Cerebral Blood Flow in the Evolution of Infarction Following Unilateral Carotid Artery Occlusion in Mongolian Gerbils

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Cerebral blood flow (CBF) was measured in gerbils 2, 4, 7, and 12 hours after unilateral irreversible carotid artery ligation to determine if the delayed ischemic damage to nerve terminals that occurs over 8 hours after stroke could be due to changes in CBF. [14C]butanol (4.5 μCi in 45 μl 0.9% saline) was injected into the femoral vein, and cpm accumulating in the cerebrum and in a catheter inserted in the abdominal aorta were measured. CBF (ml/100 g/min, mean ± SEM) in sham-operated control gerbils was 108.4 ± 37.5 in the left hemisphere and 123.8 ± 37.1 in the right. CBF in the ischemic left hemisphere was 41.0 ± 7.7 at 2 hours (n = 7), 21.6 ± 7.2 at 4 hours (n = 4), 26.2 ± 4.6 at 7 hours (n = 7), and 9.7 ± 3.1 at 12 hours (n = 6). CBF in the nonligated right hemisphere was 115.0 ± 15.3 at 2 hours, 70.4 ± 23.3 at 4 hours, 80.4 ± 14.6 at 7 hours, and 50.9 ± 20.1 at 12 hours. As expected, CBF was significantly reduced in the ischemic left cerebral hemisphere compared with the nonligated right cerebral hemisphere at each time, but CBF in the ischemic left cerebral hemisphere was also significantly lower at 12 hours than at 2 hours (p = 0.002) and at 7 hours (p = 0.014). CBF in the nonligated right cerebral hemisphere was also lower at 12 hours than at 2 hours (p = 0.02). No changes in Pco2, or blood pressure accounted for these differences. The reduction in CBF 12 hours after stroke coincides with the time of ischemic damage to nerve terminals. (Stroke 1987;18:612–615)

Irreversible unilateral carotid ligation in Mongolian gerbils produces an evolving infarction in the ipsilateral cerebral hemisphere in one-third of the animals. 1,2 In previous studies, in vitro active uptake of neurotransmitters into synaptosomes prepared from the ischemic hemisphere of gerbils with stroke had been used as a measure of irreversible ischemic damage to an energy-dependent active membrane transport system. 3,4 Synaptosomes were capable of maintaining uptake of neurotransmitters for up to 8 hours after stroke with oxygen and energy supplied in an in vitro system, even though the animals exhibited behavioral signs of stroke. By 16 hours, synaptosomes were no longer able to accumulate neurotransmitters in vitro after removal from the ischemic hemisphere, suggesting that irreversible damage to the synaptosomes occurred between 8 and 16 hours after stroke. Further studies demonstrated that the neurotransmitter dopamine (DA) continued to be metabolized to dihydroxyphenylacetic acid (DOPAC) in the ischemic striatum for up to 7 hours after stroke, 5 a process requiring molecular oxygen. 6

In the present study, cerebral blood flow (CBF) was measured in Mongolian gerbils 2, 4, 7, and 12 hours after irreversible unilateral carotid artery ligation to determine if there were any changes in hemispheric CBF that corresponded to the delayed degeneration and loss of metabolic function of nerve terminals at 16 hours. Prior studies employing hydrogen electrodes 7–10 and [14C]butanol 11,12 have shown a significant decrease in blood flow in the ischemic hemisphere for up to 2 hours after reversible carotid artery ligation and for up to 24 hours after 1 hour of unilateral carotid artery ligation followed by recirculation, 13 but CBF has not been measured for a prolonged period after unilateral irreversible carotid artery occlusion. To document further changes in CBF in brain tissue that has already been compromised, CBF was measured with [14C]butanol, which is a freely diffusible marker of tissue perfusion, resulting in high normal values of CBF (100 ml/100 g/min) in gerbils. 13 The study revealed a delayed secondary reduction in CBF in the ischemic hemisphere corresponding to the time course of degeneration of nerve terminals.

Materials and Methods

Studies were conducted with 126 gerbils. Adult male Mongolian gerbils weighing 50–70 g were anesthetized with 40 mg methohexital/kg (i.p.), with the dose titrated to the stage of surgical anesthesia for each animal. Animals breathed spontaneously without a respirator. The left common carotid artery was exposed in the paratracheal region by blunt dissection so that there was minimal blood loss. After the gerbils exhibited partial recovery from anesthesia, to the extent that they were responsive to leg pinch, the carotid artery was ligated with 2 ties. This method has been employed by Lust et al13 and in this laboratory 14 to provide animals in which behavioral changes associated with stroke could be readily observed. The behavioral criteria for designation of stroke were similar to those described by Kahn: 15 lack of movement or hemiparesis...
of the extremities contralateral to the carotid ligation, torsion of the body and circling behavior, obtundation, and a comatose-like motionless state. Gerbils that appeared otherwise normal but exhibited mild circling behavior generally associated with some lethargy were classified as "circling." This category included gerbils with mild deficits in which the final categorization of the behavioral pattern was difficult.

CBF in the cerebral hemispheres of gerbils was measured 2, 4, 7, and 12 hours after unilateral carotid ligation in animals with stroke, circling behavior, and normal behavior (unaffected). CBF was also measured in sham-operated gerbils 2 hours after surgery. Because of the fragile state of some of the stroke animals and because of the propensity to seizure activity in gerbils, CBF was measured under anesthesia with mechanical ventilation. Gerbils were anesthetized with 60 mg methohexital/kg (i.p.) and 40 mg xylazine/kg (i.m.). The total time of anesthesia, including measurement of CBF, was 20 minutes in all animals. A polyethylene endotrachéal cannula was inserted, and the gerbils were maintained on a mechanical ventilator with oxygen administered at a rate of 0.2 ml/min. Nitrous oxide (0.5 ml/min) was administered to some of the gerbils without stroke that exhibited a response to pain during surgery.

The gerbils were placed on an operating tray with a Delaphase isothermal pad (Braintree Scientific, Braintree, Mass.) to maintain body temperature at 37°C. Surgery was employed with the aid of a Zeiss dissecting microscope. A PE-50 catheter 50 cm long was inserted into the abdominal aorta, and a PE-10 catheter was inserted in the left femoral vein. Pao\textsubscript{2}, Paco\textsubscript{2}, and arterial pH were measured with a Radiometer BGA 3 blood gas analyzer on a 0.3-ml sample of blood drawn from the catheter in the abdominal aorta. The PE-50 catheter was connected to the syringe of a Sage constant withdrawal pump. After a constant withdrawal rate of 250 \mu l/min was established, 4.5 \mu Ci of \textsuperscript{14}C butanol in 45 \mu l of normal saline was injected into the femoral vein. The gerbils were quickly decapitated 30 seconds after the injection of butanol. The PE-50 catheter was detached and the blood expelled into a preweighed scintillation vial containing 0.5 ml of 1:2 Protosol:ethanol. The brain was also immediately removed, and the left and right hemispheres were separated and forced through a 25-gauge needle into preweighed vials containing 1 ml of 1:2 Protosol:ethanol. The vials were preweighed, and then 10 ml of Omnifluor in toluene were added for scintillation counting. Ten to 15 drops of 30% hydrogen peroxide were added to the vials containing blood to decolorize the samples. The vials were placed in an oven and incubated at 50°C for 24 hours to solubilize the samples. Scintillation counting was performed with a Packard Model 2450 Tricarb scintillation spectrometer. The counting efficiency of carbon-14 compared with an internal standard was 75% for samples containing decolorized blood and 80% for samples containing brain.

CBF was calculated by the equation of Van Uitert and Levy:\textsuperscript{12}:

\[
\text{Flow b/Mass b} = (\text{cpm b} \times \text{flow cath})/(\text{cpm cath} \times \text{Mass b})
\]

where Flow b/Mass b is the CBF in the cerebral hemisphere per 100 g of brain per minute, cpm b is the counts per minute of \textsuperscript{14}C butanol accumulating in the cerebral hemisphere in 30 seconds, flow cath is the milliliters of blood accumulating in the PE-50 catheter in 30 seconds, cpm cath is the counts per minute of \textsuperscript{14}C butanol accumulating in the PE-50 catheter, and Mass b is the weight of the cerebral hemisphere.

Analysis of variance and the Wilcoxon rank sum test were used to compare values at different times and conditions, and paired Student's \( t \) tests were used to compare the ligated and nonligated hemispheres of individual gerbils. Calculations were performed using the Epistat statistical program (Tracy L. Gustafson, Round Rock, Tex.) with an IBM PC-XT personal computer.

**Results**

A total of 35 gerbils suffered a stroke after unilateral carotid artery ligation. Five gerbils died after stroke or during the surgical procedure prior to measuring CBF. Mild circling behavior was observed in 12 animals. The remaining 74 gerbils were unaffected by carotid artery ligation.

The results of CBF studies in stroke, circling, and unaffected gerbils are summarized in Table 1 and Figure 1. CBF in sham-operated, control gerbils was 108.4 ± 37.5 ml/100 g/min in the left hemisphere and 123.8 ± 37.1 in the right. In gerbils with stroke, CBF in the ischemic left hemisphere fell significantly \((p < 0.001)\) to 41.0 ± 7.7, while CBF in the nonligated right hemisphere remained at 115.0 ± 15.3 ml/100 g/min. Four hours after stroke, CBF in the ischemic left hemisphere was 21.6 ± 7.2, significantly lower than CBF in the nonligated right hemisphere of 70.4 ± 23.3 ml/100 g/min. At 7 hours after stroke, CBF was 26.2 ± 4.6 in the ischemic left hemisphere and 80.4 ± 14.6 ml/100 g/min in the nonligated right hemisphere. By 12 hours after stroke, CBF in the ischemic left hemisphere had decreased further, to 9.7 ± 3.1, while CBF in the nonligated right hemisphere was 50.9 ± 20.1 ml/100 g/min. It is unlikely that changes in Paco\textsubscript{2} could account for the magnitude of the bilateral decrease in CBF observed 12 hours after carotid artery ligation since there was no linear correlation of CBF with Paco\textsubscript{2} in either hemisphere of individual gerbils with stroke. CBF in the ischemic left hemisphere at 12 hours was significantly lower than at 2 \((p = 0.02)\) and 7 hours \((p = 0.014)\). CBF in the nonligated right hemisphere 12 hours after stroke was significantly lower \((p = 0.002)\) than at 7 hours.

At 7 hours after stroke, gerbils that had exhibited no behavioral abnormality 2 and 4 hours after stroke began to develop mild circling activity, which progressed by 12 hours after stroke. These gerbils therefore were included in the unaffected category at 2 and 4 hours. CBF in the ischemic left hemisphere of gerbils with circling behavior was reduced to 42.9 ± 0.2 ml/100 g/min at 7 hours and 40.7 ± 3.6 at 12 hours, while
Table 1. Cerebral Blood Flow in Ischemic Left and Nonligated Right Cerebral Hemispheres of Gerbils With Stroke, Circling Behavior, or Normal Behavior

<table>
<thead>
<tr>
<th>Time</th>
<th>Condition</th>
<th>n</th>
<th>CBF left</th>
<th>CBF right</th>
<th>BP</th>
<th>PaCO₂</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Control</td>
<td>4</td>
<td>108.4 ± 37.5</td>
<td>123.8 ± 37.1</td>
<td>71.8 ± 8.3</td>
<td>32.3 ± 4.7</td>
<td>7.34 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>Stroke</td>
<td>7</td>
<td>41.0 ± 7.7*</td>
<td>115.0 ± 15.3</td>
<td>79.1 ± 4.8</td>
<td>50.0 ± 11.2</td>
<td>7.20 ± 0.08</td>
</tr>
<tr>
<td>4</td>
<td>Stroke</td>
<td>4</td>
<td>21.6 ± 7.2†</td>
<td>70.4 ± 23.3</td>
<td>77.3 ± 2.8</td>
<td>40.2 ± 4.0</td>
<td>7.26 ± 0.06</td>
</tr>
<tr>
<td>7</td>
<td>Stroke</td>
<td>7</td>
<td>26.2 ± 4.6†</td>
<td>80.4 ± 14.6</td>
<td>77.2 ± 4.2</td>
<td>40.4 ± 3.7</td>
<td>7.10 ± 0.06</td>
</tr>
<tr>
<td>12</td>
<td>Stroke</td>
<td>6</td>
<td>9.7 ± 3.1†‡</td>
<td>50.9 ± 20.1</td>
<td></td>
<td>75.0 ± 5.0</td>
<td>32.6 ± 1.9</td>
</tr>
<tr>
<td>7</td>
<td>Circling</td>
<td>2</td>
<td>42.9 ± 0.2</td>
<td>62.8 ± 8.5</td>
<td>75.0 ± 9.1</td>
<td>41.4 ± 4.3</td>
<td>7.20 ± 0.03</td>
</tr>
<tr>
<td>12</td>
<td>Circling</td>
<td>9</td>
<td>40.7 ± 3.6†</td>
<td>91.3 ± 4.7</td>
<td>80.3 ± 3.6</td>
<td>34.4 ± 3.1</td>
<td>7.29 ± 0.02</td>
</tr>
<tr>
<td>7</td>
<td>Unaffected</td>
<td>4</td>
<td>73.0 ± 16.0</td>
<td>70.0 ± 13.9</td>
<td>77.0 ± 2.2</td>
<td>45.7 ± 6.3</td>
<td>7.24 ± 0.05</td>
</tr>
<tr>
<td>12</td>
<td>Unaffected</td>
<td>4</td>
<td>82.7 ± 12.3</td>
<td>99.9 ± 12.5</td>
<td>73.8 ± 3.5</td>
<td>35.7 ± 3.0</td>
<td>7.28 ± 0.03</td>
</tr>
</tbody>
</table>

CBF, cerebral blood flow; BP, blood pressure; PaCO₂, arterial partial pressure of carbon dioxide; pH, arterial pH. Time is hours after stroke. Values are mean ± SEM.

*CBF in the ischemic left hemisphere significantly lower than in the right at p < 0.001, p < 0.01, and p < 0.03, respectively.
†CBF in the ischemic left hemisphere at 12 hours significantly lower than at 2 hours, p < 0.002.
‡CBF in the nonligated right hemisphere at 12 hours significantly lower than at 2 hours, p < 0.02.

Discussion

There was a bilateral decrease in hemispheric CBF 12 hours after stroke following unilateral irreversible carotid artery ligation in Mongolian gerbils. Using hydrogen clearance\(^7\)–\(^10\) and \([14C]\)butanol\(^11\)–\(^12\) techniques, other workers have noted a significant decrease in CBF in the ischemic left hemisphere 1 hour after unilateral carotid artery ligation. In the present study, CBF measured in the ischemic left hemisphere 2 hours after stroke is higher than CBF measured 1 hour after stroke by the hydrogen clearance technique, but this is to be expected since the normal values for CBF employing \([14C]\)butanol are more than twice the values for CBF measured with hydrogen clearance.\(^7\)–\(^10\) CBF in the ischemic left hemisphere of gerbils with stroke in the present study was also higher than CBF in the ischemic hemisphere measured by Levy et al\(^11\) employing \([14C]\)butanol 1 hour after stroke. This difference may be due to differences in the sampling and injection sites in the two studies, the timing of sampling, or the use of anesthesia and mechanical ventilation of the gerbils in the present study.

Levy et al\(^11\) followed the time course of changes in CBF in gerbils with stroke after 1 hour of unilateral carotid artery ligation and also noted postischemic hypoperfusion and bilateral reduction in hemispheric...
CBF 24 hours after recirculation. A similar secondary decline in CBF has been observed 27 hours after stroke in the ischemic hemisphere following middle cerebral artery occlusion in cats.19 Bilateral reduction in CBF or diaschisis has previously been observed 12–24 hours after unilateral stroke in humans,16 and bilateral reduction in glucose metabolism has been observed following focal ischemia in cats.17

The cause of the secondary reduction in CBF following ischemia is not known. It may be due to the “no-reflow phenomenon,”18 which has been attributed to encroachment of the capillaries by edematous perivascular glial cells and the formation of intracapillary blebs.19 Plugging of vessels with fibrin thrombus or platelet aggregates does not appear to be responsible for “no reflow.” It has also been hypothesized that the release of vasoactive neurotransmitters causes vasoconstriction of the cerebral vasculature.17 However, neurotransmitter release occurs almost immediately after the induction of ischemia,20 prior to the onset of diaschisis.

The secondary reduction in CBF observed 12 hours after stroke in Mongolian gerbils may be of significance in the irreversible ischemic damage of certain brain structures. It is at this time in the evolution of infarction that nerve terminals lose the capacity for active uptake of neurotransmitters when they are removed from the ischemic hemisphere and incubated in vitro with oxygen and substrate.3 Nerve terminal function may represent the response of other active transport systems of the neurons to ischemic insult or it may be part of a physiologic ischemic penumbra.21,22 In either case, there appears to be sufficient CBF in the ischemic hemisphere to maintain the viability and metabolism of nerve terminals for up to 8 hours after stroke, even though the nerve terminals do not function normally in vivo at this time. Irreversible damage to the nerve terminals occurs at the same time as the further, delayed reduction in the blood supply.

Tynson et al22 noted a correlation between the extent of morphologic ischemic neuronal damage and the degree of reduction in CBF 4 hours after middle cerebral artery occlusion in rats. The present study documents irreversible changes in neuronal membrane function coincident with greater impairment of CBF.

The irreversible unilateral carotid artery ligation model of stroke in Mongolian gerbils may not be analogous to all states of cerebral ischemia. The present study suggests that, in some instances, a considerable amount of time may exist after the onset of stroke symptoms in which the destruction of certain cerebral structures may be prevented. This may have therapeutic implications for some categories of patients with stroke.

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References


Key Words • cerebral blood flow • cerebral infarction • time course of infarction • carotid ligation
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