Acute Local Effects of Angiotensin II on the Intestinal Vasculature

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SUMMARY The vasotoxic properties of angiotensin II (All) on vascular endothelial cells have been implicated in the increased extravasation of proteins and water observed in certain forms of chronic arterial hypertension. Since microvascular permeability in the small intestine is increased in one-kidney, one clip hypertension, we tested the hypothesis that All decreases the permselectivity of intestinal microvessels to plasma proteins. The acute intestinal vascular effects of All were studied by locally infusing All into isolated canine jejunal segments. A measure of vascular permeability in control and infused segments was obtained by estimating the osmotic reflection coefficient as 1-lymph/plasma protein concentration ratio when the ratio reached a plateau at high lymph flows. Lymph flows were increased by raising the venous pressure from a control of 5 to 30-35 mm Hg in 5 mm Hg steps. Resting control blood and lymph flows were reduced by All, but these flows were not different at the higher venous pressures due to the apparent blunting of the venous-arteriolar response by All. The estimated osmotic reflection coefficient of 0.85 for the control animals was less than that obtained in the infused segments (0.93). This effect was substantiated by electrophoretic separation of protein fractions. Thus, All per se does not cause an acute increase in intestinal vascular permeability, and may, in fact reduce it. (Hypertension 6: 13-19, 1984)

KEY WORDS • angiotensin II • vascular permeability • plasma proteins • intestinal circulation

Several studies have demonstrated an increased loss of labelled plasma protein in essential human hypertension.1,2 In animal models of hypertension, the small intestine often appears as the focus of plasma protein and fluid loss.3,4 Although an increase in hydrostatic pressure in the exchange vessels may contribute to this loss, there is an indication that the intestinal microvascular permeability to plasma proteins is augmented in hypertension.5 Angiotensin II (All) has been implicated in increasing arteriolar and capillary permeability in several forms of hypertension.5 In addition, All has been shown to cause endothelial cells to contract, thereby increasing the width of the intraluminal spaces.6,7 Thus, All could increase microvascular permeability in a manner similar to histamine.8 The present study was designed to determine the specific effect of All on intestinal microvascular permeability to plasma proteins in the absence of elevated arterial pressure.

Methods

Surgical Procedure

Fasted mongrel dogs of either sex (18-30 kg) were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), intubated, and connected to a positive pressure ventilator (Model 613, Harvard Apparatus) to maintain normal blood pH (pH = 7.39 to 7.43). The intestinal preparation used was similar to that of Johnson and Richardson.9 Following a midline laparatomy, a segment of jejunum perfused by a single artery and drained by a single vein was selected. A lymphatic vessel draining the segment was cannulated with small bore polyethylene tubing (PE 10, 0.28 mm i.d.) adjacent to the main vein, and the remaining visible lymphatics (usually 1, occasionally 2) were ligated. The cannulated vessel was therefore the only exit for lymph formed within the segment. The outflow side of the PE 10 tubing was inserted into a 5 cm length of PE 50 tubing that was marked at 1 cm intervals. Lymph flow was measured by briefly separating the tubings to introduce an air bubble, and then timing the movement of the bubble through a 2 cm distance in the PE 50 tubing, a volume of 5.28 μl. A side branch of the perfusing artery was cannulated for local arterial infusion of All. Following administration of sodium heparin (500 U/kg), the vein was cannulated using wide bore polyethylene tubing (PE 320, 0.28 mm i.d.) adjacent to the main vein, and the remaining visible lymphatics (usually 1, occasionally 2) were ligated. The cannulated vessel was therefore the only exit for lymph formed within the segment. The outflow side of the PE 10 tubing was inserted into a 5 cm length of PE 50 tubing that was marked at 1 cm intervals. Lymph flow was measured by briefly separating the tubings to introduce an air bubble, and then timing the movement of the bubble through a 2 cm distance in the PE 50 tubing, a volume of 5.28 μl. A side branch of the perfusing artery was cannulated for local arterial infusion of All. Following administration of sodium heparin (500 U/kg), the vein was cannulated using wide bore polyethylene tubing (PE 320, 0.28 mm i.d.) which was connected to a section of flexible tubing (Nalgene, 4.76 mm i.d.). The outflow was directed to a reservoir system containing 100 ml Tyrodes solution.
Utilizing a pump (Model 1203, Harvard Apparatus), blood was returned to the animal through a femoral vein at a rate equal to the segment venous outflow. This system allowed alteration of venous pressure by adjusting the height of the cannula outflow-reservoir system. Segmental venous pressure was measured through a needle in the venous tubing adjacent to the site of cannulation, and blood flow was measured by timed collection of venous outflow. The ends of the intestinal segment were cut and luminal outflow tubes (Tygon, 4.89 mm i.d.) were inserted and tied to prevent bleeding yet permitting secretions to flow out. The lumen of the segment was rinsed thoroughly with warm saline and then flushed with air. The segment was moistened with warm saline and covered with plastic wrap. Segmental temperature was measured using two thermocouples (Bat 8, Bailey Instrument Company, Saddle Brook, New Jersey), one placed above and one beneath the segment, and was regulated with a heat lamp. Mean systemic arterial pressure was measured via a femoral artery. The data was recorded using a Grass Model 7D Polygraph (Grass Instrument Company, Quincy, Massachusetts).

Lymph and plasma samples were centrifuged (Microfuge B, Beckman Instruments, Inc., Palo Alto, California) and total protein concentrations were measured with a refractometer (Model TS10400, American Optical Corporation, Buffalo, New York). Steady-state lymph and plasma samples proteins were separated using polyacrylamide density gradient gel electrophoresis and scanned using a Beckman R-112 densitometer. The concentrations of seven protein fractions were calculated using total protein concentration and the percentage of total concentrations of each fraction. The molecular weights of the fractions were estimated by electrophoresis of five protein standards of known molecular weight (Pharmacia High Molecular Weight Calibration Kit, Pharmacia Fine Chemicals, Piscataway, New Jersey) with the plasma and lymph samples and construction of a standard curve using relative migration distances. The effective molecular radii were determined using the Stokes-Einstein equation and the known free diffusion coefficients of the five protein standards and their relative migration distances to form a standard curve.

Experimental Protocol

The venous pressure was set at approximately 5 mm Hg and lymph was sampled until a steady-state in protein concentration was achieved. A period of 90 to 180 minutes was usually required to obtain steady-state samples of sufficient volume for analysis. Lymph and plasma total protein concentrations were obtained and samples frozen for later electrophoresis, blood and lymph flows were measured, and the venous pressure was elevated 5 mm Hg. This process was repeated until the lymph/plasma total protein concentration ratio plateaued (venous pressure = 30–35 mm Hg). In the infused segments, All (60 ng/ml) was infused to produce an increase of 50 ng/100 ml blood for 30 minutes before measurements were begun at a venous pressure of 5 mm Hg. The infusion was continued at the same rate while the above protocol of stepwise increases in venous pressure was followed. This dose of All is somewhat higher than the elevation observed in human hypertensive patients with renal artery disease.13 The All infusion thus continued for at least 2 hours before the initial steady-state measurements were taken. Since any autoregulatory escape of the blood flow would presumably have occurred in a much shorter time, it was assumed that the response to All was a maintained response. At the termination of the experiment, the segment was stripped free of the mesentery, opened, emptied, and weighed to permit data representation as per 100 g.

Data Analysis and Interpretation

Statistics

Two-way analysis of variance using Dunnett's procedure4 was used to analyze the systemic arterial blood pressures and intestinal vascular resistances in each series. Student's t test was used for all other variable analysis.

Solute Reflection Coefficient

The solute reflection coefficient is a useful index of membrane permeability since it is dependent only on the "pore" size and the solute size. Molecules that move through the "pores" without hindrance are characterized by a reflection coefficient at or near zero. In contrast, the reflection coefficient increases with increasing interaction between the "pore" and the solute. Solutes that are too large to enter and traverse the "pore" are totally "reflected" by the membrane, and the solute reflection coefficient is 1. Thus, for a given solute, an increase in "pore" diameter is evidenced by a reduction in the solute reflection coefficient. Experimental15 and theoretical16 analyses suggest that the solute reflection coefficient can be estimated by examining the lymph/plasma solute concentration ratio at high rates of ultrafiltration. At a high lymph flow the relationship between the lymph/plasma concentration ratio and filtration rate exhibits a minimum or plateau; the solute reflection coefficient can be estimated under these conditions as 1 minus the plateau value for the lymph/plasma concentration ratio.

Results

There was no significant difference in mean arterial pressures throughout either the control (152 ± 3 mm Hg) or All-infused (123 ± 7 mm Hg) series. The apparently lower arterial pressure value in the All-infused series is due to one animal having an abnormally low arterial pressure (100 mm Hg). The fact that the systemic arterial pressure in that animal, as well as the others in the series, did not rise in the course of the experiment indicates that the dose of All used apparently did not survive long enough in the circulation to build up to a systemically active level.
In the control group, elevation of venous pressure generated a significant venous-arteriolar response, as shown in Figure 1, top panel. Blood flow initially was 51.2 ± 1.6 ml/min/100 g, a value consistent with those reported in similarly isolated canine jejunal segments.\textsuperscript{17,18} As venous pressure was raised from near 5 to 30–35 mm Hg, there was a significant increase in vascular resistance producing a steady decline in blood flow through the segment (Figure 1, bottom panel). Elevation of venous pressure increased the transvascular movement of water and solutes as indicated by the rise in lymph flow shown in Figure 2, top panel. This increased transvascular flow rate produced a sieving of macromolecules as evidenced by the decline in lymph total protein concentration depicted in Figure 2, bottom panel.

The local infusion of angiotensin II produced a higher level of vascular resistance and a lower rate of blood flow at the initial venous pressure (Figure 3). As the venous pressure was increased, however, the vascular resistance did not rise so rapidly, nor the blood flow fall so markedly, as occurred in the control segments. In the control series, the vascular resistance was significantly increased when the venous pressure was raised only 5 mm Hg, to a venous pressure of approximately 10 mm Hg. In the All-infused segments, the vascular resistance was not significantly increased until the venous pressure was raised 20 mm Hg, to a venous pressure of approximately 25 mm Hg. The increase in lymph flow and decrease in lymph total protein concentration as venous pressure was increased (Figure 4) was similar to that observed in the control segments.

Table 1 presents the initial values for blood flow, vascular resistance, lymph flow, and protein efflux for the two series, and those same values at their final measurement at a venous pressure of 30–35 mm Hg. Note that All reduced the initial blood flow by over 30% and lymph flow and the protein efflux by 50%. The lymph/plasma total protein concentration ratios at the initial venous pressures, however, were not different (0.65 ± 0.02 for control segments, and 0.64 ± 0.04 for All-infused segments). At the highest venous pressures, vascular resistances tended to be lower and

<table>
<thead>
<tr>
<th></th>
<th>Blood flow (ml/min/100 g)</th>
<th>Vascular resistance (mm Hg/ml/min/100 g)</th>
<th>Lymph flow (ml/min/100 g)</th>
<th>Lymph protein efflux (mg/min/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Pv (mm Hg)</td>
<td>5</td>
<td>2.87 ± 0.09</td>
<td>0.018 ± 0.003</td>
<td>0.76 ± 0.33</td>
</tr>
<tr>
<td>Control</td>
<td>51.2 ± 1.6</td>
<td>5.12 ± 0.48*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All   30–35</td>
<td>32.7 ± 4.0*</td>
<td>3.79 ± 0.48*</td>
<td>0.007 ± 0.002*</td>
<td>0.28 ± 0.05*</td>
</tr>
<tr>
<td>All   30–35</td>
<td>15.5 ± 1.7</td>
<td>8.25 ± 0.88</td>
<td>0.173 ± 0.048</td>
<td>1.75 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>13.3 ± 1.7</td>
<td>6.22 ± 1.11</td>
<td>0.306 ± 0.038</td>
<td>1.30 ± 0.28</td>
</tr>
</tbody>
</table>

*p < 0.05, compared to control animals.

lymph flow higher in the All-infused segments, but there were no significant differences in any of the variables listed.

Figure 5 shows how the lymph/plasma total protein concentration ratio changes as lymph flow increased due to venous pressure elevation. As indicated in the figure, the osmotic reflection coefficient (obtained by subtracting the plateau value of lymph/plasma protein concentration ratio from 1) was calculated as 0.85 for the control segments, and 0.93 for the All-infused segments. These values were significantly different and indicate that microvascular permeability to total proteins was reduced, rather than increased, in the All-infused segments. In this study, the total protein osmotic reflection coefficient of 0.85 obtained in control segments in the dog jejunum (Figure 5) is identical to that obtained in the dog colon using a similar technique. The cat ileum produced a value of 0.92 and
may represent a difference due to the section of intestine studied or to species differences.

Table 2 shows the results of electrophoretic separation of protein bands in lymph and plasma samples. Seven protein bands were consistently identified, and their molecular weight and radius estimated as shown. Fraction 6 appeared as a very wide band so the molecular weight and radius are represented as a range of values rather than as a single value. In both series of experiments, each fraction, including the smallest proteins, had two or more virtually equal values at two successive lymph flows. This indicates that each fraction had apparently reached filtration independence. The greater the estimated radius, generally, the higher was the osmotic reflection coefficient. Consistent with the total protein data, the calculated osmotic reflection coefficients in the All-treated segments were higher than those calculated for the control segments, except for the two largest fractions where the differences were not significant.

Discussion
The aim of this study was to examine the effect of All on extravasation of fluid and proteins from intestinal microvessels, with particular emphasis on testing the hypothesis that the hormone may cause widening of the transport channels in the microvascular membrane.

Surprisingly, the acute local infusion of angiotensin II produced a significant increase, not a decrease, in the calculated osmotic reflection coefficient (Figure 5). The osmotic reflection coefficient provides an indication of vascular permeability. An increase in the osmotic reflection coefficient indicates that a decrease in vascular permeability has occurred. This effect was significant in all but the two largest protein fractions, and even in those the tendency was present (Table 2). As indicated in the results, even the smallest protein fractions had apparently reached filtration independence. Thus, the selectivity of the microvascular membrane to transvascular movement of different plasma proteins may be altered.

Table 2. Protein Fraction Data from Control and Angiotensin II-Infused Intestinal Segments

<table>
<thead>
<tr>
<th>Fraction no.</th>
<th>Molecular weight (daltons)</th>
<th>Stokes-Einstein radius (Å)</th>
<th>Calculated osmotic reflection coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control All</td>
</tr>
<tr>
<td>Total protein</td>
<td></td>
<td></td>
<td>0.85±0.02 0.93±0.01*</td>
</tr>
<tr>
<td>1</td>
<td>67,000</td>
<td>35.0</td>
<td>0.77±0.04 0.89±0.01*</td>
</tr>
<tr>
<td>2</td>
<td>79,750</td>
<td>38.0</td>
<td>0.82±0.03 0.95±0.03*</td>
</tr>
<tr>
<td>3</td>
<td>109,000</td>
<td>40.0</td>
<td>0.88±0.04 0.97±0.02*</td>
</tr>
<tr>
<td>4</td>
<td>132,000</td>
<td>42.0</td>
<td>0.80±0.04 0.94±0.02*</td>
</tr>
<tr>
<td>5</td>
<td>164,000</td>
<td>44.5</td>
<td>0.84±0.02 0.94±0.03*</td>
</tr>
<tr>
<td>6</td>
<td>248,000-939,000</td>
<td>49.5-102.0</td>
<td>0.91±0.02 0.95±0.02</td>
</tr>
<tr>
<td>7</td>
<td>1,300,000</td>
<td>132.0</td>
<td>0.98±0.01 0.99±0.01</td>
</tr>
</tbody>
</table>

*p < 0.05, compared to controls.
proteins was maintained or increased during All infusion. The permeability of the membrane to the individual proteins, however, was reduced. The mechanism of this unique decrease in permeability is not clear. Angiotensin II may alter the patency of the diaphragms lining the fenestrae in the mucosal vessels, thereby reducing the size of the opening. Alternatively, All may alter the distribution of blood flow across the wall of the intestine. A redistribution of blood flow away from the area considered to contain the most permeable vasculature (the mucosa) to an area considered to contain less permeable vasculature (the muscularis) could also produce a decrease in apparent vascular permeability of the entire segment. The latter mechanism would not actually alter vascular permeability, but would give the appearance of doing so. The present study utilizing whole organ lymph analysis cannot discriminate between these two possible mechanisms.

This study only addressed the permeability of macromolecules, the plasma proteins. These macromolecules traverse the vascular wall via pathways referred to as large pores.14 Other pathways exist which are too small to allow passage of plasma proteins, but which do allow smaller molecules, ions, and water to cross the vascular wall. The effect of All on these smaller pores is unknown. There is not likely to be a major effect because the lymph/plasma protein concentration ratio at the low venous pressure was unaltered by acute All infusion. Had there been a marked increase in the mean smaller pore diameter, the increased movement of protein free filtrate would have reduced the lymph/plasma protein concentration ratio.

The increased vascular permeability associated with All observed by other investigators may result from the sharply increased arterial pressure present in many of these studies.20 In our study, arterial perfusion pressure was maintained at normotensive levels. It is also possible that the permeability effect occurs only after chronic elevations of All and was thus not evident in the present study. In any event, our results do not support the hypothesis that All per se is responsible for the increased microvascular permeability to plasma proteins observed in the small intestine in some forms of chronic arterial hypertension.

The local infusion of a vasoactive concentration of All reduced resting lymph flow (Table 1), but did not alter the lymph/plasma protein concentration ratio, despite the fact that the ratio changes very rapidly in the lower range of lymph flows (Figure 5). Therefore, the filtration rate/surface ratio must have remained constant. The decrease in filtration volume was evidently matched by a proportional decrease in available transvascular protein channels. A decrease in the number of perfused capillaries could produce this result. Angiotensin II has been shown to markedly reduce the capillary filtration coefficient measured in cat intestine,21 and this reduction of the number of perfused capillaries without a change in vascular permeability could account for the observed changes in lymph flow. Angiotensin-induced arteriolar vasoconstriction may well result in a decreased hydrostatic pressure within the exchange vessels, contributing to the decreased filtration volume. This latter mechanism alone cannot account for our observations, however, since it would also cause the lymph/plasma protein concentration ratio to rise.

An interesting finding in the study was that All also appeared to reduce the venous-arteriolar response (compare the top panels of Figures 1 and 3). That is, the "myogenic" vasoconstriction elicited by venous pressure elevation was blunted by All. Whether this phenomenon is specific for All or whether it is characteristic of any vasoconstrictor is unknown. Under normal conditions, the venous-arteriolar response also produces a reduction in the capillary filtration coefficient as venous pressure is raised,22 presumably reflecting a local capillary derecruitment in response to elevated microvascular pressure. The effect of venous pressure elevation on capillary filtration coefficient may also be reduced by All since the lymph flow tended to be even greater than in control segments at the higher venous pressures (Table 1). An alternative explanation is that the reduced venous-arteriolar response at high venous pressures allowed a greater hydrostatic pressure to develop in the exchange vessels, thus producing the greater lymph flow.

References

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