Mechanisms of Protection of the Blood-Brain Barrier During Acute Hypertension in Chronically Hypertensive Rats

WILLIAM G. MAYHAN, FRANK M. FARACI, AND DONALD D. HEISTAD

SUMMARY Spontaneously hypertensive rats are less susceptible than normotensive rats to disruption of the blood-brain barrier during acute hypertension. The purpose of this study was to examine mechanisms that protect the blood-brain barrier from disruption in chronically hypertensive rats. Normotensive Wistar-Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) were studied using intravital fluorescent microscopy and fluorescein-labeled dextran. Disruption of the blood-brain barrier was characterized by the appearance of microvascular leaky sites and quantitated by the clearance of fluorescein-labeled dextran. We measured pressure (servo null) in pial arterioles and venules 40 to 60 μm in diameter. In WKY, acute, phenylephrine-induced hypertension increased pial arteriolar pressure by 47 ± 7 mm Hg (mean ± SE) and pial venous pressure by 20 ± 2 mm Hg. Leaky sites increased from 0 to 28 ± 2. In SHRSP, acute hypertension increased pial arteriolar pressure 44 ± 8 mm Hg, but pial venous pressure increased only 6 ± 1 mm Hg and leaky sites increased from 0 to only 6 ± 1. All leaky sites were venular. In another group of WKY and SHRSP, we increased pial venous pressure passively with a neck cuff. In WKY, venous pressure increased by 22 ± 2 mm Hg, and leaky sites increased from 0 to 23 ± 2. In SHRSP, venous pressure increased by 19 ± 1 mm Hg, and leaky sites increased from 0 to 24 ± 2. Thus, when venous pressure is increased to the same level in WKY and SHRSP, disruption of the blood-brain barrier is similar. We conclude that 1) protection of the blood-brain barrier during acute hypertension in SHRSP is related to attenuation of increases in pial venous pressure, not pial arteriolar pressure, and 2) the blood-brain barrier in venules of SHRSP probably is not inherently resistant to disruption. (Hypertension 9 [Suppl III]: III-101-III-105, 1987)

KEY WORDS • stroke-prone spontaneously hypertensive rats • venous pressure • fluorescein isothiocyanate-labeled dextran

Acute hypertension produces less disruption of the blood-brain barrier in spontaneously hypertensive rats than in normotensive rats.1-4 The mechanism of protection of the blood-brain barrier in chronically hypertensive animals is unclear, but it has been postulated that hypertrophy of cerebral arteries protects arterioles and capillaries from increases in pressure.5-8 We have shown, in normotensive rats, that an acute increase in arterial pressure disrupts the blood-brain barrier primarily in venules.5,10 Disruption of the blood-brain barrier in venules apparently is the result of increases in pial venous pressure and not pial arteriolar pressure. We speculate that hypertrophy of cerebral resistance vessels in SHRSP may protect veins downstream from increases in pressure and thereby protect the blood-brain barrier.

The first goal of this study was to test the hypothesis that protection of the blood-brain barrier during acute hypertension in stroke-prone spontaneously hypertensive rats (SHRSP) may be related to attenuation of increases in pial venous pressure and not pial arteriolar pressure. The second goal of this study was to determine whether the blood-brain barrier of cerebral venules in SHRSP is inherently resistant to disruption. To test this hypothesis, we increased pial venous pressure passively by occluding cerebral venous outflow with a neck cuff.
cuff. We compared disruption of the blood-brain barrier during venous occlusion in SHRSP and normotensive Wistar-Kyoto rats (WKY). By raising venous pressure to the same level in SHRSP and WKY, we were able to determine whether the blood-brain barrier in venules of SHRSP was resistant to disruption.

**Methods**

**Preparation of Animals**

Male WKY and SHRSP (10–12 months old) were anesthetized (pentobarbital sodium, 50 mg/kg of body weight, i.p.), and a tracheotomy was performed. The animals were ventilated mechanically with room air and supplemental oxygen. Skeletal muscle paralysis was obtained with gallamine triethiodide (15–30 mg/kg, i.v.). Supplemental anesthesia was administered at a dose of 10 to 20 mg/kg/hr i.v.

A catheter was placed into a femoral vein for injection of the intravascular tracer, fluorescein isothiocyanate–dextran (FITC-dextran; molecular weight, 70,000) and for injection of phenylephrine, which was used to induce acute hypertension. A femoral artery was cannulated to obtain blood samples and for measurement of arterial blood pressure.

In one group of WKY and SHRSP, we injected phenylephrine (30 μg/kg/min i.v.) to induce acute hypertension. In another group of WKY and SHRSP, we placed a cuff around the neck to increase cerebral venous pressure passively.

To visualize the microcirculation of the cerebrum, a craniotomy was prepared over the right parietal cortex. The cranial window was suffused with artificial cerebral spinal fluid, which was bubbled continuously (pH = 7.32 ± 0.02, Pco₂ = 45 ± 2 mm Hg, Po₂ = 80 ± 6 mm Hg; values are mean ± SE). Temperature of the suffusate was maintained at 38°C. Arterial blood gases were monitored and were maintained within normal limits (pH = 7.38 ± 0.02, Pco₂ = 40 ± 1 mm Hg, Po₂ = 148 ± 13 mm Hg for WKY; and pH = 7.37 ± 0.01, Pco₂ = 41 ± 1 mm Hg, Po₂ = 135 ± 14 mm Hg for SHRSP).

**Permeability of the Blood-Brain Barrier**

Permeability of the blood-brain barrier was evaluated using methods that we have described previously. To prevent anaphylaxis to dextran, antihista mines (diphenhydramine, 10 mg/kg, and cimetidine, 15 mg/kg) were injected intravenously 15 minutes before infusion of FITC-dextran. Macromolecular leakage during interventions was indicated by extravasation of FITC-dextran, which appeared as fluorescent spots or "leaky sites" (expressed as number per 0.11 cm²). We measured the suffusate and plasma concentration of FITC-dextran, and used these measurements to calculate the clearance (ml/sec × 10⁻⁶) of FITC-dextran by pial vessels. The location of leaky sites (arterioles, capillaries, or venules) and clearance of FITC-dextran were determined under control conditions and after induction of acute hypertension, and after pial venous pressure was increased passively with a neck cuff.

To quantitate the concentration of FITC-dextran in the suffusate and plasma, we compared the percentage of transmission of unknown samples to that of known standards with the aid of a spectrophotofluorometer (Model MPF-2A; Perkin-Elmer, Norwalk, CT, USA). The clearance of FITC-dextran was calculated by multiplying the ratio of suffusate-to-plasma concentration of FITC-dextran by the suffusate flow rate, as described previously.

**Microvascular Pressure and Vessel Diameter**

Pial arteriolar and venous pressure were measured with a micropipette connected to a servo-null pressure-measuring device (Model 4A; Instrumentation for Physiology and Medicine, San Diego, CA, USA). We have used this method previously to measure microvascular pressure. Pipettes were sharpened to a beveled tip of 2 to 4 μm in diameter, filled with 1.5 M sodium chloride, and inserted into the lumen of vessels (40–60 μm) using a micromanipulator (Model MM-33; Brinkman Instruments, Westbury, NY, USA). During normotension, the pipette was first inserted into an arteriole, then withdrawn and inserted into a venule. The pipette remained in the venule during acute hypertension or venous occlusion. When pressure reached a steady state during acute hypertension (after approximately 3 minutes), the pipette was withdrawn from the venule and inserted into the original arteriole. Insertion and withdrawal of the pipette did not produce spasm or bleeding in arterioles or venules. All experiments were video-recorded, and diameters of vessels were measured using a video-image shearing device (Model 907; Instrumentation for Physiology and Medicine).

**Experimental Protocols**

**Acute Hypertension**

In 10 WKY and 10 SHRSP, mean arterial pressure was increased by intravenous infusion of phenylephrine (30 μg/kg/min for 5 minutes). Pressure and diameter of arterioles and venules were measured during control conditions and during acute hypertension. The location and number of microvascular leaky sites and clearance of FITC-dextran were determined at 5-minute intervals under control conditions and during acute hypertension.

**Venous Occlusion**

In four other WKY and seven SHRSP, we increased pial venous pressure by inflating a cuff around the neck. Pressure and diameter of pial venules was determined under control conditions and during venous occlusion. The location and number of microvascular leaky sites and clearance of FITC-dextran were determined as described above.

**Statistical Analysis**

A paired t test was used to compare control conditions with intervention. An unpaired t test was used to compare results between separate groups of animals. Bonferroni correction was used when more than two
TABLE 1. Effects of Acute Hypertension on Cerebral Hemodynamics in WKY and SHRSP

<table>
<thead>
<tr>
<th>Variable</th>
<th>WKY Control</th>
<th>WKY Acute Hypertension</th>
<th>SHRSP Control</th>
<th>SHRSP Acute Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>93±5</td>
<td>179±3*</td>
<td>188±6†</td>
<td>270±5*†</td>
</tr>
<tr>
<td>Pial arteriolar pressure (mm Hg)</td>
<td>43±4</td>
<td>91±6*</td>
<td>91±4†</td>
<td>138±6*†</td>
</tr>
<tr>
<td>Pial arteriolar diameter (μm)</td>
<td>45±3</td>
<td>71±3*</td>
<td>37±3†</td>
<td>53±5*†</td>
</tr>
<tr>
<td>Pial venous pressure (mm Hg)</td>
<td>7±1</td>
<td>27±2*</td>
<td>11±1†</td>
<td>17±2*†</td>
</tr>
<tr>
<td>Pial venous diameter (μm)</td>
<td>50±2</td>
<td>58±2*</td>
<td>48±2</td>
<td>49±2†</td>
</tr>
</tbody>
</table>

Values are means ± SE in 10 WKY and 10 SHRSP. *p<0.05 versus control using paired t test. †p<0.05 versus WKY using unpaired t test.

Results

Control Conditions

Under control conditions, mean arterial pressure, pial arteriolar pressure, and pial venous pressure were significantly higher in SHRSP than in WKY (Table 1). The pressure gradient between aorta and pial arteriole was significantly greater in SHRSP (94±6 mm Hg, mean ±SE) compared to WKY (50±3 mm Hg). Thus, although mean arterial pressure was about 95 mm Hg higher in SHRSP than in WKY, pial arteriolar pressure was only 48 mm Hg higher in SHRSP than in WKY. This finding indicates that large arteries attenuate increases in pressure in the cerebral microvasculature under control conditions.

Diameter of pial arterioles was less in SHRSP than WKY under control conditions (see Table 1). Diameter of pial venules was similar in WKY and SHRSP. Under control conditions, there were no leaky sites visible, and clearance of FITC-dextran was minimal in WKY and SHRSP. Thus, higher levels of pial arteriolar pressure and pial venular pressure in SHRSP compared to WKY under control conditions do not produce chronic disruption of the blood-brain barrier.

Response to Acute Hypertension

In WKY, pial arteriolar pressure and diameter and pial venous pressure and diameter increased significantly during acute hypertension (Figure 1; see Table 1). Acute hypertension also produced microvascular leaky sites in pial surface venules and in venules at the brain-pial surface interface, and increased clearance of FITC-dextran in WKY (Figure 2). Leaky sites occurred in small venules (25–40 μm) initially, and then in larger veins (>50 μm) within 5 minutes during acute hypertension. Occasionally, we observed diffuse extravasation of FITC-dextran from arterioles (30–60 μm) after extravasation began in venules.

In SHRSP, there were significant increases in pial arteriolar pressure and pial venous pressure during acute hypertension, but the increase in pial venous pressure was less than that observed in WKY (see Figure 1 and Table 1). Acute hypertension produced a significant increase in pial arteriolar diameter but did not increase pial venular diameter. There was only modest disruption of the blood-brain barrier during acute hypertension in SHRSP (see Figure 2).

Protection of the blood-brain barrier in SHRSP during acute hypertension cannot be explained by differences in increases in mean arterial pressure or pial arteriolar pressure between WKY and SHRSP. Mean
arterial pressure and pial arteriolar pressure increased similarly in WKY and SHRSP during acute hypertension (Figure 3). The increase in pial venous pressure, however, was significantly less in SHRSP than in WKY during acute hypertension (see Figure 3). Thus, protection of the blood-brain barrier in SHRSP during acute hypertension is not related to attenuation of increases in arteriolar pressure, but it is related to attenuation of increases in venular pressure.

The pressure gradient between pial arterioles and pial venules was greater \( p < 0.05 \) in SHRSP \((114 \pm 8 \text{ mm Hg})\) than in WKY \((64 \pm 7 \text{ mm Hg})\) during acute hypertension. Thus, small vessels contribute to protection of the blood-brain barrier in SHRSP during acute hypertension by attenuating increases in pial venous pressure.

**Response to Venous Occlusion**

The magnitude of increase in pial venous pressure was similar in WKY and SHRSP during venous occlusion \((22 \pm 2 \text{ mm Hg})\) in WKY and \(19 \pm 1 \text{ mm Hg}\) in SHRSP; \( p > 0.05 \). Diameter of pial venules also increased similarly in WKY and SHRSP \((51 \pm 4 \mu \text{m})\) to \(58 \pm 4 \mu \text{m}\) in WKY and from \(56 \pm 2 \mu \text{m}\) to \(63 \pm 1 \mu \text{m}\) in SHRSP; \( p > 0.05 \). Venous occlusion in WKY and SHRSP produced microvascular leaky sites in venules and increased the clearance of FITC-dextran (Figure 4). Disruption of the blood-brain barrier was similar in WKY and SHRSP during venous occlusion. Thus, the blood-brain barrier of venules is not inherently resistant to disruption in SHRSP.

**Discussion**

There are two major findings of this study. First, protection of the blood-brain barrier in SHRSP during acute hypertension is not accomplished by attenuation of increases in pial arteriolar pressure, but it is related to attenuation of increases in pial venous pressure. Second, the blood-brain barrier in SHRSP is not inherently resistant to disruption. During venous occlusion, there was marked disruption of the blood-brain barrier in SHRSP, which was similar in magnitude to that observed in WKY during venous occlusion.

**Consideration of Methods**

Phenylephrine was used to induce acute hypertension. Several findings suggest that infusion of \(\alpha\)-adrenergic agonists is an appropriate stimulus for studying effect of hypertension on cerebral vessels. First, \(\alpha\)-adrenergic agonists do not pass the blood-brain barrier readily.\(^{11, 12}\) Second, intravenous infusion of phenylephrine and other \(\alpha\)-adrenergic agonists has no direct effect on pial arteriolar diameter,\(^{13}\) resistance of large or small cerebral vessels,\(^{14}\) or the blood-brain barrier\(^{9}\) when hypertension is prevented. Third, disruption of the blood-brain barrier by acute hypertension may expose cerebral vessels to phenylephrine, but phenylephrine applied topically does not disrupt the blood-brain barrier (W. G. Mayhan, unpublished observation).

In addition, other studies have shown that the blood-brain barrier in chronically hypertensive rats is less susceptible to disruption using agents other than \(\alpha\)-adrenergic agonists.\(^{2}\)

We considered the possibility that the hierarchy of arterioles that were exposed by the craniotomy was different in WKY and SHRSP. If the hierarchy differed, and we studied more distal branches of arterioles in SHRSP than in WKY, this artifact could account for the smaller vessel diameter in SHRSP and the greater drop in pressure from aorta to pial arteriole. We have shown previously, however, that the number of arterial branching points between the circle of Willis and the area of the craniotomy that we studied is similar in WKY and SHRSP.\(^{15}\) Thus, arterioles that are exposed by the craniotomy are of equivalent hierarchy in WKY and SHRSP.

We also considered the possibility that disruption of the blood-brain barrier in venules and veins during acute hypertension was affected by the open-window preparation. We have shown, however, using a closed-window preparation, that disruption of the blood-brain barrier during acute hypertension occurs primarily in venules and veins.\(^{9}\)

**Consideration of Previous Studies**

We considered two mechanisms that might explain the finding that the blood-brain barrier in chronically hypertensive rats is less susceptible to disruption during acute hypertension. The first possible mechanism
is that the cerebral endothelium, which comprises the blood-brain barrier, may be altered in chronic hypertension. Evidence suggests that there is a two-fold increase in the number of tight junctional strands in the endothelial cells of spontaneously hypertensive rats. One would predict that an increase in the number of tight junctional strands between endothelial cells would make the blood-brain barrier inherently less susceptible to disruption. If that were so, results of our study could be interpreted as supporting the concept that protection of the blood-brain barrier during acute hypertension in chronically hypertensive animals is related to an increase in the number of endothelial tight junctional strands.

In our present study, however, we have shown that disruption of the blood-brain barrier during venous occlusion is similar in WKY and SHRSP. This finding suggests that when venous pressure is elevated in SHRSP, the blood-brain barrier in SHRSP is not inherently resistant to disruption. Furthermore, we have shown that SHRSP are more susceptible than WKY to disruption of the blood-brain barrier following the introduction of hyperosmolar solutions. Therefore, we have not found evidence for intrinsic resistance to disruption of the blood-brain barrier in SHRSP.

A second mechanism that might explain the finding that the blood-brain barrier of SHRSP is less susceptible to disruption than that of WKY during acute hypertension is vascular hypertrophy. Hypertrophy of large arteries might attenuate increases in perfusion pressure in arterioles and capillaries during acute increases in blood pressure. Several studies have demonstrated hypertrophy of cerebral arteries during chronic hypertension.

Findings of our present study and previous studies suggest that hypertrophy of large arteries protects the cerebral microcirculation during control conditions. The role of vascular hypertrophy in protecting the blood-brain barrier during acute hypertension is not clear. We found that pial arteriolar pressure increased similarly in WKY and SHRSP, but increases in pial venous pressure were less in SHRSP than in WKY. Thus, protection of the blood-brain barrier during acute hypertension in SHRSP is not related to attenuation of increases in arteriolar pressure, but rather to attenuation of increases in venular pressure. This finding may be explained by hypertrophy of small cerebral arterioles and/or by greater constrictor responses of small arterioles to increases in pressure. Thus, small vessels play a major role in protection of the blood-brain barrier in SHRSP during acute hypertension.

We conclude that during acute hypertension, the blood-brain barrier is less susceptible to disruption in SHRSP than in WKY. We suggest that the mechanism of protection of the blood-brain barrier in SHRSP is related to attenuation of increases in pial venous pressure. Also, based on results obtained during venous occlusion, we suggest that the blood-brain barrier in SHRSP is not inherently resistant to disruption.

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