Transient and sustained increases in glomerular permeability following ANP infusion in rats

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Axelsson J, Rippe A, Rippe B. Transient and sustained increases in glomerular permeability following ANP infusion in rats. Am J Physiol Renal Physiol 300: F24–F30, 2011. First published October 13, 2010; doi:10.1152/ajprenal.00347.2010.—The present study was performed to investigate the effects of systemic atrial natriuretic peptide (ANP) infusion on the glomerular permeability to macromolecules in rats. In anesthetized Wistar rats (250–280 g), the left urether was cannulated for urine collection while simultaneously blood access was achieved. Rats were continuously infused intravenously with ANP [30 ng·kg⁻¹·min⁻¹ (Lo-ANP, n = 8) or 800 ng·kg⁻¹·min⁻¹ (Hi-ANP; n = 10)] or 0.9% NaCl (SHAM; n = 16), respectively, and with polydisperse FITC-Ficoll-70/400 (molecular radius 13–90 Å) and ⁵¹Cr-EDTA for 2 h. Plasma and urine samples were taken at 5, 15, 30, 60, and 120 min of ANP infusion and analyzed by high-performance size-exclusion chromatography (HPLC) for determination of glomerular sieving coefficients (⁵¹Cr-EDTA). In Hi-ANP, there was a rapid (within 5 min), but bimodal, increase in glomerular permeability. θ to high-molecular-weight Ficoll thus reached a maximum at 15 min, after which θ returned to near control at 30 min, to again increase moderately at 60 and 120 min. In Lo-ANP, there was also a rapid, reversible increase in glomerular θ, returning to near control at 30 min, followed by just a tendency of a sustained increase in permeability, but with a significant increase in “large-pore” radius. In conclusion, in Hi-ANP there was a rapid increase in glomerular permeability, with an early, partly reversible permeability peak, followed by a (moderate) sustained increase in permeability. In Lo-ANP animals, only the initial permeability peak was evident. In both Lo-ANP and Hi-ANP, the glomerular sieving pattern observed was found to mainly reflect an increase in the number and radius of large pores in the glomerular filter.

Capillary permeability; Ficoll; glomerular filtration; glomerular sieving coefficient

Plasma volume overload, in conjunction with e.g., congestive heart failure, induces an increased secretion to the circulation of atrial natriuretic peptide (ANP) from the heart upon atrial stretch. By its natriuretic, diuretic, and vasodilating properties, ANP is believed to act physiologically as a compensatory hormone protecting against plasma volume expansion (9, 11, 29). Furthermore, in extrarenal tissues ANP induces a fluid shift from the vascular to the extravascular space, causing increases in hematocrit and plasma protein concentration in nephrectomized rats (2). Although these effects can be related to vasodilatation, and hence, to increases in capillary surface area and in microvascular pressure, there is now good evidence that ANP directly affects capillary permeability to proteins (11, 12, 25, 29, 34) and microvascular hydraulic conductance (16).

Microalbuminuria (i.e., moderately elevated albumin excretion rates, ranging from 20 to 200 μg·min⁻¹ in humans) is a common feature during exercise, posttrauma (4, 14) or after surgery (21), and in systemic inflammation (6), but also in congestive heart failure and following myocardial infarction (15, 23). In the latter conditions, microalbuminuria has been attributed to permeability effects mediated via ANP. Intravenous (iv) infusion of ANP in anesthetized rats thus resulted in moderate increases in urinary albumin/Inulin concentration ratios for the dose of 0.5 μg·min⁻¹·kg⁻¹ body wt (BW) and in marked albuminuria for a dose of 1 μg·min⁻¹·kg⁻¹ (22). The exact (path)physiology of ANP-induced albuminuria is not known. Some of the proteinuric actions of ANP have been attributed to reductions in the proximal tubular reabsorption of albumin, but whether, or to what extent, glomerular permeability per se is affected, either via charge-selective or size-selective glomerular changes, needs to be further elucidated.

On that background, we investigated the functional behavior of the glomerular filtration barrier in response to continuous iv infusion of ANP in rats. A low, “physiological” (Lo-ANP) and a high, “supraphysiological” ANP (Hi-ANP) dose were investigated. Glomerular size selectivity was assessed in vivo using FITC-Ficoll 70/400, a neutral polysaccharide which is not significantly reabsorbed by the proximal tubules, to assess the glomerular sieving coefficient (θ), i.e., the filtrate-to-plasma concentration ratio, for a broad spectrum of molecular radii, with emphasis on the glomerular sieving pattern of molecules of high molecular weight (MW ~400,000). A rapid, reversible increase in glomerular permeability was noted for both Lo-ANP and Hi-ANP animals, in the latter followed by a moderate, but sustained increase in glomerular permeability.

Materials and Methods

Animals and surgery. Experiments were performed in 34 male Wistar rats (Möllegård, Lille Stensved, Denmark) with an average BW of 267.7 ± 2.8 g. The rats were kept on standard chow and had free access to water until the day of the experiment. The animal Ethics Committee at Lund University approved the animal experiments. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium, 60 mg/kg, and the animals were placed on a thermostatically controlled heating pad to keep body temperature at 37°C. A tracheotomy was performed to facilitate breathing. The tail artery was cannulated (PE-50 cannula) for blood pressure monitoring and registration of heart rate (HR) on a polygraph (model 7B; Grass Instruments, Quincy, MA) and for repeated injections of maintenance anesthesia (pentobarbital sodium). The left carotid artery and the left and right external jugular veins were cannulated (PE-50 cannulas) for blood sampling and infusion purposes, respectively. Access to the left urether was obtained through a small (6–8 mm) abdominal incision. Furosemide (0.375 mg/kg, Furosemid, Recip, Sweden) was administered in the tail artery to increase urine production and facilitate cannulation of the urether, which was used for urine sampling. The latter was dissected free, and a PE-10 (connected to a PE-50) cannula was inserted and secured by a ligature.
Experimental procedures: ANP infusion. All experiments started with an initial resting period of 10–15 min following the cannulation of the left urether. ANP (A8208, lot no. 049K4809, Sigma-Aldrich, St. Louis, MO) was infused iv by either a low dose (30 ng·kg⁻¹·min⁻¹; Lo-ANP, n = 8) (34) or a high dose (800 ng·kg⁻¹·min⁻¹; Hi-ANP; n = 10). The Lo-ANP dose, chosen not to affect mean arterial pressure (MAP) or glomerular filtration rate (GFR), was selected based mainly on two previous studies (30, 34). Tucker et al. (34) found that 20 ng·kg⁻¹·min⁻¹ infused iv in rats resulted in a plasma concentration of 190 µg/ml, which caused moderate increases in the extravasation of radiolabeled albumin to the gut (colon and jejunum), while MAP remained unaltered, despite a reduction in heart rate. Salazar et al. (30) found in dogs that 50 ng·kg⁻¹·min⁻¹ of ANP infusion iv was the highest dose that could be given without a drop in MAP or an increase in GFR. In a pilot study, we found that 50 and 60 ng·kg⁻¹·min⁻¹ of ANP iv slightly reduced MAP. We therefore chose a moderately lower dose, 30 ng·kg⁻¹·min⁻¹, for the Lo-ANP group, yielding a calculated plasma ANP level of 200 pg/ml, which is approximately twice the physiological plasma ANP level. From the data of Tucker et al. (34), we could calculate that an iv ANP infusion rate of 800 ng·kg⁻¹·min⁻¹ would produce a plasma concentration of 1,740 µg/ml, which is in the (lower) range of 2,000–3,000 µg/ml, which have been observed after 25% blood volume expansion in rats with experimental heart failure (10), but higher than observed after 20% plasma volume expansion in rats by another group (24). Furthermore, 800 ng·kg⁻¹·min⁻¹ is about intermediate between the medium and the high doses of ANP infused in rats for studies of ANP-induced albuminuria by Nielsen et al. (22).

For the Lo-ANP dose, an initial bolus (4.75 µl of a 0.04 µg/µl ANP solution) was given iv followed by a constant infusion (2.5 µl/min of a 0.0033 µg/µl solution) throughout the experiment (2 h). Also for the Hi-ANP dose, an initial bolus (75 µl of a 0.04 µg/µl solution) was given iv followed by a continuous infusion (5 µl/min of a 0.04 µg/µl solution). For Ficoll sieving experiments, sampling of urine and blood (2 × 70 µl at a time for Ficoll determinations, using hematocrit capillaries, plus 25 µl for 51Cr-EDTA assessments, using precision Micro-caps) was performed sequentially, at 5, 15, 30, 60, and 120 min, respectively. The blood sampling in hematocrit tubes allowed for simultaneous plasma retrieval and hematocrit determination. In SHAM (n = 16), 0.9% NaCl in a bolus and infusion, mimicking the volume load of the ANP experiments, was given during 2 h with measurements performed at the start (0–5 min; SHAM-5) and at 60 min (SHAM-60) and 120 min (SHAM-120). The total blood volume withdrawn during 120 min was 825 µl (4% of total blood volume) for ANP animals and 495 µl for SHAM animals.

GFR. GFR was measured in the left kidney during the experiment, using 51Cr-EDTA. A priming dose of 51Cr-EDTA (0.3 MBq in 0.2 ml iv, Amersham Biosciences, Buckinghamshire, UK) was administered and followed by a continuous infusion (10 ml·kg⁻¹·h⁻¹) of 51Cr-EDTA (0.37 MBq/ml in 0.9% NaCl) throughout the experiment, which yielded a stable plasma concentration of 51Cr-EDTA over time. Urine was collected from the left urether repeatedly during the experiment, and blood samples, using microcapillaries, 25 µl at a time (see above), were taken to be able to assess GFR, approximately every 20 min. Radioactivity in blood and urine was measured in a gamma counter (Wizard 1480, LKP, Wallac, Turku, Finland). Hematocrit (see above) was assessed throughout the experiments to be able to convert blood radioactivity into plasma radioactivity. During the FITC-Ficoll sieving periods (see below), GFR was also assessed from the urine clearance of FITC-Inulin. The urinary excretion of 51Cr-EDTA and FITC-Inulin per minute (U₀ × V₀) divided by the concentration of tracer in plasma (P₀) was used to calculate GFR where U₀ represents the tracer concentration in urine, and V₀ the flow of urine per minute. Since the variability (coefficient of variation) for FITC-Inulin-assessed GFR was slightly higher than that for 51Cr-EDTA-assessed GFR, we have presented the latter consistently throughout this study.

Glomerular sieving of FITC-Ficoll. A mixture of FITC-Ficoll-70 (10 mg/ml) and FITC-Ficoll-400 (10 mg/ml) (TDB Consultancy, Uppsala, Sweden) in a 1:24 relationship was administered as a bolus dose together with FITC-Inulin (10 mg/ml, TDB Consultancy). The bolus dose [40 µg (FITC-Ficoll-70); 960 µg (FITC-Ficoll-400); and 500 µg (FITC-Inulin)] was followed by a constant infusion of 10 ml·kg⁻¹·h⁻¹ (FITC-Ficoll-70, 20 µg/ml; FITC-Ficoll-400, 0.48 mg/ml; FITC-Inulin, 0.5 mg/ml; and 54Cr-EDTA, 0.3 MBq/ml) for at least 20 min before sieving measurements, after which urine from the left kidney was collected for 5 min, with a midpoint (2.5 min) plasma sample collected. During the constant infusion of FITC-Ficoll 70/400, Ficoll molecules >50 Å in radius slightly increased their concentration, while Ficoll molecules <30 Å decreased their concentrations over the course of the infusion. During a 5-min period, however, these changes were <1%. The midpoint plasma sample taken during the 5 min of urine collection would thus rather accurately reflect the average plasma concentration of Ficoll during each urine sampling period.

High-performance size-exclusion chromatography. Plasma and urine samples were assessed on a size-exclusion high-performance chromatography system (Waters, Milford, MA) with an Ultrahydrogel 500 column (Waters) and calibrated as described in detail previously (5). The mobile phase was driven by a pump (Waters 1525), and fluorescence was detected with a fluorescence detector (Waters 2475) with an excitation wavelength at 492 nm and an emission wavelength at 518 nm. The samples were loaded to the system with an autosampler (Waters 717 plus), and the system was controlled by Breeze Software 3.3 (Waters).

Glomerular Ficoll θ vs. Stokes-Einstein radius. The θ is defined as the concentration of solute in the ultrafiltrate over that in plasma, i.e., a filtrate-to-plasma concentration ratio. Ficoll θ were obtained by analyzing HPLC-curves, i.e., Ficoll concentration vs. elution time (translated into the relative distribution volumes in the column of the different size Ficoll molecules) from the plasma (Cₚ) and urine samples for each experiment. The urine Ficoll concentration vs. the Stokes-Einstein radius (αₛ) curve was divided by the Inulin concentration to obtain the primary urine concentration of Ficoll (Cₚₙ). For each αₛ, the data were then calculated by dividing Cₚₙ by Cₚ.

Two-pore analysis. A two-pore model (19, 26, 28) was used to analyze the θ data for Ficoll (molecular radius 15–80 Å). A nonlinear least-squares regression analysis was used to obtain the best curve fit, using scaling multipliers, as described at some length previously (27). The major parameters of the two-pore model are 1) the small-pore radius (rₛ), 2) the large-pore radius (rₐ), 3) the unrestricted pore area over unit diffusion path-length (Aₛ/Dₛ), and 4) the fraction of the glomerular ultrafiltration-coefficient accounted for by the large pores (αₛ). The latter parameter reflects the abundance of “large pores” in the glomerular filter and is calculated from the fractional GFR diverted through the large pores, i.e., Jᵥₛ/FGR. For stable GFRs, this parameter may be regarded as a more “robust” parameter than αₛ, αₛ, and Jᵥₛ/FGR are mathematically obtained by extrapolating the “flat” portion of the Ficoll sieving curve for molecules >50 Å in radius, i.e., the rₛ curve, back to the ordinate scale (i.e., to 0 Å). This imparts some uncertainty to αₛ, especially if rₛ is markedly altered. After a large increment in rₛ, αₛ (Jᵥₛ/FGR) may tend to be slightly underestimated. By contrast, if rₛ is reduced, then αₛ and Jᵥₛ/FGR may be overestimated. The parameter, rₛ, is mainly dependent on sieving data (θ) close to the inflection point between the rₛ curve and the rₛ curve, i.e., for θ values for αₛ ranging between ~40 and ~46 Å. Aₛ/Dₛ is a diffusive parameter reflecting the surface area of the small pores. A high Aₛ/Dₛ (or a low GFR) will displace the sieving curve for Ficoll radii between 20 and 40 Å to the right, thereby causing a “steeper” rₛ curve with a sharp cut-off. A low Aₛ/Dₛ (or a high GFR) will displace the rₛ curve to the left, creating a more “shallow” rₛ curve (27).

Statistical analysis. Values are presented as medians and ranges (25th-75th percentile). Differences among groups were tested using nonparametric analysis of variance with the Kruskal-Wallis test and statistical analysis.
post hoc tested using the Mann-Whitney U-test. Bonferroni corrections for multiple comparisons were made. Significance levels were set at *P < 0.05, **P < 0.01 and ***P < 0.001. All statistical calculations were made using SPSS 18.0 for Windows (SPSS, Chicago, IL).

RESULTS

MAP and HR. SHAM and Lo-ANP animals showed a stable MAP and HR (not shown) throughout the experiment. Hi-ANP animals showed an early decrease in MAP just after start of the infusion of ANP from 97.5 (85–111.3) to 77.5 (73.8–87.5) mmHg, the MAP remaining depressed during the rest of the experiment, reaching 65.0 (65–75) mmHg at 120 min. A decrease in HR from 340 (320–385) to 275 (248–303) beats/min; *P < 0.01 was also seen in the Hi-ANP group at 120 min of the experiment.

GFR. GFR (Fig. 1 and Table 1) remained more or less stable in all groups, but tended to slightly increase in SHAM from 0.65 (0.62–0.82) to 0.84 (0.73–0.93) ml·min⁻¹·g⁻¹ (kidney)⁻¹; not significant.

θ of FITC-Ficol. Figure 2 shows the θ for Ficoll70Å in Hi-ANP (top), Lo-ANP (middle), and SHAM (bottom), respectively, as a function of time, i.e., at 5, 15, 30, 60, and 120 min. There was a bimodal permeability response with an early permeability peak, i.e., an increase in θ for Ficoll70Å vs. SHAM (horizontal line) at 5 and 15 min, reversing to near SHAM values at 30 min. This was later followed by a more sustained, low-grade increase in θ Ficoll70Å at 60 and 120 min, which was statistically significant in Hi-ANP (at 60 min).

Figure 3 and Table 2 demonstrate the median θ vs. a_e curves for Ficoll molecules ranging in radius from 15 to 80 Å for Lo-ANP and Hi-ANP vs. SHAM at the (first) permeability peak at 15 min. Median and ranges for Ficoll60Å, Ficoll70Å, and Ficoll80Å, are shown in Table 2. In the a_e range 50–80 Å, both Lo-ANP and Hi-ANP showed a clearly increased θ. For Ficoll70Å, θ thus increased from 2.19 × 10⁻⁵ [1.67 × 10⁻⁵, 3.09 × 10⁻⁵ (SHAM-60 min) to 1.14 × 10⁻⁴ (7.15 ×

Table 1. Median and 1st–4th quartile ranges for GFR as a function of time in Fig. 1

<table>
<thead>
<tr>
<th>Time, min</th>
<th>SHAM</th>
<th>Time, min</th>
<th>Lo-ANP</th>
<th>Hi-ANP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.65 (0.62–0.82)</td>
<td>0</td>
<td>0.72 (0.43–0.92)</td>
<td>0.72 (0.64–0.80)</td>
</tr>
<tr>
<td>5</td>
<td>0.70 (0.63–0.73)</td>
<td>10</td>
<td>0.62 (0.57–0.73)</td>
<td>0.71 (0.55–0.85)</td>
</tr>
<tr>
<td>10</td>
<td>0.59 (0.57–0.76)</td>
<td>20</td>
<td>0.63 (0.45–0.71)</td>
<td>0.65 (0.52–0.79)</td>
</tr>
<tr>
<td>30</td>
<td>0.69 (0.59–0.87)</td>
<td>30</td>
<td>0.74 (0.53–0.84)</td>
<td>0.58 (0.49–0.69)</td>
</tr>
<tr>
<td>60</td>
<td>0.83 (0.78–0.87)</td>
<td>60</td>
<td>0.68 (0.54–0.79)</td>
<td>0.71 (0.61–0.79)</td>
</tr>
<tr>
<td>120</td>
<td>0.84 (0.73–0.93)</td>
<td>120</td>
<td>0.74 (0.54–0.84)</td>
<td>0.71 (0.60–0.78)</td>
</tr>
</tbody>
</table>

GFR, glomerular filtration rate; ANP, atrial natriuretic peptide; SHAM, control animals; Lo-ANP and Hi-ANP, low-dose ANP- and high-dose ANP-treated animals, respectively.
animals, there were indications of increases in $r_L$ throughout the intervention in this group. Thus, according to a two-pore model of glomerular permeability, the sieving patterns observed during the first and second phase of ANP infusion were compatible with an increase in the number and/or radius of large pores in the glomerular filter without any primary alterations in glomerular charge selectivity. Furthermore, a dose dependence of ANP action on glomerular permeability was evident.

ANP is a small peptide secreted by the heart upon atrial stretch and/or myocardial ischemia. The acute effects of ANP are both renal and nonrenal. The renal effects include an increased GFR and an increased renal excretion of sodium and water, preferentially in the distal part of the nephron (9). The nonrenal effects of ANP include vasodilation, by relaxation of vascular smooth muscle, and an acute increase in vascular permeability via receptors in the microvascular endothelium (25). Due to the diuretic and natriuretic effects of ANP, it would cause plasma protein upconcentration and increases in plasma oncotic pressure that would mobilize fluid from the interstitium, counteracting any reductions in plasma volume. However, due to the nonrenal effects of ANP, mainly the increases in vascular permeability, plasma volume overload is counteracted by permitting an increased flux of fluid and proteins to the interstitium. Both ANP and the closely related B-type natriuretic peptide (BNP) act on the ANP/BNP receptor guanylyl cyclase-A (GC-A) signaling via a guanylyl cyclase pathway. Deletion of this receptor has been found to result in various degrees of hypertension, plasma volume expansion, and cardiac hypertrophy. In endothelial cell-specific GC-A knockout mice, iv ANP failed to increase endothelial permeability and failed to cause a hypovolemic and hypotensive response, despite intact renal responses to ANP (29). This strongly indicates that increases in endothelial permeability are critically involved in ANP-induced reductions of plasma volume during volume overload.

While the rationale for systemic ANP actions on vascular permeability is logical, the effects on glomerular permeability are more enigmatic. Previous studies have indeed demonstrated that systemic ANP infusion in anesthetized rats can markedly increase urinary albumin excretion, although the exact mechanisms of this proteinuric action have not been elucidated (22). The direct assessment of glomerular permeability in the present study supports the concept that ANP-induced albuminuria is a consequence of a direct effect on the glomerular filtration barrier, leading to a partly reversible increase in the radius and number of large glomerular pores without any primary effects on glomerular charge selectivity.

We have previously demonstrated that anaphylaxis (8) and hyperglycemia (7) can induce rapid, reversible changes in glomerular permeability similar to those induced by ANP. The hyperglycemic permeability response could be abrogated by a

Table 2. Median and ranges of $\theta$ for Ficoll60Å, Ficoll70Å, and Ficoll80Å in SHAM, Lo-ANP, and Hi-ANP, respectively

<table>
<thead>
<tr>
<th></th>
<th>SHAM 60 min</th>
<th>Lo-ANP</th>
<th>Hi-ANP</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 Å</td>
<td>3.28 $\times$ 10^{-5} (2.94 $\times$ 10^{-5}–4.03 $\times$ 10^{-5})</td>
<td>3.15 $\times$ 10^{-4} (8.11 $\times$ 10^{-5}–1.75 $\times$ 10^{-4})</td>
<td>3.10 $\times$ 10^{-4}† (1.29 $\times$ 10^{-5}–5.85 $\times$ 10^{-5})</td>
</tr>
<tr>
<td>70 Å</td>
<td>2.19 $\times$ 10^{-4} (1.67 $\times$ 10^{-5}–3.09 $\times$ 10^{-5})</td>
<td>1.14 $\times$ 10^{-4} (7.15 $\times$ 10^{-5}–1.62 $\times$ 10^{-4})</td>
<td>2.86 $\times$ 10^{-4} (9.93 $\times$ 10^{-5}–5.40 $\times$ 10^{-4})</td>
</tr>
<tr>
<td>80 Å</td>
<td>1.91 $\times$ 10^{-4} (1.71 $\times$ 10^{-5}–2.97 $\times$ 10^{-5})</td>
<td>9.98 $\times$ 10^{-5} (6.83 $\times$ 10^{-5}–1.49 $\times$ 10^{-4})</td>
<td>2.72 $\times$ 10^{-4} (9.43 $\times$ 10^{-5}–5.33 $\times$ 10^{-4})</td>
</tr>
</tbody>
</table>

Statistical difference between SHAM and experimental groups: *$P < 0.01$, †$P < 0.001$. 

Fig. 3. Median curves depicting $\theta$ vs. Stokes-Einstein radius ($a_e$) for SHAM-60 and Lo-ANP and Hi-ANP, respectively, at 15 min after start of the ANP infusion. Medians and ranges of $\theta$ values for Ficoll60Å, Ficoll70Å, and Ficoll80Å, respectively, are given in Table 2. The increase in glomerular permeability to ANP occurred in a more or less dose-dependent fashion during the initial (0–30 min) permeability peak.

$10^{-5}$-1.62 $\times$ 10^{-4} ($P < 0.01$) and to 2.86 $\times$ 10^{-4} (9.93 $\times$ 10^{-5}–5.49 $\times$ 10^{-4}) ($P < 0.001$) at 15 min for Lo-ANP and Hi-ANP, respectively.

Two-pore modeling. The best curve fits of $\theta$ vs. $a_e$ for Ficoll according to the two-pore model were obtained using the parameters listed in Table 3 (Lo-ANP) and Table 4 (Hi-ANP). The fractional fluid flow through the large pores (large-pore volume flow/GFR) was markedly increased, nearly 10-fold at 15 min, in Hi-ANP and then remained elevated compared with SHAM-60 even at 30 min ($P < 0.05$). For Lo-ANP, the fractional volume flow through the large pores was seen to be increased (more than doubled) at 5 ($P < 0.05$) and 15 min ($P < 0.05$), but not thereafter. The fractional hydraulic conductance accounted for by the large pores ($\alpha_L$) increased almost three-fold in Hi-ANP at 5 and 15 min compared with SHAM-60 ($P < 0.01$), clearly indicating the formation of more large pores in the glomerular filter during infusion of high doses of ANP. Furthermore, there was an increase in $r_L$ in Hi-ANP at 5 and 15 min. In Lo-ANP, such an increment was actually significant at all time points ($P < 0.05$) except at 30 min.

**DISCUSSION**

This is the first direct assessment of the dynamics of glomerular permeability alterations occurring during infusions of ANP in the rat. The essential result of the study is that both low (34) and high ANP concentrations caused a rapid (within 5 min), partly reversible increase in glomerular permeability. This was followed by a very moderate, sustained increase in barrier permeability, which was significant for the high ANP dose. Even though the second, more sustained increase in permeability did not reach statistical significance in Lo-ANP
### Table 3. Two-pore parameters, Lo-ANP

<table>
<thead>
<tr>
<th></th>
<th>SHAM 60 min</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small-pore radius ($r_s$), Å</td>
<td>45.96 (45.66–46.29)</td>
<td>46.02 (45.79–46.18)</td>
<td>45.94 (45.81–46.31)</td>
<td>45.79 (45.28–45.86)</td>
<td>45.61 (45.27–45.77)</td>
<td>45.73 (45.73–45.73)</td>
</tr>
<tr>
<td>Large-pore radius ($r_L$), Å</td>
<td>122.36 (116.59–128.37)</td>
<td>192.78 (150.74–205.44)</td>
<td>183.03 (163.79–203.59)</td>
<td>108.63 (103.24–139.54)</td>
<td>146.11* (134.45–161.86)</td>
<td>159.74† (158.10–164.49)</td>
</tr>
<tr>
<td>$J_v/L/GFR \times 10^6$</td>
<td>6.87 (6.05–8.85)</td>
<td>22.5† (13.54–68.57)</td>
<td>18.6† (10.3–26.5)</td>
<td>8.05 (6.09–16.9)</td>
<td>8.13 (6.63–9.94)</td>
<td>6.67 (6.54–6.82)</td>
</tr>
<tr>
<td>$A_0/X_{\text{cm/g}} \times 10^{-5}$</td>
<td>3.95 (3.70–4.84)</td>
<td>3.67 (2.93–4.13)</td>
<td>4.95 (2.84–5.76)</td>
<td>3.21 (2.66–4.94)</td>
<td>4.14 (2.98–4.55)</td>
<td>3.98 (3.61–5.95)</td>
</tr>
</tbody>
</table>

Median values are given together with ranges (1st–4th quartile); $n$ = no. of rats. Fractional ultrafiltration coefficient accounted for by large pores; $J_v/L/GFR$, fractional fluid flow through large pores; $A_0/X$, effective pore area over unit diffusion path length. Statistical difference between SHAM and experimental groups: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

### Table 4. Two-pore parameters, Hi-ANP

<table>
<thead>
<tr>
<th></th>
<th>SHAM 60 min</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small-pore radius ($r_s$), Å</td>
<td>45.96 (45.66–46.29)</td>
<td>46.40 (46.37–46.61)</td>
<td>46.84 (46.58–47.07)</td>
<td>46.56 (46.47–46.97)</td>
<td>46.24 (46.12–46.78)</td>
<td>46.35 (46.02–46.46)</td>
</tr>
<tr>
<td>Large-pore radius ($r_L$), Å</td>
<td>122.36 (116.59–128.37)</td>
<td>146.66† (140.01–163.02)</td>
<td>154.72† (135.55–172.12)</td>
<td>128.94 (113.59–141.78)</td>
<td>131.06 (124.10–139.85)</td>
<td>125.78 (128.39–131.78)</td>
</tr>
<tr>
<td>$J_v/L/GFR \times 10^6$</td>
<td>6.87 (6.05–8.85)</td>
<td>56.1‡ (29.3–114.2)</td>
<td>58.3‡ (23.8–102.3)</td>
<td>14.3* (9.39–29.5)</td>
<td>18.3† (14.5–22.1)</td>
<td>22.6† (17.7–38.3)</td>
</tr>
<tr>
<td>$A_0/X_{\text{cm/g}} \times 10^{-5}$</td>
<td>3.95 (3.70–4.84)</td>
<td>3.39 (3.12–4.19)</td>
<td>3.30 (3.07–4.21)</td>
<td>2.94 (2.48–4.20)</td>
<td>3.18 (3.35–4.13)</td>
<td>3.86 (3.57–5.12)</td>
</tr>
</tbody>
</table>

Median values are given together with ranges (1st–4th quartile); $n$ = no. of rats. Statistical difference between SHAM and experimental groups: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. 

**Translational Physiology**

F28 ANP-INDUCED INCREASES IN GLOMERULAR PERMEABILITY

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Rho-kinase inhibitor, influencing the contractile F-actin cytoskeleton in podocytes and endothelial cells. Even though previous studies from this group have indicated that the ultimate sieving barrier to proteins is not at the podocyte slit diaphragm (19, 27), podocyte interactions with the rest of the glomerular filtration barrier seem crucial for its integrity. It is thus speculated that the rapid alterations in glomerular permeability observed in this study may actually be a consequence of actions on podocyte actin dynamics. Endothelial cells may also be involved, in a way similar to how they react to, for example, histamine, substance P, or thrombin, or in anaphylaxis in nonfenestrated endothelium (20, 26). The first permeability peak induced by ANP actually showed a time cycle which is similar to that following massive histamine release in anaphylactic shock (0–30 min) (8).

On podocytes there are ANP binding receptors, which have been localized mostly to the foot processes (32). ANP receptors signal through a family of particulate guanylate cyclases (pGC) (18), which can induce cGMP formation in a dose-dependent fashion (3). The functional consequences of increases in cGMP in podocytes are, however, poorly understood. In undifferentiated rat podocytes, 1 μM ANP (3 × 10⁶ ng/ml) produced a decreased intensity of F-actin fluorescence and rearrangements of actin in sparse, parallel bundles (31). This suggests that ANP might produce podocyte relaxation (31). It is thus speculated that such changes in the F-actin cytoskeleton may alter the shape of the podocytes, and hence, the tension they exert on the glomerular basement membrane, thereby affecting glomerular permeability.

In the present study, the Hi-ANP animals showed a clear-cut second steady-state phase of increased glomerular permeability, but in the Lo-ANP group this phase was less evident. However, Lo-ANP animals still seemed to have been moderately affected by ANP over 2 h, because the r_L had actually increased significantly at both 60 (P < 0.05) and 120 min (P < 0.01) in this group. Therefore, also low concentrations of ANP seemed to have produced sustained alterations in glomerular size selectivity, which may partly explain the microproteinuria occurring in states of plasma volume expansion.

In Hi-ANP (800 ng·kg⁻¹·min⁻¹ of ANP infusion), there was a marked fall in systemic MAP, but despite that, there was a well-maintained GFR. ANP has been shown to be able to stimulate unmyelinated vagal receptors in the myocardium to induce vagal afferent and efferent excitation, leading to reductions in heart rate and contractility (1). In the present animal model, reductions in MAP would normally cause reductions in GFR, but this was completely prevented in the Hi-ANP group. Dunn et al. (13) found in rats infused with 500 ng·kg⁻¹·min⁻¹ of ANP that MAP was only moderately affected. In this situation, GFR increased, which resulted from relaxation of the afferent arteriole and a slight contraction of the efferent arteriole by ANP, raising the capillary glomerular pressure. In the present study, any reductions in glomerular capillary pressure seem to have been counteracted by such mechanisms, despite the reduction in MAP. In fact, it has been shown that infusions of human ANP at moderate supraphysiological doses positively affect renal dysfunction (GFR reductions) after complicated cardiac surgery and decreases the probability of dialysis (33), although this is slightly controversial (17). In the Lo-ANP group (30 ng·kg⁻¹·min⁻¹), systemic MAP was not affected, and the intrarenal hemodynamic effects were not large enough to produce any significant increments in GFR. This is consistent with data of Salazar et al. (30), who found that intrarenal infusion of ANP at a dose of 50 ng·kg⁻¹·min⁻¹ in anesthetized dogs did not produce any changes in either GFR or MAP, while the natriuretic effect was similar to that of higher doses of ANP (300 ng·kg⁻¹·min⁻¹). Due to the rather unchanging levels of GFR throughout the study for all animal groups, the experimental conditions in the present experiment, at least during the assessment of glomerular Ficoll sieving curves, should have been similar among the groups.

In the present study, we chose not to assess θ for albumin, because of unexpectedly high levels of free iodine and denaturated protein in the radiolabeled albumin preparation (¹²⁵I-albumin) at our disposal. Especially, the high concentrations of free label yielded abnormally high θ values in tissue uptake studies under control (SHAM) conditions. However, we have previously demonstrated a near-perfect coupling between alterations in θ for high-molecular-weight Ficoll and θ for radiolabeled albumin under conditions of increased permeability (6, 8, 28). Thus, given the high correlation between θ for albumin and θ for Ficoll50–80Å, we considered it quite safe in this study to rely upon the glomerular sieving of high-molecular-weight Ficoll as an indicator of glomerular permeability.

In conclusion, the present study, especially designed to assess θ for large Ficoll molecules, demonstrated a rapid, reversible increase in glomerular permeability for both low-dose and high-dose ANP infusions in rats. A more sustained action of ANP following the initial peak of permeability was especially noted for the high ANP dose. Also, for low ANP concentrations, there were indications of a sustained phase of changes in glomerular selectivity, manifested by an increase in the glomerular r_L. The early permeability peak, especially in Hi-ANP animals, can be explained by an increase in glomerular large pore number and r_L without invoking any changes in glomerular charge selectivity. The actions of ANP on the glomerular filter may be responsible for the microalbuminuria observed in plasma volume overload associated with e.g., congestive heart failure.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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