Acute occlusion of a middle cerebral artery (MCA) reduces cerebral blood flow in normotensive Wistar-Kyoto rats (WKY) and in stroke-prone spontaneously hypertensive rats (SHRSP). The goal of this study was to determine whether MCA occlusion produces a sustained reduction in cerebral blood flow or whether collateral vessels restore blood flow to normal levels. We measured blood flow through cerebral collateral vessels to the territory of the occluded MCA and to homologous tissue of the other hemisphere in WKY 1 month after occlusion of the MCA. Cerebral blood flow, measured with microspheres, was restored to normal levels under control conditions in the territory of the occluded MCA. During vasodilatation produced by seizures, blood flow and vascular conductance were increased to similar levels in tissue distal to the site of MCA occlusion and in the homologous tissue of the other hemisphere. MCA occlusion did not produce infarction in any of the WKY. In contrast, 1 month after MCA occlusion in SHRSP, a large atrophic infarct was invariably present in the territory of the occluded MCA. The number of collateral vessels to the territory of the MCA do not differ in SHRSP and WKY. Internal diameter and orientation of the anastomotic vessels differ in SHRSP and WKY. We conclude that, after 1 month of MCA occlusion, changes in the collateral vessels supplying the territory of the occluded MCA in WKY were sufficient to restore blood flow to normal under control conditions and to virtually normal levels during vasodilatation. We suggest that, in normotensive rats, protection against infarction after occlusion of the MCA may relate, at least in part, to effective dilator reserve in collateral vessels. (Stroke 1987;18:407–411)

DISTAL branches of the 3 major cerebral arteries anastomose to form an extensive collateral network in rats.1,2 Occlusion of the middle cerebral artery (MCA) increases the diameter of these anastomoses3,4 and, in 5-week-old normotensive Wistar-Kyoto rats (WKY), occlusion of the MCA does not produce infarction.5 In contrast to WKY, in stroke-prone spontaneously hypertensive rats (SHRSP), major cerebral vessels are smaller in lumenal diameter,6 cerebral vascular resistance is greater,7 and occlusion of the MCA produces infarction.5

Five minutes after MCA occlusion, blood flow through collateral vessels to the territory of the occluded MCA is significantly less in SHRSP than in WKY.8 Furthermore, vasodilatation produced by seizures after MCA occlusion produces minimal increases in blood flow to the territory of the occluded MCA in SHRSP and WKY. If the anastomoses in WKY enlarge progressively after occlusion of the MCA,3,4 blood flow through the collateral vessels should increase toward normal levels and if sufficient, protect tissue distal to the occlusion from infarction. In this study, we measured blood flow through cerebral collateral vessels in WKY after 1 month of MCA occlusion. Flow was measured during control conditions and during vasodilatation induced by seizures to evaluate vasodilator reserve of the collateral vessels and the vessels downstream from the collaterals. Our goal was to determine whether MCA occlusion produces a sustained reduction in cerebral blood flow, or whether collateral vessels restore cerebral flow and vasodilator reserve toward normal.

Materials and Methods
We studied 12 male WKY. When the rats were 5–7 months old, they were anesthetized with ketamine (130 mg/kg wt, i.m.). The left MCA was exposed by a transtemporal approach.9 Occlusion of the MCA was accomplished with a monofilament nylon ligature, about 35 μm in diameter. After the skin was sutured, bicillin (30,000 units) was injected i.m.

All rats survived the operation. After 32 days the rats were again anesthetized with ketamine, and a tracheostomy was performed. A PE-50 cannula was inserted into a femoral artery to monitor mean arterial blood pressure (MAP) and to obtain blood samples for measurement of blood gases. A catheter was placed in each brachial artery to obtain reference arterial blood samples. Gallamine triethiodide (Flaxedil 10–15 mg,
i.v.) was administered, and the rat was ventilated with room air supplemented with oxygen, delivered by a Harvard small animal respirator. A thoracotomy was performed, and the tip of a flanged cannula made from PE-50 tubing was inserted through an incision in the left atrial appendage and secured with a ligature. Before the experimental protocol was begun, 0.3 ml of arterial blood was withdrawn for analysis of pH and blood gases. The blood was returned to the rat or an equal volume of 3% ficoll in saline was administered. PaO₂ was 165 ± 11 mm Hg, PaCO₂ 31 ± 3 mm Hg, and pH 7.33 ± 0.04.

Male SHRSP (n = 6) were also studied. When the rats were 7-8 months old, they were anesthetized with ketamine and the left MCA was ligated. At 37 ± 3 days, the rats again were anesthetized with ketamine, and a cannula was inserted into the femoral artery to measure blood pressure. We did not measure cerebral or collateral blood flow in SHRSP because all of the rats had large atrophic infarcts in the region normally supplied by the left MCA.

To quantify the size of the infarcts we perfusion-fixed the brain. A thoracotomy was performed, and the tip of a cannula was secured with a ligature in the ascending aorta. About 180 ml of 10% neutral buffered formalin was injected into the ascending aorta after clamping the aorta above the diaphragm. An incision was made in the right atrium to allow drainage of the formalin. The brain was removed, the olfactory bulbs and hindbrain were excised, and the remaining portion of the brain was weighed after fixation. Infarct size was computed after digitizing the perimeter of the lesion, and adjustments were made for brain curvature. Ratios of infarct area to hemisphere area were computed.¹⁹

**Measurement of Blood Flow with Microspheres**

We measured blood flow with microspheres 3 times in each WKY. About 100,000 microspheres, 15 μm in diameter, labelled with either gadolinium-153, strontium-85, or scandium-46 (New England Nuclear), were shaken vigorously for 3–5 minutes, drawn into a syringe, and the volume was adjusted to 0.5 ml with saline. Microspheres were injected into the left atrium during the control period and seizure. Probability values < 0.05 were considered to be significant.

**Experimental Protocol**

Microspheres were injected 1 month after occlusion of the MCA under control conditions and during a seizure induced with bicuculline (1 mg/kg body wt). Seizure was induced to produce maximal vasodilatation.¹¹ After the last measurement of blood flow, neutral red dye (1% in 1 ml saline) was injected i.v. The rat was killed with ketamine i.v., and the brain was removed. Neutral red is an intravital dye¹² that stainsnormally perfused brain tissue intensely red. The dye was injected to determine whether tissue receiving blood through collateral vessels was less intensely stained than tissue receiving blood through normal routes.

**Tissue Samples and Blood Flow**

The left MCA cortical tissue distal to the occlusion and the homologous tissue on the right (unoccluded) side were dissected and weighed after formalin perfusion fixation. The dissection followed a map of the territory of the occluded MCA demonstrated by reduced intensity of neutral red staining after acute occlusion of the MCA.⁸ After acute MCA occlusion, the cortex distal to the occluded MCA in WKY is less intensely stained than normal cortex. In contrast, we found that 1 month after MCA occlusion the cortex distal to the site of occlusion was stained with similar intensity to cortex receiving blood supply by normal routes. Tissue sample weights for the territory of the occluded MCA obtained after acute occlusion⁷ and after chronic occlusion in this study were the same (mean ± SEM, 0.13 ± 0.01 g). Paraffin sections stained with basic fuchsin showed no evidence of tissue infarction distal to the site of MCA occlusion. A gamma counter was used to count radioactivity in tissue and reference blood samples. Nuclides were separated by standard procedures.¹³ Blood flow (BF) was calculated as $BF = \frac{(C_R \times 100 \times RBF)}{C_B}$, where $RBF$ = reference blood flow (rate of withdrawal of blood samples from reference brachial arteries) in ml/min, $C_B$ = total counts in brain tissue sample, and $C_R$ = reference blood samples. Vascular conductance was calculated as $BF/MAP$. In the region of cortex ($n = 24$), supplied by the MCA, the estimated number of microspheres during control measurements was $419 \pm 86$ (mean ± SEM).

**Statistical Procedures**

A paired t test was used to compare values obtained during the control period and seizure. Probability values < 0.05 were considered to be significant.

**Visualization of Anastomotic Vessels**

In 4 anesthetized, unoccluded WKY and 3 SHRSP maximal vasodilatation was produced with papaverine hydrochloride (1 ml/rat, saturated solution in water, i.v.). Warm Vultex (Chicago Latex Products, No. 563), a latex material, was injected via a cannula in the aorta to demonstrate the course of collateral vessels on the surface of the brain. Branches of the MCA were differentiated from rami of the anterior (ACA) or posterior cerebral artery (PCA) by branch angle orientation. An anastomosis joining an MCA with an ACA branch was identified as the site of smallest vessel diameter or one-half the distance between the most distal MCA and ACA branch points. Details of the procedure are given elsewhere.¹ Internal diameters of the anastomoses were measured from photographs with a digitizer connected to a microcomputer.
Results

Effect of Chronic Occlusion of the MCA in WKY

During control conditions 1 month after occlusion, blood flow to the territory of the occluded MCA was essentially the same as blood flow to the homologous tissue of the other hemisphere (Figure 1). During seizure, blood flow and vascular conductance to tissue distal to the site of MCA occlusion increased significantly (Figure 1; Table 1) but were not significantly different from blood flow and conductance to homologous tissue of the other hemisphere.

Incidence of Infarction 1 Month after MCA Occlusion

Without exception, SHRSP had large (Table 2), grossly visible (Figure 2), atrophic cortical infarcts in the territory of the occluded MCA 1 month after occlusion. The left forebrain ipsilateral to the occluded MCA weighed significantly less than the right forebrain. In contrast, none of the WKY had evidence of infarction 1 month after MCA occlusion. A small cortical lesion was present at the site of occlusion in WKY (Figure 2), presumably a result of local trauma during exposure and ligation of the MCA.59 Weights of the left and right forebrain were similar (Table 2).

Collateral Vessels in SHRSP and WKY

There were 27 ± 3 (mean ± SEM) ACA-MCA anastomoses in SHRSP and 26 ± 1 in WKY. Two features that differentiate collaterals in SHRSP from collaterals in WKY are the internal diameter and configuration of the vessels at the site of the anastomosis. Mean internal diameter of anastomoses in SHRSP were 33 ± 1 and for WKY 50 ± 1 μm. In relation to the configuration of collateral vessels, internal diameter of the bridging collateral was often 50% or less than that of the upstream arteriole. Such “bottleneck” anastomoses may present high resistance. In WKY there was less taper in the internal diameter of the collaterals.

Discussion

The major new findings of this study are, first, 1 month after occlusion of the MCA in WKY, blood flow and vascular conductance to the territory of the occluded MCA and to homologous tissue of the other hemisphere were similar during control conditions. Second, during seizure after chronic occlusion of the MCA, the increase in blood flow and vascular conductance to the territory of the occluded MCA were similar to that of homologous tissue of the other hemisphere. Thus, collateral vessels not only restored control blood flow to normal levels after MCA occlusion, but vasodilator reserve also was restored to virtually normal levels.

Earlier studies on collateral circulation in the brain provided evidence that vascular responses are impaired by acute occlusion of the MCA as autoregulation is impaired over a wide range of arterial pressures. A secondary vascular adjustment occurs with time after the occlusion.1415 Meyer and Denny-Brown14 reported that, after proximal occlusion of the MCA, there was progressive dilatation of pial arteries with a time course of at least several hours. Halsey and Clark16 demonstrated that a normalization of vascular responses to hypercapnia occurs progressively during days and weeks after MCA occlusion in cats. Thus, these studies demonstrated that blood flow to the cor-

Table 2. Effects of Chronic Occlusion of the Middle Cerebral Artery

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stroke-prone spontaneously hypertensive rats (n = 6)</th>
<th>Wistar-Kyoto rats (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemisphere weight (g)</td>
<td>0.69 ± 0.01*</td>
<td>0.84 ± 0.05</td>
</tr>
<tr>
<td>Left</td>
<td>0.76 ± 0.00</td>
<td>0.84 ± 0.04</td>
</tr>
<tr>
<td>Right</td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Lesion produced by exposure</td>
<td>53 ± 7</td>
<td>34</td>
</tr>
<tr>
<td>of MCA (mm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarct size (mm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarct/hemisphere area (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>206 ± 8</td>
<td>89 ± 5</td>
</tr>
</tbody>
</table>

*p < 0.001 left vs. right.
Seizure produces maximal or near maximal dilatation of cerebral vessels and allowed us to determine whether structural changes or differences in vascular tone might account for differences in collateral blood flow. Blood flow and vascular conductance to the territory of the occluded MCA increased significantly during seizure, which indicates that the collateral vessels were not maximally vasodilated during control conditions. This finding is in striking contrast to our finding that vasodilatation produced by seizures 20 minutes after occlusion produced minimal increases in blood flow to the territory of the occluded MCA. Thus, the vasodilator reserve for the territory of the occluded MCA increased greatly during the month after occlusion of the artery.

The mechanism by which vasodilator reserve increases after MCA occlusion probably involves structural remodelling of existing pial surface vessels rather than growth of new vessels either on the pial surface or within the cortex. Three weeks after MCA occlusion, latex-filled vessels that supply the territory of the occluded MCA are larger in diameter than control vessels. There was no evidence of an increase in the number of collateral vessels, even 90 days after MCA occlusion.

Twenty minutes after occlusion of the MCA there is significantly less blood flow to the territory of the occluded MCA in SHRSP than in WKY. SHRSP, but not WKY, developed cerebral infarction, and 1 month after MCA occlusion the infarct had atrophied. The atrophic tissue in SHRSP precluded meaningful measurement of blood flow in the territory of the occluded MCA.

Impairment of collateral blood flow clearly contrib-
utes to cerebral infarction in SHRSP. There is no difference in the number of ACA–MCA anastomoses, but these anastomoses are smaller in diameter in SHRSP than in WKY. Cerebral vessels hypertrophy in spontaneously hypertensive rats\textsuperscript{7} and SHRSP,\textsuperscript{6,18} and changes occur in the vascular wall\textsuperscript{19–21} that may reduce vasodilator reserve. As a consequence of high resistance of the collateral vessels and impaired vasodilator reserve of cerebral vessels, SHRSP are more susceptible to infarction than WKY after MCA occlusion.

**Implications of Findings**

Previous studies provide evidence that, after occlusion of the MCA, changes occur in the collateral vessels that provide blood flow to the territory of the occluded MCA.\textsuperscript{3,4} Our findings indicate that the dilator capacity of these vessels, which is minimal immediately after MCA occlusion in WKY,\textsuperscript{4} is increased 1 month after the occlusion so that the vasodilator reserve is virtually normal. MCA occlusion invariably produces cerebral infarction in SHRSP but not WKY. We suggest that protection against infarction in WKY after occlusion of the MCA may be related, at least in part, to important differences in the anastomosing collateral vessels in WKY and SHRSP and to the greater vasodilator reserve of collateral vessels in normotensive rats.

**Acknowledgments**

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**References**


**Key Words** • collateral blood flow • microspheres • Wistar-Kyoto rat • cerebral anastomoses • stroke-prone spontaneously hypertensive rat • middle cerebral artery
Blood flow through cerebral collateral vessels one month after middle cerebral artery occlusion.

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