Increased Susceptibility to Osmotic Disruption of the Blood-Brain Barrier in Chronic Hypertension

Kinya Tamaki, Seizo Sadoshima, and Donald D. Heistad

SUMMARY We examined the effects of chronic hypertension and acute reduction of arterial pressure on the susceptibility of the blood-brain barrier (BBB) to disruption. The BBB was disrupted with an intracarotid injection of 1.6 M arabinose in spontaneously hypertensive rats (SHR), stroke-prone SHR (SHRSP), and normotensive Wistar-Kyoto (WKY) rats. Permeability of the BBB was determined from the ratio of 125I-albumin in brain to 125I-albumin in blood. When the BBB was intact, permeability was less than 0.4%. After hypertonic arabinose, permeability of the BBB was greater (mean ± SE) in SHRSP (17.6% ± 1.6%) and in SHR (21.1% ± 3.1%) than in WKY (10.3% ± 2.4%) (p < 0.05). When arterial pressure of SHRSP was reduced acutely with nitroprusside before arabinose, the BBB permeability to albumin was not reduced (21.5% ± 1.5%). In other rats, we examined survival after osmotic disruption. In SHRSP, 14 of 15 rats died within 1 day after osmotic disruption with marked cerebral edema. In WKY, four of 15 rats died (p < 0.05 vs SHRSP). When arterial pressure of SHRSP was reduced before arabinose, mortality was reduced to six of 15 (p < 0.05 vs untreated SHRSP). We conclude that the BBB in SHRSP has enhanced vulnerability that is detrimental to survival. Reduction of arterial pressure improves survival in SHRSP without affecting BBB permeability to albumin. The findings suggest that 1) there is an inherent defect in the BBB to albumin in SHRSP; 2) the increased susceptibility of the BBB is not simply a function of elevated arterial pressure at the time of disruption of the BBB; and 3) the higher mortality in SHRSP after disruption of the BBB is pressure-dependent and readily prevented by reduction of arterial pressure.

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Key Words • permeability to albumin • stroke-prone rats • spontaneously hypertensive rats • acute hypotension • cerebral edema

The blood-brain barrier (BBB), which is relatively impermeable to albumin, ions, and many other substances, can be transiently disrupted by intracarotid injection of hyperosmolar solutions or by acute increases in arterial pressure. Several studies have suggested that disruption of the BBB is important in the pathogenesis of hypertensive encephalopathy. The purpose of this study was to examine factors that affect susceptibility of the BBB to disruption in chronic hypertension.

Hazama et al. have suggested that there is intrinsic vulnerability of the BBB under basal conditions in chronic hypertension. Acute increases in arterial pressure, however, produce less disruption of the BBB in spontaneously hypertensive rats (SHR) than in normotensive rats. We have attributed this difference to cerebral vascular hypertrophy during chronic hypertension. We suggested that vascular hypertrophy during chronic hypertension promotes greater autoregulatory vasoconstriction and thus protects against cerebral edema during increases in pressure. Vascular hypertrophy and, perhaps, decreased distensibility of cerebral vessels might mask enhanced vulnerability of the BBB during hypertension. To test this hypothesis, we have disrupted the BBB with a stimulus that does not produce vasoconstriction, and compared effects in hypertensive and normotensive rats.

The first purpose of this study was to compare susceptibility of the BBB to disruption by a hyperosmolar solution in normotensive Wistar-Kyoto (WKY) rats, stroke-prone SHR (SHRSP), and SHR that are not stroke-prone. Susceptibility to disruption was evaluated by measurement of BBB permeability to labeled albumin and by survival rate after disruption of the BBB. Our hypothesis was that, in contrast to reduced susceptibility of SHRSP to disruption of the BBB by acute hypertension, SHRSP and SHR might be more susceptible to disruption by hyperosmolar solutions.

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**Methods**

We studied 60 male SHRSP, eight male SHR, and 28 male WKY. When the rats were 5 to 7 months old, they were anesthetized with pentobarbital (5 mg/100 g i.p.). After an external carotid artery was exposed, its branches were ligated, and the artery was cannulated retrogradely with a PE 50 catheter for injection of arabinose or saline into the common carotid artery. The rats breathed spontaneously, except for three SHRSP that became apneic after injection of arabinose and therefore were ventilated mechanically. Rectal temperature was maintained at 37°C by a heating pad.

**Permeability of the Blood-Brain Barrier to Albumin**

We studied 18 SHRSP (mean weight ± se, 343 ± 5 g), eight SHR (370 ± 11 g), and 11 WKY rats (345 ± 11 g). A femoral artery was cannulated for recording systemic blood pressure and for sampling arterial blood, and a femoral vein was cannulated for injection of drugs. A No. 25 needle was inserted into the lumen of the catheter tip in the external carotid artery to measure carotid pressure during injection of arabinose. Heparin (100 μ/100 g) was injected i.v.

Arabinose 1.6 M in 0.9 M saline was warmed to 37°C and injected into the common carotid artery at 3.88 ml/min in eight SHRSP, eight SHR, and eight WKY rats. In eight other SHRSP, systemic blood pressure was reduced by sodium nitroprusside (4-10 µg/min i.v.) before and after intracarotid injection of arabinose, to assess the effect of acute reduction of blood pressure on the susceptibility of the BBB to disruption. Saline (0.9 M, 3.88 ml/min) instead of arabinose was injected into the common carotid artery in eight SHRSP and three WKY rats.

Because nitroprusside dilates large arteries, it might reduce aortic pressure without reducing microcirculatory pressure. To examine this possibility, we measured pressure in small pial arteries in two other SHRSP during infusion of nitroprusside i.v. A micropipette was inserted into pial arteries approximately 60 µm in diameter, and pressure was measured with a servonull device.20

Carotid arterial pressure increased transiently after injection of arabinose and then decreased transiently. Maximal increases in carotid artery pressure after injection of arabinose were 23 ± 10 mm Hg in SHRSP (n = 5), 26 ± 9 mm Hg in SHR (n = 5), and 40 ± 20 mm Hg in WKY rats (n = 7) (p > 0.05). Blood gases and pH were determined before injection of arabinose.

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the rat had survived for 1 week, the animal was anesthetized with pentobarbital and killed with KCl i.v. The brain was removed and examined.

Statistical Methods

Values for BBB permeability index were compared by analysis of variance and Dunnett’s test. Survival of three groups of rats after disruption of the BBB was compared with Fisher’s exact test.

Results

Permeability of the Blood-Brain Barrier

In all animals that were killed 1 hour after injection of hypertonic arabinose into one carotid artery, the brain was markedly edematous on gross examination and darkly stained with Evans blue dye. The staining and edema occurred predominantly in the hemisphere ipsilateral to the injection site of arabinose, but the contralateral cerebrum also had some staining with blue dye, primarily in the distribution of the anterior and posterior cerebral arteries. The cerebellum and upper pons sometimes were stained, but the lower pons and medulla were not stained. In contrast to the brains of rats that had received hypertonic arabinose, the brains of those that had received normal saline showed no edema on gross examination or staining with Evans blue dye.

The permeability index was low in control SHRSP (0.4% ± 0.2%) and WKY rats (0.1% ± 0.0%). There was a marked increase in the permeability index in the ipsilateral cerebral hemisphere in hypertensive and normotensive rats that received arabinose (Figure 1). The permeability index after arabinose was greater in SHR and SHRSP than in WKY rats (p < 0.05). Although arterial pressure in SHR was lower than that in SHRSP, the values for permeability index were not lower in SHR than in SHRSP.

Reduction of blood pressure with nitroprusside in SHRSP did not reduce permeability to albumin (Figure 1). Infusion of nitroprusside reduced both arterial and microvascular pressure: in two SHRSP, nitroprusside reduced mean systemic arterial pressure by 48 and 55 mm Hg, and reduced pial artery pressure by 15 and 24 mm Hg.

Mortality After Disruption of the Blood-Brain Barrier

Among rats that died within 1 week after injection of arabinose, all died within 1 day. The brains of these rats had marked swelling with tonsillar herniation, and the cerebrum, especially the ipsilateral side, was heavily stained with Evans blue dye.

Survival rate was significantly less in SHRSP than in WKY rats after arabinose (Figure 2). Survival rate increased significantly in the SHRSP that had received nitroprusside (Figure 2), in comparison with SHRSP that had not received nitroprusside, even though reduction of arterial pressure with nitroprusside did not reduce permeability to albumin (Figure 1).

Discussion

The major findings of this study are 1) permeability of the BBB to albumin after injection of intracarotid arabinose is greater in hypertensive rats than in normotensive rats; 2) death from cerebral edema after disruption of the BBB by arabinose occurs more frequently in SHRSP than in WKY rats; and 3) acute reduction of systemic pressure in SHRSP does not reduce permeability.
ability to albumin but does reduce the mortality rate after arabinose. We suggest that increased permeability of the BBB to albumin in SHRSP and SHR reflects an inherent defect in the barrier, because reduction of arterial pressure does not reduce the permeability to albumin. We also suggest that increased mortality after disruption of the BBB in SHRSP reflects an increase in cerebral vascular filtration, and that improved survival after reduction of arterial pressure in SHRSP indicates that this phenomenon is preventable.

This discussion will focus on several points: first, the methods that we used to disrupt the BBB and to quantitate permeability to albumin; second, effects of chronic hypertension on permeability of the BBB to albumin and on mortality after arabinose; third, effects of acute reduction in pressure on permeability and mortality in hypertensive rats; and fourth, the implications of this study.

Methods

Disruption of the BBB occurs after intracarotid injection of hypertonic solutions of mannitol,8 urea,20 or arabinose.7,9 Intracarotid injection of hypertonic solutions has been reported to produce no change in cerebral blood flow (CBF) or transient cerebral vasodilation,21 22 but not vasoconstriction. We found, as have previous investigators,9 that intracarotid injections of isotonic solutions do not disrupt the BBB. The mechanism of disruption of the BBB by hypertonic solutions is not clear, but may involve both an increase in vesicular transport23 24 and the opening of tight endothelial junctions.5,8

We have used the ratio of labeled albumin in the brain to labeled albumin in the blood as an index of permeability of the BBB in this and previous studies.12 26 The method appears to be a valid index of permeability after disruption of the BBB. When the BBB is not disrupted, however, the method probably provides only a semiquantitative estimate of permeability. Because we cannot be certain that postmortem perfusion with saline removes all intravascular labeled albumin and because the amount of albumin in the brain parenchyma normally is very low, the error that is introduced by residual intravascular albumin may be sizable when the BBB is intact. This error becomes minimal when the BBB is disrupted and the amount of extravascular albumin becomes large.

The values for permeability index are determined by the amount of albumin that passes the BBB and the volume of distribution of albumin in the brain. Our calculations depend on two major assumptions. First, it is likely that albumin is confined to the extracellular space and does not enter brain cells, at least during the 1 hour after injection of 131I-albumin. Second, we assume that the extracellular space is similar in the different strains of rats. It would be necessary for brain extracellular space to be twice as large in WKY as in SHR and SHRSP to account for our findings. Thus, it is likely that the differences in permeability index reflect differences in permeability of the BBB rather than differences in the volume of distribution of albumin.

We have used survival rate as an index of the functional consequences of disruption of the BBB. Rats that died after arabinose had marked cerebral edema, usually with tonsillar herniation. Although death from cerebral edema is not a sensitive endpoint, the functional consequences of disruption of the BBB were sufficiently different in normotensive and hypertensive rats to be reflected in a change in survival.

Effects of Chronic Hypertension

Endothelial craters, an increase in the number of microvilli, and plasmalemmal pits have been described in cerebral vessels of SHRSP25 and in cats26 after acute hypertension. Plasmalemmal pits, but not microvilli or endothelial craters, appear to be related to increased permeability of the BBB.26 Thus, plasmalemmal pits may provide a morphological basis for an increase in permeability of the BBB in SHRSP. Increased transport of horseradish peroxidase across cerebral endothelium in SHRSP25 and the presence of plasmalemmal pits25 suggest an increase in permeability of the BBB under basal conditions. In the absence of encephalopathy, however, brain water content is normal in chronic hypertension,13 which implies that abnormalities of the BBB are not of great functional importance under basal conditions.

In renal hypertensive rats, there is increased permeability of the BBB under basal conditions25 and during increases in arterial pressure.28 It is likely that focal disruption of the BBB and cerebral edema play an important role in the pathogenesis of hypertensive encephalopathy in renal hypertensive rats.13 Thus, increased permeability of the BBB in renal hypertensive rats may be important in their susceptibility to hypertensive encephalopathy.

The focus of this study is not on the permeability of the BBB under basal conditions, but on the susceptibility of the BBB to disruption. The findings clearly indicate that SHRSP and SHR are more susceptible than WKY to osmotic disruption of the BBB. In addition, two findings suggest that the BBB of SHRSP and SHR is more susceptible to disruption because of an intrinsic defect, not simply as the result of elevated arterial pressure at the time of BBB disruption.

The first finding is that arterial pressure is higher in SHRSP than in SHR, but susceptibility to osmotic disruption is not greater in SHRSP than in SHR. This suggests that increased susceptibility to disruption is characteristic of SHRSP and SHR and is not closely related to the level of arterial pressure at the time of disruption. Parenthetically, the fact that the defect is seen in SHR, as well as in SHRSP, suggests that susceptibility to stroke in SHRSP is not primarily a function of the BBB susceptibility to disruption.

The second finding is that acute reduction of arterial pressure by nitroprusside in SHRSP did not reduce susceptibility to osmotic disruption. This suggests that augmented permeability to albumin in SHRSP is not a convective, pressure-dependent phenomenon, but instead reflects an intrinsic vulnerability of the BBB. An alternative interpretation might be that large cerebral
arteries, which play an important role in regulating the cerebral circulation, dilate during infusion of nitroprusside. One might then speculate that cerebral microvascular pressure is not reduced, despite reduction of arterial pressure, and one would not predict a decrease in susceptibility to osmotic disruption. This possibility seems to be excluded by our finding that nitroprusside reduces cerebral microvascular pressure in SHRSP.

Effects of Acute Reduction in Pressure

Because reduction of arterial pressure by nitroprusside did not decrease permeability to albumin in SHRSP, one might expect that mortality from cerebral edema would not be reduced. We found, however, that nitroprusside produced a marked reduction in mortality after osmotic disruption in SHRSP. This protective effect may be related to the Starling hypothesis. A decrease in microvascular pressure secondary to reduction in arterial pressure would be expected to decrease the rate of water movement from the vascular to extravascular space.

If permeability to albumin is not reduced by nitroprusside, the concentration of albumin in the extravascular space presumably would not be altered. If concentration of albumin in the extracellular space is unchanged, the Starling hypothesis would predict that decreases in microvascular pressure by nitroprusside would reduce the rate of water movement but would not affect the total amount of edema formed at steady state. Why then did nitroprusside reduce mortality from cerebral edema in SHRSP? The most likely explanation is that, because hypertonic solutions produce only transient (<30 minutes) disruption of the BBB, the rate of movement of water into the extravascular space becomes a critical determinant of the total amount of edema at steady state.

Thus, the response to acute reduction in arterial pressure in SHRSP has two important implications. First, it suggests an intrinsic susceptibility of the BBB in SHRSP that is not simply a function of elevated arterial pressure at the time of disruption of the BBB. Although the abnormality of the BBB in SHRSP may not be related to the level of the prevailing blood pressure, it may be a consequence of chronic hypertension. Second, the higher mortality in SHRSP than in WKY after disruption of the BBB is dependent on the level of pressure at the time of disruption, since this difference in mortality is readily preventable by reduction of arterial pressure.

Implications of the Study

The major implication of this study relates to evidence for increased intrinsic susceptibility to disruption of the BBB in SHR and SHRSP. During acute increases in arterial pressure, it appears that vascular hypertrophy in SHR and SHRSP produces augmented autoregulatory vasoconstriction and protects the BBB. Thus, increased susceptibility of the BBB in SHR and SHRSP, as demonstrated in this study, is masked by vascular hypertrophy. We have suggested that hypertensive encephalopathy in SHRSP may be related to disruption of the BBB. Thus, the finding of intrinsic susceptibility of the BBB to disruption in SHRSP may have implications for susceptibility of SHRSP to hypertensive encephalopathy.

A second implication of this study relates to the effects of reduction in arterial pressure. Despite evidence for an intrinsic abnormality of the BBB to albumin in hypertensive rats, the findings suggest that acute reduction of arterial pressure may decrease susceptibility to cerebral edema. Thus we suggest that, when the BBB is disrupted, reduction in arterial pressure may be effective in reducing edema formation, even without reducing permeability to albumin.

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