Permeability of blood-brain barrier to various sized molecules

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MAYHAN, WILLIAM G., AND DONALD D. HEISTAD. Permeability of blood-brain barrier to various sized molecules. Am. J. Physiol. 248 (Heart Circ. Physiol. 17): H712-H718, 1985.—We studied disruption of the blood-brain barrier (BBB) by acute hypertension and a hyperosmolar solution. The goals were to determine whether 1) disruption of the BBB occurs primarily in arteries, capillaries, or veins, and 2) transport of different-sized molecules is homogeneous or size dependent. Sprague-Dawley rats were studied using intravital fluorescent microscopy of pial vessels and fluorescein labeled dextrans (FITC-dextran, mol wt = 70,000, 20,000, and 4,000 daltons). The site of disruption was determined by the appearance of microvascular leaky sites. Transport of different-sized molecules was calculated from clearance of FITC-dextran. During gradual hypertension and osmotic disruption, all leaky sites were venular. Rapid hypertension produced venular leaky sites and, in some experiments, diffuse arteriolar extravasation of FITC-dextran. Clearance of different-sized molecules was homogeneous during acute hypertension. In contrast, clearance of molecules during osmotic disruption was size dependent. The findings suggest that 1) venules and veins are the primary sites of disruption following acute hypertension and a hyperosmolar solution; 2) transport of different sized molecules is homogeneous following acute hypertension, which suggests a vesicular mechanism; and 3) transport following hyperosmolar disruption is size dependent, which suggests that hyperosmolar disruption may involve formation of pores as well as vesicular transport.

rats; acute hypertension; arabinose; vesicles; pores; fluorescent microscopy

THE BLOOD-BRAIN BARRIER (BBB) minimizes entry of water-soluble molecules into brain tissue. This restriction is accomplished by tight junctions between adjacent endothelial cells and paucity of pinocytotic vesicles in cerebral arteries, capillaries, and veins (21).

Acute hypertension produces transient disruption of the BBB. Disruption of the BBB following acute hypertension is assumed to be due to an increase in the number and turnover rate of pinocytotic vesicles within endothelial cells (7, 14, 15, 24-26). It is not clear whether disruption occurs in arterioles, capillaries, or veins. Some studies (7, 14, 15, 24, 26) suggest that the BBB is disrupted in arterioles and capillaries, but not veins, following acute hypertension. Other studies (1, 17), however, suggest that venules and veins are the site of disruption following acute hypertension.

Hyperosmolar solutions also disrupt the BBB. Rapoport et al. (19, 20) and Brightman et al. (2) postulated that hyperosmolar solutions (3.0 M urea and 1.6 M arabinose) produce shrinkage of endothelial cells. They suggested that this process allows large gaps to form between adjacent endothelial cells and provides a pathway for the movement of large molecules. On the other hand, several studies (8, 9, 24) suggest that osmotic disruption of the BBB does not produce separation of tight junctions but increases the number of pinocytotic vesicles within endothelial cells. The site of disruption following osmotic disruption is also uncertain and has been reported to involve arterioles (8, 24), capillaries (2, 4, 9), and venules (19, 20).

The first goal of this study was to examine the site of disruption of the BBB following acute hypertension and a hyperosmolar solution. Using intravital fluorescent microscopy and fluorescein-labeled dextran, we were able to visualize the site of disruption following acute hypertension and a hyperosmolar solution.

The second goal of this study was to examine mechanisms of disruption of the BBB following acute hypertension and a hyperosmolar solution. To accomplish this, we used fluorescein-labeled dextrans of different sizes. We postulated that if disruption of the BBB is due to an increase in vesicular transport, molecules of different sizes would be transported relatively homogeneously. If disruption of the BBB is due primarily to separation of tight junctions, transport of smaller molecules would be much greater than that of larger ones. This hypothesis is based on a theoretical study by Garlick and Renkin (6) and on results obtained in experiments in the limb by Carter et al. (3).

METHODS

Preparation of animals. Male Sprague-Dawley rats (n = 59) weighing between 350 and 400 g were anesthetized (pentobarbital sodium, 50 mg/100 g body wt ip), and a tracheotomy was performed. The femoral vein was cannulated for injection of the intravascular tracer, fluorescein isothiocyanate (FITC) dextran (mol wt 70K, 20K, or 4K daltons), and for injection of phenylephrine, which was used to induce acute hypertension. To prevent anaphylaxis to dextran, antihistamines (Benadryl, 10 mg/kg, and Cimetidine, 15 mg/kg) were injected intravenously 15 min prior to infusion of FITC-dextran. The femoral artery was cannulated to obtain blood samples and to measure systemic arterial pressure.

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To visualize the cerebral microvasculature, a cranial window was prepared over the right parietal cortex using a modification of a technique described by Forbes (5) and others (10). A 1-cm incision was made in the skin to expose the skull. The skin was retracted with sutures and served as a "wall" for the suffusate fluid. Outlets were made in the well to allow for constant inflow and outflow of suffusate. In some experiments, the well was connected to an infusion pump that allowed for superfusion with hyperosmolar arabinose. Finally, a craniotomy was performed, the dura was incised, and the cerebral vessels were exposed.

The suffusate fluid [artificial cerebrospinal fluid (CSF)] (22) was bubbled continuously with 5.0% CO₂, 7.5% O₂, and 7.5% N₂. The pH (7.36 ± 0.02), PO₂ (60 ± 2 mmHg), PCO₂ (45 ± 1 mmHg), and temperature (38°C) of the suffusate were maintained constant throughout the experiment. Blood gases were monitored and were maintained within normal limits.

Cerebral vessels were epiphase with a vertical illuminator and viewed through an Olympus microscope (Jewelmont Microscope, Minneapolis, MN). Fluorescent microscopy was accomplished with filters that matched the spectral characteristics of FITC-dextran. An excitation filter (490 nm) and a heat filter were positioned between the light source and the objective (x4.0). A barrier filter (510 nm) was positioned between the objective and the eyepiece.

Determination of permeability to FITC-dextran. Macromolecular leakage was indicated by extravasation of FITC-dextran, which appeared as fluorescent spots or "leaky sites," similar to those that we have observed previously in the hamster cheek pouch (11). The location, i.e., arterioles, capillaries, or venules, of leaky sites in the area of the cranial window (0.11 cm²) was noted under control conditions and after induction of acute hypertension or superfusion with hyperosmolar arabinose. We used several criteria to distinguish arterioles from veins. First, under light microscopy, blood is much darker in veins than arterioles. Second, direction of blood flow is from small vessels to large vessels in veins and from large vessels to small vessels in arterioles. Third, the velocity of blood flow is slower in veins than in arterioles. Fourth, veins usually join at <90° angles, whereas arterioles usually branch at 90° angles. Finally, veins are more tortuous than arterioles. Capillaries are distinguishable from arterioles and veins by flow of single cells and by diameters of <10 μm. The diameter of vessels in which leaky sites occurred was measured with a Vickers image splitter (Vickers Instruments, Malden, MA).

After surgery was completed, the cerebral vessels were suffused with artificial CSF for a 30-min equilibration period. FITC-dextran (70K, 20K, and 4K; 1.0 ml/400 g body wt of a 5% solution) was injected slowly into the suffusate fluid at a rate of 0.35 ml/min. The site of disruption of the BBB was visible. The site of disruption was determined at 2-min intervals initially and at 10- to 20-min intervals until 60 min after superfusion of arabinose.

In 38 rats, mean arterial pressure (MAP) was increased by intravenous infusion of phenylephrine. In 20 rats, pressure was increased gradually by infusion of 5 μg/min phenylephrine for 5 min. This dose produced a maximum MAP of 172 ± 3 (SE) mmHg, with peak pressure occurring in 2.9 ± 0.2 min. In 18 rats, pressure was increased rapidly by infusion of 10 μg/min phenylephrine for 5 min. This dose produced a maximum pressure of 167 ± 2 mmHg, and the maximum pressure was reached in 1.7 ± 0.2 min. The location of leaky sites was determined at predetermined intervals.

To determine whether pretreatment with antihistamine affected the results, four Wistar-Furth rats were subjected to osmotic disruption of the BBB without pretreatment with antihistamines. This strain of rats does not have an anaphylactic reaction to dextran (12, 23).

Statistical analysis. Comparison of differences in permeability of the BBB to FITC-dextran 70K, 20K, and 4K was performed using one-way analysis of variance (ANOVA) with Tukey's test for critical differences. An unpaired t test was used to compare permeability of the BBB in rats pretreated with antihistamines (Sprague-Dawley) to untreated rats (Wistar-Furth). A P value of less than 0.05 was considered to be significant.

RESULTS

Site of disruption. Under control conditions, no leaky sites were visible. The site of disruption of the BBB...
during gradual acute hypertension and superfusion with 1.4 M arabinose always was venular (Fig. 1). Arteriolar and capillary leaky sites were not observed. Leaky sites occurred initially in venules with a diameter of 15–30 μm within 3–5 min. More diffuse leaky sites were observed in discrete areas of larger veins, 50–100 μm within 5–10 min. Rapidly induced acute hypertension always produced venular leaky sites initially, but in 8 of 18 experi-

**FIG. 1.** Photograph of cranial window following injection of FITC-dextran 70K. **Upper:** control conditions, with no leaky sites visible. **Lower:** 5 min after injection of phenylephrine. Leaky sites are visible and all are located around veins.
HYPERTENSIVE AND OSMOTIC DISRUPTION OF THE BBB

Table 1. Disruption of blood-brain barrier during gradual acute hypertension

<table>
<thead>
<tr>
<th>FITC-70K (n = 7)</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Time to Peak Pressure, min</th>
<th>[FITC]plasma, mg/ml</th>
<th>[FITC]superfusate, ng/ml</th>
<th>Clearance, ml/s × 10⁻⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89 ± 4</td>
<td>2.9 ± 0.3</td>
<td>5.4 ± 0.5</td>
<td>13 ± 3</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>178 ± 5</td>
<td></td>
<td>4.7 ± 0.4</td>
<td>199 ± 26</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>FITC-20K (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>87 ± 3</td>
<td>3.3 ± 0.6</td>
<td>4.0 ± 0.2</td>
<td>13 ± 2</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>168 ± 6</td>
<td></td>
<td>3.5 ± 0.2</td>
<td>178 ± 24</td>
<td>1.10 ± 0.10</td>
</tr>
<tr>
<td>FITC-4K (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>101 ± 5</td>
<td>2.6 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>7 ± 1</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>170 ± 4</td>
<td></td>
<td>3.2 ± 0.2</td>
<td>396 ± 60†</td>
<td>3.00 ± 0.60†</td>
</tr>
</tbody>
</table>

Values are means ± SE. FITC, fluorescein-isothiocyanate-labeled dextrans; 70K, 20K, 4K, mol wts of 70,000, 20,000, and 4,000.

* P < 0.05 vs. 70K dextran using ANOVA. † P < 0.05 vs. 20K dextran using ANOVA.

Table 2. Disruption of blood-brain barrier during rapid acute hypertension

<table>
<thead>
<tr>
<th>FITC-70K (n = 8)</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Time to Peak Pressure, min</th>
<th>[FITC]plasma, mg/ml</th>
<th>[FITC]superfusate, ng/ml</th>
<th>Clearance, ml/s × 10⁻⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88 ± 1</td>
<td>1.8 ± 0.2</td>
<td>5.1 ± 0.3</td>
<td>11 ± 3</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>168 ± 3</td>
<td></td>
<td>3.6 ± 0.3</td>
<td>663 ± 66</td>
<td>4.30 ± 0.50</td>
</tr>
<tr>
<td>FITC-20K (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>91 ± 7</td>
<td>1.9 ± 0.4</td>
<td>4.3 ± 0.4</td>
<td>5 ± 1</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>165 ± 3</td>
<td></td>
<td>3.1 ± 0.3</td>
<td>640 ± 110</td>
<td>4.80 ± 0.80</td>
</tr>
<tr>
<td>FITC-4K (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>89 ± 8</td>
<td>1.5 ± 0.4</td>
<td>3.8 ± 0.1</td>
<td>9 ± 2</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>165 ± 5</td>
<td></td>
<td>3.4 ± 0.2</td>
<td>696 ± 32</td>
<td>5.20 ± 0.50</td>
</tr>
</tbody>
</table>

Values are means ± SE. Abbreviations as in Table 1.

Response to arabinose. Under control conditions, there was minimal transport of each size FITC-dextran molecule (Table 3). Values for clearance represent peak responses, which occurred between 5 and 10 min after hyperosmolar arabinose. After superfusion with arabinose, transport of FITC-dextran 20K was two- to threefold greater than that of 70K dextran. Transport of FITC-dextran 4K was two- to threefold greater than that of 20K dextran.

Table 3. Disruption of blood-brain barrier during superfusion with 1.4 M arabinose

<table>
<thead>
<tr>
<th>FITC-70K (n = 7)</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>[FITC]plasma, mg/ml</th>
<th>[FITC]superfusate, ng/ml</th>
<th>Clearance, ml/s × 10⁻⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>91</td>
<td>±1.4</td>
<td>±0.1</td>
<td>±0.01</td>
</tr>
<tr>
<td>1.4 M Arabinose</td>
<td>91</td>
<td>±5</td>
<td>±0.1</td>
<td>±0.03</td>
</tr>
<tr>
<td>FITC-20K (n = 9)</td>
<td></td>
<td>±3</td>
<td>±0.2</td>
<td>±3</td>
</tr>
<tr>
<td>Control</td>
<td>99</td>
<td>±3</td>
<td>±0.2</td>
<td>±3</td>
</tr>
<tr>
<td>1.4 M Arabinose</td>
<td>103</td>
<td>±2</td>
<td>±0.3</td>
<td>±90</td>
</tr>
<tr>
<td>FITC-4K (n = 5)</td>
<td></td>
<td>±5</td>
<td>±0.2</td>
<td>±6</td>
</tr>
<tr>
<td>Control</td>
<td>101</td>
<td>±5</td>
<td>±0.2</td>
<td>±6</td>
</tr>
<tr>
<td>1.4 M Arabinose</td>
<td>110</td>
<td>+2</td>
<td>+0.9</td>
<td>+298</td>
</tr>
</tbody>
</table>

Values are means ± SE. Abbreviations as in Table 1. * P < 0.05 vs. 70K dextran using ANOVA. † P < 0.05 vs. 20K dextran using ANOVA.

Response to osmotic disruption. There was diffuse extravasation of FITC-dextran from arterioles (40-60 μm) in 5-10 min, in addition to venular leaky sites.

Response to acute hypertension. The effect of gradual acute hypertension on permeability of the BBB is shown in Table 1. Values for clearance represent peak responses, which occurred between 5 and 10 min after the onset of acute hypertension. Under control conditions, there was minimal clearance of each sized FITC-dextran molecule. After acute hypertension, there was a similar increase in the transport of FITC-dextran 70K and 20K, as indicated by increased clearance of FITC-dextran, but transport of FITC-dextran 4K was greater than that of 70K and 20K dextran. Thus, after a gradual increase in arterial pressure, transport of small molecules tends to be greater than that of large molecules.

We speculated that if transport of molecules is size dependent after disruption of the BBB by acute hypertension, a more rapid elevation of arterial pressure might amplify the modest size-dependent transport that we observed during gradual acute hypertension. To examine this possibility, we produced more rapid hypertension.

During rapid acute hypertension (Table 2), transport of 70K, 20K, and 4K FITC-dextran molecules was similar (P > 0.05). Thus acute hypertension produced a nearly homogeneous increase in transport of different-sized molecules.

Clearance of FITC-dextran decreased slowly from the peak values following gradual and rapid acute hypertension but did not return to control values during the 60-min collection period.
Capillaries, but rarely in veins. In contrast, others, using intravital microscopy and horseradish peroxidase (HRP), reported that the BBB is disrupted in arterioles and small veins. Several investigators (7, 14, 15, 18), using electron microscopy and HRP, observed that the BBB is disrupted in venules rather than collecting veins. Therefore, we have considered the possibility that the mode of application of hyperosmolar solutions could affect disruption of the BBB to different-sized molecules. If an arachnoid barrier contributes importantly to the BBB, topical application of hyperosmolar solutions presumably disrupts this barrier, as well as the endothelial barrier, while intracarotid infusion may disrupt only the endothelial barrier. Therefore, differences in transport of different-sized molecules could be explained by a size selectivity in the arachnoid membrane. The arachnoid, however, has discontinuous as well as tight junctions between cells (13) and does not provide a size-selective barrier. Also experiments using intracarotid infusion of arabinose have shown a size-selective transport of molecules (27). Thus our study, using topical application, and a study that used intracarotid infusion of arabinose (27) both demonstrate size selectivity after hyperosmolar disruption of the BBB.

Our results are in agreement with those (1, 17, 20) indicating that the primary site of disruption of the BBB by acute hypertension or a hyperosmolar solution is in small veins.

It is possible that the site of disruption of the BBB differs in pial and parenchymal vessels. We and others (1, 17, 20) have examined pial vessels while other investigators (7, 14, 15, 18) have examined parenchymal vessels. Therefore, we have considered the possibility that there are structural differences between vessels in the two regions. Rapoport et al. (20) suggest that the morphological feature that is common to pial and parenchymal vessels is the presence of tight junctions between endothelial cells. Reese and Karnovsky (21) report that pial and parenchymal vessels contain HRP within their lumen to a similar degree. Nevertheless, we cannot exclude the possibility that there are differences in structure or responses to injury in pial and parenchymal vessels.

A recent study by Nagy et al. (16) suggests that the barrier is discontinuous in collecting veins under normal conditions. This finding implies that the BBB may be more vulnerable in large veins. The observation does not correspond closely to our findings, however, because we found disruption primarily in venules rather than collecting veins.

**Figure 2.** Clearance of various sized FITC-dextran molecules following gradual acute hypertension (open circles), rapid acute hypertension (triangles), and superfusion with 1.4 M arabinose (closed circles).

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**DISCUSSION**

There are three major findings in this study. First, veins are the primary site of disruption of the BBB during acute hypertension and superfusion with arabinose. Second, transport of different-sized FITC-dextran molecules during acute hypertension is relatively homogeneous. Third, transport of different-sized FITC-dextran molecules during osmotic disruption of the BBB is size dependent.

**Site of disruption of the BBB.** Previous studies have led to conflicting conclusions concerning the primary site of disruption of the BBB during acute increases in arterial pressure. Several investigators (7, 14, 15, 18), using electron microscopy and horseradish peroxidase (HRP), reported that the BBB is disrupted in arterioles and capillaries, but rarely in veins. In contrast, others (1, 17),
The frequency distribution of different types of vessels could contribute to differences in findings in this study and other studies (1, 17, 20) that examined pial vessels and those of other investigators (7, 14, 15, 18) that examined parenchymal vessels. For example, capillary density is much greater in the parenchyma than on the surface of the brain. Thus, although we did not observe disruption of the BBB in any pial capillaries, we cannot exclude the possibility that the barrier is disrupted in some parenchymal capillaries.

Conflicting results concerning the site of disruption of the BBB following acute hypertension and hyperosmolar solutions may also be related to the methods that were used to evaluate the increase in permeability. Studies that have used histological methods, i.e., electron microscopy and HRP, have shown clearly that disruption of the BBB occurs in arterioles and capillaries, rarely in veins. Because this approach limits the area that can be sampled, however, marked disruption of the BBB in discrete areas of veins might not be detected. The presence of reaction product can be used to guide sampling sites with electron microscopy, but the authors cited (7, 14, 15, 18) do not indicate that this approach was used to evaluate the site of disruption. In those studies in which we observed disruption of the BBB in arterioles, extravasation of FITC-dextran did not occur in discrete areas but was diffuse over the entire segment of the arteriole. Thus one would expect that most sections of the arteriole, but not venule, would show increased permeability using histological approaches.

Other factors that may affect conclusions regarding the primary site of disruption of the BBB following acute hypertension are the rate of rise of arterial pressure and severity of hypertension. Morphological studies (14, 15, 26), which have indicated that the primary site of disruption is in arterioles, have obtained peak increases in mean arterial pressure of 190–230 mmHg in 0.5–1.5 min. In experiments in which we rapidly raised pressure and in which we have observed arteriolar disruption, the maximum blood pressure (167 ± 3 mmHg) was less than that observed in histological studies, but the rate of rise in pressure was similar (1.7 ± 0.2 min). Thus, although our study suggests that venules and veins are the primary site of disruption of the BBB following acute hypertension, our data also suggest that the site of disruption of the BBB is dependent on the rate of rise of arterial pressure.

We have considered the possibility that the open cranial window preparation might alter responses to acute hypertension. In two rats, therefore, we implanted a Perspex cover over the cranial window and observed the site of disruption during acute hypertension in a closed-skull preparation. In these experiments we also observed leakage of FITC-dextran primarily from venules and veins.

On the basis of our studies and those of others (1, 17, 20) in vivo, we suggest that the initial site of disruption of the BBB may be discrete focal leaky sites in venules. One would expect that these venular sites would not be detected in histological samples taken randomly for electron microscopy. We suggest, however, based on histological studies (14, 15, 26) and on our findings during rapid acute hypertension, that a more sustained period of hypertension or more severe hypertension may produce diffuse arteriolar disruption of the BBB. It is not clear from our study or previous studies whether the focal venular leaky sites or diffuse arteriolar extravasation are of greater quantitative importance in disruption of the BBB during acute hypertension or hyperosmolar solutions.

Mechanism of disruption of the BBB. Recent evidence suggests that disruption of the BBB during acute hypertension is the result of an increase in the number and turnover rate of pinocytotic vesicles within endothelial cells (7, 14, 15, 24–26). The mechanism of disruption of the BBB following a hyperosmotic stimulus, however, is not fully understood. It has been proposed that topical application or injection of hyperosmolar solutions produces shrinkage of endothelial cells (2, 19, 20). These experiments (2, 19, 20) suggested that shrinkage of endothelial cells allowed large gaps to form between adjacent endothelial cells and provided a pathway for the movement of large molecules. Other investigators (4, 8, 9, 16, 24) have failed to observe separation of tight junctions following hyperosmolar solutions. These investigators (4, 8, 9, 24) have, on the other hand, observed an increase in the number of pinocytotic vesicles during osmotic disruption of the BBB. Farrell and Shivvers (4) recently observed an increased formation of pinocytotic vesicles and fusion of vesicles, which led to formation of transendothelial channels.

We used different-sized FITC-dextran molecules in an attempt to determine whether osmotic and hypertensive disruption of the BBB increases transport of molecules by vesicles or pores. The rationale for using different-sized molecules to determine if transport is via vesicles or pores is based on a theoretical study by Garlick and Renkin (6), which indicates that vesicular transport is relatively independent of molecular size of the molecule, but transport via pores is size dependent, with more transport of small molecules than large molecules. Results obtained in a study of Carter et al. (3), using dog hindlimb, support this concept. Carter et al. (3) found that the transport of different-sized molecules following stimulation with histamine to be relatively independent of molecular size. This finding, according to Carter et al. (3), could be explained by an increase in vesicular transport and not by an increase in transport through pores. The concept that size-dependent transport indicates separation of junctions, whereas equal transport of small and large molecules indicates a vesicular mechanism, is speculative, however, and will require histological confirmation.

The concept of size-dependent (pores) and size-independent (vesicles) transport systems in cerebral vessels has been examined in preliminary experiments conducted by Ziylan et al. (27). Results from these experiments, which measured permeability-surface area products for various regions of the brain, indicated that intracarotid injection of 1.8 M arabinose caused a size-dependent increase in transport of molecules 5, 15, and 65 A. The investigators (27) concluded that increased
transport after a hyperosmolar solution is due to separation of tight endothelial junctions and an increase in a size-independent transport mechanism, such as vesicular transport. Although we are unable to directly compare data of Ziylan et al. (27), which examined regional permeability of the BBB to different-sized molecules, both studies indicate a size-dependent increase in permeability of the BBB following osmotic disruption of the BBB. Our data extend those of Ziylan et al. (27) with the finding that transport of different-sized molecules during acute hypertension is homogeneous.

In conclusion, transport of different-sized molecules during acute hypertension is relatively homogeneous. This finding suggests that the mechanism of transport is vesicular. In contrast, transport of different-sized molecules during superfusion with arabinose is size-dependent. This finding suggests that the primary mechanism of transport may be separation of tight junctions between endothelial cells to form functional "pores" in the membrane.

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