Atriopeptin Does Not Augment the Transvascular Flux of Macromolecules in the Hamster Cheek Pouch (43068)

DEBORA M. GAWLOWSKI, BRUCE A. BENJAMIN,[1] THOMAS V. PETERSON, NICHOLAS R. HARDING, AND HARRIS J. GRANGER

Microcirculation Research Institute and Department of Medical Physiology, College of Medicine, Texas A&M University, College Station, Texas 77843

Abstract. **Natriuretic peptides elaborated by atrial myocytes promote marked renal sodium and water excretion as a mechanism for fluid and electrolyte balance. Recent evidence suggests that atriopeptin (ANP) also targets the non-renal vasculature as a site for enhanced fluid exchange. It remains unclear whether ANP alters microvascular integrity to facilitate the efflux of both plasma and proteins across the endothelial barrier, or if fluid exchange is selectively enhanced. This study evaluated the influence of ANP on macromolecular transport through the direct observation of microvessels in the hamster cheek pouch using fluorescent intravital microscopy. Fluorescein isothiocyanate conjugated to either bovine serum albumin or dextran 150,000 $\bar{M}_w$ was utilized as a permeability probe. Macromolecular efflux was quantified as fluorochrome clearance. The clearance of fluorescein-conjugated bovine serum albumin (57.94 ± 7.03) or fluorescein-conjugated dextran 150 (4.09 ± 1.35) remained unaltered by intravascular injection of 1 μg/kg ANP. Topical application of 40 ng to cheek pouch microvessels produced similar results. All pouches demonstrated positive leakage response to histamine 2.5 × 10^{-6} *M*, increasing fluorochrome clearance approximately 2- to 11-fold. Bolus injection of 1 μg/kg ANP reduced mean arterial pressure, increased urine flow from 6.63 ± 2.59 μl/min to 8.20 ± 6.13 μl/min, and elevated sodium excretion from 1.37 ± 0.49 μEq/min to 2.54 ± 0.99 μEq/min. These results suggest that ANP fails to significantly alter the integrity of the protein-transporting channels in the microvascular exchange barrier.** [P.S.E.B.M. 1990, Vol 194]

Recent evidence (1) supports the existence of a novel cardioendocrine system which may be involved in the regulation of fluid and electrolyte homeostasis. Extracts from atrial tissue have been found to possess potent natriuretic, diuretic, and spasmolytic properties and to elicit a reduction in mean arterial pressure when infused systemically in the monkey, dog, and rat (2, 3). Biologic activity appears to parallel the distribution and relative density of specific granules localized to specialized myoendocrine cells in mammalian atrial tissues. Although the inhibition of aldosterone biosynthesis and renal renin release most likely contribute to the natriuretic response associated with chronic atriopeptin (ANP) release, it is likely that acute reductions in plasma volume result from the direct action of ANP on renal hemodynamics and tubular transport, and, possibly, through influences mediated at non-renal vascular target sites (4).

A number of indirect studies support the hypothesis that ANP shifts fluid from the intravascular compartment to the interstitial space, decreasing plasma volume and increasing hematocrit and protein concentration via a mechanism(s) which precludes urinary fluid loss (5, 6). Considering the vast surface area over which fluid exchange occurs in the various circulatory networks of the body, this would prove a rapid and highly efficient mechanism for acute plasma volume reduction. In turn, translocation of fluid into the extravascular compartment would facilitate the reductions in mean arterial pressure, cardiac output, and venous return which have been reported following ANP administration (6–8).

[1]Present address: Department of Cell Biology, Division of Physiology, Duke University Medical Center, Durham, NC 27710.

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Specific ANP vascular receptors have been demonstrated in rat mesenteric artery and rabbit aorta, cultured rat aortic smooth muscle cells (9, 10), bovine aortic smooth muscle and endothelial cells (11), and human platelets (12). In a recent study, direct analysis of transvascular fluid movement in single, isolated microvessels of the frog mesenteric circulation perfused with ANP indicated a 4-fold augmentation in hydraulic conductivity (13). Hyperfiltration in the absence of any change in surface area or hydrostatic pressure indicates that the selectivity of the microvascular membrane for solvent has been modified by the direct action of ANP at the level of the endothelial cell. It remains to be elucidated whether ANP's actions on endothelial cells could likewise diminish the intimal meshwork's capacity to sieve macromolecules, thereby enhancing protein flux across the barrier. The purpose of the present study was to evaluate the influence of ANP on macromolecular transport across the hamster cheek pouch microvascular exchange barrier. A modified cheek pouch preparation in conjunction with fluorometric analysis of fluorescein-labeled bovine serum albumin (FITC-BSA) or dextran $\bar{M}_w$ 150 (FITC-Dx 150) efflux rate was used to quantify the impact of ANP on macromolecular exchange. The results of this study indicate that ANP fails to modulate the transcapillary exchange of macromolecules despite its reported capacity to enhance water movement across the vascular membrane.

Materials and Methods

General Animal Preparation. Male Syrian golden hamsters *(Mesocricetus auratus)* weighing 80–150 g were used in both permselectivity and renal studies. Hamsters were anesthetized with sodium pentobarbital (65 mg/kg i.p.) and positioned on a heating mat regulated to maintain body temperature at 37°C. A tracheal cannula facilitated spontaneous respiration. The left external carotid artery and jugular vein were cannulated to enable blood collection and administration of pharmacologic agents, respectively.

Microvascular Preparation. The right cheek pouch was prepared for fluorescent intravital microscopic analysis using a chamber technique similar to that previously described by Greenblatt and Choudari (14) with the modifications of Gawlowski and Durán (15). Briefly, a Lucite base plate was inserted through the buccal cavity into the orifice of the pouch, and the overlying skin was incised along the midline of the cheek and retracted. The loose connective tissue layer was dissected away to expose microvessels and to improve visualization of the microcirculation. A chamber capable of retaining a 1-ml reservoir was attached to the base with the pouch interposed as a single layer. A purse string suture about the incision secured the chamber. Microvascular preparations were suffused with bicarbonate buffer at a constant flow rate of 1 ml/min. Chamber effluent was continuously collected over 5-min intervals for subsequent fluorometric analysis.

Analysis of Microvascular Macromolecular Efflux. The cheek pouch microvasculature was observed with a Zeiss ACM intravital microscope equipped with a vertical Ploem episcopic fluorescent attachment and the appropriate excitation/emission filters for fluorescein. One of two macromolecular tracers were utilized in each ANP study. Dextran, $\bar{M}_w$ 150,000, labeled with fluorescein isothiocyanate (FITC-Dx 150) was purchased from Sigma Chemical Company and FITC-tagged bovine serum albumin (FITC-BSA), $\bar{M}_w$ 67,000, was prepared in a manner similar to that previously described by McDonagh and Williams (16), with two modifications: unconjugated fluorescent material was extracted from the protein sample using column chromatography (Sephadex G-25) in addition to charcoal adsorption, and the final product was lyophilized to prolong storage. Fluorochromes were prepared fresh daily and administered intravenously at doses of 100 or 150 mg/kg for FITC-Dx 150 and FITC-BSA, respectively. The fluorochrome concentration of suffusate and plasma samples was determined with a Perkin Elmer LS-5 fluorescence spectrophotometer. Standard curves of concentration versus emission were generated from known concentrations of fluorescein-labeled compounds, and unknown samples were determined from standard curves. Macromolecular efflux was quantified as fluorochrome clearance (Cl), calculated as the ratio of suffusate (S, ng/ml) to plasma (P, mg/ml) concentration multiplied by suffusate flow rate:

$$Cl\ (\text{nl/min}) = (S/P)\ (1\ \text{ml/min})\ (10^6\ \text{nl/ml})$$

For each hamster the average clearance (mean ± SE) was calculated 20 min before and 20 min after ANP administration. Rat ANP_{1-28} was administered either intravascularly (i.v. or i.a.) at a dose (1 μg/kg) calculated to give plasma levels in the pathophysiologic range or applied topically for 4 min at a concentration (40 ng/ml) estimated to expose the cheek pouch vessels to levels 8–10 times higher than this. At the end of the study, a positive control for each preparation was obtained by topically applying histamine (1×10^{-6} M) to ensure that clearance could increase.

A paired t test was used to test the null hypothesis using a confidence limit of 5%.

Renal Preparation. Eight hamsters were anesthetized and tracheotomized as described above. The left carotid artery was cannulated for measurement of arterial blood pressure. The right and left jugular veins were cannulated for constant infusion of saline (5 μl/min) and administration of an rat ANP_{1-28} bolus injection. The urinary bladder was exposed through a midline abdominal incision and cannulated with an 18-gauge catheter. After an equilibration period of 30 min, urine was continuously collected 30 min before and 30

min after ANP administration into precalibrated volumetric pipettes. The renal response to ANP was quantified as mean change in urine volume and sodium excretion. Sodium concentration was measured by flame photometry (Instrumentation Laboratories, 643). Data are expressed as mean (±SE) μl/min for volume flow and mean (±SE) μEq/min for sodium excretion.

Results

Efflux Studies. ANP, whether administered intravascularly or topically, did not influence the flux of macromolecules across the exchange barrier (Table I). The mean clearance of FITC-BSA during control conditions, 57.94 ± 7.03 (SEM) nl/min, was no different after ANP injection, 56.70 ± 7.6 nl/min ($P > 0.48$). Hamsters treated topically with ANP had clearance values of 60.59 ± 6.04 nl/min, which were also similar to their respective controls, 63.32 ± 4.99 nl/min ($P > 0.33$). Topical application of histamine 1 x 10^{-6} M did, however, elicit extravasation of fluorochrome in all animals tested, increasing FITC-BSA clearance 1.6- to 6.7-fold, thus confirming the viability of the preparation.

Similar results were also obtained using FITC-Dx 150 as macromolecular fluorochrome (Table II). Fluorochrome clearance (4.46 ± 1.41 nl/min) in hamsters injected with ANP was not significantly different from control (4.09 ± 1.35 nl/min, $P > 0.17$). Topical application of ANP also did not augment FITC-Dx 150 clearance, 4.30 ± 1.66 nl/min vs 4.31 ± 1.92 nl/min in control ($P > 0.98$). Histamine again increased FITC-Dx 150 clearance, the range in this group being from 3.6- to 10.7-fold.

Renal Studies. As ANP failed to modulate macromolecular flux across the exchange barrier, we examined the blood pressure and renal responses to ANP administration in order to (i) verify that hamsters are capable of responding to rat atrial natriuretic peptide, i.e., possess specific receptors for ANP and (ii) validate the efficacy of the ANP used in the study. Intravenous bolus injection of ANP (1 μg/kg) elicited a reduction in mean arterial pressure by 11 +3 mm Hg ($n = 10$).

Intravenous injection of this ANP bolus also caused a significant increase in urinary excretion over the 30-min postinjection interval. Urine flow rose from 5.63 ± 2.59 μl/min in control to 8.20 ± 6.13 μl/min ($P < 0.01$) following ANP injection. Sodium excretion nearly doubled, rising from 1.37 ± 0.49 μEq/min to 2.54 ± 0.99 μEq/min ($P < 0.01$). Increasing concentrations of ANP further increased both sodium excretion and urinary volume.

Discussion

In independent studies, we utilized either of two fluorescent tracers to assay for ANP-mediated alterations in macromolecular flux across the exchange barrier. This approach was undertaken to optimize our ability to evaluate potential changes in pore size and better assess the degree to which such changes influence the transvascular passage of different sized molecules. However, neither intravascular administration of ANP nor its direct application to hamster cheek pouch exchange vessels augmented macromolecular efflux. These results indicate that the sieving characteristics of the endothelial barrier regulating protein leakage from plasma to interstitial fluids remain unaltered upon exposure to ANP. Although *Huxley et al.* (13) have reported that atrial natriuretic peptides augment fluid flux across single, perfused microvessels, the dose of ANP used in their study was quite high and, more importantly, ANP's capacity to simultaneously modulate protein efflux was not assessed. To the best of our knowledge, ours is the first report to address this issue and we found that ANP does not augment protein flux across the microvasculature.

Aside from renal effects, the hypovolemic actions

Table I. Effect of ANP on Clearance (nl/min)[a] of FITC-BSA

Hamster	CON	ANP (1 μg/kg)	CON	ANP (40 ng/ml)	CON	Histamine[b] (2.5 × 10^{-6} M)
1	77.19 ± 7.26	72.85 ± 4.49	—[c]	—	101.16	272.80
2	67.96 ± 0.09	67.43 ± 3.88	72.89 ± 4.65	75.60 ± 3.52	69.89	177.33
3	53.33 ± 1.93	56.08 ± 2.74	53.89 ± 1.92	54.03 ± 2.27	54.03	160.53
4	28.04 ± 5.35	18.79 ± 3.50	—	—	15.86	50.71
5	40.44 ± 4.85	39.47 ± 1.36	48.24 ± 3.66	48.84 ± 11.19	87.45	189.62
6	52.25 ± 7.31	49.17 ± 2.85	61.17 ± 2.53	49.33 ± 9.94	49.19	78.69
7	—	—	81.59 ± 6.03	82.94 ± 5.29	88.09	167.20
8	89.64 ± 5.58	89.44 ± 4.57	—	—	82.86	207.56
9	54.10 ± 10.41	60.34 ± 7.68	62.14 ± 1.28	52.79 ± 2.64	—	—
Mean ± SE	57.94 ± 7.03	56.70 ± 7.60	63.32 ± 4.99	60.59 ± 6.04	68.57 ± 9.80	163.06 ± 24.90[c]

[a] Data from individual experiments are expressed as mean clearance ± SD measured during a 20-min interval before and after ANP administration.
[b] Histamine as a positive control, values represent clearance of FITC-BSA measured during the 5-min interval before and after exposure.
[c] Significantly different from control.

Table II. Effect of ANP on Clearance (nl/min)[a] of FITC-Dx 150

Hamster	CON	ANP (1 μg/kg)	CON	ANP (40 ng/ml)	CON	Histamine[b] (2.5×10^{-6} M)
1	8.25 ± 0.98	8.77 ± 1.42	10.46 ± 0.80	9.87 ± 1.25	6.70	23.81
2	5.44 ± 0.60	6.02 ± 0.65	7.00 ± 0.55	5.71 ± 0.29	5.77	24.51
3	4.23 ± 0.23	4.02 ± 0.27	3.18 ± 0.83	3.86 ± 0.67	4.05	21.77
4	2.17 ± 0.38	3.15 ± 3.08	0.85 ± 0.70	0.42 ± 0.01	3.90	18.54
5	0.38 ± 0.15	0.32 ± 0.01	0.36 ± 0.01	1.64 ± 0.46	1.59	17.01
Mean ± SE	4.09 ± 1.35	4.46 ± 1.41	4.31 ± 1.92	4.30 ± 1.66	4.40 ± 0.88	21.13 ± 1.46[c]

[a] Data from individual experiments are expressed as mean clearance ± SD measured during a 20-min internal before and after ANP administration.
[b] Histamine as a positive control, values represent clearance of FITC-Dx 150 measured during the 5-min interval before and after exposure.
[c] Significantly different from control.

of ANP and reductions in vascular filling pressures (6–8) are through currently undefined mechanisms which may facilitate the rapid efflux of plasma into extracellular spaces. Reports of hemoconcentration and hence plasma volume reduction (5, 6) following ANP administration in nephrectomized rats clearly support alterations in fluid transfer as an immediate means of volume regulation. However, it remained to be elucidated if fluid shifts occur secondary to a generalized increase in vascular permeability resulting from reduced sieving of plasma proteins, or through a selective enhancement in fluid efflux. Our data suggest that ANP fails to significantly alter the pathways of protein transport across the microvascular exchange barrier since the flux of macromolecules remained unchanged. Perhaps ANP acts to widen small water-conducting channels that exclude plasma proteins and larger macromolecules. Increases in capillary hydrostatic pressure occurring as a consequence of alterations in pre- to postcapillary resistance ratios may also promote the hyperfiltration observed (17), and elevations in capillary hydraulic conductivity would further support fluid exchange (13).

Our findings support preliminary studies in the rat mesenteric microcirculation in which no extravasation was observed following 30 min of ANP infusion (18). However, whole animal studies (19) report that despite no change in plasma protein concentration, an increased escape of radiolabeled albumin from the circulation occurs following long-term (5 days) administration of ANP in conscious sheep. This rate of albumin disappearance increased from 9.7 to 12.4%/hr. We suggest that this could have occurred as a permeability-independent phenomenon since initially, plasma volume decreased, body weight and water intake both increased, but urinary volume excretion remained unchanged. This scenario supports a model of interstitial fluid expansion despite a reduction in plasma volume. Thus, there would be an increased dissipative flux of albumin because its concentration has risen in plasma but decreased in the interstitium.

The possibility still exists that the golden hamster may prove an inappropriate model in which to assess the biological response to a hormone which facilitates water and sodium excretion. Specific receptors for ANP may not be present in a desert animal whose survival is dependent upon water conservation. In addition, because of our negative clearance results with ANP, efficacy of the rat atrial peptide we utilized needed to be verified. As such, we evaluated the renal effects of ANP and monitored arterial blood pressure before and after bolus injection. The systemic injection of ANP transiently reduced mean carotid artery pressure and elicited both natriuresis and diuresis. These results provide functional evidence supporting the presence of specific renal and vascular receptors for atrial peptide hormones in the hamster, which, when stimulated, facilitate a biologic response typical of that reported in other species.

Experiments were carried out to determine the effect of ANP on macromolecular efflux across the hamster cheek pouch microvessels. Results from this study demonstrate that physiologically relevant doses of ANP enhance urinary sodium and volume excretion in the absence of any alterations in protein flux across the microvasculature. If ANP causes an intravascular fluid shift in the hamster, it does not appear to be due to an increase in vascular permeability.

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