Microcirculation of the Area Postrema
Permeability and Vascular Responses
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The area postrema is a circumventricular organ that plays an important role in neurohumoral regulation of the circulation. We have developed a method to examine permeability and vascular responses of the microcirculation of the area postrema in vivo. A craniotomy was performed over the dorsal brain stem in anesthetized rats, and blood vessels to the area postrema were visualized with fluorescent microscopy. Extravasation of sodium fluorescein (MW, 386), but not 150 kDa (MW) fluorescein isothiocyanate-dextran, occurred in the area postrema under control conditions. There was no extravasation of fluorescein or dextran in the brain stem under control conditions. Acute hypertension produced marked disruption of the barrier to 150 kDa dextran in the area postrema, compared with minimal disruption in the brain stem. We tested the hypothesis that the area postrema has greater permeability to small molecules than the brain stem and that this permeability might be accompanied by distinctive vascular responses. Topical suffusion of adenosine and ADP produced similar dose-related dilation of arterioles to area postrema and dorsal brain stem. Topical and intravenous vasopressin produced similar dose-related constriction of vessels to area postrema and brain stem. Electron microscopy in rats demonstrated that a barrier to horseradish peroxidase, which is absent in capillaries in the area postrema, is present in arterioles that supply the area postrema. Thus, 1) the microcirculation of the area postrema is permeable to relatively small molecules under normal conditions and is more susceptible than the brain stem to disruption of the barrier by large molecules during acute hypertension and 2) regulation of vascular tone in response to several stimuli is similar in area postrema and brain stem, despite marked differences in capillary permeability. (Circulation Research 1989;65:417–425)

The area postrema is one of the specialized circumventricular organs of the brain that lacks a blood-brain barrier.\textsuperscript{1–3} It is located on the floor of the fourth ventricle of the dorsal brain stem. The area postrema appears to play an important role in autonomic control of the cardiovascular system and may function as a sensing organ for circulating peptides such as angiotensin and vasopressin.\textsuperscript{4–6}

The area postrema is supplied by a dense capillary network that lacks tight junctions between endothelial cells and is characterized by the presence of large sinusoidal capillaries with fenestrated endothelium.\textsuperscript{7–9} There are no studies, to our knowledge, concerning regulation of the microcirculation of the area postrema. Studies of other circumventricular organs, such as the median eminence, neurohypophysis, and the choroid plexus, suggest that mechanisms that regulate blood flow to these regions of the brain may differ from mechanisms that regulate blood flow to regions in which the blood-brain barrier is present. For example, blood vessels of the pituitary and choroid plexus are very sensitive to humoral stimuli presumably because circulating agonists can readily reach smooth muscle in these relatively permeable vessels.\textsuperscript{10–12}

In the present study, we developed a method to examine the microcirculation of the area postrema in vivo by using a modification of our method to study blood vessels of the brain stem.\textsuperscript{13} The first goal of the study was to examine permeability of the area postrema to different-sized molecules under...
normal conditions and to examine effects of acute hypertension on permeability of the area postrema to large molecules.

Vascular responses to many stimuli are modulated by the endothelium. The observation that endothelium is morphologically and functionally different in the area postrema than in regions of the brain that have a blood-brain barrier suggests that vascular responses also may differ in the two types of vessels. Thus, the second goal of the study was to test the hypothesis that arterioles that supply the area postrema are more sensitive to circulating vasoactive stimuli and respond differently to endothelium-dependent agonists than vessels of the brain stem that have a blood-brain barrier.

Materials and Methods

Animal Preparation

Thirty-seven male Sprague-Dawley rats (300–500 g) were used in these experiments. Animals were anesthetized with pentobarbital sodium (50 mg/kg i.p.) supplemented at a rate of approximately 10–20 mg/kg/hr i.v. A catheter was placed into a femoral vein for injection of drugs and fluorescent tracers. Catheters were also placed into both femoral arteries for measurement of arterial pressure and to sample arterial blood. The trachea was cannulated, and the rats were mechanically ventilated with air and supplemental oxygen. Paralysis of skeletal muscle was produced with gallamine triethiodide (5–10 mg/kg). Arterial blood gases were measured and maintained within the normal range (pH, 7.36±0.01; \(P_{\text{CO}_2}\), 38±1 mm Hg; and \(P_{\text{O}_2}\), 168±12 mm Hg). Body temperature was monitored and maintained at 38±1°C with a heating pad.

The animals were placed in a head holder with the head down at approximately a 45° angle. A midline incision was made through the skin and dorsal neck muscles to expose the atlanto-occipital membrane. The neck muscles were retracted, and inflow and outflow lines for artificial cerebrospinal fluid were sutured to the muscle. The atlanto-occipital membrane and dura were incised with ophthalmic scissors to expose the dorsal medulla and area postrema. With this preparation, approximately the caudal two thirds of the triangular-shaped area postrema can be visualized (Figure 1). The most cranial aspect of the area postrema is obscured by the overlying cerebellum. The exposed brain surface was suffused with cerebrospinal fluid at a temperature of 37°–38°C and bubbled continuously to maintain normal gases and pH (\(P_{\text{O}_2}\), 69±5 mm Hg; \(P_{\text{CO}_2}\), 42±1 mm Hg; and pH, 7.35±0.005).

Permeability of the brain stem and area postrema was evaluated by fluorescent microscopy as described previously. To prevent anaphylaxis to fluorescein isothiocyanate (FITC)-dextran, antihistamines (diphenhydramine [10 mg/kg] and cimetidine [15 mg/kg]) were administered intravenously 15 minutes before injection of dextran.

Diameter of arterioles to the area postrema and the dorsal medulla were measured with a video image-shearing device (model 907, Instrumentation for Physiology and Medicine, San Diego, California).

Experimental Protocol

Permeability to fluorescent tracers. In initial experiments, we examined permeability of the area pos-
trema to different-sized fluorescent molecules (sodium fluorescein [MW, 386]; 4, 10, 20, 40, 70, and 150 kDa [MW] FITC-dextran) under normal conditions. Fluorescein (1 ml of a 2.5 mg/ml solution i.v.) was injected in eight rats, and each of the dextran molecules (1 ml of a 50 mg/ml solution) was injected in two rats. An individual animal received only one size FITC-dextran. There was visible leakage in the area postrema of sodium fluorescein, 4, 10, 20, and 40 kDa FITC-dextran with the smaller molecules appearing to have the greatest degree of leakage. There was no visible leakage of 70 and 150 kDa dextran in the area postrema, so the largest molecule (150 kDa dextran) was used in all subsequent experiments. In evaluation of microvascular permeability, the observer was not "blinded."

In eight rats, we examined effects of acute hypertension on permeability of the microcirculation of the area postrema to 150 kDa FITC-dextran. Systemic pressure was increased by intravenous infusion of phenylephrine (5–15 μg/min) for 5 minutes. All experiments were videotaped for later analysis of formation of microvascular leaky sites and extravasation of fluorescent tracer in the area postrema.

To provide a semiquantitative evaluation of disruption of the barrier to dextran, each animal was assigned a number based on the degree of dextran extravasation: 0, no visible extravasation; 1, minimal extravasation consisting of one or two leaky sites; 2, moderate extravasation; and 3, extensive extravasation.

In five rats, we examined effects of hyperosmolar arabinose on permeability of the brain stem and area postrema. Arabinose (1.6 M) in cerebrospinal fluid was suffused over the craniotomy for 10 minutes in rats in which 150 kDa dextran had been injected.

**Vascular responses.** In nine rats, we examined effects of vasoactive agents on diameter of arterioles that supply the area postrema and other arterioles to the dorsal brain stem. The area postrema in the rat is a single midline structure that is supplied from the posterior inferior cerebellar arteries. These blood vessels typically enter the dorsal aspect of the caudal portion of the area postrema and appear to be the only arterial blood supply to this structure. The diameter of arterioles to the area postrema was measured and compared with vessels that supplied other regions of the dorsal brain stem.

We examined responses to topical suffusion of an endothelium-independent vasodilator (adenosine, 10⁻⁷–10⁻⁴ M), an endothelium-dependent vasodilator (adenosine diphosphate [ADP], 10⁻⁷–10⁻⁴ M), and a vasoconstrictor agonist (arginine vasopressin, 10⁻⁸–10⁻⁴ M). All compounds were mixed in artificial cerebrospinal fluid and then suffused over the exposed portion of the brain. Applications of agonists were randomized. Application of vehicle did not affect diameter of arterioles. Diameter of vessels was measured before and 3–5 minutes after application of agonists. Steady-state responses were reached in 1–2 minutes, and diameter of arterioles returned to control values before a subsequent agent was tested.

In addition, we examined responses to intravascular vasopressin in five rats. Arginine vasopressin was infused intravenously at 2 and 10 milliunits/kg/min for 5 minutes. These doses of vasopressin have marked effects on blood flow to the choroid plexus. Arterial pressure tended to increase during infusion of vasopressin, but pressure was maintained at control levels by withdrawal of blood (0.5–3 ml). We measured diameter of arterioles that supply the area postrema and other regions of the dorsal brain stem.

**Electron Microscopy**

In five rats, the ultrastructural characteristics of the microcirculation of the area postrema were examined by electron microscopy. The rats were anesthetized and ventilated as described above. Blood pressure was monitored, and it did not change significantly during injection of horseradish peroxidase (Type II [MW, 40,000], Sigma Chemical, St. Louis, Missouri). Horseradish peroxidase was injected intravenously and allowed to circulate for 1 (n=3), 5 (n=1), or 15 (n=1) minutes. After the rat was killed with intravenous potassium chloride, the thoracic aorta was cannulated and the upper body was perfused with 2.25% glutaraldehyde in 0.1 M sodium cacodylate for 30 minutes. The brain stem was removed, embedded in agar, and cut into 50–70 μm sections with a TC-Sorvall tissue chopper (DuPont Instruments, Newtown, Connecticut). Sections of brain stem were incubated for 1 hour in a solution of 3,3′-diaminobenzidine tetrahydrochloride (0.05%) in 0.05 M Tris buffer with 1% H₂O₂. The sections were rinsed in cacodylate buffer, postfixed in osmium tetroxide, dehydrated, and embedded in Spurr’s resin. Ultrathin sections were prepared for electron microscopy with an ultratrac microtome (Reichert-Jung, Buffalo, New York).

**Statistical Analysis**

A paired t test was used to compare values for vessel diameter in area postrema versus brain stem. The ranked data for permeability were evaluated by Wilcoxon’s signed-ranks test. A p value of 0.05 was considered to be significant.

**Results**

**Permeability of the Area Postrema**

In rats in which 150 kDa MW FITC-dextran was injected, the dextran remained within the vessels, and there was no visible extravasation or leaky sites in either the brain stem or the area postrema (Figure 1, left panel). In eight rats in which sodium fluorescein (MW, 386) was injected, there was no visible extravasation of the fluorescein in the dorsal brain stem, but there was rapid and extensive extravasa-
tion throughout the area postrema (Figure 1, right panel). Extravasation was confined within the margins of the area postrema. There was no leakage of fluorescein visible from arterioles that supply the area postrema.

Effects of Acute Hypertension and Hyperosmolar Arabinose

Intravenous infusion of phenylephrine increased systemic pressure from 86±7 to 196±3 mm Hg and produced only a modest degree of extravasation (formation of a few leaky sites) of 150 kDa dextran in the brain stem, as we have reported previously.16 In contrast, there was a moderate to marked degree of extravasation of dextran in the area postrema. During acute hypertension, permeability (graded from 0 to 3 for each animal) was greater in area postrema than in brain stem (1.9±0.4 versus 0.8±0.3, p<0.05). Thus, during acute hypertension, the microcirculation of the area postrema was more susceptible than the surrounding brain stem to disruption of the barrier to a large molecule.

In two rats, we injected phenylephrine intravenously but maintained systemic pressure at control levels by withdrawal of blood from a second femoral artery catheter. In these animals, there was no visible extravasation of 150 kDa dextran in the area postrema or the brain stem. This finding suggests that extravasation of dextran in the area postrema during acute hypertension is due to the acute rise in systemic pressure and is not due to a direct effect of phenylephrine.

In contrast to effects of acute hypertension, hyperosmolar arabinose produced marked disruption of the blood-brain barrier to 150 kDa dextran in the brain stem but only a minimal amount of extravasation in the area postrema. After hyperosmolar arabinose, permeability to 150 kDa dextran (graded from 0 to 3) was 2.8±0.2 in brain stem and 0.6±0.3 (p<0.05) in area postrema.

Vascular Responses

In rats in which we examined vascular responses, systemic pressure was 92±5 mm Hg, and it did not change during application of agonists. The average diameter of arterioles that supply the area postrema was 37±3 µm. For comparison, we selected similarly sized arterioles (41±4 µm) that supplied other areas of the dorsal brain stem away from the area postrema. Topical application of adenosine and ADP produced dose-related dilatation of arterioles to the area postrema and brain stem (Figure 2). Dilatation was similar in the two groups of vessels. Arginine vasopressin produced dose-related constriction that was similar in magnitude in arterioles to the brain stem and area postrema (Figure 2).

In rats in which we examined responses to circulating vasopressin, average diameter of arterioles to area postrema and brain stem under control conditions was 43±4 and 41±4 µm, respectively. In these rats, systemic pressure was 90±8 mm Hg under control conditions, 87±9 mm Hg during infusion of the low dose of vasopressin, and 94±9 mm Hg during infusion of the high dose of vasopressin. Infusion of vasopressin produced a modest dose-related constriction of arterioles to area postrema and brain stem (Figure 3). Responses to circulating vasopressin were similar in the area postrema and brain stem.

Permeability to Horseradish Peroxidase

The area postrema contained numerous large sinusoidal capillaries that were surrounded by a prominent perivascular space containing fibroblasts, collagen, and a few macrophages. Endothelium of these sinusoidal capillaries was characterized by numerous fenestrations (Figure 4). Horseradish peroxidase was found predominantly around sinusoidal capillaries and occasionally in the neuropil and adjacent to other vessels (arterioles, venules, and nonfenestrated capillaries) within the area postrema (Figure 5). In contrast, arterioles and venules immediately adjacent to, and supplying, the area postrema were devoid of horseradish peroxidase. Endothelial cells of arterioles and venules that supply the area postrema exhibited tight junctions without fenestrations (Figure 6).
Discussion

The present study describes a new method to examine permeability and regulation of the microcirculation of the area postrema. The results demonstrate in vivo that capillaries of the area postrema are relatively permeable under normal conditions; thus, previous morphological findings are extended. A new concept is that disruption of the barrier to large molecules occurs more readily during acute hypertension in the area postrema than in the brain stem.

We also examined responses to vasoactive stimuli. Although endothelium of capillaries in the area postrema has properties that are distinctly different from capillaries in other regions of the brain, responses to topical and blood-borne vasoactive stimuli are not different in area postrema and brain stem. A likely explanation for this finding is that, although capillaries in the area postrema have a permeable endothelium, arterioles that supply the area postrema appear to have an intact blood-brain barrier.

Consideration of Methods

In this study, we used fluorescent microscopy to examine permeability of the area postrema. We and others have used fluorescent microscopy to examine changes in permeability of the blood-brain barrier either by visualizing the formation of microvascular leaky sites or by quantifying the clearance of various molecules from blood to cerebrospinal fluid. We were not able to measure clearance of molecules in the present study, as we have previously in the cerebrum, because the area postrema is small and it is located in the middle of the dorsal brain stem. Thus, it is not possible with this method to selectively collect cerebrospinal fluid that suffuses only the area postrema.

Although we did not quantify clearance of fluorescent molecules, we were able to provide a semi-quantitative estimate of permeability by direct visualization. With this method, there was no visible extravasation of 150 kDa dextran in the area postrema under normal conditions, but the area postrema was permeable to small molecules under control conditions and to large molecules during acute hypertension. We cannot exclude the possibility that the greater vascularity of the area postrema may contribute to the visual image of extravasation of fluorescein. However, the observation that large molecules do not give the same image suggests that increased vascularity cannot account for the appearance of extravasation of fluorescein in the area postrema.

The technique of direct measurement of diameter of pial vessels on the surface of the brain has been
used widely. These arterioles are important resistance vessels and are therefore important determinants of blood flow. A major assumption of the technique is that changes in vessel diameter are representative of changes in local blood flow. In the present study, we measured the diameter of arterioles that supply the area postrema. The arterioles that were selected appear to provide all of the blood flow to the area postrema in the rat. After these arterioles enter the area postrema, they divide immediately to form a dense capillary network. We rarely observed any arterioles within the area postrema in vivo, and arterioles were seen only occasionally in sections of the area postrema prepared for electron microscopy. Although the arterioles that were observed in this study were very responsive to vasoactive stimuli, they did not respond differently from arterioles in other parts of the dorsal brain stem. We cannot exclude the possibility, however, that very small distal arterioles, which could not be visualized with this technique, may have different responses.

Consideration of Previous Studies

Responses of pial vessels on the dorsal brain stem have not been examined previously. The results of the present study indicate that arterioles in both brain stem and area postrema are very responsive to several vasoactive stimuli. The responses that we observed to adenosine and ADP in the present study are similar to those reported previously for pial arterioles on the cerebrum. In the present study, arginine vasopressin was a very potent constrictor of pial arterioles on the brain stem. These findings differ from a previous study, in which topical application of lysine vasopressin had no effect on pial vessels on the cerebrum in the rat. It is possible that responses to arginine and lysine vasopressin differ in cerebral blood vessels. Arginine vasopressin is the form produced in mammals with the exception of the pig and hippopotamus. It is unlikely that regional differences account for the different findings because we have observed previously that pial arterioles on the cerebrum in rats constrict in response to arginine vasopressin.

Intravenous vasopressin did not have a selective effect on the diameter of arterioles to the area postrema, but it did produce modest constriction of arterioles in the area postrema and dorsal brain stem. This finding is surprising because vasopressin does not readily cross the blood-brain barrier. The modest constrictor response of arterioles could be a response to the small fraction of vasopressin that passes cerebral endothelium. In addition, circulating vasopressin decreases resistance of large cerebral arteries (vessels greater than 200 μm diameter) and increases cerebral microvascular pressure, but circulating vasopressin does not change blood flow,
because small vessels constrict as an autoregulatory response to the increase in microvascular pressure. It is possible that autoregulatory responses of small vessels may account for the small constriction of arterioles that we observed in the brain stem. In the dorsal brain stem preparation that we used in this study, large pial arteries are not present, so we were not able to compare changes in diameter of large and small vessels.

To our knowledge, there has been only one previous study of vascular responses in the area postrema. Blood flow to the area postrema was measured with iodoantipyrine in rats and was similar to blood flow to the cerebrum under control conditions. Blood flow to the area postrema was not affected by intravenous infusion of angiotensin and increased by approximately 40% during infusion of peptide YY. Blood pressure increased during infusion of both peptides, whereas vascular resistance in the area postrema was not significantly changed; so it is difficult to separate direct effects of the peptides from autoregulatory responses to increases in arterial pressure. The previous findings are difficult to interpret because recent evidence suggests that iodoantipyrine may not be an appropriate tracer for measurement of blood flow to circumventricular organs. Iodoantipyrine appears to greatly underestimate the rate of blood flow to the pituitary, and the validity of measurements of blood flow to the area postrema with iodoantipyrine is not clear.

Effects of Acute Hypertension

We have previously examined effects of acute hypertension on permeability of the blood-brain barrier to various molecules. Acute hypertension produces an increase in microvascular pressure, a passive increase in cerebral blood flow, and disruption of the blood-brain barrier. Disruption of the barrier occurs primarily in venules, occasionally in arterioles, but is not observed in capillaries. Thus, it is surprising that the area postrema, which consists of a dense capillary bed, was more susceptible to disruption during acute hypertension. It is possible that sinusoidal capillaries, which are present throughout the area postrema (Figure 4), are the primary site of disruption. The law of Laplace suggests that wall stress in these vessels may be higher than in other capillaries during acute hypertension because the radius of the capillaries is unusually large. It is also possible that the fenestrations in the sinusoidal capillaries are more susceptible to disruption during acute hypertension than tight junctions between endothelium in other regions of the brain. It seems likely, therefore,
that the barrier in sinusoidal capillaries may be very susceptible to disruption during acute hypertension.

This study does not provide evidence about effects of moderate levels of hypertension on permeability of the barrier in the area postrema. Nevertheless, the levels of systemic pressure that were produced during acute hypertension in this study (approximately 200 mm Hg) are within the range of pressures that are observed under some normal conditions. For example, weight-lifting exercises in humans have been reported to produce levels of mean systemic pressure that approach 300 mm Hg.31

In contrast to acute hypertension, hyperosmolar arabinose produced marked disruption of the brain stem but minimal disruption of the barrier in area postrema. At least two possible mechanisms may account for this difference. First, arabinose may produce more shrinkage of endothelial cells of venules, the primary site of disruption to hyperosmolar solutions in the brain stem, as compared with the ependymal cells that overlie the area postrema and are joined by tight junctions.2 Second, since capillaries of the area postrema are relatively permeable, it is also possible that only a minimal osmotic gradient is established during topical application of arabinose.

**Implications for Regulation of Blood Flow**

Previous studies have suggested that blood vessels in both the pituitary and the choroid plexus respond selectively to humoral stimuli.10–12 Circulating agonists produce marked changes in blood flow to these organs without changing cerebral blood flow. Selective vascular responses in the pituitary and choroid plexus provide functional evidence to suggest that endothelium of resistance vessels is more permeable in these than in typical brain vessels in which a blood-brain barrier is present. Morphological studies suggest that the barrier in arterioles to the median eminence32 and the choroid plexus33 is relatively permeable.

It had been demonstrated previously that the blood-brain barrier is absent in capillaries of the area postrema.2,7–9 Findings of the present study suggest that the blood-brain barrier is present in arterioles that supply the area postrema. Thus, we found no functional or morphological evidence for selective permeability of arterioles in the area postrema. These findings suggest that humoral regulation of blood flow to the area postrema may be similar to regulation of blood flow to the surrounding brain stem.

We speculate that differences in regulation of blood flow to the area postrema and to other circumventricular organs may relate to differences in the physiological roles of the structures. Filtration and transport by the choroid plexus and secretion by the pituitary depend in part on blood flow to these regions. The area postrema apparently functions as a sensing region in the brain for substances such as circulating peptides, and it is possible that selective modulation of blood flow to the area postrema by circulating hormones would compromise the sensing function of the region.

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