Blood Flow Through Cerebral Collateral Vessels in Hypertensive and Normotensive Rats

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SUMMARY We tested the hypothesis that blood flow through cerebral collateral vessels is lower in stroke-prone spontaneously hypertensive rats (SHRSP) than in normotensive Wistar-Kyoto rats (WKY) after occlusion of the middle cerebral artery and during maximal vasodilatation. Cerebral blood flow, measured with microspheres, was similar in adult male SHRSP and WKY under control conditions. In both strains, occlusion of the middle cerebral artery reduced blood flow and vascular conductance to the territory of the occluded artery, as compared with homologous tissue on the side contralateral to the occlusion. The territory distal to the site of occlusion was identified by intravital demarcation with neutral red dye. In both strains, vasodilatation produced by seizures after occlusion of the middle cerebral artery produced minimal increases in blood flow to the territory of the occluded artery. Blood flow and vascular conductance to the territory of the occluded MCA were significantly lower in SHRSP than in WKY (p < 0.05) after occlusion and during seizure after occlusion. We conclude that the lower blood flow through collateral vessels in SHRSP may be related to structural differences in those vessels. Thus, the tendency toward infarction after occlusion of the middle cerebral artery in SHRSP may be related, at least in part, to a more limited dilator reserve of cerebral collateral vessels in SHRSP.

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KEY WORDS • collateral blood flow • microspheres • stroke-prone spontaneously hypertensive rats • Wistar-Kyoto rats • anastomoses

STUDIES have demonstrated that distal branches of the three major cerebral arteries anastomose in the rat and thus form an extensive collateral network. Occlusion of the middle cerebral artery (MCA) increases the diameter of these anastomoses, and in normotensive Wistar-Kyoto rats (WKY), occlusion of the MCA does not produce infarction. In contrast, the major cerebral vessels have a smaller luminal diameter, cerebral vascular resistance is greater, and occlusion of the MCA produces infarction in stroke-prone spontaneously hypertensive rats (SHRSP). If the resistance of collateral vessels is greater in SHRSP than in WKY, then compromised collateral circulation could be an important determinant of the susceptibility of SHRSP to stroke.

In this study, we tested the hypothesis that blood flow through cerebral collateral vessels is lower in SHRSP than in WKY. We measured blood flow in the territory of the occluded MCA. Flow was measured during control conditions and during maximal vasodilatation induced by seizure after MCA occlusion, to evaluate the vasodilator reserve of collateral vessels in SHRSP and WKY.

Methods

Animal Preparation

Male SHRSP (n = 9) and WKY (n = 7) were studied. The SHRSP were 266 ± 8 (mean ± SEM) days old at the time of study, and they weighed 371 ± 9 g. The WKY were 248 ± 10 days old and weighed 423 ± 20 g.

The rats were anesthetized with sodium pentobarbital (30 mg per kilogram of weight, i.p.). After tracheostomy, a PE 50 cannula was inserted into a femoral artery and advanced toward the aorta to monitor mean
arterial blood pressure and to obtain blood samples for measurement of pH, \( P_{\text{CO}_2} \), and \( P_{\text{O}_2} \). A femoral vein was cannulated for injection of supplemental doses (7 mg) of anesthetic approximately every hour. A catheter was placed in each brachial artery to obtain reference arterial blood samples. Before left thoracotomy, gallamine triethiodide (Flaxedil) (10 to 15 mg, i.v.) was administered, and the rat was ventilated with room air supplemented with oxygen, delivered by a small animal respirator (Harvard Bioscience, South Natick, MA, USA). 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.

The left MCA was exposed by a transtemporal approach.\(^8\) Occlusion of the MCA was accomplished with a monofilament nylon suture, about 35 \( \mu \)m in diameter, or by coagulation with a silver nitrate crystal followed by transection of the vessel with iridectomy scissors. Before initiation of the experimental protocol, 0.3 ml of arterial blood was withdrawn for analysis of \( \text{pH} \) and blood gases. The blood was returned to the rat, or an equal volume of 3% Ficoll was administered. In SHRSP \( P_{\text{O}_2} \) was 136 ± 9 mm Hg, \( P_{\text{CO}_2} \) was 35 ± 4 mm Hg, and \( \text{pH} \) was 7.34 ± 0.04; in WKY \( P_{\text{O}_2} \) was 126 ± 13 mm Hg, \( P_{\text{CO}_2} \) was 31 ± 4 mm Hg, and \( \text{pH} \) was 7.43 ± 0.03.

Measurement of Blood Flow with Microspheres

We measured blood flow with microspheres three times in each rat. About 100,000 microspheres, 15 \( \mu \)m in diameter, labeled with \( ^{153}\text{Gd} \), \( ^{48}\text{Sr} \), or \( ^{48}\text{Sc} \) (New England Nuclear, Boston, MA, USA) were shaken vigorously for 3 to 5 minutes, drawn into a syringe, and adjusted to a volume of 0.5 ml with saline. The microspheres were injected into the left atrium in about 30 seconds. Beginning 5 to 10 seconds before the injection and continuing for 60 seconds afterward, blood was withdrawn from each brachial artery at a rate of 0.21 ml/min with a Harvard withdrawal pump. During the injection of microspheres, blood pressure was monitored continuously. Saline (0.5 ml) was flushed into the atrial cannula immediately after the injection. Blood that was withdrawn was replaced with an equal volume of 3% Ficoll.

Experimental Protocol

Microspheres were injected and blood samples were taken 5 minutes before MCA occlusion, after 5 minutes of occlusion, and after 20 minutes of occlusion, during a seizure induced with bicuculline (1 mg per kilogram of body weight). Seizure was induced to produce maximal vasodilatation.\(^9\) After the last measurement of blood flow, neutral red dye (1% in 1 ml of saline), an intravital dye that stains brain tissue,\(^9\) was injected intravenously. The rat was killed with intravenous ketamine, and the brain was removed. The right (intact) cerebral hemispheres were stained relatively homogeneously with neutral red dye. The territory of the left MCA distal to the occlusion was less intensely stained in WKY and was unstained in SHRSP on gross inspection.

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. Great care was taken in excising the cortical tissue distal to the MCA occlusion to avoid removing normal tissue, which was intensely stained with neutral red. The olfactory bulbs were removed, and each forebrain was weighed.

A gamma counter was used to count radioactivity in tissue and reference blood samples. Nuclides were separated by standard procedures.\(^1\) Blood flow was calculated as follows: \( BF = (C_B \times 100 \times RBF)/C_R; \) where \( BF \) is blood flow (ml/min per 100 g), \( C_B \) denotes counts per gram of brain tissue sample, RBF is reference blood flow (rate of withdrawal of blood samples from reference brachial arteries in ml/min), and \( C_R \) denotes total counts in the reference blood samples. Vascular conductance was calculated as blood flow divided by MAP. The estimated number of microspheres in the forebrain samples under control conditions was 848 ± 129 in SHRSP and 922 ± 143 in WKY. In the smallest tissue samples, the area of cortex supplied by the MCA, the estimated number of microspheres during control measurements was 201 ± 24 for SHRSP and 214 ± 42 for WKY.

Statistical Procedures

A paired \( t \) test was used to compare values for samples from the left hemisphere (ipsilateral to MCA occlusion) and the right hemisphere. An unpaired \( t \) test was used to compare values in SHRSP and WKY. \( P \) values under 0.05 were considered to be significant.

Results

Blood Flow and Vascular Conductance in WKY

Occlusion of the MCA in WKY decreased blood flow to the distal cortical tissue. Blood flow and vascular conductance were significantly lower to ipsilateral cortical tissue than to the homologous tissue on the contralateral unoccluded side (Table 1). Blood flow and vascular conductance on the side contralateral to the occlusion did not change appreciably after MCA occlusion.

During seizure in WKY, blood flow and vascular conductance to the cortex distal to MCA occlusion were significantly lower than blood flow and vascular conductance to the homologous tissue on the right (unoccluded) side (see Table 1). A fivefold to sixfold increase in flow occurred bilaterally in the forebrain during seizure (see Table 1).

Blood Flow and Vascular Conductance in SHRSP

After MCA occlusion, blood flow and vascular conductance were lower on the left (occluded) side than in homologous tissue of the hemisphere contralateral to the occlusion. During seizure in SHRSP, blood flow and vascular conductance to the territory of the occluded MCA were lower than the flow and conductance to the right (unoccluded) side (see Table 1).
Comparison of WKY and SHRSP

Before occlusion of the MCA, cerebral blood flow was similar in SHRSP and WKY (Figure 1). After occlusion, blood flow and vascular conductance to the territory distal to the occlusion were significantly lower in SHRSP than in WKY (see Figure 1; Figure 2). During seizure, blood flow and vascular conductance to the region normally supplied by the MCA remained significantly lower in SHRSP than in WKY (see Figures 1 and 2).

Discussion

Two major new findings are documented in this study. First, after occlusion of the MCA, blood flow and vascular conductance to the territory of the occluded artery were lower in SHRSP than in WKY, which suggests that blood flow through collateral vessels to the brain is lower in SHRSP than in WKY. Second, during seizure after occlusion of the MCA, blood flow remained lower in the territory of the occluded vessel, which suggests that minimal resistance of the anastomoses and distal vessels is greater in SHRSP than in WKY.

Consideration of Methods

Neutral red dye clearly demarcated the region with reduced flow after MCA occlusion. This was essential to exclude normally perfused tissue receiving blood flow by normal routes, not by the collateral pathways. Normal tissue was apparently excluded from the tissue supplied by collateral vessels, because there was only a minimal increase in vascular conductance in this region during seizure. Vascular conductance of normal
Vascular conductance to the territory of the middle cerebral artery. Statistical comparison was by Student's t test.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control</th>
<th>Occlusion</th>
<th>Seizure</th>
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<tr>
<td>WKY</td>
<td>0.60</td>
<td>0.60</td>
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<tr>
<td>SHRSP</td>
<td>0.60</td>
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*p < 0.05 vs WKY

Values for cerebral blood flow under control conditions were lower than those obtained in most studies in rats. Although several factors could explain the low values (e.g., head surgery or open chest preparation), the anesthetic is probably the primary explanation. We used ketamine, a dissociative anesthetic, in other experiments in rats (unpublished observations, 1985), and values for cerebral blood flow were considerably higher (94 ± 11 ml/min per 100 g in 12 rats).

Seizure produces maximal dilatation of cerebral vessels and allowed us to determine whether differences in the smooth muscle "tone" of collateral vessels might account for differences in collateral blood flow in SHRSP and WKY. Flow and vascular conductance to the territory of the occluded MCA remained lower in SHRSP than in WKY, which suggests that differences in collateral blood flow are determined by structural factors rather than differences in neurohumoral effects on the collateral vessels. This conclusion corresponds with previous morphological data providing evidence of vascular structural changes secondary to established hypertension. These structural changes include hyaline degeneration, fibrinoid necrosis, thrombotic vascular occlusions, subintimal deposition of fibrinoid materials, and fibrous thickening of the vessel wall involving only distal ramifications and small muscular arteries.

In SHRSP vascular conductance to the region supplied by the MCA did not increase significantly during seizure activity. Thus, the increase in collateral supply was the result of increased blood pressure during the seizure. The cortex distal to MCA occlusion was probably not ischemic in WKY, with an impaired response to seizure, because WKY do not undergo infarction after MCA occlusion. Therefore, the evidence suggests that after MCA occlusion, collateral vessels were almost maximally dilated in WKY and that any increase in collateral blood flow during seizure was largely a passive response to increased perfusion pressure.

Implications of Findings
Previous studies provide evidence that cerebral vessels undergo hypertrophy in SHRSP and that changes secondary to hypertension occur in the vascular wall. In addition, there may be important differences in the structure of anastomosing vessels necessary for collateral blood flow in SHRSP and WKY. Our findings provide support for the concept that the vasodilator capacity of these collateral vessels is more limited in SHRSP than in WKY. These findings may also be of pathophysiological importance. Ligation of the MCA produces cerebral infarction in SHRSP but not in WKY. We suggest that the tendency toward infarction after occlusion of the MCA in SHRSP may be related, at least in part, to a more limited dilator reserve of cerebral collateral vessels in SHRSP.

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