Role of Molecular Charge in Disruption of the Blood-Brain Barrier During Acute Hypertension

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Acute hypertension disrupts the blood-brain barrier and may neutralize the negative charge on cerebral endothelium. The goal of this study was to determine the effects of molecular charge on permeability of the blood-brain barrier during acute hypertension. Intravital fluorescent microscopy and fluorescein-labeled dextrans were used to evaluate disruption of the blood-brain barrier during acute hypertension in rats. Disruption of the blood-brain barrier was quantitated by calculating clearance of neutral dextran and of anionic dextran sulfate in two groups of rats.

Pressure in pial venules, which are the primary site of disruption of the blood-brain barrier during acute hypertension, was measured using a servo-null device. When systemic arterial pressure was increased from 87±5 (mean±SEM) to 189±5 mm Hg, clearance of neutral dextran increased from 0.04±0.01 to 4.38±0.72 ml/sec×10⁻⁶. When systemic arterial pressure was increased from 91±4 to 181±3 mm Hg, clearance of anionic dextran sulfate increased from 0.02±0.01 to only 0.70±0.23 ml/sec×10⁻⁶. Increases in pial venular pressure were similar in the two groups. Thus, similar increases in systemic arterial pressure and pial venular pressure during acute hypertension produce less disruption of the blood-brain barrier to anionic dextran sulfate than neutral dextran. The findings suggest that 1) the net negative charge of cerebral vessels may be preserved during acute hypertension, and 2) molecular charge is an important determinant of the severity of disruption of the blood-brain barrier during acute hypertension. (Circulation Research 1989;64:658–664)

The luminal surface of cerebral endothelium is coated with an anionic glycocalyx that imparts a negative charge to the endothelium. Sialyl residues account for most of the negative charge, with a small contribution by amines, carboxylic groups of proteins, and phospholipids. Distribution and movement of the negative endothelial surface charge may influence membrane processes and transport of molecules across the blood-brain barrier.

We and others have suggested that acute hypertension disrupts the blood-brain barrier primarily in venules. Previous studies suggest that disruption of the blood-brain barrier during acute hypertension may be associated with an alteration of endothelial surface charge. Furthermore, intracarotid infusion of protamine sulfate or the polycation lysine, which neutralize anionic groups, disrupts the blood-brain barrier. These studies suggest that neutralization of the negative endothelial surface charge may be an important determinant of disruption of the blood-brain barrier during acute hypertension. No studies have been conducted, however, to examine or quantitate disruption of the blood-brain barrier to molecules of different charge during acute hypertension.

In this study, our goal was to determine whether severity of disruption of the blood-brain barrier during acute hypertension is affected by molecular charge. We used neutral dextran and anionic dextran sulfate, with similar molecular weights, to determine the effect of molecular charge on disruption of the blood-brain barrier during acute hypertension. We anticipated that, if the negative charge on cerebral endothelium is neutralized during acute hypertension, disruption of the blood-brain barrier to neutral dextran and anionic dextran sulfate should be similar. However, if the negative charge on...
cerebral endothelium is preserved during acute hypertension, disruption of the blood-brain barrier to neutral dextran should be greater than that for anionic dextran sulfate.

Materials and Methods

Preparation of Animals

We studied 40 male Sprague-Dawley rats. The rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and a tracheotomy was performed. The animals were mechanically ventilated with room air and supplemental oxygen. Paralysis of skeletal muscle was obtained with gallamine triethiodide (15–30 mg/kg i.v.). Supplemental anesthesia was administered intravenously at a dose of 10–20 mg/kg/hr.

A catheter was inserted into a femoral vein for injection of the intravascular tracer, fluorescein isothiocyanate (FITC)-dextran, and for injection of phenylephrine hydrochloride (15–30 μg/kg/min), which was used to induce acute hypertension. To prevent anaphylaxis to dextran, antihistamines (10 mg/kg diphenhydramine and 15 mg/kg cimetidine) were injected intravenously 15 minutes before infusion of FITC-dextran. A femoral artery was cannulated to measure arterial blood pressure.

To visualize the microcirculation of the cerebrum, a craniotomy was prepared over the right parietal cortex. An incision was made in the skin to expose the skull. The skin was retracted and served as a reservoir for the suffusate fluid. Openings were made for constant inflow and outflow of suffusate. A window was made in the skull, and the dura was incised to expose the cerebral microcirculation.

The suffusate fluid (artificial cerebrospinal fluid) was heated (38–39°C) and bubbled continuously to maintain gases within normal limits (pH, 7.38±0.01; Pco2, 42±2 mm Hg; Po2, 67±3 mm Hg). Blood gases were monitored and maintained within normal limits (pH, 7.38±0.01; Pco2, 39±1 mm Hg; Po2, 125±4 mm Hg).

Permeability of the Blood-Brain Barrier

Permeability of the blood-brain barrier was evaluated using two methods that we have described previously. First, extravasation of FITC-dextran was indicated by the appearance of fluorescent spots or “leaky sites.” We visually counted the number of leaky sites in the area of the craniotomy (0.11 cm²) under control conditions and during acute hypertension.

Second, we calculated the clearance (ml/sec x 10^-6) of FITC-dextran by pial vessels. The suffusate fluid was collected in glass test tubes at 5-minute intervals with the aid of a fraction collector. We determined the concentration of FITC-dextran in the suffusate fluid before and during acute hypertension. Arterial blood samples were drawn 10 minutes before injection of FITC-dextran and 15, 40, and 60 minutes thereafter to determine the concentration of FITC-dextran in plasma.

To quantify the concentration of FITC-dextran in the suffusate fluid and plasma samples, we obtained standard curves for concentration of FITC-dextran versus percent transmission with a spectrofluorometer (Perkin-Elmer, Eden Prairie, Minnesota). The standards (neutral FITC-dextran and anionic FITC-dextran sulfate) were prepared on a weight per volume basis. The suffusate concentration was used as background, a standard curve was generated for each experiment, and each curve was subjected to linear regression analysis. The percent transmission for unknown samples (suffusate and plasma) was measured on the fluorometer, and the concentration was calculated from the standard curve. The clearance of FITC-dextran was calculated by multiplying the ratio of suffusate-to-plasma concentration by the flow rate of suffusate, which was maintained constant in all experiments. The clearance of FITC-dextran was determined under control conditions and after induction of acute hypertension.

Induction of Acute Hypertension

We used intravenous infusion of phenylephrine to induce acute hypertension. Several findings suggest that infusion of an α-adrenergic agonist has minimal direct effects on cerebral vessels and therefore is an appropriate stimulus for studying effects of hypertension on cerebral vessels. First, α-adrenergic agonists do not pass the blood-brain barrier readily. Second, intravenous infusion of phenylephrine and other α-adrenergic agonists have no direct effect on pial arteriolar diameter resistance of large or small cerebral vessels, or the blood-brain barrier when hypertension is prevented.

It seemed possible, however, that disruption of the blood-brain barrier by acute hypertension might expose cerebral vascular muscle to phenylephrine, and that phenylephrine might then affect the blood-brain barrier. Thus, in four rats we evaluated permeability of the blood-brain barrier, by counting the number of microvascular leaky sites, during topical application of phenylephrine (10 μg/min for 10 minutes). There were no leaky sites under control conditions or during topical application of phenylephrine. Thus, neither intravascular phenylephrine nor extravascular phenylephrine produce disruption of the blood-brain barrier in the absence of acute hypertension. It seems clear, therefore, that disruption of the blood-brain barrier during infusion of phenylephrine is a response to hypertension per se and not a direct effect of phenylephrine on cerebral vessels.

Microvascular Pressure

Pressure in pial venules was measured with a micropipette connected to a servo-null pressure measuring device (model 4A, Instrumentation for Physiology and Medicine, San Diego, California). Pipettes were sharpened to a beveled tip of 2–4 μm in diameter, filled with 1.5 M sodium chloride and then inserted into the lumen of pial venules (40–60
FIGURE 1. Contrast enhanced thin-layer electrophoresis of neutral fluorescein isothiocyanate (FITC)-dextran and anionic FITC-dextran sulfate. Neutral FITC-dextran did not migrate towards the anode or cathode. In contrast, anionic FITC-dextran sulfate and orange G, which was used as a reference of anionic charge, migrated towards the anode to a similar degree.

Preparation of Neutral FITC-Dextran and Anionic FITC-Dextran Sulfate

Neutral FITC-dextran (50 mg/ml) was dissolved in saline and dialyzed for 2 days. After dialysis, the neutral FITC-dextran solution was lyophilized for 24 hours. The powder of neutral FITC-dextran was again dissolved in saline (50 mg/ml) before use. Anionic FITC-dextran sulfate was prepared by conjugation of sulfate groups to neutral FITC-dextran as described previously. After conjugation, anionic FITC-dextran sulfate was dialyzed and lyophilized as described for neutral FITC-dextran.

Molecular charge of neutral FITC-dextran and anionic FITC-dextran sulfate was determined using thin-layer electrophoresis. Neutral FITC-dextran did not migrate toward the anode or cathode (Figure 1). Anionic FITC-dextran sulfate migrated toward the anode to a similar degree as orange G, which was used as a reference for anionic charge.

Molecular weight of neutral FITC-dextran and anionic FITC-dextran sulfate was determined using gravity flow gel filtration (Sephadex G-75 or G-100, column dimensions were 50 cm by 1 cm). We compared neutral FITC-dextran and anionic FITC-dextran sulfate with known standards (FITC-dextran 4K, 10K, 20K, and 70K and FITC-albumin) for determination of molecular weight. The molecular weights of neutral FITC-dextran and anionic FITC-dextran sulfate were 20,000 daltons.

Thus, in these experiments we compared disruption of the blood-brain barrier during acute hypertension to molecules of similar molecular weight, but different molecular charge.

Binding of Neutral FITC-Dextran and Anionic FITC-Dextran Sulfate to Plasma Proteins

We examined the possibility that neutral and anionic FITC-dextran bind to plasma proteins. In three rats, we injected neutral FITC-dextran intravenously and obtained blood samples after approximately 15 minutes. Plasma was separated from red
of the blood-brain barrier unrelated to charge. To test this possibility, we infused anionic dextran sulfate which was not fluorescent labeled, and then infused neutral FITC-dextran, in order to examine disruption of the blood-brain barrier to neutral FITC-dextran. We anticipated that if anionic dextran sulfate affected transport properties of the blood-brain barrier unrelated to charge, clearance of neutral FITC-dextran during acute hypertension should be altered. If dextran sulfate did not affect transport properties of the blood-brain barrier, however, clearance of neutral FITC-dextran would be similar to that observed in rats without prior injection of anionic dextran sulfate.

**Statistical Analysis**

Statistical analysis was performed using paired t tests to compare values during control conditions and acute hypertension. Unpaired t tests were used to compare values between different groups of rats. Bonferroni correction was used for multiple comparisons. A value of p<0.05 was considered significant.

**Results**

Under control conditions, no leaky sites were visible, and clearance of neutral FITC-dextran and anionic FITC-dextran sulfate was minimal (Table 1). During acute hypertension, there was marked disruption of the blood-brain barrier to neutral FITC-dextran (Table 1; Figure 2). As reported previously, acute hypertension increased cerebral venous pressure (Figure 2), and leaky sites occurred primarily around venules.

Disruption of the blood-brain barrier to anionic FITC-dextran sulfate was less severe than to neutral FITC-dextran during acute hypertension (Table 1; Figure 2). The magnitude and time course for increases in arterial pressure, and the magnitude and time course for increases in pial venous pressure during acute hypertension were similar in rats that received neutral FITC-dextran and anionic FITC-dextran sulfate (Table 1; Figure 2). Thus, there was less disruption of the blood-brain barrier to anionic FITC-dextran sulfate than to neutral FITC-dextran during acute hypertension, and the findings cannot be explained by differences in cerebral hemodynamics.

Disruption of the blood-brain barrier to neutral FITC-dextran during acute hypertension, after injection of non–fluorescein-labeled anionic dextran sulfate (Table 2), was similar to that observed for neutral FITC-dextran alone during acute hypertension (Table 1). Thus, less severe disruption of the blood-brain barrier to anionic FITC-dextran sulfate during acute hypertension cannot be explained by an effect of dextran sulfate on transport properties of the blood-brain barrier that is unrelated to molecular charge.

**Discussion**

This is the first study to quantitate disruption of the blood-brain barrier to molecules of different molecular charge.
TABLE 1. Effects of Acute Hypertension on the Blood-Brain Barrier to Neutral and Anionic Dextran

<table>
<thead>
<tr>
<th></th>
<th>Neutral dextran (n=13)</th>
<th>Anionic dextran (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>87±5</td>
<td>187±5*</td>
</tr>
<tr>
<td>Time to half-maximum pressure (minutes)</td>
<td>1.15±0.06</td>
<td>1.21±0.14</td>
</tr>
<tr>
<td>Pial venous pressure (mm Hg)</td>
<td>8±1</td>
<td>25±1*</td>
</tr>
<tr>
<td>Time to half-maximum pressure (minutes)</td>
<td>1.25±0.05</td>
<td>1.27±0.03</td>
</tr>
<tr>
<td>Leaky sites (No./0.11 cm²)</td>
<td>0</td>
<td>21±2*</td>
</tr>
<tr>
<td>Clearance (ml/sec x 10⁻⁶)</td>
<td>0.04±0.01</td>
<td>4.38±0.72*</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
* p<0.05 vs. control using paired t test.
† p<0.05 vs. neutral dextran using unpaired t test and Bonferroni correction for multiple comparisons.

molecular charge during acute hypertension. The major finding of this study is that the severity of disruption of the blood-brain barrier during acute hypertension is greater for neutral FITC-dextran than for anionic FITC-dextran sulfate. This finding suggests that the net negative charge of cerebral vessels may be preserved during acute hypertension. Thus, molecular charge is an important determinant of the severity of disruption of the blood-brain barrier during acute hypertension.

Consideration of Methods

In this study, we examined permeability of pial vessels during acute hypertension. We considered the possibility that permeability is different in pial and parenchymal vessels during acute hypertension. Previous studies have shown that both pial and parenchymal vessels possess tight junctions between endothelial cells, and confine horseradish peroxidase within their lumen to a similar degree. Thus, these findings suggest that the barrier is morphologically similar in pial and parenchymal vessels. Nevertheless, we cannot exclude the possibility that there are differences in responses to injury in pial and parenchymal vessels.

It was critical to determine whether the stimulus for disruption of the blood-brain barrier was similar in rats that received neutral and anionic dextran. We have suggested that the primary stimulus to disruption of the blood-brain barrier during acute hypertension is an increase in cerebral venous pressure. We considered the possibility that acute hypertension might produce less severe disruption of the blood-brain barrier to anionic FITC-dextran sulfate because increases in pial venous pressure were less in rats that received anionic FITC-dextran sulfate. We found, however, that the increase in pial venous pressure during acute hypertension was similar in rats that received anionic and neutral FITC-dextran. Thus, less severe disruption of the blood-brain barrier to anionic FITC-dextran sulfate cannot be explained by attenuation of increases in pial venous pressure.

We considered the possibility that acute hypertension produces less disruption of the blood-brain barrier to anionic dextran than to neutral dextran by an effect of anionic dextran on cerebral endothelium that is unrelated to molecular charge. To test this possibility, we examined effects of acute hypertension on the blood-brain barrier to neutral dextran after injection of nonfluorescent anionic dextran. We anticipated that, if anionic dextran affected the blood-brain barrier by an effect that is unrelated to charge, clearance of neutral FITC-dextran during acute hypertension would be less in rats that received an injection of unlabeled anionic dextran than in rats without prior injection of anionic dextran. If dextran sulfate did not affect transport properties of the blood-brain barrier, however, clearance of neutral FITC-dextran would be similar to that observed in rats without prior injection of anionic dextran sulfate. We found that clearance of neutral FITC-
The charge on both sides of the endothelium. No studies have examined the effects of acute hypertension on the distribution of charge on cerebral endothelium. Thus, less disruption of the blood-brain barrier to anionic dextran during acute hypertension may be related to preservation of the net (luminal plus abluminal) negative charge on cerebral endothelium during acute hypertension. The precise role of the charge on luminal and abluminal endothelial surfaces in restricting the transport of anionic molecules across the blood-brain barrier during acute hypertension, however, is not clear.

Consideration of Previous Studies

Other investigators have examined the role of molecular charge on permeability of the blood-brain barrier. Permeability of the blood-brain barrier was examined after neutralization of the negative surface charge on cerebral endothelium by protamine or poly-L-lysine. Intracarotid infusion of protamine or poly-L-lysine produced disruption of the blood-brain barrier to horseradish peroxidase and Evans blue dye. The studies suggest that the negative surface charge on cerebral endothelium plays an important role in regulating permeability of the blood-brain barrier under normal conditions. It is possible, however, that protamine and poly-L-lysine produce disruption of the blood-brain barrier independently of neutralization of the endothelial surface charge. Protamine may impair blood-brain barrier function by a direct cytotoxic effect on cerebral endothelium. Poly-L-lysine produces pulmonary edema, which may be the result of a direct toxic effect of poly-L-lysine on pulmonary endothelium.

Acute increases in arterial pressure produce marked disruption of the blood-brain barrier. Recently, we have suggested that disruption of the blood-brain barrier during acute hypertension occurs primarily in venules and is associated with, and presumably due to, increases in cerebral venous pressure. Studies by other investigators have suggested that the negative surface charge on cerebral endothelium may be altered during acute hypertension. Binding of cationized ferritin to cerebral endothelium and permeability of the blood-brain barrier to horseradish peroxidase was examined during control conditions and during acute hypertension. During control conditions, no disruption of the blood-brain barrier was observed, and cationized ferritin was bound to cerebral endothelium. Acute hypertension produced marked disruption of the blood-brain barrier to horseradish peroxidase. Associated with disruption of the blood-brain barrier, the authors observed a reduction or in some instances a loss of binding of cationized ferritin to cerebral endothelium. When arterial pressure returned to control levels, binding of cationized ferritin to the cerebral endothelium was restored. Thus, the authors concluded that disruption of the blood-brain barrier during acute hypertension is associated with alteration of endothelial surface charge. The functional implications of this finding,

### Table 2. Effect of Anionic Dextran Sulfate on the Blood-Brain Barrier to Neutral FITC-Dextran During Acute Hypertension

<table>
<thead>
<tr>
<th>Control</th>
<th>Acute hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>81±12</td>
</tr>
<tr>
<td>Time to half maximum pressure (minutes)</td>
<td>1.55±0.49</td>
</tr>
<tr>
<td>Leaky sites (No./0.11 cm²)</td>
<td>0</td>
</tr>
<tr>
<td>Clearance of FITC-dextran (ml/sec×10⁻⁶)</td>
<td>0.04±0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*p<0.05 vs. control using paired t test.

dextran during acute hypertension was similar in rats with and without prior injection of anionic dextran sulfate. Thus, less severe disruption of the blood-brain barrier to anionic FITC-dextran sulfate cannot be explained by effects of anionic dextran sulfate on transport properties of the blood-brain barrier that are unrelated to charge.

We considered the possibility that differences in molecular weight or size of neutral FITC-dextran and anionic FITC-dextran sulfate may account for differences in disruption of the blood-brain barrier during acute hypertension. Although molecular weight of neutral and anionic dextran were similar, it is conceivable that there is a small difference in the molecular size of anionic and neutral dextran. We have shown previously however that over a wide range of molecular weight and size, disruption of the blood-brain barrier during acute hypertension is independent of molecular size. Thus, differences in molecular weight and size do not account for less severe disruption of the blood-brain barrier to anionic dextran during acute hypertension.

We also considered the possibility that less disruption of the blood-brain barrier during acute hypertension to anionic dextran than to neutral dextran may be related to binding of anionic dextran to plasma proteins. To test this possibility, we examined the ability of neutral and anionic dextran to bind to plasma proteins. We found, as suggested previously, that neutral dextran does not bind to plasma proteins. Furthermore, our findings suggest that anionic dextran does not bind to plasma proteins. Thus, less disruption of the blood-brain barrier to anionic dextran than to neutral dextran cannot be explained by binding of anionic dextran to plasma proteins.

Endothelium appears to be the primary site of negative charge in cerebral vessels. It is possible that the distribution of anionic sites across the endothelial cell may affect transport of molecules across the blood-brain barrier. Cationized ferritin binds to both the luminal and abluminal surfaces of endothelial cells. This finding suggests that transport of anionic molecules across the blood-brain barrier may be affected by the negative surface charge on both sides of the endothelium. No studies
however, are not clear. The authors examined disruption of the blood-brain barrier to neutral horseradish peroxidase, but did not examine disruption of the blood-brain barrier to anionic molecules. Thus, it is not clear whether a reduction in binding of cationized ferritin to cerebral endothelium represents an impairment of the ability of the blood-brain barrier to discriminate between neutral and charged molecules. Previous studies do not exclude the possibility that the endothelial anionic charge is reduced during acute hypertension, but the charge may remain sufficient to represent a significant barrier to transport of anionic molecules across the blood-brain barrier. Although we did not specifically examine the endothelial surface charge in the present study, our findings suggest that charge on cerebral vessels remains sufficient during acute hypertension to significantly affect transport of anionic molecules across the blood-brain barrier.

In summary, we found that disruption of the blood-brain barrier to neutral molecules was greater than disruption to anionic molecules during acute hypertension. This finding suggests that the negative charge on cerebral endothelium may not be functionally altered during acute hypertension. Thus, a barrier to the transport of anionic molecules across the blood-brain barrier is preserved during acute hypertension and may play an important role in regulating the severity of disruption of the blood-brain barrier during acute hypertension.

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References


Key Words: cerebral endothelium • pial venular pressure • FITC-dextran • rats
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