

Effect of Chronic Hypertension on the Blood-Brain Barrier

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SUMMARY Disruption of the blood-brain barrier (BBB) during acute hypertension may contribute to hypertensive encephalopathy. In this study we tested the hypothesis that, in chronic hypertension, vascular changes might influence the susceptibility of the BBB to disruption. Spontaneously hypertensive rats (SHR) and normotensive rats (WKY), 3–4 months of age, were anesthetized and acute hypertension was produced by infusing phenylephrine intravenously (i.v.). Permeability of the BBB was studied with radioactive iodine serum albumin (RISA) injected i.v. The ratio of brain-to-blood RISA was used as an index of permeability of the BBB expressed as protein transfer. In both SHR and WKY at resting arterial pressure, the protein transfer was $< 0.10\%$. In WKY exposed to acute hypertension (mean arterial pressure increased by 87 ± 7 mm Hg), the protein transfer was $2.77 \pm 0.60\%$. In SHR with acute hypertension superimposed on chronic hypertension (arterial pressure increased by 80 ± 7 mm Hg), the protein transfer was $1.16 \pm 0.45\%$ ($p < 0.05$, SHR vs WKY). These data suggest that cerebral vessels are less susceptible to disruption of the BBB by acute hypertension in SHR than in WKY. We speculate that the finding of reduced susceptibility to BBB disruption in chronic hypertension may explain, in part, the apparent susceptibility of previously normotensive patients to acute hypertensive encephalopathy. (Hypertension 2: 809–812, 1980)

KEY WORDS • blood-brain barrier • hypertensive encephalopathy • brain • cerebrovascular permeability • protein transfer

HYPERTROPHY of vascular muscle is a common finding in chronic hypertension.^{1, 2} Although most studies have been performed in skeletal muscle beds, recent work suggests that these changes also occur in cerebral vessels.^{3, 4} The increase in vascular muscle results in an increase in wall-to-lumen ratio.⁵ During constrictor stimuli, hypertrophied vessels constrict more than normal vessels.⁶

The physiological significance of structural changes may not be the same in all vascular beds. Folkow and others^{6, 7} attribute, in part, the maintenance of hypertension to these structural alterations. In this

light, structural alterations may be harmful. In the brain, however, structural changes might be “protective.” During acute increases of arterial pressure when the autoregulatory capacity of cerebral vessels is exceeded, there is an increase in cerebral blood flow (CBF) and disruption of the blood-brain barrier (BBB).⁸ During chronic hypertension, cerebral vascular hypertrophy might lead to increased vascular resistance and attenuation of the increase in CBF and disruption of the BBB. The objective of this study was to determine if chronically hypertensive animals would manifest less disruption of the BBB during superimposed acute hypertension than normotensive animals.

Methods

Surgical Preparation

We studied 3–4 month-old spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY) and Wistar rats. Eight SHR and seven WKY were studied during drug-induced hypertension (experimental group); five Wistar and five SHR served as controls. Four SHR and Wistar were used to determine residual hemoglobins in the brain after perfusion of the brain with saline. All rats were fed standard rat chow.

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Terminal Study

The animals were anesthetized with nembutal, 50 mg/kg intraperitoneally and artificially ventilated with room air and supplemental oxygen via tracheal intubation. Polyethylene catheters (PE-60, thin-walled) were inserted into the femoral artery and vein for measurement of arterial pressure and drug injection, respectively. Heparin, 1000 units/kg, was injected intravenously. Decamethodium bromide 0.3 mg/kg, intravenously, was used for skeletal muscle paralysis when needed. Arterial blood gases and pH were maintained in the physiological range. Arterial $p\text{CO}_2$, $p\text{O}_2$, and pH were 38 ± 3 , 183 ± 19 , and 7.39 ± 0.04 in the WKY and 38 ± 3 , 176 ± 39 and 7.31 ± 0.01 in the SHR. Phenylephrine ($35 \mu\text{g}/\text{min}$) was infused intravenously to induce acute hypertension in the experimental groups.

Assessment of Blood-Brain Barrier Permeability

A quantitative determination of permeability of the BBB to albumin was obtained by using ^{125}I human serum albumin (RISA, Mallinckrodt Nuclear). The method is similar to that which we have used previously^{8,9}. Approximately $10 \mu\text{Ci}$ RISA was injected intravenously. The RISA was allowed to circulate for ten minutes prior to withdrawal of the first reference arterial RISA sample. A second reference sample was drawn at 15 minutes during basal conditions (controls) or 5 minutes after induction of hypertension (experimental group) to determine the rate of degradation of RISA.

The rat was killed with KCl injected intravenously. Immediately after death the ascending aorta of the animal was cannulated through the left ventricle and the descending aorta was ligated. To remove RISA from the lumen of cerebral vessels, the brain was perfused through the cannula in the ascending aorta with 0.9% saline for ten minutes. After perfusion of the brain, the cerebrum was excised and divided at the midline. Brain and blood samples were weighed and radioactivity was determined in a gamma counter. The counts in the blood sample obtained at 10 and 15 minutes were averaged to give counts blood/g blood. Permeability to albumin was expressed as protein transfer and calculated using this formula:

$$\frac{\text{counts in tissue/g tissue}}{\text{counts in blood/g blood}} \times 100 = \% \text{ protein transfer.}$$

The unpaired *t* test was used for statistical comparison between the two groups.

Effectiveness of Perfusion of Brain

To assess the effectiveness of brain perfusion with saline in removal of RISA from the lumen of cerebral vessels, samples of saline effluent from the right atrium were examined for RISA radioactivity. Radioactivity in the last sample of effluent was always less than $0.50 \pm 0.09\%$ (mean \pm SE) of the RISA activity in arterial blood.

We determined whether efficacy of perfusion of the brain was similar in SHR and WKY by assessing residual hemoglobin content in aliquots of SHR and WKY brain homogenates. In each group (4 SHR, MAP = 178 ± 4 (mean \pm SE) and 4 WKY, MAP = 123 ± 1), the ascending aorta was perfused with saline as described above. Each hemisphere was homogenized with 3 ml of diluant, and 40 λ of this mixture was added to 20 ml of diluant. Six drops of zaptoglobin were added, the mixture was shaken, and readings were obtained with a hemoglobinometer. There were no significant differences in hemoglobin content between hemispheres in the SHR and WKY or between the two groups. (Lt and Rt SHR 1.1 ± 0.1 and 1.2 ± 0.1 ; WKY 1.1 ± 0.2 and 1.3 ± 0.0 units. There are no absolute units for these readings.

Results

Permeability of Cerebral Vessels During Basal Conditions

The mean arterial pressure of the normotensive (Wistar) and chronically hypertensive animals (SHR) was 115 ± 10 and 171 ± 9 mm Hg (mean \pm SE) respectively. The resting protein transfer was $< 0.10\%$ in each group (fig. 1). Thus, although the resting blood pressures were markedly different, the protein transfer in each group was similar and minimal.

Permeability of Cerebral Vessels During Acute Hypertension

The basal blood pressure of the normotensive (WKY) and the chronically hypertensive animals (SHR) was 110 ± 3 and 178 ± 7 mm Hg respectively. During phenylephrine-induced hypertension, the

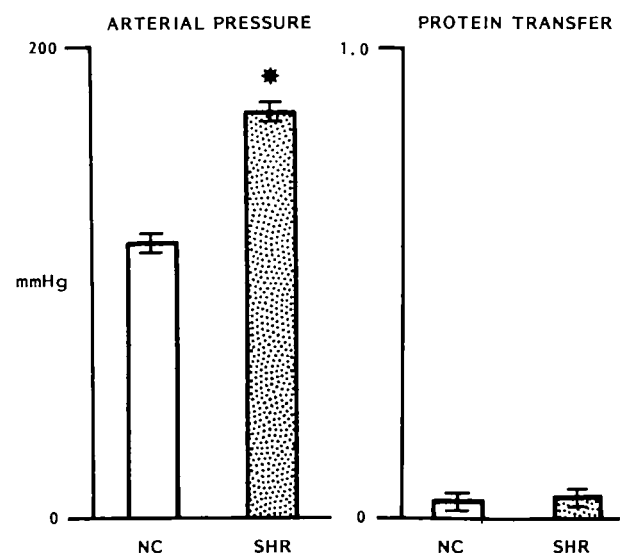


FIGURE 1. Protein transfer during basal conditions. Arterial pressure = systemic arterial pressure. Values are mean \pm SE in five normotensive rats (NC) and 5 SHR. * = $p < 0.05$.

blood pressure increased by 87 ± 7 (110 ± 3 to 197 ± 6 mm Hg) in the WKY and by 80 ± 7 (178 ± 7 to 258 ± 14 mm Hg) in the SHR. The level of arterial pressure reached in the SHR was higher, but the increase in arterial pressure was similar in WKY and SHR. In WKY, protein transfer during acute hypertension was $2.77 \pm 0.60\%$, and in SHR protein transfer was $1.16 \pm 0.45\%$ (fig. 2). This represents a 2.5 greater increase in BBB permeability in WKY than in SHR during acute hypertension ($p < 0.05$). Thus, in spite of a similar increase in mean arterial pressure in normotensive and chronically hypertensive animals, and a higher absolute level of pressure in SHR, there was significantly less protein transfer across the cerebral vessel wall of SHR.

RISA Clearance

During control conditions, when arterial pressure was not acutely elevated, the clearance of RISA from the vascular compartment in both SHR and normotensive rats was minimal and similar (SHR $2.0 \pm 6.0\%$ and WKY $-0.4 \pm 2.5\%$). During acute hypertension, RISA clearance tended to be greater in SHR, although there was not a significant difference between SHR and WKY (SHR $-18.8 \pm 6.5\%$ and WKY -2.8 ± 7.9). To determine whether more rapid clearance of RISA in SHR might account for the lower protein transfer in SHR, we used the 15-minute blood count in this group to calculate protein transfer, rather than using the averaged 10 and 15 minute counts. With these values, SHR permeability was 1.23 ± 0.44 and WKY remained 2.77 ± 0.60 ($p < 0.05$).

Discussion

The major finding in this study is that cerebral vessels of SHR are more resistant to BBB disruption during acute hypertension than the vessels of normotensive rats. Decreased permeability during acute increases in blood pressure in SHR might be due to several factors.

First, the BBB might be less permeable in SHR than WKY. Our data during basal conditions do not support this possibility, but our method probably is not sufficiently sensitive to examine permeability of the BBB under basal conditions. In another subset of SHR (stroke-prone SHR), an increase, rather than decrease, in BBB permeability to horseradish peroxidase has been reported.¹⁰

Second, hypertrophy of large arteries in SHR might attenuate increases in perfusion pressure in distal vessels during acute increases in blood pressure, thereby reducing disruption of the BBB. Several studies^{11, 12} have demonstrated that large arteries participate actively in regulation of cerebral blood flow during changes in arterial pressure. Thus, if large cerebral arteries undergo hypertrophy in SHR, it is likely that increases in pressure in distal vessels would be smaller in SHR than WKY during similar increases in systemic arterial pressure.

Third, hypertrophy of small arteries and arterioles might lead to reduction in wall stress of these vessels during acute increases in blood pressure. Recent morphological studies support this possibility. Nordborg and Johansson⁹ found that cerebral arterial vessels of 15- and 200-day-old SHR had a thicker media in relation to radius than did normal controls. Hart et al.,⁴ reported that the wall/lumen ratio of

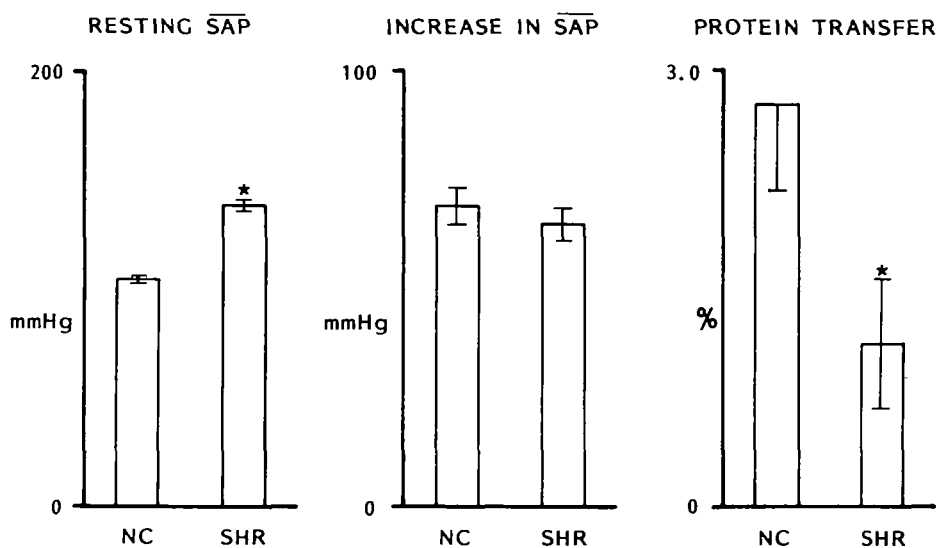


FIGURE 2. Protein transfer during acute hypertension. \bar{SAP} = mean systemic arterial pressure. Values are mean \pm SE in seven normotensive rats (NC) and eight SHR. * = $p < 0.05$.

cerebral vessels in fourteen month old stroke-prone SHR was 0.14 compared to 0.08 in age matched WKY.

Fourth, surface area of SHR cerebral vessels might be decreased due to rarification or closure of vessels. Hutchins and Darnell¹³ found that the number of perfused arterioles in cremaster muscle is smaller in SHR than in WKY at age 12–30 weeks. The potential role of vessel closure in our study of cerebral vessels is difficult to assess. We are not aware of studies comparing the number of capillaries or arterioles per gram of brain in SHR and WKY. Nevertheless, this possibility cannot be excluded, especially since the protein transfer is dependent on surface area available, as well as on the permeability of the barrier.

Permeability of the blood-brain barrier to protein has also been examined using quantitative methods by other investigators. Johansson¹⁴ used RISA in SHR to examine permeability of the blood-brain barrier to protein during a marked increase in metabolism and a modest increase in arterial pressure induced by amphetamines. The author found that permeability was greater in normotensive rats than in 6-month-old SHR. In Johansson's study, maximal or near-maximal cerebral vasodilatation was induced by amphetamines, so that a modest increase in arterial pressure produced pronounced disruption of the BBB. Our experiments support the findings of Johansson and, in addition, suggest that cerebral vessels are more resistant in SHR than in normotensive rats to an acute increase in arterial pressure, in the absence of metabolic vasodilation.

We did not detect a difference in the rate of clearance of RISA in SHR and WKY during control conditions. In contrast, Parving and Gyntelberg¹⁵ found that transcapillary escape of albumin was greater in hypertensive patients than in normotensive men. Because their observations were made over one hour, and our determination was made over only 5 minutes, their study was more likely to detect a difference in rate of clearance of RISA. It is of interest, however, that in our study the rate of clearance of RISA tended to be greater in SHR than in WKY during acute hypertension. The explanation for this finding is not clear, but it may be related to the higher level of arterial pressure in SHR than in WKY. Parving and Gyntelberg¹⁵ found a significant correlation between transcapillary escape rate of albumin and arterial pressure in hypertensive men.

In this study we used phenylephrine to induce hypertension. We cannot exclude the possibility that the lesser disruption of the BBB in SHR than in WKY might be due to greater constrictor responses of the hypertrophic cerebral vessels to phenylephrine. Because alpha-adrenergic agonists have minimal direct constrictor effect on cerebral vessels,^{16, 17} however, it is unlikely that direct constrictor effects of phenylephrine on cerebral vessels are sufficient to account for our findings.

In conclusion, during acute hypertension SHR are more resistant to dysfunction of the blood-brain

barrier than are normotensive rats. This resistance may be due to cerebral vascular hypertrophy that develops during chronic hypertension and results in attenuation of the increase in wall stress during acute hypertension. We speculate that this finding may explain, at least in part, the impression¹⁸ that previously normotensive individuals are more prone to cerebral complications from acute blood pressure elevations than patients with chronic hypertension.

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References

1. Furuyama M: Histometrical investigations of arteries in reference to arterial hypertension. *Tohoku J Exp Med* **76**: 388, 1962
2. Suwa N, Takahashi T: Morphological and morphometric analysis of circulation in hypertension and ischemic kidney. Munchen-Berlin-Wein, Urban and Schwarzenberg, 1971
3. Nordborg C, Johansson BB: The ratio between media thickness and radius in cerebral arteries and arterioles of SHR and NR. *Jap Heart J*, **20** (1): 290, 1979
4. Hart MN, Heistad DD, Brody MJ: Effect of hypertension and sympathetic denervation on wall to lumen ratio of cerebral vessels. *Fed Proc* **38**: 1375, 1979
5. Short D: Morphology of the intestinal arterioles in chronic human hypertension. *Brit Heart J* **28**: 184, 1966
6. Folkow B: Hemodynamic consequences of adaptive structural changes of the resistance vessels in hypertension. *Clin Sci* **41**: 1, 1971
7. Folkow B, Grimby G, Thulesius O: Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance. *Acta Physiol Scand* **44**: 255, 1958
8. Heistad DD, Marcus ML: Effect of sympathetic stimulation on permeability of the blood-brain barrier to albumin during acute hypertension in cats. *Circ Res* **45**: 331, 1979
9. Mueller SM, Heistad DD, Marcus ML: Effect of sympathetic nerves on cerebral vessels during seizures. *Am J Physiol* **237** (2): H178, 1979
10. Hazama F, Amano S, Haebara S, Okamoto K: Changes in vascular permeability in the brain of stroke-prone spontaneously hypertensive rats studied with peroxidase as a tracer. *Acta Patho Jap* **25** (5): 565, 1975
11. Heistad DD, Marcus ML, Abboud FM: Role of large arteries in regulation of cerebral blood flow in dogs. *J Clin Invest* **62**: 761, 1978
12. Kontos HA, Wei EP, Navari RM, Levasseur JE, Rosenblum WI, Patterson JL: Responses of cerebral arteries and arterioles to acute hypotension and hypertension. *Am J Physiol* **3**: H371, 1978
13. Hutchins PM, Darnell AE: Observation of a decreased number of small arterioles in spontaneously hypertensive rats. *Circ Res* **34** and **35** (1): 161, 1974
14. Johansson BB: Cerebrovascular permeability to protein after bicuculline and amphetamine administration in spontaneously hypertensive rats. *Acta Neurol Scand* **56**: 397, 1977
15. Parving HH, Gyntelberg F: Transcapillary escape rate of albumin and plasma volume in essential hypertension. *Circ Res* **32**: 643, 1973
16. Duckles SP, Bevan JA: Pharmacological characterization of adrenergic receptors of a rabbit cerebral artery in vitro. *J Pharm Exp Ther* **197**: 371, 1976
17. Mackenzie ET, McCulloch J, O'Keane M, Pickard JD, Harper HM: Cerebral circulation and norepinephrine: relevance of the blood-brain barrier. *Am J Physiol* **231**: 483, 1976
18. Pickering G: *High Blood Pressure*. New York, Grune and Stratton, Inc. 596, 1968

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