**Short Communications**

**Sequential Cerebral Blood Flow Changes in Short-term Cerebral Ischemia in Gerbils**

Hiroyuki Kato, MD, DMSc, Tsutomu Araki, PhD, Kyuya Kogure, MD, DMSc, Matsutaro Murakami, PhD, and Kazuo Uemura, MD, DMSc

Using quantitative autoradiography, we studied sequential changes in regional cerebral blood flow during and after 2 minutes of bilateral common carotid artery occlusion in 18 gerbils. Occlusion (n=4) led to severe ischemia in the forebrain (regional cerebral blood flow <5% of control [ft=4]) and midbrain (regional cerebral blood flow <10% of control), but was morphologically nonlethal. Reperfusion of the brain was complete, and regional cerebral blood flow was not different from control 1 minute after ischemia (n=4), but hypoperfusion (regional cerebral blood flow 30-50% of control) occurred at 5 minutes (n=3) and was pronounced at 1 hour (n=4); at this stage blood flow was inhomogeneous. Hypoperfusion had disappeared at 4 hours (n=3). Our results indicate that the well-documented sequence of cerebral blood flow changes (i.e., ischemia, initial recovery of blood flow, and delayed hypoperfusion) takes place even after nonlethal cerebral ischemia. *(Stroke 1990;21:1346–1349)*

B ilateral common carotid artery occlusion in gerbils leads to severe forebrain ischemia in >90% of animals because of a unique anomaly of the circle of Willis.1-3 Five minutes of ischemia damages only neurons in the hippocampus,2,4,5 whereas 10-15 minutes of ischemia causes extensive neuronal damage to selectively vulnerable regions.5 This damage occurs not only in forebrain regions (such as the hippocampal CA1 subfield, the striatum, thalamus, and neocortical layers 3 and 5) but also in midbrain structures (i.e., the medial geniculate body, substantia nigra, and inferior colliculus).5

On the other hand, repeated brief bilateral carotid artery occlusions in gerbils produce cumulative damage to the brain. Tomida et al6 found that damage following three 5-minute ischemic insults at 1-hour intervals is greater than that following a single 15-minute ischemic insult and concluded that postischemic hypoperfusion plays an important role. We have reported that repeated 2-minute ischemic insults, each of which is nonlethal to the brain when given singly, produce severe neuronal damage.7,8 However, whether hypoperfusion takes place after nonlethal 2-minute ischemic insults is not known.

The purpose of this study was to visualize and quantify using autoradiography the extent and degree of regional cerebral blood flow (rCBF) changes during and after 2 minutes of cerebral ischemia in gerbils.

**Materials and Methods**

We used a total of 33 adult male Mongolian gerbils weighing 70–90 g. Anesthesia was induced with 2% halothane and maintained with 1% halothane in 30% O₂ and 70% N₂O. A midcervical skin incision was made, and both common carotid arteries were gently exposed. Nine control gerbils received no occlusion, but in the remaining 24 experimental gerbils the arteries were occluded with aneurysm clips for 2 minutes. In 14 gerbils for autoradiography the carotid arteries were reperfused by removing the clips for 1 (n=4) or 5 (n=3) minutes or 1 (n=4) or 4 (n=3) hours. In all gerbils rectal temperature was maintained at around 37° C using a heating pad and a lamp.

For the autoradiographic determination of rCBF, a femoral artery and vein were cannulated for arterial blood sampling and radioisotope injection, respectively, in four control and 18 experimental gerbils. At the indicated time, 10 μCi of [14C]iodoantipyrine (Amersham, Tokyo, Japan) dissolved in approximately 0.7 ml saline was injected intravenously over 30 seconds, and six to eight-mu timed arterial blood samples were collected. The gerbils were then decapitated, and the brains were quickly removed and frozen in powdered dry ice. Frozen sections 20 μm thick were cut in a cryostat at −18° C,
mounted on cover glasses, dried at 60°C, and exposed to Kodak NMC-1 film (Rochester, N.Y.) for 14 days with carbon-14 standards (Amersham). Radioactivity of the arterial blood samples (10–20 μl) was determined with a liquid scintillation counter, and rCBF was determined using the autoradiographic method of Sakurada et al using a brain-blood partition coefficient of 0.85.

Because removal of a large volume of blood may affect the systemic and cerebral circulations in these small animals, we monitored mean arterial blood pressure and arterial blood gases (Paco2, Pao2, and pH) in three other experimental gerbils. Arterial blood gases were measured only before and 1 and 4 hours after ischemia.

We made histologic observations in 10 gerbils, five experimental gerbils (three with and two without ischemia) and five controls. Each gerbil was anesthetized with 50 mg/kg i.p. pentobarbital, and the entire brain was examined for ischemic neuronal damage, and the neuronal density (i.e., the number of intact pyramidal cells per 1 mm length) of the hippocampal CA1 subfield was also determined.

Significant differences were analyzed using one-way analysis of variance and Dunnett’s test and Student’s paired t test.

**Results**

Retrospectively, one experimental gerbil was considered not to have been rendered severely ischemic and was excluded from autoradiographic study. Values for the physiologic variables in three gerbils are shown in Table 1. No significant changes were observed.

Values of rCBF are summarized in Table 2, and representative autoradiographs are shown in Figure 1. Severe ischemia was induced in the forebrain structures, and rCBF during occlusion in these regions was as low as <5% of control. Calculated rCBF values were often 0 ml/100 g/min, although slight blackening of the autoradiographs was visible. Midbrain structures were also rendered severely ischemic, but to a lesser degree (<10% of control). The

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**Table 1. Physiologic Variables in Three Gerbils Before, During, and After 2 Minutes of Bilateral Common Carotid Artery Occlusion**

<table>
<thead>
<tr>
<th></th>
<th>Before (n=4)</th>
<th>During (n=4)</th>
<th>1 min (n=4)</th>
<th>5 min (n=3)</th>
<th>1 hr (n=4)</th>
<th>4 hr (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP (mm Hg)</td>
<td>91±0.9</td>
<td>109±9.4</td>
<td>91±5.0</td>
<td>92±2.9</td>
<td>96±5.1</td>
<td>89±1.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.33±0.02</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>7.30±0.01</td>
<td>7.26±0.01</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>181±36.2</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>186±28.1</td>
<td>153±28.2</td>
</tr>
<tr>
<td>PaCO2 (mm Hg)</td>
<td>40±2.3</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>44±2.6</td>
<td>41±3.1</td>
</tr>
</tbody>
</table>

MABP, mean arterial blood pressure. Values are mean±SEM.

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**Table 2. Regional Cerebral Blood Flow in Gerbils Before, During, and After 2 Minutes of Bilateral Common Carotid Artery Occlusion**

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Before (control)</th>
<th>During (n=4)</th>
<th>1 min (n=4)</th>
<th>5 min (n=3)</th>
<th>1 hr (n=4)</th>
<th>4 hr (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>215±43</td>
<td>0±0*</td>
<td>201±11</td>
<td>95±21†</td>
<td>72±13*</td>
<td>182±10</td>
</tr>
<tr>
<td>Sensorimotor cortex</td>
<td>207±37</td>
<td>1±1*</td>
<td>217±10</td>
<td>92±21*</td>
<td>73±11*</td>
<td>173±2</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>201±20</td>
<td>1±1*</td>
<td>201±13</td>
<td>106±29†</td>
<td>92±12*</td>
<td>194±35</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>216±22</td>
<td>0±0*</td>
<td>158±11</td>
<td>101±19†</td>
<td>97±19†</td>
<td>195±13</td>
</tr>
<tr>
<td>Visual cortex</td>
<td>188±30</td>
<td>1±1*</td>
<td>157±9</td>
<td>91±15*</td>
<td>90±16*</td>
<td>194±14</td>
</tr>
<tr>
<td>Caudoputamen</td>
<td>178±22</td>
<td>1±1*</td>
<td>159±6</td>
<td>95±24*</td>
<td>57±10*</td>
<td>146±5</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>143±13</td>
<td>4±3*</td>
<td>147±11</td>
<td>76±28†</td>
<td>64±13*</td>
<td>143±13</td>
</tr>
<tr>
<td>Thalamus</td>
<td>187±10</td>
<td>9±5*</td>
<td>232±20</td>
<td>104±28†</td>
<td>89±20*</td>
<td>202±9</td>
</tr>
<tr>
<td>Internal capsule</td>
<td>79±3</td>
<td>0±0*</td>
<td>95±10</td>
<td>65±3</td>
<td>48±13</td>
<td>71±7</td>
</tr>
<tr>
<td>Medial geniculate body</td>
<td>238±21</td>
<td>12±4*</td>
<td>236±9</td>
<td>104±20*</td>
<td>101±17*</td>
<td>223±9</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>148±8</td>
<td>35±14*</td>
<td>185±26</td>
<td>77±11</td>
<td>85±15</td>
<td>169±11</td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>228±27</td>
<td>8±5*</td>
<td>209±5</td>
<td>134±28†</td>
<td>148±16†</td>
<td>216±16</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>202±26</td>
<td>15±5†</td>
<td>220±14</td>
<td>181±41</td>
<td>171±33</td>
<td>211±7</td>
</tr>
<tr>
<td>Pons</td>
<td>181±26</td>
<td>184±30</td>
<td>182±13</td>
<td>164±39</td>
<td>198±36</td>
<td>209±13</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>121±13</td>
<td>108±12</td>
<td>108±11</td>
<td>108±23</td>
<td>141±20</td>
<td>134±14</td>
</tr>
</tbody>
</table>

Values are mean±SEM ml/100 g/min.

* †p<0.01, 0.05, respectively, different from control by ANOVA and Dunnett’s test.
substantia nigra was rendered moderately ischemic (24% of control). Areas supplied by branches and perforators of the basilar and vertebral arteries (i.e., the cerebellum, medulla oblongata, pons, and part of the diencephalon) were well perfused.

All ischemic areas were well reperfused after removal of the clips, rCBF not differing from control at 1 minute. However, hypoperfusion occurred in all previously ischemic areas except the inferior colliculus, substantia nigra, and internal capsule at 5 minutes and was pronounced at 1 hour. rCBF was 30–50% of control in forebrain structures and 50–60% of control in midbrain structures and inhomogeneous among structures. Hypoperfusion had disappeared at 4 hours, and rCBF did not differ from control in any area.

No definite ischemic neuronal damage was observed throughout the brain in any gerbil. Mean±SD neuronal density of the hippocampal CA1 subfield in the five experimental gerbils (232±19.8/mm) did not differ significantly from that of the five controls (229±20.9/mm).

Discussion

We show that the well-documented sequence of rCBF changes after ischemia (i.e., initial hyperemia and delayed hypoperfusion\(^{11,12}\)) occurs after nonlethal cerebral ischemia, although we observed no significant increase in rCBF during hyperemia.

Severe ischemia was induced not only in the forebrain but also in the midbrain by bilateral common carotid artery occlusion. Neuronal damage following transient global ischemia occurs in specific neuronal populations.\(^{13,14}\) The selectively vulnerable regions following 10–15 minutes of bilateral common carotid artery occlusion in gerbils are distributed among forebrain structures (neocortical layers 3, 5, and 6; the dorsolateral part of the striatum, the hippocampal CA1 and CA4 subfields, and the thalamus) and midbrain structures (the medial geniculate body, the substantia nigra, and the inferior colliculus).\(^{5}\) Thus, all vulnerable regions are rendered severely ischemic by bilateral common carotid artery occlusion in gerbils.

The brain was well reperfused after removal of the clips. The no-reflow phenomenon\(^{15}\) was not observed. No overshoot of rCBF (hyperemia) was observed at 1 minute. Two possibilities may explain the absence of hyperemia: 1) it did not occur, or 2) it was very brief and had resolved by 1 minute.

Then postischemic hypoperfusion took place; rCBF was 30–60% of control. Hypoperfusion had already appeared at 5 minutes, was pronounced at 1 hour, and disappeared at 4 hours. The time course was shorter and the damage was less severe than that after 5 or 15 minutes of ischemia; rCBF is approximately 20% of control 1 hour after 5 minutes of ischemia and hypoperfusion persists for >6 hours after 15 minutes of ischemia.\(^{6}\)

It is unlikely that the hypoperfusion was induced by decreases in blood pressure or Paco\(_2\) because no such reductions were observed. It is unlikely that the hypoperfusion was induced by prolonged halothane anesthesia because rCBF returned to normal after 4 hours. It is also unlikely that the hypoperfusion was induced by withdrawal of blood during radiotracer infusion because a comparable volume of saline was infused at the same time and because, if this were the case, rCBF of the control gerbils would also have been reduced.
Two minutes of ischemia in gerbils is nonlethal because no morphologic brain damage results, as reported earlier. Therefore, hypoperfusion per se may not be directly connected with ischemic neuronal death. On the other hand, 2 minutes of ischemia in gerbils decreases cerebral metabolism; cerebral protein synthesis is depressed for several hours after 2 minutes of ischemia. Therefore, the decrease in rCBF may be coupled with depressed metabolism. We have reported that severe neuronal damage results if such nonlethal ischemic insults are repeated at 1-hour intervals while hypoperfusion is maximum. All these pieces of evidence suggest that the brain is vulnerable to insult during postischemic hypoperfusion.

In conclusion, we show that, following even brief and nonlethal cerebral ischemia, conspicuous rCBF changes and hypoperfusion occur. This suggests that even brief cerebral ischemia has a considerable impact on neurons and that the insult can be lethal if the postischemic course is modified by some harmful events.

References
2. Kirino T: Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res 1982;239:57–69

Key Words • cerebral blood flow • cerebral ischemia • reperfusion • gerbils
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