Brief Communication

Increased Capillary Hydraulic Conductivity Induced by Atrial Natriuretic Peptide

Virginia H. Huxley, Vicky L. Tucker, Kenneth M. Verburg, and Ronald H. Freeman

The small molecular weight peptide, atrial natriuretic peptide (ANP), produces marked sodium and water excretion. The peptide, extracted from several species of vertebrate heart, also has been shown to increase glomerular filtration and reduce plasma volume. Several mechanisms have been proposed to account for the action of the peptide but remain undefined. In the present report, the ANP-induced alterations in transcapillary water movement were directly assessed. The modified Landis technique was used to measure single capillary hydraulic conductivity ($L_p$) of vessels from the frog mesenteric circulation. In 6 individual microvessels, $L_p$ was measured under control conditions and again during perfusion with $10 \times 10^{-4}$ M ANP. The $L_p$ increased in each vessel by a mean of $3.79$-fold ($\pm 2.09$ SD). In 4 of these vessels, an additional measurement of $L_p$ was repeated under control conditions; the capillary filtration coefficient returned to control levels. It was concluded that ANP directly and reversibly elevates capillary hydraulic conductivity; this response is independent of changes in capillary hydrostatic pressure or surface area. (Circulation Research 1987;60:304-307)

There is compelling evidence to suggest that cardiac myocytes synthesize and secrete a peptide hormone involved in body fluid volume regulation. These specialized cardiac myoendocrine cells contain specific secretory granules and appear to be localized in the atria of mammals. However, secretory granules have been identified in both the atrial and ventricular cardiac tissues of nonmammalian vertebrate species, including the frog. The natriuretic activity of cardiac extracts from different species appears to parallel the distribution and relative abundance of these cardiac-specific granules. Small molecular weight peptides with natriuretic activity have been isolated from mammalian atrial tissue. These peptides have been sequenced and synthesized and share a near-homologous amino acid sequence at the carboxy terminus featuring a 17-residue disulfide-bonded loop. Specific renal and vascular receptors for these atrial natriuretic peptides (ANP) have been identified, and infusions of synthetic ANPs promote sodium and water excretion and increase glomerular filtration. Recent studies also suggest that infusion of ANP may increase fluid efflux across nonrenal systemic capillaries to reduce plasma volume.

The mechanisms by which ANPs act to increase capillary filtration are as yet undefined, and studies have been limited primarily to the glomerular capillary bed. Renal micropuncture studies have reported ANP-induced increments both in the mean glomerular capillary hydrostatic pressure and in proximal tubular stop-flow pressures. These observations are consistent with the concept that hyperfiltration induced by infusion of ANPs is related partially to an increase in the capillary filtration pressure. Further, synthetic ANP increased the capillary ultrafiltration coefficient in the in vitro perfused dog glomerulus, but whether it resulted from an alteration in the capillary hydraulic conductivity or the surface area is not known. The purpose of the present study was to assess directly ANP-induced alterations in the capillary hydraulic conductivity ($L_p$) of the frog mesenteric microcirculation.

The modified Landis technique for quantifying $L_p$ was used in this study. This technique assesses surface area and hydrodynamic independent changes in transcapillary water filtration. Paired determinations of $L_p$ on single perfused capillaries of the frog mesentery were made under control conditions and following addition of ANP to the perfusate. In a subset of these vessels, $L_p$ was measured again following washout of the atrial peptide. The results of this initial study demonstrate an ANP-induced increase in microvascular hydraulic conductivity that is independent of surface area or pressure changes in the intact circulation. These results further support the supposition that vasoreactive peptides, such as ANPs, can exert a direct action on the endothelial cell to modulate transcapillary movement of water.

Materials and Methods

The experiments were conducted on single, perfused microvessels of the male frog (rana pipiens, length 6.3-7.6 cm) purchased in the spring from J.M. Hazen, Alburg, Vt. The frog brain was destroyed by pithing, and care was taken to keep the spinal cord intact. The skin of the right side was dissected free and the abdominal wall opened. Following exposure of the gut, the intestines were floated out with frog-Ringer's...
solution over a polished quartz pillar. The tissue was continuously superfused with room-air-equilibrated frog-Ringer’s solution at pH 7.4 ± 0.1 at 15 ± 1° C.

The vessels chosen for study were selected from different portions of the microvasculature. Vessel classification was based on the definitions of Chambers and Zweifach. In all cases, vessels were long (>900 μm), unbranched, with brisk flow and absence of white cell sticking or rolling on the vessel wall. Long vessels were chosen to accommodate the repeated cannulations and occlusions required by this study. The site of occlusion was moved approximately 30 μm toward the micropipette after 3 to 4 separate occlusions of the vessel.

**Measurement of Hydraulic Conductivity**

The methods for capillary perfusion and the measurement of hydraulic conductivity have been described elsewhere in detail. All measurements are based on the modified Landis technique, which provides a measure of the volume flow of water across the capillary wall immediately following occlusion of the vessel. In this method, each microvessel serves as its own control, and only 1 vessel is used per experimental animal. The initial transcapillary flow of water per unit of capillary wall \( (Jv/S) \) is calculated from the initial cell velocity \( (d\ell/dt)i \), the capillary radius \( r \), and the distance of the marker red cell from the point of occlusion:

\[
(Jv/S)i = (d\ell/dt)i \times (r/2\ell)
\]

The initial cell velocity is taken from the tangent drawn to the point of cell position \( (\ell) \) and time \( (t) \). The initial tangent line is drawn either by eye or by linear regression. \( (Jv/S)i \) was measured at a minimum of 3 hydrostatic pressures, and \( Lp \) was determined from the slope of the relationship between \( (Jv/S)i \) and capillary hydrostatic pressure:

\[
(Jv/S)i = Lp \times (\Delta P - \sigma \Delta \pi)
\]

The absolute value of \( Lp \), measured using human erythrocytes as flow markers, may overestimate the true value of \( Lp \) by 1.75-fold. Compliance effects in the capillaries may also result in overestimation of true hydraulic conductivity, but this factor reportedly is less important in venular capillaries. In this paper, the hydraulic conductivity measurements are reported in two ways. First, the uncorrected, measured values of \( Lp \) will be given. Second, the ratio of \( Lp \) during experimental conditions to \( Lp \) under control conditions will be reported. This normalization of data is useful because the tendencies for overestimation of the true hydraulic conductivity will cancel out.

**Solutions and Red Cell Suspensions**

Frog-Ringer’s solutions were prepared daily and used for the dissection of the mesentery, superfusion of the tissue, and preparation of the perfusion solutions. The composition of the frog-Ringer’s solution was (mM): NaCl 111, KCl 2.4, MgCl₂ 1.0, CaCl₂ 2.0, NaHCO₃ 0.2, glucose 5.5, N₂-hydroxymethylpipеразине-N₂'-ethanesulfonic (HEPES) acid and Na-HEPES 5.0. The pH of the frog-Ringer’s solution at 15° C was 7.5 and was determined by the ratio of HEPES acid to Na-HEPES. The solution osmolality was measured by freezing point depression (Osmette A; Precision Systems, Natick, Mass.).

The protein solutions for perfusion were prepared from a stock solution of 100 mg/ml bovine serum albumin (Sigma Chemicals, St. Louis, Mo., Sigma A-7638, γ-globulin free, lot # 25F-9405). The albumin was dialyzed against 4 liters of frog-Ringer’s solution in 2-liter amounts over 48 hours in 20,000 MW cut-off dialysis tubing (Spectrapor). The final albumin concentration in the perfusate solutions, checked by absorption spectroscopy \( (A_{280nm} = 10 \text{ mg/ml bovine serum albumin (BSA) = 0.667}) \), was 10 mg/ml. The test solution used in the present study was prepared by the addition of synthetic human atrial natriuretic peptide (Bachem, 28 amino acids, MW 3000, lot #140A) from a 333 μM stock suspension in frog-Ringer’s solution. The final concentration of ANP used in this preliminary set of experiments was 10 μM. Although the peptide structure of frog ANP has not been elucidated, recent studies on the immunological and chromatographic properties of endogenous frog ANP suggest that it is similar to the mammalian atrial peptides. All solutions were filtered through 0.2-μm pore diameter Millipore (13 mm, Gelman) membranes; the ANP suspension was filtered through 0.2-μm pore diameter 44 mm diameter Millex (4 mm, Millipore) membranes.

A small number of human red blood cells, washed in frog-Ringer’s solution and buffy-coat-free, were suspended in each of the perfusion solutions as flow markers. The superfusate contained frog-Ringer’s solution alone.

**Experimental Design**

In each vessel in the set of 6 paired determinations of \( Lp \), \( (Jv/S)i \) was measured a minimum of 3 times at each of 3 capillary hydrostatic pressures above 15 cm H₂O. The sequence of pressures was usually 25, 35, and 15 cm H₂O. \( Lp \) was first measured in the vessel perfused with frog-Ringer’s solution containing 10 mg/ml BSA and measured again following the addition of 10 μM ANP to the frog-Ringer’s-albumin solution. This dose of ANP was chosen to elicit a maximal response. Four of the 6 vessels were successfully recannulated with control frog-Ringer’s solution containing BSA alone; a recovery measurement of \( Lp \) was performed in this set of vessels. The time of perfusion of the microvessel with ANP solution was noted but no minimum time of exposure to the peptide was set in these experiments. An average of 60 (range 20–180) seconds elapsed prior to measurement of \( (Jv/S)i \). Following recannulation with the albumin-control solution, 60 seconds or longer elapsed prior to the first determination of \( Jv \) in 3 of the vessels, and 15 seconds in the fourth.
Results

Figure 1 illustrates an experiment in which the response of hydraulic conductivity to the addition of ANP to the solution perfusing the capillary lumen is determined. The capillary was first perfused with frog-Ringer’s solution containing 10 mg/ml dialyzed albumin. The baseline Lp (open circles), measured from the slope of the relation between transcapillary water flux per unit capillary surface area (Jv/S), and capillary pressure (P), was 6.6 x 10^-7 cm/(sec cm H2O). Next, the capillary was perfused with frog-Ringer’s solution containing 10 mg/ml dialyzed albumin and 10 μM ANP (closed circles) and Lp redetermined. The capillary hydraulic conductivity increased greater than six-fold to 40.4 x 10^-7 cm/(sec cm H2O) in the presence of ANP. Finally, the capillary was reperfused (closed triangles) with frog-Ringer’s solution containing 10 mg/ml albumin alone. The Lp in this recovery period fell back to control levels of 6.9 x 10^-7 cm/(sec cm H2O). The same sequence was followed in an additional 5 vessels, but it was not possible to make the final recovery measurement of Lp in 2 vessels. Measured Lp’s for each capillary are given in Table 1.

The results for the 6 capillaries are plotted as filled circles in Figure 2. Although a fortyfold range was observed in the magnitude of the baseline Lp, the addition of ANP to the perfusate induced a mean increase of 3.79 ± 0.94 (SE). The open circles in Figure 2 are the recovery values of Lp following removal of the peptide. In this case, the recovery Lp’s are within 30% of their original control values [± 32% (SE)].

In none of the 6 microvessels was there a change in vessel diameter either during or after perfusion with ANP. As noted in Table 1, the vessels were classified according to anatomical position and flow patterns prior to cannulation. Two vessels were arterial capillaries with divergent flow at both ends of the vessel and a high initial downstream balance pressure (>18 cm H2O); 3 vessels were true capillaries with divergent flow at one end and convergent flow at the other end, with initial balance pressures between 15 cm H2O and 6 cm H2O. Only 1 vessel was classified as a venular capillary with convergent flow at both ends and a balance pressure of 5 cm H2O. The response of the vessel hydraulic conductivity to the presence of ANF clearly was not coupled to vessel classification or diameter. It was noted, however, in 4 vessels not included in this study, that vessels of venular classification may be more sensitive to an irreversible alteration in Lp during perfusion with ANP. In those 4 vessels, transcapillary water flux (Jv/S) increased dramatically (> 10 fold) with the addition of ANP to the perfusate; marker red blood cells tracked to the vessel wall and in most cases

<table>
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<tr>
<th>Vessel</th>
<th>D</th>
<th>Class</th>
<th>Lp BSA I</th>
<th>Lp ANP BSA II</th>
<th>Lp ANP/II</th>
<th>Lp III</th>
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<tr>
<td>4-23 a</td>
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</tr>
<tr>
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<td>22</td>
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<td>6.95</td>
<td>6.10</td>
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<tr>
<td></td>
<td></td>
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<td>67.10</td>
<td>20.83</td>
<td>4.44</td>
</tr>
</tbody>
</table>

AC = arteriolar capillary; TC = true capillary; VC = venular capillary. Diameter measure is in m; Lp measurements are 10^-7 cm/(sec cm H2O).

- Figure 1. Measurement of transcapillary water flux (Jv/S), as a function of pressure with and without ANP on a single capillary. The vessel was first perfused with 10 mg/ml dialyzed bovine serum albumin (BSA) in frog-Ringer’s solution (O) and Jv/S was measured at each of 3 pressures. The mean flux ± standard deviation of the mean is plotted. Mean slope of the relation between Jv/S and capillary pressure is the filtration coefficient (Lp) 6.62 x 10^-7 cm/(sec cm H2O) at 15°C. The vessel was perfused with 10 μM ANP (*) and Jv/S increased dramatically (>10 fold) with the addition of ANP to the perfusate; marker red blood cells tracked to the vessel wall and in most cases
- Figure 2. Microvessel hydraulic conductivity during ANP perfusion (●) and following ANP removal (○) as a function of the control Lp measurement of microvessel. The solid line is the line of identity. This plot summarizes data for 6 vessels treated by the protocol described for Figure 1. The description of the vessel type and perfused diameter is given in Table 1.
Thus, ANPs can produce capillary hyperfiltration, in addition. Further studies also suggest that atrial peptides increase a remarkable increase in renal glomerular filtration pressures in the intact circulation. It also appears to be capable of increasing capillary hydraulic conductivity and enhances transcapillary fluid movement in the frog microcirculation. The large capillary surface area present in the frog mesentery microcirculation. The large capillary surface area available for filtration throughout the systemic circulation thus provides a potentially important site of action for the pharmacological alteration of intravascular volume during the infusion of ANPs. Therefore, the present study demonstrates for the first time that ANP increases capillary hydraulic conductivity, which enhances transcapillary fluid movement in the frog mesentery microcirculation. This action of ANP to increase single capillary hydraulic conductivity and filtration is independent of changes in surface area and pressures in the intact circulation. It also appears to be readily reversible, as indicated by the complete recovery to control values of the hydraulic conductivity following washout of the perfusate containing ANP. Thus, ANPs can produce hyperfiltration, in part at least, by a direct action on the endothelial cells to increase hydraulic conductivity and transcapillary movement of water out of the vascular space.

It has been suggested that a decrease in intravascular volume may contribute to the reported decrement in cardiac output produced by infusion of ANPs. The pharmacologic dose of atrial natriuretic peptide used in the present study produced a nearly fourfold increase in the capillary hydraulic conductivity of the mesenteric microcirculation. The large capillary surface area available for filtration throughout the systemic circulation thus provides a potentially important site of action for the pharmacological alteration of intravascular volume during the infusion of ANPs. Therefore, the present study support the hypothesis that ANPs might reduce the intravascular volume not only by promoting renal natriuresis and diuresis, but also by increasing capillary hydraulic conductivity and systemic transcapillary fluid filtration.

**References**

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**Key Words** • atrial natriuretic factor • capillary water movement • endothelial cell • single perfused capillary
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