The Blood-Brain Barrier
In Renovascular Hypertension

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SUMMARY  Cerebral vessels of the spontaneously hypertensive rat (SHR) are less susceptible to disruption of the blood-brain barrier to proteins during acute hypertension than normotensive controls. This protective adaptation in SHR during acute hypertension is thought to be due to cerebral vascular hypertrophy which leads to increased vascular resistance and attenuation of the increase in cerebral blood flow and blood-brain barrier disruption during acute hypertension. To determine if a similar relationship is present in the renovascular form of chronic hypertension, we studied blood-brain barrier protein transfer in Sprague-Dawley normotensive and renovascular hypertensive rats. During acute hypertension, the rise in arterial pressure in the renovascular hypertensive group (Δ36 ± 7 mm Hg) did not differ from the renovascular hypertensive group (Δ49 ± 4 mm Hg), but protein transfer across the cerebral vasculature was increased significantly in the renovascular hypertensive group (NT 0.08 ± 0.00, RV 0.40 ± 0.14%, *p < 0.05). These results suggest that cerebral vessels of renovascular hypertensive rats are more susceptible to blood-brain barrier disruption during acute hypertension than normotensive controls. Thus, susceptibility of the blood-brain barrier to disruption during acute hypertension differs between the two forms of animal hypertension, renovascular and the previously studied SHR. This observation may relate to the clinical impression that hypertensive encephalopathy (associated with enhanced blood-brain barrier permeability) is more frequent in patients with hypertension of renal origin than in those patients with hypertension from other causes.

Methods

Animals
A total of 29 male Sprague-Dawley rats (14 normotensive and 15 renovascular hypertensive) were used in this study. All rats were fed standard rat chow. Renovascular hypertension (two-kidney, one clip model) was induced in 15 rats at one month of age. The hypertensive and normotensive animals were studied between 3–4 months of age. Both the normotensive and hypertensive rats were subdivided into two further groups — those studied during control conditions and those studied during an acute elevation in blood pressure. The control and acute hypertension groups were studied in succession using an animal from each group alternately. Protein transfer during control conditions (in the absence of induction of acute hypertension) was determined in 15 rats (7 normotensive and 8 renovascular hypertensive animals). Protein transfer during acute hypertension was assessed in 14 rats (7 normotensive and 7 renovascular hypertensive animals).

Experimentally Produced Renal Hypertension
We used the two-kidney, one clip model of hypertension. At four weeks of age the left main renal artery was ligated in the area of the renal hilus in the renal pelvis. At eight weeks of age, blood pressure was determined by indirect tail-cuff plethysmography. The left kidney was isolated from the renal artery and transplanted into the left iliac artery of a normal Sprague-Dawley recipient rat. The transplanted kidney was immediately perfused with saline to ensure patency of the renal artery. The transplanted kidneys were removed at 4 weeks of age and replaced with saline-filled empty capsules. The experimental animals were studied 1 week later. Protein transfer was determined after the experimental 4 weeks period.
artery of male Sprague-Dawley rats was partially occluded with a 0.4 diameter silver clip through a flank incision. The right renal artery was left untouched. In a preliminary experiment using an identical surgical technique, we followed the awake systolic pressures using a tail sphygmomanometer and found that the abrupt rise in pressure takes place within four weeks of the surgical procedure (fig. 1) after which the systolic pressure remains stable.

Assessment of Protein Transfer Across the Blood-Brain Barrier

At 3–4 months of age, the rats were anesthetized with pentobarbital (40 mg/kg) intraperitoneally and artificially ventilated with room air and supplemental oxygen via tracheal intubation. Polyethylene catheters (PE-50, thin-walled) were inserted into the femoral artery and vein for measurement of the anesthetized arterial pressure and drug injection respectively. Heparin, 1000 units/kg, was injected intravenously. Arterial blood gases and pH were maintained in the physiological range. Phenylephrine was used to induce similar degrees of elevated blood pressure in the acute hypertension groups. The volume of phenylephrine that was used was small (0.25–0.50 ml).

A quantitative determination of permeability of the blood-brain barrier (BBB) to albumin was obtained by using radioactive $^{125}$I human serum albumin (RISA) (Mallinckrodt Nuclear). The method is similar to that which we have used previously. Ten uCi of RISA was injected intravenously. The RISA was allowed to circulate for ten minutes prior to the withdrawal of the first reference arterial RISA sample. A second reference sample was taken five minutes later during control conditions, or five minutes after induction of acute hypertension, to determine the rate of clearance of RISA.

The rat was then 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 the RISA from the lumen of the cerebral vessels, the brain was perfused through the cannula in the ascending aorta with 0.9% saline for 12 minutes by one of us (SMM). After perfusion of the brain, the cerebrum was removed and divided at the midline. These samples plus the blood samples were weighed and radioactivity counts were determined in a gamma counter. The counts in the blood sample at ten minutes and 15 minutes were averaged to give counts in the blood/gm blood. Permeability to albumin was expressed as protein transfer and calculated using this formula:

\[
\text{counts in tissue/gm tissue} \times 100 = \text{protein trans.}
\]

Figure 1. Systolic arterial blood pressure (mean ± SEM) in 19 awake rats using a tail sphygmomanometer technique. A renal clamp was applied at week 0 (clamp) in one month-old Sprague-Dawley rats. Systolic blood pressure increased steadily through week four after application of the clamp. Over the next two months systolic blood pressure remained at the level present at week four.
fer in %. The unpaired t-test was used for statistical comparison between the groups.

### Results

#### Arterial Pressure During Control Conditions and Acute Hypertension

During control conditions, the anesthetized mean arterial pressure of the normotensive rats was 131 ± 6 mm Hg (mean ± SE) and that of the renovascular hypertensive rats was 189 ± 11* (*p < 0.05) (table 1). During the induction of acute hypertension, the mean arterial pressure of the normotensive rats increased 49 ± 4 mm Hg (142 ± 6 to 191 ± 3) while the renovascular hypertensive rats increased 36 ± 7 mm Hg (177 ± 4 to 213 ± 9) (table 2). Thus, during acute hypertension, the rise in mean arterial pressure was greater in the normotensive rats than in the renovascular hypertensive animals, but the difference was not significant. No appreciable differences in mean values for blood gases or pH were detected between the groups.

#### Protein Transfer During Control Conditions and Acute Hypertension

During control conditions, cerebral hemisphere protein transfer in both the normotensive and hypertensive rats was low and not different (fig. 2). During acute hypertension, there was no appreciable change in the cerebral hemisphere protein transfer in the normotensive animals (fig. 3). In the renovascular hypertensive rats, however, protein transfer was significantly elevated in the cerebral hemispheres during acute hypertension (fig. 3). This increase in protein transfer represents a five-fold elevation over the renovascular controls.

#### RISA Clearance

During control conditions, when arterial pressure was not acutely elevated, the clearance of RISA from the vascular compartment in both the normotensive and renovascular hypertensive rats was minimal and not different [normotensive -3.1 ± 5.2%, hypertensive +1.9 ± 1.4% (NS)]. During acute hypertension, RISA clearance again was minimal and not different [normotensive +2.3 ± 4.6% and hypertensive +0.8 ± 11.0% (NS)]. Thus, the RISA clearance in each group was similar and not different one from the other.

### Discussion

The major finding in this study is that cerebral vessels of renovascular hypertensive rats are more susceptible to blood-brain barrier disruption during superimposed acute hypertension than vessels of normotensive rats. This observation is in contrast to previous studies in which reduced susceptibility to blood-brain barrier disruption was found in another form of chronic hypertension, the spontaneously hypertensive rat, compared to normotensive controls. Therefore, the factors influencing blood-brain barrier permeability appear to differ between the two different forms of chronic hypertension, the spontaneously hypertensive model and the renovascular hypertensive model.

Three methodologic points should be made in support of the conclusion that cerebral vessels of renovascular hypertensive rats are more susceptible to blood-brain barrier disruption during acute hypertension than normotensive controls. First, our results could be related to enhanced RISA degradation in renovascular hypertensive compared to normotensive rats. If degradation were greater in the hypertensive animals, the average reference arterial samples would be lower, thereby leading to an increased protein transfer. However, radioactive degradation was not different in the renovascular compared to the normotensive rats. A second consideration in analyzing these results is the consistency in flushing the blood and residual RISA from the brain. Inadequate flushing of the brain of the renovascular hypertensive group during acute hypertension could have resulted in the data we are reporting. The control and acute hypertension groups were studied alternately using the same technique which makes the possibility of this artifact improbable. In addition, all brain flushes were performed by a single individual (SMM). A third consideration might be that the absolute increase in systemic arterial pressure during acute hypertension could be higher in the hypertensive than in the norm-
motensive group and this would lead to increased blood-brain barrier disruption and enhanced protein transfer. However, the absolute increase in arterial pressure was not significantly different between the normotensive and the hypertensive group (in fact, in absolute numbers the rise in arterial pressure in the normotensive group was greater than the renovascular group). Therefore, it appears that methodologic problems have not contributed to these results.

The results of this study differ from our previous study\(^9\) in which blood-brain barrier permeability was increased in both SHR and their normotensive controls during a marked acute elevation in arterial pressure (80 mm Hg), but the disruption was significantly less in the SHR. In this study, blood-brain barrier permeability did not significantly increase in the control group during blood pressure elevation. This is because the increase in arterial pressure in this study (50
mm Hg) was considerably less than that in our previous study (80 mm Hg). Thus, arterial pressure levels above the upper end of the autoregulatory curve were not achieved in the normotensive rats in this study and blood-brain barrier disruption did not occur. In spite of the lack of blood-brain barrier disruption in the control group, blood-brain barrier disruption did occur in the renovascular hypertensive group even though the level of arterial pressure elevation was relatively low. Thus, the blood-brain barrier in renovascular hypertensive rats appears to be acutely sensitive to even moderate increases in arterial pressure. This finding is in contrast not only to the control animals in this study, but also to the SHR in our previous study which were less susceptible to blood-brain barrier disruption during acute hypertension than the normotensive controls.

Increased protein transfer during moderate acute increases in blood pressure in renovascular hypertensive rats may be due to one or more factors. One possibility is that structural alterations may not be present in cerebral vessels of renovascular hypertensive animals. Although structural changes have been demonstrated in the skeletal vasculature in renovascular hypertension, they have not been studied in the cerebral vessels. Even if present, they may not be as severe as those found in spontaneous hypertension. Another possible explanation for the enhanced BBB permeability of renovascular hypertensive rats is that circulating substances such as renin and angiotensin (that are associated with renovascular hypertension) lead to accelerated vascular damage during acute hypertension. In contrast to SHR which is a low renin model of hypertension, the two-kidney, one clip model of renovascular hypertension is a high renin model of hypertension. The renin-angiotensin system has been associated with enhanced vascular permeability.

Fujishima and colleagues have studied cerebral function in normotensive, SHR and renovascular hypertensive rats during superimposed acute hypertension. They measured cerebral lactate, pyruvate, and adenosine triphosphate concentrations as indicators of cerebral metabolism. The results of their study indicated that cerebral hemispheres lactate was significantly increased to 135% of control in the renovascular hypertensive rats, but was unchanged in the SHR and normotensive animals. From their study it was not apparent if the ischemic metabolic changes in renovascular hypertensive rats were primarily due to increased blood-brain barrier permeability leading to brain edema and reduced cerebral blood flow or to a greater reduction in cerebral blood flow with hypertension. Our results indicate that enhanced blood-brain barrier permeability during acute hypertension contributed importantly to the results reported by Fujishima et al.

Protein leakage in the brain of two-kidney, one clip renovascular hypertensive rats has been studied previously using radioactive iodine serum albumin by Johansson. In that study, male Sprague-Dawley rats were studied early during the hypertensive course, i.e. 3–5 weeks after constriction of the renal artery and cerebral vascular permeability was slightly but significantly (p < 0.01) increased in the renovascular hypertensive rats, even in the absence of further blood pressure manipulation. Since it is during this early state of renovascular hypertension that circulating humoral substances are thought to be maximal, it is possible that damage to the blood-brain barrier may be present during control conditions in the early hypertensive state. Our experimental results that do not indicate an elevated protein transfer in renovascular hypertensive rats during control conditions are not in conflict with those of Johansson since we studied a later stage of chronic renovascular hypertension in which renin levels have been reported to revert toward normal. Thus, the stimulus necessary for vascular damage in late chronic two-kidney, one clip renovascular hypertension may be greater than is necessary during early renovascular hypertension, i.e. an insult such as acute hypertension in conjunction with circulating factors associated with the renovascular hypertension.

In conclusion, during superimposed acute hypertension, rats with chronic elevation of arterial pressure secondary to two-kidney, one clip renovascular hypertension are less resistant to dysfunction of the blood-brain barrier than are normotensive rats or SHR. This predisposition to blood-brain barrier dysfunction may be related to the presence of circulating humoral substances in renovascular hypertension which could predispose cerebral vessels to damage during acute elevations in blood pressure. These described results are referable to the clinical observation that patients with hypertension of renovascular origin are more susceptible to hypertensive encephalopathy than are those patients with hypertension from other causes.

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References

Factors Limiting Regeneration of ATP following Temporary Ischemia in Cat Brain

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SUMMARY  Cerebral ischemia was induced in cats using bilateral carotid artery occlusion coupled with hemorrhagic hypotension. Thirty minutes of ischemia, which depleted levels of ATP and phosphocreatine throughout the cerebral cortex, was followed by 2-4 hours of recirculation. During the recovery period, cortical perfusion and NADH fluorescence were monitored through a cranial window. Postischemic perfusion, as indicated by transit time, was initially higher than control, but declined to subnormal levels by 60 minutes. NADH fluorescence transients, induced by brief anoxia, also decreased steadily during recirculation, indicating a failure of oxidation-reduction capability. The disappearance of anoxic-NADH transients usually preceded the decline of flow, suggesting that O$_2$ delivery was not the factor limiting redox reactions. Furthermore, tissue levels of NADH, which were nearly normal after 2-4 hours of recirculation, did not indicate tissue hypoxia.

In spite of normalization of NADH, resynthesis of high energy phosphates was severely impaired. The degree of ATP recovery varied widely in different cortical regions; however, there were two general groups of ATP values — one at 5% and the other at 70% of control levels. In the energy-depleted areas, NADH levels were normal, but the total pool of NAD (NADH + NAD$^+$) and the tissue content of K$^+$ were 43% lower than control. In contrast, the NAD pool and K$^+$ content were only slightly diminished in the regions with greater ATP restitution. The results suggest that postischemic resynthesis of ATP may be limited not by inadequate delivery of O$_2$, but rather by defective production of NADH.

PERMANENT DISRUPTION of energy metabolism occurs in several models of temporary cerebral ischemia.$^{1,2}$ Two distinct mechanisms may account for this irreversible energy failure. First, postischemic blood flow may not be adequate for energy restitution even though the capability for ATP resynthesis remains. Alternatively, there may be a primary lesion within the cell which prevents resynthesis of ATP regardless of blood flow. Although postischemic hypoperfusion has been well demonstrated,$^{3,4}$ it is not known whether flow plays a limiting role in ATP regeneration. If blood flow were delivering insufficient amounts of O$_2$, then the resulting tissue hypoxia should be accompanied by increased levels of NADH. However, tissue NADH levels were not higher than normal in regions with permanent energy depletion.$^{4,1}$ In the present study we have measured the time-course of postischemic blood flow and of redox transients of surface NADH fluorescence. In addition, we have determined regional levels of NADH, NAD$^+$, and K$^+$ in recirculated brain and correlated these levels with postischemic restitution of ATP.

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