Deafferentation Versus Cortical Ischemia in a Rabbit Model of Middle Cerebral Artery Occlusion

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A two-site middle cerebral artery occlusion model in rabbits was developed. Platinum electrodes served for simultaneous recordings of regional cerebral blood flow, auditory evoked potentials, and electroencephalogram in the left and right auditory cortex and in the left medial geniculate body. Auditory evoked potentials and regional cerebral blood flow were also recorded in the subcortical white matter, and regional cerebral blood flow was recorded in the internal capsule. Distal segment occlusion of the middle cerebral artery caused severe cortical ischemia in four of 11 rabbits (Group I), accompanied by abolition of the auditory evoked potential in the left auditory cortex and white matter and severe reduction of the left electrocorticogram. Deep subcortical regions were affected either little or not at all. In the remaining seven rabbits (Group II) with only mild disturbance of cortical perfusion after distal middle cerebral artery occlusion, additional clamping of the proximal middle cerebral artery stem reduced thalamocortical tract blood flow and abolished cortical auditory evoked potentials. Spontaneous electrocorticogram was less affected in Group II than in Group I; thalamic regional cerebral blood flow and auditory evoked potentials were not altered. Histologically, ischemic lesions predominated in the cortex of Group I and in the subcortical structures of Group II rabbits. While correlated reductions in regional cerebral blood flow and auditory evoked potentials indicate effective cortical ischemia, the impairment of auditory evoked potentials in Group II rabbits must be due to cortical deafferentation by ischemia in the afferent tract. This model permits the investigation of the effects of predominantly cortical or subcortical ischemia in one functional system. (Stroke 1989;20:1071-1078)

Structural and functional damage in focal ischemia has been attributed mainly to the severity of local blood flow reduction. A threshold concept has been suggested assuming approximately 18 ml/100 g/min as the lower limit for functional and 12 ml/100 g/min as the lower limit for structural integrity.1,2 Evidence shows that the difference between these thresholds lessens with prolonged ischemia3-5 and that the thresholds vary depending on the intrinsic selective vulnerability of neuronal networks, neurons, or neuronal constituents involved.6-9

Beyond these local aspects, remote deactivation such as diaschisis or deafferentation may play an important role in functional impairment under conditions of focal ischemia.10-12 It may well be that in a considerable number of cases pathologic changes leading to remote disturbances have a better prognosis than functional loss in severely ischemic areas. In the cat middle cerebral artery (MCA) occlusion model, it has been shown that somatosensory evoked potentials in the hind limb cortical projection area are abolished due to subcortical white matter ischemia.5-12 A higher tolerance of ischemia for white matter was indicated by measurements of regional cerebral blood flow (rCBF) in the thalamocortical tract (internal capsule: 5-10 ml/100 g/min during reversible ischemia13).

So far, studies of the role of deafferentation in other ischemia models have not been attempted. The purpose of our study was to modify existing rabbit MCA occlusion models14,15 in such a way that either cortical or subcortical ischemia developed in one afferent system, namely the auditory pathway.
Materials and Methods

Eleven adult Dutch belted rabbits weighing 1.5–2.5 kg were used in these acute experiments. General anesthesia was induced with 30 mg/kg i.m. ketamine hydrochloride. The left femoral artery and vein were cannulated for continuous recording of arterial blood pressure, for intermittent blood sampling, and for intravenous injections, and a tracheotomy was performed. After being paralyzed with 0.15 mg/kg pancuronium, the rabbits were artificially ventilated with 1–2% halothane in 30% oxygen and 70% nitrous oxide. Xylocaine was used for local pain reduction.

The left MCA was exposed via the transorbital route. An incision was made at the dorsal ridge of the orbit. The eyeball was then retracted ventrally, and a craniotomy was performed around the optic foramen. After directomy, the MCA was carefully prepared. Nylon thread (6-0) loops were positioned around the artery at its origin from the circle of Willis (proximal segment) and at the level of the olfactory tract (distal segment, Figure 1). Guided in polyethylene tubing, the threads were brought outside the orbit for later ligation. Absorbable gelatin sponge was placed on the exposed brain surface, and the orbit was closed.

Craniotomies were made to implant stereotactically platinum/iridium macroelectrodes (electrolytically sharpened and glass-insulated 250-μm wires with 0.5-mm exposed tips) into the left and right auditory cortices, the left thalamus (medial geniculate body), the left auditory white matter radiation directly beneath the auditory cortex, and the left internal capsule (Figure 1). Exposed brain was covered with absorbable gelatin sponge. The electrodes were fixed, and the craniotomies were sealed with dental cement. In the cortex and thalamus, the electrodes simultaneously recorded auditory evoked potentials stimulated by a click (square wave, 100 μsec duration, supramaximal intensity), electroencephalogram (EEG), and hydrogen clearance curves. Auditory evoked potentials and hydrogen clearance curves were obtained in the subcortical white matter, and only hydrogen clearance curves were obtained in the internal capsule. Fifty auditory evoked potentials were averaged, and EEG was analyzed after active high- (0.1 Hz) and low- (35 Hz) pass filtering with a fast Fourier routine by calculating the sum of the square roots of Fourier coefficients in the range of 0.5–20 Hz on a laboratory computer (MINC, Digital Equipment Corp., Marlboro, Massachusetts). rCBF as milliliters per 100 grams per minute was calculated from the initial 2 minutes of the hydrogen clearance curves as $rCBF = 69.3 + T_{1/2}$, where $T_{1/2}$ is the time to reach half-saturation in minutes.

The experimental protocol included two steps after control measurements. All rabbits underwent occlusion of the distal MCA segment (Site I, inset in Figure 1) and were observed for 1 hour. Rabbits with severe cortical ischemia (Group I, $n=4$) were followed for 5 hours. The remaining rabbits with only mild rCBF reduction (Group II, $n=1$) subsequently had the proximal MCA (Site II, inset in Figure 1) occluded and were then observed for 5 hours. The experiments were terminated by perfusion-fixation, and hematoxylin-and-eosin– or

| Table 1. Physiologic Variables in 11 Rabbits Before and After Middle Cerebral Artery Occlusion |
|---------------------------------|---------|---------|---------|----|
| Time after occlusion (min) | MABP (mm Hg) | $P_{\text{A}0_2}$ (mm Hg) | $P_{\text{ACO}_2}$ (mm Hg) | pH |
| Before | 83.55±2.08 | 157.82±5.65 | 27.00±1.28 | 7.39±0.02 |
| 60 | 84.45±2.08 | 152.64±6.58 | 27.45±1.76 | 7.36±0.02 |
| 120 | 83.00±1.99 | 162.45±4.39 | 27.00±2.26 | 7.35±0.02 |
| 300 | 82.09±1.88 | 161.18±7.51 | 27.27±1.85 | 7.32±0.01 |

Values are mean±SEM. MABP, mean arterial blood pressure.
TABLE 2. Measurements of rCBF, ECoG, and Amplitude and Latency of EP in 11 Rabbits Before Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Region</th>
<th>rCBF (ml/100 g/min)</th>
<th>ECoG intensity</th>
<th>Amplitude</th>
<th>P1 (msec)</th>
<th>N1 (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>79.36±7.31</td>
<td>11,393.55±1,068.73</td>
<td>171.36±30.16</td>
<td>8.26±0.25</td>
<td>14.51±0.6</td>
</tr>
<tr>
<td>Ac</td>
<td>67.63±9.02</td>
<td>24,252.25±5,114.72</td>
<td>97.38±23.38</td>
<td>8.44±0.38</td>
<td>15.85±0.83</td>
</tr>
<tr>
<td>GM</td>
<td>66.91±5.92</td>
<td>8,387.45±1,714.31</td>
<td>22.30±3.2</td>
<td>5.52±0.06</td>
<td>9.41±0.36</td>
</tr>
<tr>
<td>WM</td>
<td>42.90±5.12</td>
<td>—</td>
<td>39.90±4.09</td>
<td>5.52±0.12</td>
<td>8.49±0.15</td>
</tr>
<tr>
<td>IC</td>
<td>38.67±4.10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

rCBF, regional cerebral blood flow; ECoG, electrocorticogram; EP, auditory evoked potential; A, left primary auditory complex; Ac, right auditory complex; GM, medial geniculate body; WM, subcortical white matter; IC, internal capsule. Values are mean±SEM.

Klüver-Barrera-stained brain cross-sections at different levels were histologically examined. Statistical analysis included parametric and nonparametric tests (analysis of variance, Kruskal-Wallis test). A significance level of p<0.01 was chosen.

Results

Physiologic variables stayed within control values throughout the entire experiment (Table 1), and no significant differences were found between groups (data not shown). Examples of auditory evoked potentials recorded in the auditory cortex, the medial geniculate body, and the subcortical auditory radiation are shown in Figure 1. Absolute values of rCBF, spontaneous left electrocorticogram, and auditory evoked potential amplitude and latency before occlusion are presented in Table 2. No significant differences were found between groups (data not shown). For analysis of the electrophysiologic parameters, percent changes relative to control were used. Interindividual variations of the values are probably due to differences in electrode location in the various rabbits.

Measurements in the auditory cortex ipsilateral to the occluded MCA clearly distinguished the effects of the two types of occlusion. After ligation of the distal MCA segment, rCBF decreased in all 11 rabbits, to <10 ml/100 g/min in four of 11 (Group I). This severe reduction in rCBF was followed by immediate and persistent decrease in auditory evoked potentials and electrocorticogram (Figure 2, Group I). In the other seven rabbits (Figure 2, Group II), auditory evoked potentials were not significantly decreased during the first hour even though rCBF was reduced to approximately 20 ml/100 g/min. Electroccorticogram total power was less reduced than in Group I. After additional occlusion of the proximal MCA segment in Group II, auditory evoked responses dropped drastically, while rCBF and electrocorticogram were not significantly altered. Correlations between rCBF and percent changes in auditory evoked potentials or electrocorticograms (Figure 3) demonstrate the difference between Groups I and II. Auditory evoked potential impairment was related to cortical rCBF reduction in Group I but not in Group II, and the positive correlation of electrocorticogram to rCBF was significant only in Group I.

In Group I rabbits, rCBF was severely disturbed in the subcortical white matter directly underneath the auditory cortex and was mildly lowered in the thalamus. In the internal capsule and in the contralateral auditory cortex, rCBF remained at control levels (Figure 4). Auditory evoked potentials in the cortical and subcortical white matter were similarly impaired, while responses in the medial geniculate body were not altered or were even increased in amplitude. Spontaneous EEG activity in the contra-
lateral auditory cortex and in the thalamus was slightly disturbed.

In Group II rabbits, after proximal MCA occlusion, rCBF in the internal capsule decreased significantly, while no further rCBF changes were observed in other regions. Ipsilateral cortical and subcortical auditory evoked potentials were abolished, while those in ipsilateral thalamic and contralateral cortical areas stayed nearly unchanged. Spontaneous EEG activity in the thalamus and contralateral auditory cortex did not differ significantly from that during the control period.

Histologic examination after perfusion-fixation revealed infarcted areas in all rabbits. Ischemic alterations in the auditory cortex, caudate nucleus, and internal capsule are documented in Figure 5. Drawings of the cross sections (Figure 6) schematically show examples of infarcts in rabbits in Groups I and II. In the Group I rabbit infarction was nearly restricted to cortical areas, whereas in the Group II rabbit infarction occurred predominantly in subcortical regions. A similar pattern of either predominantly cortical or predominantly subcortical infarction was found in the other rabbits. All Group I rabbits developed severe infarcts in the frontoparietal and temporal cortex; in only one rabbit were slight ischemic lesions also found in the anterior caudate nucleus. In contrast, all Group II rabbits showed severe infarcts in the anterior caudate nucleus and putamen, and only two slight ischemic lesions were found in cortical areas.

Discussion

Rabbits have recently become the animal of choice for focal ischemia studies. This is partly due to the increased expense of other species such as baboons, dogs, and cats, which had been the most frequently used ischemia models. For electrophysiologic studies involving multiple electrode implantations, large animals are superior to small.
FIGURE 4. Bar graphs of cerebral blood flow (CBF), auditory evoked potentials (EP), and electroencephalographic activity (EEG) in various brain regions of rabbits after distal (Group I, n=4) or distal and proximal middle cerebral artery (MCA) occlusion (Group II, n=7). Values are mean±SD. * Significantly different from preceding values. A, primary auditory cortex; Ac, right auditory complex contralateral to occluded MCA; GM, medial geniculate body; WM, subcortical white matter; IC, internal capsule.
FIGURE 5. Histologic appearances of cortex, caudate nucleus, and internal capsule of rabbits in Groups I and II after distal or distal and proximal middle cerebral artery occlusion, respectively. Note signs of severe damage (shrunken cells, vacuolation) in cortex of Group I rabbit and in caudate nucleus of Group II rabbit and vacuolation in internal capsule of Group II rabbit. Klüver-Barrera stain; cortex, ×246; caudate nucleus, ×156; internal capsule, ×246.

FIGURE 6. Schematic drawings of cross sections of rabbit brain at levels a, b, and c (see Figure 1) with infarcts (black areas) in Group I and Group II rabbit after distal or distal and proximal middle cerebral artery occlusion, respectively. Survival time after occlusion was ≥6 hours.

animals such as rats and gerbils, in which MCA occlusion models have also recently been described.20,21 Another advantage of the rabbit focal ischemia model is the relative ease of transorbital artery preparation due to the orbit being less narrow and less deep than in, for example, cats. It has been suggested that the rabbit model is less variable in its pathologic and pathophysiologic outcome15 since the cortical circulation does not receive a collateral supply from the external carotid artery22 and does not possess a rete system as do the cortical circulations of other species.23 For the study of afferent systems it is of particular interest that the MCA territory, except for the thalamus,24 includes cortical and subcortical nuclei. Therefore, ischemic disturbances spare the thalamic relay nuclei. Despite these peculiarities, variable outcomes do occur after MCA occlusion in rabbits and are probably related to collateralization from the anterior and posterior circulations. One strategy for overcoming this problem has been to occlude additional arteries, for example, the azygos anterior cerebral artery and the internal carotid artery14 or the distal A1 segment of the anterior cerebral artery and the internal carotid artery.16 Such occlusions reliably produce cortical ischemia and infarction. However, site and size of the infarct remain subject to variation.

Another strategy for overcoming the variability is to separate different pathophysiologic patterns into different experimental groups. For example, it has been shown that at least five patterns of transient or permanent blood flow disturbances can be distinguished after MCA occlusion in cats.25 We demonstrate two major types of ischemia in rabbits that can be easily discriminated by electrophysiologic and blood flow criteria within the first hour after distal MCA occlusion. Subsequent occlusion of the proximal MCA stem produces white matter ischemia and cortical deafferentation. Thus, the efficiency of experiments increases considerably since cortical and subcortical ischemia can be studied.

The recording techniques we used have been discussed in detail.5 Platinum black electrodes and appropriate filtering techniques provide repeat measurements and direct correlation of rCBF values and electrophysiologic parameters. One must consider, however, that the assessment of rCBF with the hydrogen clearance technique becomes increasingly unreliable at low values. Furthermore, invasive techniques tend to result in lowered rCBF values due to traumatization by the electrode. Additionally, monopolar recordings required for polarographic rCBF determinations impair the spatial resolution of electrophysiologic data.
The standard histologic techniques used were adequate for the identification of gray matter but not of white matter lesions. Structural white matter alterations were therefore only grossly determined (e.g., by a spongy appearance documenting edema). Electron microscopy is better suited to study white matter ischemia.

As in various other experimental studies on focal cortical ischemia,\(^{15}\) MCA occlusion led to an immediate decrease of rCBF in Group I rabbits. Accordingly, auditory evoked potentials were abolished, and electrocorticographic intensity was largely reduced. Since a similar abolition of cortical auditory evoked potentials was accomplished in Group II rabbits despite noncritical ischemia, other than local reasons for functional impairment must be considered.

Oclusion of the proximal MCA in Group II rabbits decreased rCBF in the internal capsule, while rCBF in all other sites (including the cortex) did not change. Even though residual blood flow did not fall to extremely low values, we interpret the abolition of cortical auditory evoked potentials in Group II rabbits as being caused by ischemic lesions in the auditory radiation at the level of the internal capsule. The changes in cortical auditory evoked potential cannot be caused by damage to the thalamic relay nuclei since neither significant rCBF changes nor electrophysiologic alterations were recorded in the thalamus, which is not supplied by the MCA in rabbits.\(^{24}\) Occasional increases in thalamic auditory evoked potentials possibly reflect diminished cortical inhibition. The slight reduction of electrocorticogram intensity does not contrast with our hypothesis since the total power was significantly less reduced than in Group I rabbits.

Furthermore, EEG is disturbed not only in the ischemic location but also in other regions of the ipsilateral and contralateral hemisphere.\(^{27-29}\) Synergistic effects of local mild ischemia and disturbances in subcortical sites may also play a considerable role.

Histologic examinations support our interpretation. Distal MCA occlusion with severe cortical symptoms in Group I resulted in almost pure cortical infarction as previously described.\(^{15,30}\) In contrast, two-site MCA occlusion in Group II rabbits caused subcortical infarcts in the caudate nucleus and the internal capsule as a substrate of remote cortical deactivation by ischemic lesions in the auditory radiation.

Our model of focal cerebral ischemia in rabbits supplements existing models. It provides the opportunity to study both local ischemic effects and remote cortical deactivation in one afferent, namely the auditory system, and it offers the possibility of studying white matter ischemia.

Acknowledgments

We were very fortunate to have had the excellent technical assistance of Doris Lattacz, Uschi Wittkamp, and Bernd Radermacher.

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KEY WORDS • animal models • cerebral ischemia • rabbits
Deafferentation versus cortical ischemia in a rabbit model of middle cerebral artery occlusion.
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Stroke. 1989;20:1071-1078
doi: 10.1161/01.STR.20.8.1071
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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http://stroke.ahajournals.org/content/20/8/1071

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