Permeability of Intestinal Microvessels in Chronic Arterial Hypertension

GLEN A. LAINE, PH.D., AND HARRIS J. GRANGER, PH.D.

SUMMARY We studied changes in intestinal microvascular permeability resulting from chronic arterial hypertension. Normotensive dogs and dogs made chronically hypertensive utilizing the one-kidney, one-clip Goldblatt technique were used to obtain values for: arterial pressure, portal pressure, intestinal lymph flow, and the lymph-to-plasma protein concentration ratio (CL/CP). Values for the normotensive dogs were 111 mm Hg, 7.1 mm Hg, 6.2 ml/hr, and 0.64, respectively, while values for the chronically hypertensive dogs were 165 mm Hg, 7.3 mm Hg, 12.5 ml/hr, and 0.66, respectively. Control lymph flow in the hypertensives was 100% greater than in the normotensives, while there was no significant difference in control CL/CP between the two groups. When portal venous pressure was acutely increased to 30 mm Hg, lymph flow increased to approximately the same maximum value in both groups. This represents an eightfold increase in normotensive and a fourfold increase in hypertensive lymph flows. The reflection coefficient determined as 1 – (CL/CP) for total proteins at maximum lymph flow was 0.78 for the normotensives and 0.56 for the hypertensives. An electrophoretic analysis indicated sieving of large molecular weight protein fractions was considerably reduced in the hypertensives when compared to the normotensives. Our results indicate a significant increase in intestinal microvascular permeability to macromolecules resulting from the one-kidney, one-clip Goldblatt model of chronic arterial hypertension. (Hypertension 5: 722-727, 1983)

KEY WORDS • Goldblatt hypertension • lymph flow • reflection coefficient • plasma proteins

ALTERATIONS in the walls of the vasculature, resulting from chronic elevations of systemic arterial pressure, have long been associated with the pathogenesis of hypertensive disease.1 Although changes take place in both large and small vessels, we shall concentrate on alterations of the microvasculature, with special attention to transcapillary exchanges of water and proteins. In hypertension, the rate of plasma and protein escape from the circulation is increased above the values obtained for normotensive patients.2-3 The deposition of normally impermeable macromolecules in the subintimal space of hypertensive microvessels has also been noted and confirmed utilizing several methods.4-6 Some investigators believe that protein flux is increased in the hypertensive state simply due to augmented microvascular fluid filtration and solute convection concomitant with an elevation in microvascular hydrostatic pressure.3,7 Alternatively, the permeability of the endothelial barrier to water and proteins may be increased by processes involved in the genesis of hypertension. For example, an increased microvascular hydrostatic pressure could lead to a stretching of existing transport pathways resulting in a reduced microvascular selectivity. Yet another group of investigators believe that the permeability of the microvascular exchange barrier is altered in hypertension due to the direct effect of vasoactive hormones on endothelial cells.8-10

The splanchnic bed receives 25% of the total cardiac output and produces 70% of the body’s lymph flow and transvascular protein flux.11 The microvasculature of some splanchnic organs, such as the liver and spleen, only slightly impede the movement of protein out of the circulation.12 The intestine on the other hand normally restricts the movement of proteins across the exchange barrier.13 In view of the restrictive role of intestinal microvessels in systemic water and protein exchange, alterations of the microvasculature in this tissue could contribute in a substantial manner to the
accelerated extravasation of solvent and solutes observed in hypertension. Therefore, we used the intestine as an experimental organ to pursue the objectives of this study, which were twofold: 1) to quantify the alterations in transmicrovascular solute and water exchange induced by chronic elevation of systemic arterial pressure, and 2) to elucidate the mechanisms responsible for the acceleration of transmicrovascular protein leakage in the hypertensive state.

Methods

Animal Preparation
To produce a chronic one-kidney, one clip renal hypertensive model, mongrel dogs with body weights exceeding 17 kg were anesthetized with sodium pentobarbital (30 mg/kg). A midline incision was made into the abdominal cavity. The renal artery and vein from one kidney were double-tied and cut; this kidney was then removed. An electromagnetic flow probe was placed around the renal artery of the remaining kidney. Flow through the renal artery was reduced by 50% with one of several ligatures or clips. After surgery, all dogs were immediately placed on a regime of intramuscular antibiotics for a period of 7 days. Arterial pressures were monitored on all animals at differing time intervals. Animals with excessive renal artery occlusion usually died within 3 days. Once the animals had become hypertensive (mean arterial pressure of 150 mm Hg or higher) and remained so for 4 weeks, they were prepared for the acute experiments.

On the day of the experiment, the animals were again anesthetized with sodium pentobarbital. The left femoral artery and vein were exposed and cannulated. The femoral vein catheter was used to obtain blood samples as well as for the administration of additional anesthetic. The abdomen was entered through a midline incision and tissues retracted to form an optimal working field. The splenic vein was cannulated using a fluid-filled Swan-Ganz balloon catheter, which was advanced to a position within the portal vein. PE-50 tubing filled with sodium heparin was used to cannulate a large lymphatic draining the intestine and located near the mesenteric lymph nodes. The acute procedure was carried out on a series of normotensive control animals as well as the animals having chronic arterial hypertension. At the conclusion of each experiment, the dogs were euthanized with 10 ml of saturated solution of potassium chloride injected intravenously.

Physiological Measurements and Experimental Protocol
Statham (p23Db) pressure transducers and a Grass recorder were used to record all pressures. Arterial pressure was monitored through the left femoral artery catheter. Pressure in the portal vein was recorded through the Swan-Ganz catheter. In the chronic preparation, renal blood flow was monitored using a square-wave electromagnetic flowmeter. Lymph flow rate and lymph samples were obtained simultaneously and automatically by interfacing a phototastic drop counter tachograph with an automatic fraction collector. Caution was taken to collect lymph samples without applying a positive or negative hydrostatic pressure head to the lymphatic catheter. Protein concentrations in the lymph and plasma were measured with a refractometer. Lymph samples were obtained from the fraction collector, and plasma samples were obtained by centrifugation of venous blood. Lymph and plasma samples were fractionated with polyacrylamide gradient gel electrophoresis and quantified utilizing a scanning densitometer.

At the beginning of each acute experimental procedure, for both normotensive and hypertensive animals, a steady-state control recording of arterial pressure, portal venous pressure, and the lymph flow rate were obtained. Protein concentration in both lymph and plasma were also recorded during the control period. The Swan-Ganz occlusion catheter then was inflated with normal saline solution in incremental steps. Desired step sizes were obtained by observing increases in portal venous pressure. All pressures and flows were allowed to come to equilibrium at each incremental step in portal pressure. Steady-state lymph and plasma protein concentrations were also recorded at several of the incremental pressure steps.

Data Interpretation and Analysis
In the steady state, total solute transport (Jv) is the sum of the convective (Jv,c) and diffusive (Jv,d) mechanisms, or

\[ J_v = J_{v,c} + J_{v,d} = (1 - \sigma) \cdot C_p \cdot J_{s,m} + P^* \cdot S \cdot (C_p - C_L) \]  

(1)

where \( J_v \) is the transcapillary filtration rate, \( \sigma \) is the solute reflection coefficient, \( P^* \cdot S \) is the solute permeability - surface area product, and \( C_p \) and \( C_L \) are the plasma and lymph solute concentrations. Since \( J_v \) is also defined as \( C_p \cdot J_{s,m} \), substituting into Equation (1) and solving for the ratio \( C_L/C_p \) yields

\[ C_L/C_p = (1 - \sigma + \lambda)(1 + \lambda) \]  

(2)

where \( \lambda \) is \( P^* \cdot S/J_v \). Thus, when diffusion and convection interact to govern solute transport, \( C_L/C_p \) is a function of \( \sigma \) and the permeability - surface area product relative to filtration rate (i.e., \( \lambda \)). The sieving curve predicted by Equation 2 is shown in figure 1. At zero filtration rate, solute separation is precluded and \( C_L/C_p \) is 1 for all finite values of \( P^* \cdot S \) and \( \sigma \leq 1 \). For a given \( \sigma < 1 \) and \( P^* \cdot S > 0 \), \( C_L/C_p \) decreases with increasing filtration rate until a minimum plateau value is reached. At the plateau, \( J_v \) is large compared to \( P^* \cdot S \) (i.e., \( \lambda \to 0 \)) and Equation 2 simplifies to \( C_L/C_p = 1 - \sigma \).

Thus, as \( J_v \) increases to high levels, the contribution of diffusive mechanisms to net transmicrovascular protein flux is minimal. Moreover, if the sieving curve exhibits a plateau, the solute reflection coefficient can be calculated as 1 minus the minimum \( C_L/C_p \).

All data analysis was carried out on an AMDAHL 470 V/6 computer. Regression lines were generated from data points using the SAS Program STEPWISE. All means were compared using a one-way analysis of variance and the Bonferroni method of multiple comparison.
TABLE 1. Comparison of Normotensive and Hypertensive Animals at Control Venous Pressure and Maximum Venous Pressure Elevation

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Arterial pressure (mm Hg)</th>
<th>Portal pressure (mm Hg)</th>
<th>Lymph flow (ml/hr)</th>
<th>[prot]L (g/dl)</th>
<th>Protein flux (g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>111 ± 14.8</td>
<td>7.30 ± 1.02</td>
<td>12.5 ± 2.7</td>
<td>4.2 ± 0.26</td>
<td>0.53</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>165 ± 18.4</td>
<td>10.2 ± 1.33</td>
<td>5.3 ± 0.5</td>
<td>1.45</td>
<td></td>
</tr>
</tbody>
</table>

Comparison (p < 0.01) NS (p < 0.05) NS (p < 0.01)

Values are means ± the standard deviation.

NS = not significantly different at p = 0.05.

Results

The impact of one-kidney, one clip Goldblatt hypertension on systemic and intestinal circulatory dynamics is summarized in the left half of table 1. By comparison to the normotensive group, systemic arterial pressure was 50% higher in the hypertensive animals. In contrast, portal venous pressure was unchanged. With respect to transmicrovascular exchanges, intestinal lymph flow was 100% higher in the hypertensive state. Although the protein concentration in intestinal lymph was similar in the two groups, the rate of protein escape across intestinal microvessels was 100% higher in the hypertensive dogs.

The right half of table 1 summarizes the effect of acute portal venous pressure elevation on transmicrovascular exchanges in the intestine of normotensive and hypertensive animals. Elevation of portal venous pressure to 30 mm Hg elicited an acceleration of lymph flow to slightly over 50 ml/hr in both groups. This represents an eightfold increase in the normotensive animals. Because control lymph flow was twofold higher in the hypertensive state, elevation of portal venous pressure caused only a fourfold rise in lymph flow. At maximum lymph flow, sieving of plasma proteins was greater in the normotensive group, as evidenced by the greater reduction of total lymph protein concentration (1.5 vs 2.7 g/dl). The relative difference between the two groups with respect to transvascular protein flux persisted at elevated portal venous pressure, although the hypertension/normotension flux ratio was slightly reduced in comparison to its value at control venous pressure (1.88 vs 2.12).

The global sieving characteristics of the microvascular membrane for plasma proteins can best be appreciated by plotting the lymph/plasma protein concentration ratio (C_L/C_p) as a function of net transmembrane water flux, as represented by the lymph flow rate. Figure 2 shows the sieving curve with respect to total protein for normotensive and hypertensive animals. In both groups, C_L/C_p falls as transmicrovascular filtration rate is augmented by stepwise elevation of portal venous pressure. This phenomenon is known as "washdown" and reflects the exchange properties of the barrier. By comparison with normotensive microvasculature, the intestinal exchange vessels of hypertensive animals exhibit a shift of the sieving curve upward and to the right. Consequently, total lymph protein concentration and C_L/C_p are higher in the hypertensive state for a given ultrafiltration rate. Moreover, the extent of washdown at maximum lymph flow is diminished in the hypertensive group; the minimum values for C_L/C_p for total protein, are 0.22 and 0.44 in normotensive and hypertensive intestine, respectively. These alterations in the sieving curve suggest that the exchange characteristics of the blood/lymph barrier are altered by the hypertensive process.

To probe the sieving process in greater detail, sieving curves also were generated for specific plasma proteins of different molecular size, namely albumin and β-lipoprotein. As illustrated in figure 3, C_L/C_p for albumin in normotensive intestine was 0.87 at control lymph flow and 0.65 at the maximum filtration rate. By contrast, the hypertensive microvessels allowed a minimal washdown to a C_L/C_p of 0.85 at the highest lymph flow. As shown in figure 4, the sieving curve for β-lipoprotein is shifted upward and to the right in

![Graph depicting control curve and curves resulting from a decreased reflection coefficient (s) and an increased exchange surface area (S) when (C_L/C_p) is plotted as a function of J_v.](image-url)
chronic arterial hypertension. At maximum lymph flow, the lipoprotein C₄/C₆ for normotensive and hypertensive exchange vessels of the intestine are 0.05 and 0.25, respectively. In summary, the sieving curves for total protein, albumin and β-lipoprotein suggest that the microvascular barrier in the intestine is altered in chronic one-kidney, one clip renal hypertension.

Discussion

The major aims of our study were to test the hypothesis that fluid and protein effluxes from the intestinal microvasculature are augmented in chronic arterial hypertension, and if the hypothesis proved to be correct, to delineate the specific physical basis of altered barrier function. To achieve these goals, the canine one-kidney, one clip Goldblatt model of chronic renal hypertension was utilized. Our study clearly demonstrated an acceleration of transcapillary water and macromolecular extravasation from intestinal exchange vessels after 4 weeks of arterial hypertension.

Permeability of Intestinal Microvessels in Hypertension

Estimates of the reflection coefficient (σ) for total protein and β-lipoprotein obtained as 1 - (C₄/C₆) at high filtration rates (figs. 2 and 4)⁹,¹⁰ indicate that the hypertensive processes causes opening of the microvascular barrier in intestine. More specifically, the
reflection coefficient for total protein falls from 0.78 in normotension to 0.56 in renal hypertension. For the large β-lipoprotein molecule, hypertension reduces the reflection coefficient from 0.95 to 0.75. Due to the weak washdown of albumin at maximum filtration rates, the conditions required for generating a reasonable estimate of σ for this solute are not met. The estimate of the reflection coefficient for β-lipoprotein is the most reliable since a plateau of $C_L/C_p$ is achieved at high filtration rates in both normotensive and hypertensive intestine. The major physical determinants of the solute reflection coefficient are the size of the solute molecule and the dimensions of the pore. If we assume cylindrical pore geometry and spherical molecules, the relationship is:

$$\sigma = \left[1 - \left(1 - \frac{r_s}{r_p}\right)^2\right]$$  \hspace{1cm} (3)

where $r_s$ and $r_p$ are the solute and pore radii respectively. Thus, with literature values of solute size and experimental determinations of the solute reflection coefficient, application of Equation 3 provides a gross estimate of effective pore radius. As shown in figure 5, application of the cylindrical pore model to the reflection coefficient of β-lipoprotein indicates that the impact of the hypertensive process can be viewed as being caused by an increase in effective pore radius for intestinal microvessels from 149 to 197 Å.

Barrier opening may be caused by endothelial contraction elicited by renin, angiotensin II or other angiotensin-like substances, or physical stretch of membrane channels secondary to chronic elevation of distension pressure in exchange vessels. For the one-kidney, one clip Goldblatt model, the participation of renin or angiotensin is difficult to reconcile with the short duration of the transient increase in plasma levels of these hormones. In any case, our data cannot discriminate the direct pathophysiologic cause of reduced macromolecular selectivity in chronic renal hypertension. The underlying mechanisms remain to be elucidated.

Movement of Fluid Across Intestinal Microvessels in Hypertension

The net rate of transmicrovascular fluid flow ($J_v$) is governed by hydrostatic (P) and oncotic (σ) forces operating across the endothelial membrane; in mathematical terms

$$J_v = L_p \cdot S \cdot [(P_c - P_i) - σ(π_c - π_i)]$$  \hspace{1cm} (4)

where $L_p$ is the hydraulic conductivity and the subscripts c and i denote the capillary and interstitial compartments, respectively. Our data suggest that, due to a decreased σ, the effective oncotic pressure difference $[σ(π_c - π_i)]$ across intestinal microvessels is reduced in one-kidney one clip Goldblatt hypertension. Also, $L_p$ is probably increased as a result of the increased pore size. Thus, the reduced σ and elevated $L_p$ may contribute, at least in part, to an acceleration of transcapillary filtration and protein flux. In turn, a consequent elevation of interstitial pressure could provide the motive force for the rise in lymph flow. This schema is consistent with other studies of hypertensive intestinal microcirculation that have shown enhanced extravasation of marker particles across microvascular endothelium25 and expanded interstitial spaces.26 Another implication of our study is that the maximum potential for oncotic buffering in response to augmented filtration across intestinal microvessels is reduced in one-kidney, one clip Goldblatt hypertension (fig. 2). A full characterization of transcapillary fluid movement in hypertensive intestine will require quantification of capillary hydrostatic pressure, microvascular surface area, and interstitial fluid pressure under a variety of conditions.

In summary, the rate of fluid and protein transfer across intestinal exchange microvessels is accelerated in the canine one-kidney, one clip Goldblatt model of chronic arterial hypertension. The augmentation of transport is due, at least in part, to reduced perselectivity of the intestinal blood/lymph barrier to the plasma proteins. The consequent changes in effective oncotic gradient operating across the microvascular membrane produce significant alterations in transmicrovascular fluid balance in control hypertensives and in hypertensives exposed to additional edemagenic stresses.

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Permeability of intestinal microvessels in chronic arterial hypertension.
G A Laine and H J Granger

Hypertension. 1983;5:722-727
doi: 10.1161/01.HYP.5.5.722

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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