Disruption of the Blood–Brain Barrier in Open and Closed Cranial Window Preparations in Rats

William G. Mayhan, PhD

The goal of this study was to determine whether the severity of disruption of the blood–brain barrier during acute hypertension is similar in open and closed cranial window preparations. Intravital fluorescent microscopy and fluorescein-labeled albumin were used to evaluate disruption of the blood–brain barrier under control conditions and during acute arterial hypertension in 10 rats equipped with an open cranial window and in six rats equipped with a closed cranial window. Permeability of the blood–brain barrier was quantified by calculating the clearance of fluorescein-labeled albumin and by counting the number of microvascular leaky sites under control conditions and during acute hypertension. Pressure in cerebral venules and intracranial pressure were measured in rats equipped with an open cranial window and a closed cranial window, respectively, under control conditions and during acute hypertension. In rats equipped with an open cranial window, arterial pressure increased from 118±6 to 189±3 mm Hg (mean±SEM) and pial venous pressure increased from 7±1 to 22±3 mm Hg during acute hypertension induced with 30 μg/kg/min phenylephrine for 5 minutes. In addition, the clearance of fluorescent albumin increased from 0.11±0.03 to 1.2±0.4 ml/sec×10^-4 and the number of microvascular leaky sites increased from 0 to 25±1 during phenylephrine infusion. In rats equipped with a closed cranial window, arterial pressure increased from 122±5 to 187±7 mm Hg and intracranial pressure increased from 3±1 to 12±1 mm Hg during the intravenous infusion of 30 μg/kg/min phenylephrine for 5 minutes. Clearance of fluorescent albumin increased from 0.08±0.04 to 1.0±0.2 ml/sec×10^-4 and the number of microvascular leaky sites increased from 0 to 25±1 during phenylephrine infusion. Thus, these findings suggest that the severity of disruption of the blood–brain barrier during acute hypertension is similar in open and closed cranial window preparations. (Stroke 1991;22:1059–1063)

Under normal conditions, the blood–brain barrier minimizes the entry of molecules into brain tissue. This restriction is accomplished by tight junctions between adjacent endothelial cells and a paucity of pinocytic vesicles in the endothelium of arterioles, capillaries, and veins.1,2 Acute severe increases in arterial blood pressure produce an increase in cerebral blood flow, passive dilatation of cerebral blood vessels, and disruption of the blood–brain barrier.3–6 We have reported that disruption of the blood–brain barrier during acute hypertension, in an open cranial window preparation, occurs primarily in cerebral veins and venules.7,8

It is possible that disruption of the blood–brain barrier in venules during acute hypertension may be an artifact of the open cranial window preparation. Furthermore, the severity of disruption of the blood–brain barrier in venules during acute hypertension may be influenced by the open cranial window preparation. In an open cranial window preparation, transmural pressure across cerebral venules during acute hypertension increases dramatically, whereas with the skull intact, transmural pressure across cerebral venules presumably is less than that observed in an open cranial window preparation because of an increase in intracranial pressure. Thus, the relative attenuation of transmural pressure with the skull intact may shift the site of disruption of the blood–brain barrier from venules to arterioles and capillaries and may influence the severity of disruption of the blood–brain barrier during acute hypertension.

The goal of this study was to examine the site and severity of disruption of the blood–brain barrier
FIGURE 1. Bar graphs of mean arterial pressure (left panel), pial venous pressure (middle panel), and intracranial pressure (right panel) under control conditions and during phenylephrine-induced acute hypertension in rats equipped with open cranial window (open bars) and in rats equipped with closed cranial window (filled bars). Values are mean±SEM. *p<0.05 versus control conditions.

Materials and Methods

Sixteen male Sprague-Dawley rats were anesthetized with 50 mg/kg body wt i.p. pentobarbital sodium, and a tracheotomy was performed. The animals were ventilated mechanically with room air and supplemental oxygen. Skeletal muscle paralysis was obtained with 10 mg/kg i.v. gallamine triethiodide. Supplemental anesthesia was administered at a dose of 10–20 mg/kg/hr i.v.

A catheter was placed into a femoral vein for injection of the intravascular tracer, fluorescein isothiocyanate albumin (FITC-albumin; molecular weight=69,000), and for injection of phenylephrine (30 µg/kg/min for 5 minutes) to induce acute hypertension. A femoral artery was cannulated for measurement of arterial blood pressure and to obtain blood samples for determination of the FITC-albumin concentration in plasma.

In 10 rats an open cranial window was prepared to visualize the cerebral microcirculation using methods described previously.7,8

In six other rats a closed cranial window was prepared using methods described previously.7,9 An incision was made in the skin to expose the skull. Bone wax was used to form a dam for the suffusion fluid. Outlets were made in the bone wax to allow for the constant suffusion of fluid and for the measurement of intracranial pressure. Then, a craniotomy was performed to expose the cerebral microcirculation, a plastic coverslip was fixed into position over the preparation with additional bone wax, and the window was sealed with dental acrylic.

The open and closed cranial windows were suffused with artificial cerebrospinal fluid (2 ml/min), which was bubbled continuously. Temperature of the suffusate was maintained at 38°C. Arterial blood gases were monitored and were maintained within normal limits.

Permeability of the blood–brain barrier was evaluated using two methods described previously.7,8,10 First, extravasation of FITC-albumin was indicated by the appearance of fluorescent spots or "leaky sites." The numbers of leaky sites in the area of the craniotomy (0.11 cm²) were counted under control conditions and during acute hypertension.

Second, the clearance (in milliliters per second×10⁻⁶) of FITC-albumin by pial vessels was calculated. The suffusate was collected in glass test tubes at 5-minute intervals with the aid of a fraction collector. The concentration of FITC-albumin in the suffusate before and during acute hypertension was determined with the aid of a spectrophotofluorometer (650-10LC Fluorescence Spectrophotometer, Perkin-Elmer Corp., Norwalk, Conn.). Arterial blood samples were drawn 10 minutes prior to the injection of FITC-albumin, 5 minutes prior to the induction of acute hypertension, and 5 and 30 minutes thereafter to determine the concentration of FITC-albumin in plasma. The clearance of FITC-albumin under control conditions and during acute hypertension was calculated by multiplying the ratio of the suffusate to the plasma concentrations by the flow rate of the
Mayhan  Blood–Brain Barrier During Acute Hypertension  1061

Leaky Sites

Clearance of FITC-Albumin

FIGURE 2. Bar graphs of number of microvascular leaky sites per 0.11 cm² (left panel) and clearance of fluorescein isothiocyanate albumin (FITC-albumin) in ml/sec×10⁻⁶ (right panel) under control conditions and during phenylephrine-induced acute hypertension in rats equipped with open cranial window (open bars) and in rats equipped with closed cranial window (filled bars). Values are mean±SEM. *p<0.05 versus control conditions.

suffusate, which was maintained constant in all experiments at 2.0 ml/min.

In rats equipped with an open cranial window, pial venous pressure was measured under control conditions and during acute hypertension with a micropipette connected to a servo-null pressure-measuring device (Model 5A, Instrumentation for Physiology and Medicine, Inc., San Diego, Calif.).8,11

In rats equipped with a closed cranial window, intracranial pressure was measured under control conditions and during acute hypertension via an outlet from the cranial window to a pressure transducer.

In rats equipped with an open cranial window, mean arterial pressure was increased by the intravenous infusion of 30 μg/kg/min phenylephrine for 5 minutes. The location and number of microvascular leaky sites were determined at 5-minute intervals under control conditions, at 1-minute intervals for 5 minutes during the induction of acute hypertension, and at 5- to 10-minute intervals thereafter for 50 minutes. Clearance of FITC-albumin was determined at 5-minute intervals under control conditions, during acute hypertension, and for 50 minutes following acute hypertension.

In rats equipped with a closed cranial window, a similar protocol was followed for the induction of acute hypertension and for the determination of permeability of the blood–brain barrier under control conditions and during acute hypertension.

A paired t test was used to compare results under control conditions and during acute hypertension in the same animals. An unpaired t test was used to compare results between groups of animals. A probability value of less than 0.05 was considered to be significant.

Results

Mean arterial pressure was similar in rats equipped with an open and a closed cranial window under control conditions (Figure 1, left panel). Pial venous pressure was 7±1 mm Hg in rats equipped with an open cranial window (Figure 1, middle panel), and intracranial pressure was 3±1 mm Hg in rats equipped with a closed cranial window (Figure 1, right panel).

Under control conditions, no leaky sites were visible and the clearance of FITC-albumin was minimal in rats equipped with an open or a closed cranial window (Figure 2).

Intravenous infusion of phenylephrine produced a marked increase in mean arterial pressure (Figure 1, left panel) and pial venous pressure (Figure 1, middle panel) in rats equipped with an open cranial window.

In rats equipped with a closed cranial window, intravenous infusion of phenylephrine also produced a marked increase in mean arterial pressure (Figure 1, left panel) and intracranial pressure (Figure 1, right panel).

In rats equipped with an open cranial window, phenylephrine infusion produced marked disruption of the blood–brain barrier. The primary site of disruption of the blood–brain barrier during acute hypertension in the open cranial window preparation was venules 20–40 μm in diameter. As hypertension progressed, more diffuse leaky sites were observed in larger venules and veins and occasionally diffuse extravasation was observed in arterioles as well as venules. This finding is similar to that reported previously.7,8 The number of leaky sites increased from 0 to 25±1 in the area exposed by the craniotomy, and the clearance of FITC-albumin increased from 0.11±0.03 to 1.2±0.4 ml/sec×10⁻⁶ (Figure 2).

In rats equipped with closed cranial window, phenylephrine infusion also produced marked disruption of the blood–brain barrier. The primary site of disruption of the blood–brain barrier during acute hy-
pertension in rats equipped with a closed cranial window was similar to that observed in rats with an open cranial window, that is, the veins and venules. The number of leaky sites increased from 0 to 25±1 in the area exposed by the craniotomy, and the clearance of FITC-albumin increased from 0.08±0.04 to 1.0±0.2 ml/sec×10⁻⁶ (Figure 2).

The magnitude of changes in mean arterial pressure and disruption of the blood–brain barrier during acute hypertension (number of leaky sites and clearance of FITC-albumin) was similar in rats equipped with an open and a closed cranial window (Figure 3).

Discussion

This is the first study to examine the site and severity of disruption of the blood–brain barrier during acute hypertension in rats equipped with an open or a closed cranial window. There are two major findings of the present study. First, the primary site of disruption of the blood–brain barrier during acute hypertension (number of leaky sites and clearance of FITC-albumin) was similar in rats equipped with an open and a closed cranial window. Second, the severity of disruption of the blood–brain barrier during acute hypertension appears to be similar in open and closed cranial window preparations. We found that veins and venules are the primary sites of disruption of the blood–brain barrier during acute hypertension in rats equipped with an open or a closed cranial window. Second, the severity of disruption of the blood–brain barrier during acute hypertension appears to be similar in open and closed cranial window preparations. We found that the number of microvascular leaky sites and the clearance of FITC-albumin were similar in rats equipped with an open and a closed cranial window during acute hypertension.

Permeability of the blood–brain barrier under control conditions and during acute hypertension was quantified using two techniques that we7,8,10,12 and others13,14 have described previously. First, we counted the number of leaky sites in the area exposed by the craniotomy (0.11 cm²) under control conditions and during acute hypertension. Second, we calculated the clearance of FITC-albumin by pial vessels in rats equipped with an open or a closed cranial window. Thus, two methods were used to quantify the permeability of the blood–brain barrier under control conditions and during acute hypertension.

In the present study, we examined permeability of the blood–brain barrier in pial vessels under control conditions and during acute hypertension. It is possible that disruption of the blood–brain barrier during acute hypertension may differ in pial and parenchymal vessels. It is possible that there are structural differences between pial and parenchymal vessels and that disruption of the blood–brain barrier during acute hypertension may differ in these regions. However, Rapoport et al1 suggest that the morphological feature that is common to pial and parenchymal vessels is the presence of tight junctions between endothelial cells. In addition, Reese and Karnovsky2 report that pial and parenchymal vessels confine horseradish peroxidase within their lumens to similar degrees. Furthermore, Olsen15 reports that disruption of the blood–brain barrier in pial veins and venules during acute hypertension was similar to that found in veins and venules located in the gray and white substances of the brain. Thus, evidence suggests that the structure of the blood–brain barrier under control conditions may be similar in pial and parenchymal vessels1,2 and that disruption of the blood–brain barrier in veins and venules is similar in the pial and parenchymal circulations during acute hypertension.15

We7,8 and others16 have shown that disruption of the blood–brain barrier during acute arterial hypertension occurs primarily in veins and venules. In addition, the mechanism of disruption of the blood–brain barrier in veins and venules during acute hypertension appeared to be related to increases in cerebral venous pressure.8 These studies7,8 however, examined disruption of the blood–brain barrier dur-
ing acute hypertension in an open cranial window preparation. Thus, it is possible that the open cranial window preparation might alter the site and the severity of disruption of the blood–brain barrier during acute hypertension.

In the present study and in previous studies, we have shown dramatic increases in the transmural pressure across cerebral venules during acute hypertension in rats equipped with an open cranial window. With the skull intact, however, the increase in transmural pressure across cerebral venules during acute hypertension presumably would be less than that observed in an open cranial window preparation because of an increase in intracranial pressure. Other studies, using the closed cranial window technique, have shown significant increases in intracranial pressure during acute arterial hypertension. Thus, findings from these previous studies support the concept that increases in transmural pressure across cerebral venules during acute hypertension is less in a closed cranial window preparation than in an open cranial window preparation. These previous studies, however, did not examine disruption of the blood–brain barrier in an open and a closed cranial window preparation during acute hypertension. In light of these previous studies, we considered the possibility that the relative attenuation of transmural pressure with the skull intact may shift the site of disruption of the blood–brain barrier from venules to arterioles and capillaries and/or may influence the severity of disruption of the blood–brain barrier during acute hypertension.

In the present study, however, we found that the primary site of disruption of the blood–brain barrier during acute hypertension in rats equipped with an open or a closed cranial window was the veins and venules. In addition, the severity of disruption of the blood–brain barrier during acute hypertension was similar in rats equipped with an open and a closed cranial window.

Thus, the findings of the present study do not support the concept that the site of disruption of the blood–brain barrier (veins and venules) and the severity of disruption of the blood–brain barrier in veins and venules during acute hypertension in an open cranial window preparation are an artifact of the open window preparation.

References


Key Words: Blood–brain barrier • Intracranial pressure • Microcirculation • Rats
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W G Mayhan

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