked potential; VPL, ventral posterior lateral nucleus; GM, medial geniculate body; baseline, before injection of horseradish peroxidase or during initial 30 minutes of occlusion. Grades of staining indicate neuronal labeling in thalamus: -, no neurons; +, few neurons; ++, many neurons.
Two aspects, the tolerance to ischemia of gray and white matter and the vascular supply of the different compartments, seem to be of particular relevance for white matter infarction. Both aspects may have implications for prognosis after ischemia in different locations.

A higher tolerance to ischemia of white matter with regard to the development of ischemic lesions and to the recovery of neurologic symptoms has been discussed, and thresholds of approximately 12 ml/100 g/min for failure of electrical conduction in white matter have been reported compared with thresholds of approximately 20 ml/100 g/min for electrical failure in gray matter. In our experiments, white matter rCBF was far below this critical threshold and the thalamocortical tract was therefore severely ischemic. In light of the low residual rCBF, the specific high tolerance of white matter to ischemia is documented by the delayed abolition and the good recovery of somatosensory EPs. In contrast, the rapid abolition and poor recovery of auditory EPs are probably due to characteristics of cortical ischemia even though the auditory fiber tract was also affected. From studies of peripheral nerves, it is known that ischemic periods lasting up to 5 hours can be tolerated by axons, and differing vulnerabilities of various axon types (small and large, myelinated and unmyelinated) to ischemia/anoxia have been suggested. Similarly, axoplasmatic transport has been shown to be tolerant to >1 hour of anoxia, and the disappearance and reappearance after blockade was closely related to failure and recovery of electrical conduction, with a slightly higher ischemic tolerance for the latter. In our experiments, a similar tolerance was documented by the persistence of at least partial retrograde flow of HRP in brains deteriorating progressively during recirculation. Interestingly, a close correlation between electrical function and axonal flow was also seen in our present study since retrograde thalamic HRP labeling was found in cats with relatively little recovery of EPs.

Differences in the vascular supplies of the cortex and the white matter may also be important. Given the higher tolerance to ischemia of white matter, little would be gained if the chance for recirculation were poor. The vascular supply of fiber tracts is indeed not as good by far as that of the cerebral cortex. End-arteries dominate white matter compartments, and only little collateralization exists. Therefore, the higher white matter tolerance may be
of questionable value because of the finite character of lesions appearing after relatively long periods of ischemia. In humans, deep white matter infarcts are commonly found, and such lesions are typical of large-artery occlusions.

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References


Key Words: cerebral blood flow • cerebral ischemia • evoked potentials • cats
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