Collateral Development After Carotid Artery Occlusion in Fischer 344 Rats

Peter Coyle, PhD, and Maret J. Panzenbeck, PhD

Mortality following permanent occlusion of both common carotid arteries decreases as the time between the first and second occlusions increases in Fischer 344 rats. Our goal was to examine the possibility that collaterals develop after unilateral carotid artery occlusion. During temporary occlusion of both carotid arteries in nine ketamine-anesthetized male rats, mean±SEM blood flow in both parietal cortices was 23±4% of the preocclusion (control) blood flow (120±7 ml/min/100 g) measured by laser Doppler flowmetry (p<0.05). After permanent occlusion of one carotid artery for either 1-2 days (n=10) or 6 weeks (n=7), mean±SEM blood flow was 16±2% and 30±3% of control, respectively, during a temporary test occlusion of the other carotid artery. During the test occlusion, blood flow in the cortex ipsilateral to the 6-week occlusion was 170% that in the contralateral cortex (which was similar to the blood flow immediately after temporary occlusion of both carotid arteries) and twice that after 1-2 days of occlusion. Mean luminal diameter of the basilar-carotid anastomosis ipsilateral to the 6-week occlusion was 186% that of the contralateral anastomosis, which showed only minimal change, and 145% that after 1-2 days of occlusion. We conclude that during 6 weeks of permanent carotid artery occlusion the anastomosis enlarges and its blood flow or reserve increases. Thus, collaterals developed ipsilateral to, but not contralateral to, the 6-week carotid artery occlusion, which suggests the possibility of greater collateral protection on the permanently occluded side. (Stroke 1990;21:316–321)

Simultaneous occlusion of the right and left common carotid arteries produces 100% mortality in Fischer 344 rats. Survival rate rises to 100% as the time between the two occlusions increases to 1 month. During unilateral carotid artery occlusion, blood flow through the ipsilateral basilar–carotid anastomosis may increase to provide greater protection against mortality after the second occlusion. If blood flow or reserve increases slowly after unilateral carotid artery occlusion, then blood flow should be noticeably greater during temporary occlusion of the second carotid artery several weeks later. Objectives of our inquiry were to examine the anastomosis for a change in luminal diameter and to study blood flow 1–2 days and 6 weeks after unilateral carotid artery occlusion in Fischer 344 rats.

Materials and Methods

Male Fischer 344 rats weighing 200–250 g were purchased from Charles River Laboratories, Inc. (Wilmington, Massachusetts). All rats were anesthetized with 130 mg/kg i.m. ketamine during surgery and blood flow measurements. In nine rats (Group I), snare ligatures were used to temporarily (15–45 seconds) occlude the right or left (in random order) common carotid artery or both arteries by compression against the sternohyoid muscle. Blood flow sample times were 1 second before (control) and 15 seconds after its initiation, and 15 seconds after occlusion release. In 17 additional rats, either the right (n=8) or the left (n=9) common carotid artery was permanently occluded by double ligatures and vessel transection between the ligatures. All 17 rats survived the first occlusion without an observable gross neurologic lesion or deficit except for ptosis in 14. In 10 rats (Group II) the other common carotid artery was temporarily occluded and blood flow was measured bilaterally 1–2 days after the first occlusion. In the remaining seven rats (Group III) the other artery was temporarily occluded and blood flow was measured 6 weeks (41–45 days) after the first occlusion. In both Groups II and III, blood flow sample times were 1 second before the second occlusion (control), 15 seconds after its initiation, and 15 seconds after its release.

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Supported by National Institutes of Health National Heart, Lung, and Blood Institute grant HL-18575 and by the Bristol-Myers Company.

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Received May 30, 1989; accepted September 26, 1989.
After dissection of the carotid arteries and placement of the ligatures, the rat's head was placed in a stereotactic frame and cranial windows were made in the parietal bone bilaterally to allow blood flow measurement. After a dorsal midline incision and skin reflection, the periosteum was removed by scraping with a scalpel blade. A window 3–4 mm wide × 5–6 mm long was made by removing the outer table of parietal bone and medullary substance with a number 6 dental bur. Bone of the inner table was thinned to 100–200 µm, but the cranial cavity was not penetrated. Physiological saline kept the bone moist and prevented its overheating during removal. The cranial window was covered with Aquasonic (Parker Laboratories, Inc., Orange, New Jersey) to couple the blood flow probe to the rat's head.

The rats were not paralyzed, no mechanical ventilation was used, and no respiratory gases were administered. Blood pressure and blood gases in Fischer 344 rats are within the physiologic range of normotensive rats. Blood flow was monitored bilaterally using two Model BPM 403A Laserflo Blood Perfusion Monitors, each equipped with an 800-µm-diameter probe and a time constant of 0.5 seconds (Thermo-System Inc., St. Paul, Minnesota). Parameters used to estimate blood flow by laser Doppler flowmetry have been presented elsewhere. Blood flow was recorded continuously at three or four parietal locations. Instantaneous values were averaged to yield one blood flow value per sample time per hemisphere.

Blood flow was expressed in absolute terms as milliliters of blood per minute per 100 g tissue because the laser Doppler instrument was calibrated to such units by the manufacturer. We know of no published report that validates this calibration for rat brain; thus, blood flow was also expressed in relative terms as a percentage of blood flow 1 second before the temporary occlusion to control for possible calibration error.

Saturated papaverine hydrochloride in water was infused into a jugular vein after blood flow was measured. Latex rubber was injected through a cannula inserted into the ascending aorta to visualize the luminal diameter of the arteries (Number 563, Chicago Latex Products, Schaumburg, Illinois).

The internal carotid and basilar arteries supply the circle of Willis, which is complete in Fischer 344 rats. To avoid confusing terminology of the posterior cerebral artery and the posterior communicating artery in rats, we designated the measured branch as the posterior communicating anastomosis (PC) in Figure 1. A calibrated eyepiece micrometer in a stereozoom dissecting microscope was used to measure the luminal diameter at the arrow in Figure 1.

Blood flows and luminal diameters were expressed as mean±SEM. Analysis of variance was used to compare blood flows and diameters in the three groups. The paired t test was used to compare blood flows and diameters within a group. An α error of <0.05 (i.e., p<0.05) was considered significant.

Results
The posterior communicating anastomosis was present bilaterally (Figure 1) in all 26 rats. In Group I, mean±SEM luminal diameters of the left and right anastomoses were 153±10 and 151±12 µm, respectively. However, for each rat one anastomosis was larger than the other. Luminal diameters for the large and small anastomoses were significantly different (169±11 vs. 134±6 µm, p<0.05).

In Group II, the anastomosis ipsilateral to the first occlusion was 74 µm larger than the contralateral anastomosis (p<0.05, Table 1). In Group III, the anastomosis ipsilateral to the first occlusion was 158 µm larger than the contralateral anastomosis (p<0.05, Table 1). The anastomosis ipsilateral to the first occlusion was significantly larger at 6 weeks than at 1–2 days (p<0.05, Table 1); diameter of the contralateral anastomosis at 6 weeks and 1–2 days did not differ.

In Group I, control blood flow was 120±7 ml/min/100 g (Table 2). Within 5 seconds after the first occlusion, ipsilateral blood flow fell to a minimum and then began to rise. After 15 seconds of unilateral occlusion, ipsilateral blood flow was significantly less than control (p<0.05, Table 2). During the first occlusion, contralateral blood flow (116±5 ml/min/100 g) was essentially unchanged from that before the occlusion (117±5 ml/min/100 g). Within 5 seconds after the second occlusion blood flow ipsilateral and contralateral to the first occlusion fell to a minimum and remained there for the duration of the occlusion (Figure 2, A and B). After 15 seconds of bilateral occlusion, mean blood flow was 23% of control (p<0.05, Table 2), but there was no significant difference between the two sides (data not shown). By 15 seconds after the release of the occlusions, blood flow was restored to the control level (Table 2).

In Group II, after 1–2 days blood flow ipsilateral to the first occlusion was not significantly different from the contralateral control (Table 2) nor from resting blood flow in rats without occlusion (control, Group I). During the second occlusion, mean blood flow was 16% of control (Table 2), not significantly different on the two sides (Table 2, Figure 3). By 15 seconds after the release of the second occlusion, blood flow was restored to the control level (Table 2).

In Group III, after 6 weeks blood flow ipsilateral to the first occlusion was not significantly different from the contralateral control (Table 2) nor from resting blood flow in rats without occlusion (control, Group I). During the second occlusion, mean absolute blood flow was less than control (p<0.05, Table 2); both absolute and relative blood flows were significantly greater ipsilateral to the first occlusion than contralateral to it (p<0.05, Table 2; Figure 3). During the second occlusion, blood flow ipsilateral to the 6 weeks' occlusion was twice that ipsilateral to the 1–2 days' occlusion (p<0.05, Table 2). Following release of the second occlusion,
blood flow was restored to control levels by 15 seconds (Table 2). Patterns of blood flow restoration included an initial hyperemia and a slow return to control (Figure 2, C and D, respectively). Thus, blood flow restoration was variable, with no single consistent pattern.

During the second (bilateral) occlusion, the cortex with higher blood flow was ipsilateral to the larger anastomosis in seven (78%) of the nine Group I rats, in eight (80%) of the 10 Group II rats, and in all seven (100%) Group III rats. The null hypothesis that the higher collateral blood flow was related only...
by chance to the cortex of either side was rejected ($\chi^2=12.5, df=1, p<0.001$).

**Discussion**

Our major findings of greater blood flow and a larger communicating anastomosis at 6 weeks suggest collateral development following permanent occlusion of a carotid artery. Blood flow and the anastomosis contralateral to the permanent occlusion were essentially unchanged from their control levels, which suggests no or only minimal collateral development contralateral to the occlusion. Rather, the larger anastomosis and increased blood flow were lateralized to the side of the permanently occluded artery.

Absolute blood flow values for resting brain measured by laser Doppler flowmetry in our ketamine-anesthetized rats (120±7 ml/min/100 g) were similar to those measured with radioactive labeled microspheres in awake rats $^9$ (117±13 ml/min/100 g) or microspheres in rats given nitrous oxide $^{10}$ (128±10 ml/min/100 g). Because of the high spatial and temporal resolution of laser Doppler flowmetry and the relatively low resolution of the microsphere method, comparisons may be valid only if laser Doppler readings are made at multiple tissue sites $^{11}$ in each rat and then averaged, as we did.

Previous studies $^9,10,12,13$ suggest that blood flow is restored to normal resting levels during unilateral carotid artery occlusion in some strains of rats. After 15 seconds of unilateral carotid artery occlusion, blood flow returns to control levels in normotensive Wistar rats $^{13}$ but not in Fischer 344 rats or hypertensive stroke-prone rats. $^{13}$ The mechanism responsible for a blood flow less than control after 15 seconds of unilateral carotid artery occlusion in Fischer 344 rats is unknown. Because blood flow 15 seconds after release of the second occlusion was at control levels, the mechanism appears to involve the collateral vessels, possibly at the anastomosis. Stroke-prone rats are known to have a defective collateral circulation, probably due to narrower anastomosing vessels. $^{14,15}$ An endothelium-dependent dilator response appears to be impaired in hypertensive stroke-prone rats. $^{16}$
vascular wall remodeling could alter the diameter of which depends on a large dilator reserve.

levels sooner than blood flow during hypercapnia, of occlusion, the larger anastomosis was probably diameter due to muscle contraction. After 1-2 days

Blood flow was not evaluated for a bilateral decrease,9,12 which is possible because the distal unpaired segment of the anterior cerebral artery receives blood from each internal carotid artery in rats. However, after 1-2 days of unilateral carotid artery occlusion, ipsilateral blood flow was similar to that in rats without occlusion, thus suggesting resting blood flow was restored before 1-2 days.

During hypercapnia after 5 days of permanent unilateral carotid artery occlusion in Wistar rats, ipsilateral blood flow was 63% of that on the nonoccluded side, but the percentage difference diminished over 1 month.10 Thus, after unilateral carotid artery occlusion, resting blood flow returns to normal levels sooner than blood flow during hypercapnia, which depends on a large dilator reserve.

We found that the posterior communicating anastomosis was wider following permanent occlusion of a carotid artery. The luminal diameter may have increased by different mechanisms. Dilatation or contraction, change in vascular wall compliance, or vascular wall remodeling could alter the diameter of an anastomosis. We used papaverine to relax the smooth muscle and prevent variability in luminal diameter due to muscle contraction. After 1-2 days of occlusion, the larger anastomosis was probably more compliant because the time for structural rearrangement was minimal. During 6 weeks of occlusion, structural remodeling of the vascular wall most likely produced an anastomosis having larger outer and inner diameters. Further study may define these possible mechanisms.

Despite a wider anastomosis after 1-2 days of unilateral carotid artery occlusion, blood flow during bilateral occlusion was not increased. One possible explanation of this discrepancy was that contracted smooth muscle prevented widening of the anastomosis for increased blood flow and that the anastomosis widened only following muscle relaxation caused by papaverine or latex filling. Another possibility was that a steal phenomenon diverted blood from the monitored cortical site to elsewhere in the brain, to the eye, or to skeletal muscle.

After 6 weeks of unilateral carotid artery occlusion, ipsilateral blood flow was increased twofold during bilateral occlusion, thus indicating either less steal or, more likely, greater blood flow through a

TABLE 1. Luminal Diameter of Posterior Communicating Anastomosis in Fischer 344 Rats After Common Carotid Artery Occlusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Time after first occlusion</th>
<th>Diameter ipsilateral to occluded side</th>
<th>Diameter second occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Same day</td>
<td>152±11</td>
<td>164±8</td>
</tr>
<tr>
<td>II</td>
<td>1-2 days</td>
<td>238±9*</td>
<td>164±8</td>
</tr>
<tr>
<td>III</td>
<td>6 weeks</td>
<td>342±22**</td>
<td>184±3</td>
</tr>
</tbody>
</table>

Bonferroni correction used for multiple comparisons. Data are mean±SEM μm of n anastomoses (9 rats in Group I).
*p<0.05 different from second occlusion by paired t test.
*tp<0.05 different from Group II by analysis of variance.

![Fig. 3](http://stroke.ahajournals.org/DownloadedFrom/Stroke.ahajournals.org/content/21/2/320/F2.large.jpg)

**FIGURE 3. Bar graph. Effect of occlusion of both common carotid arteries on mean±SEM blood flow in Fischer 344 rats.**

Group I: n=9 (18 hemispheres), temporary occlusion; Group II: n=10, 1-2 days' occlusion; Group III: n=7, 6 weeks' occlusion. Probability determined using paired t test without Bonferroni correction.

TABLE 2. Blood Flow in Parietal Cortex of Fischer 344 Rats

<table>
<thead>
<tr>
<th>Time</th>
<th>Group I (15 seconds) (n=18)</th>
<th>Group II (1-2 days) (n=10)</th>
<th>Group III (6 weeks) (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SEM (ml/min/100 g)</td>
<td>%</td>
<td>Mean±SEM (ml/min/100 g)</td>
</tr>
<tr>
<td>Before occlusion (control)</td>
<td>120±7</td>
<td>100</td>
<td>112±6</td>
</tr>
<tr>
<td>After first occlusion</td>
<td>69±8*†</td>
<td>59±6*</td>
<td>124±5</td>
</tr>
<tr>
<td>15 seconds after second occlusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral to first occlusion</td>
<td>22±3</td>
<td>19±3</td>
<td>46±5*†</td>
</tr>
<tr>
<td>Contralateral to first occlusion</td>
<td>15±3</td>
<td>14±3</td>
<td>27±4</td>
</tr>
<tr>
<td>Mean of two sides</td>
<td>27±5†</td>
<td>23±4</td>
<td>19±2†</td>
</tr>
<tr>
<td>After release of second occlusion</td>
<td>113±6</td>
<td>103±11</td>
<td>117±7</td>
</tr>
</tbody>
</table>

n: number of hemispheres (9 rats in Group I). Bonferroni correction used for multiple comparisons.
*p<0.05 different from Group II by analysis of variance.
*tp<0.05 different from control by paired t test.
*tp<0.05 different from contralateral by paired t test.
wider anastomosis. That the side of the greater blood flow and the side of the wider anastomosis were related, not by chance, suggests that the anastomosis diameter was one factor, perhaps a major one, limiting blood flow during the second occlusion. We propose that during the days and weeks following unilateral carotid artery occlusion the anastomosis undergoes structural enlargement, thus allowing increased blood flow.

Others have found that blood flow to the cerebrum after 2, 3, 4, and 5 hours of bilateral carotid artery occlusion was appreciably less than flow after 5 minutes of occlusion. After simultaneous occlusion of both carotid arteries in Fischer 344 rats for 4 hours, cerebral edema is present, which may compromise blood flow to the cerebrum.

After 6 weeks of unilateral carotid artery occlusion, the ipsilateral posterior communicating anastomosis is wider and more blood flows through it. When substantial time elapses between permanent occlusion of the first and second carotid arteries, mortality after the second occlusion is reduced appreciably, possibly due to protection from edema. Our study suggests that protection may be greater on the side having the wider communicating anastomosis and greater collateral blood flow in Fischer 344 rats.

Acknowledgment

We thank Dr. Donald Heistad for review and critical comments on an early version of the manuscript.

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KEY WORDS • carotid arteries • collateral circulation • cerebral blood flow • rats
Collateral development after carotid artery occlusion in Fischer 344 rats.
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Stroke. 1990;21:316-321
doi: 10.1161/01.STR.21.2.316

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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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