

The renal action of atrial natriuretic peptide during control of glomerular filtration

JOHN C. BURNETT, JR., TERRY J. OPGENORTH, and JOEY P. GRANGER

Departments of Medicine and Physiology, Mayo Medical School, Rochester, Minnesota, USA

The renal action of atrial natriuretic peptide during control of glomerular filtration. Studies were performed in anesthetized dogs ($N = 7$) to determine the effects of synthetic atrial natriuretic peptide (ANP) on sodium excretion in the presence and absence of control of glomerular filtration rate produced by suprarenal aortic clamping. Intrarenal infusion of synthetic atrial natriuretic peptide ($0.3 \mu\text{g}/\text{kg}/\text{min}$) significantly increased glomerular filtration rate from 29.3 ± 3.0 to 43.2 ± 4.4 ml/min, urinary sodium excretion from 20.1 ± 10.3 to $223.3 \pm 52.3 \mu\text{Eq}/\text{min}$, fractional sodium excretion from 0.47 ± 0.19 to $3.75 \pm 0.59\%$. In contrast, aortic clamping blocked the increase in glomerular filtration rate in association with an attenuated natriuresis. Urinary sodium excretion increased from 6.3 ± 2.3 to $68.3 \pm 23.4 \mu\text{Eq}/\text{min}$ and fractional sodium excretion increased from 0.15 ± 0.04 to $0.90 \pm 0.30\%$. Despite this differential response in glomerular filtration rate and sodium excretion, whole kidney fractional delivery of sodium from the proximal tubule as estimated by the fractional excretion of lithium increased during both unclamped (17.7 ± 1.8 to $30.4 \pm 0.8\%$) and clamped (12.9 ± 2.1 to $23.9 \pm 2.7\%$) periods. These studies demonstrate that atrial natriuretic peptide-induced natriuresis is importantly mediated by an increase in glomerular filtration rate and decrease in tubular reabsorption.

Recent investigations employing both crude atrial extract and newly available synthetic atrial natriuretic peptide have repeatedly demonstrated that the natriuretic response to this peptide is associated with an increase in glomerular filtration rate [1-3]. While some investigations have suggested an effect of atrial peptides on tubular reabsorption [1, 4, 16], most studies support the hypothesis that atrial natriuretic peptide-induced natriuresis is mediated by an enhancement of the filtration process [1-3]. No studies have been performed, however, to establish the dependency of atrial natriuretic peptide-induced natriuresis on glomerular filtration rate in the normally functioning kidney employing a specific synthetic atrial natriuretic peptide fragment.

The present study was therefore designed to elucidate the contribution of glomerular filtration rate in the mechanism of atrial natriuretic peptide-induced natriuresis. Aortic clamping was used to block the predicted increase in glomerular filtration rate during administration of synthetic atrial natriuretic peptide in anesthetized dogs. This experimental maneuver has been employed in previous investigations which established the role

of glomerular filtration rate in the natriuretic response to the peptide glucagon [6, 7].

Methods

Experiments were performed on seven mongrel dogs of either sex (16 to 22 kg body wt). The dogs were fasted overnight prior to the acute experiment but allowed access to water. Dogs were anesthetized with sodium pentobarbital (30 mg/kg) and maintained with supplemental doses as necessary. The trachea was intubated with a cuffed endotracheal tube and the dogs were ventilated with a Harvard Apparatus respirator. A femoral vein was cannulated for infusions and obtaining blood samples. Two catheters were inserted via the femoral artery and placed below and above the aortic clamp to assess systemic blood pressure and renal perfusion pressure. The left kidney and left renal artery were isolated via a retroperitoneal flank incision. The dogs were supported in a metal frame that held them in a position approximating their normal upright posture. A noncannulating flow probe (Carolina Medical Electronics, King, North Carolina, USA) was placed around the left renal artery and a small needle was inserted into the artery distal to the flow probe. The left ureter was cannulated for collection of urine from the left kidney only. An infusion of saline (1 ml/min) was initiated to maintain the patency of the needle. A variable-resistance Blalock clamp was placed around the aorta just proximal to the renal arteries without causing any constriction.

After the surgical preparation, an inulin infusion was begun in the femoral vein at 1 ml/min at a concentration calculated to produce a plasma concentration of 50 mg/dl. At the end of this equilibration period, two 15 min clearances were performed, with a blood sample obtained either in the middle of a single clearance or between the two clearances. The saline infusion into the renal artery was then replaced by an infusion of synthetic ANP (8-33, Peninsula Lab., Belmont, California, USA) at a rate of $0.3 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for a total of 45 min. Fifteen min after initiation of synthetic ANP infusion, two 15 min clearances were obtained. The infusion was stopped. Following a 45 min washout period, two 15 min recovery clearances were obtained. After the recovery clearance, the clamp was tightened to decrease renal blood flow to the lowest level of renal blood flow autoregulation determined by that pressure at which renal blood flow began to fall. Following a 30 min equilibration period, the above protocol was repeated. In

four of the seven dogs, the clamping protocol preceded the unclamped protocol.

Glomerular filtration rate was measured by the clearance of inulin. Inulin concentrations were measured by the anthrone method [8]. Concentrations of sodium and potassium were measured using ion-selective electrodes (Beckman E2A analyzer, Beckman Instruments, Fullerton, California, USA).

Whole kidney proximal tubular reabsorption of sodium was estimated by the lithium-clearance technique. This technique has been shown to be a reliable method for estimating delivery of sodium from the proximal tubule, since lithium is reabsorbed exclusively by the proximal tubule [9, 10]. This method is based on evidence indicating that lithium is reabsorbed almost exclusively in the proximal tubule, including the pars recta, in parallel with sodium and water. Micropuncture studies have shown that fractional lithium reabsorption correlates very well with proximal sodium reabsorption over a wide range of proximal reabsorption rates. In addition, diuretics that predominantly inhibit sodium transport in the ascending limb of the loop of Henle and the distal tubule and collecting duct do not increase urinary lithium excretion [5]. In contrast, maneuvers known to inhibit proximal sodium reabsorption, such as acetazolamide and osmotic diuretics, increase lithium excretion [11]. The dogs were given 300 mg of lithium (orally) the night before each experiment. Lithium concentration in plasma and urine was measured by flame emission spectrophotometry (model 357, Instrumentation Laboratory, Lexington, Massachusetts, USA).

All data from the two clearances at baseline, atrial natriuretic peptide, and recovery periods were averaged and are expressed as means \pm SE. The data were analyzed using Dunnett's paired *t*-test for simultaneous multiple comparisons.

Results

Table 1 and Figure 1 summarize the renal hemodynamic and excretory response data during control, intrarenal infusion of synthetic atrial natriuretic peptide, and recovery for seven dogs in the presence and absence of control of glomerular filtration rate.

In the absence of suprarenal aortic constriction, intrarenal ANP infusion resulted in a significant increase in glomerular filtration rate (29.3 ± 3.0 to 43.2 ± 4.4 ml/min, $P < 0.02$), urine flow (0.37 ± 0.17 to 2.46 ± 0.68 ml/min, $P < 0.05$), urinary sodium excretion (20.1 ± 10.3 to 223.3 ± 52.3 μ Eq/min, $P < 0.005$), fractional sodium excretion (0.47 ± 0.17 to $3.75 \pm 0.59\%$, $P < 0.005$), and fractional lithium excretion (17.7 ± 1.8 to $30.4 \pm 0.8\%$, $P < 0.005$). Although renal blood flow did not significantly change (168 ± 21 to 175 ± 23 ml/min), renal vascular resistance decreased (0.76 ± 0.06 to 0.68 ± 0.06 mm Hg \cdot (ml \cdot min) $^{-1}$, $P < 0.05$) in association with a decrease in mean arterial pressure (119 ± 3 to 109 ± 3 mm Hg, $P < 0.05$). Urine osmolality decreased from 932 ± 265 to 383 ± 58 mOsmol/kg H₂O ($P < 0.05$). Filtration fraction increased from 0.33 ± 0.05 to 0.46 ± 0.06 , $P < 0.02$.

During aortic clamping, glomerular filtration rate did not significantly increase from 27.4 ± 1.9 ml/min. Despite the absence of a significant increase in glomerular filtration rate during ANP infusion, whole kidney proximal tubular delivery of sodium as estimated from the clearance of lithium increased. Fractional lithium excretion increased from 12.9 ± 2.1 to 23.9

Table 1. Renal hemodynamic and excretory responses to intrarenal infusion of synthetic atrial natriuretic peptide in the presence and absence of control of glomerular filtration rate ($N = 7$)

	Unclamped		
	Baseline	ANF (0.3 μ g/kg/min)	Recovery
MAP, mm Hg	119 \pm 3	109 \pm 3*	112 \pm 3*
RBF, ml/min	168 \pm 21	175 \pm 23	159 \pm 18
GFR, ml/min	29.3 \pm 3.0	43.2 \pm 4.4**	30.6 \pm 3.3
RVR,			
mm Hg \cdot (ml/min) $^{-1}$	0.76 \pm 0.06	0.68 \pm 0.06*	0.73 \pm 0.05
FF	0.33 \pm 0.05	0.46 \pm 0.06**	0.35 \pm 0.03
V, ml/min	0.37 \pm 0.17	2.46 \pm 0.68*	0.44 \pm 0.14
U _{osmol} , mOsmol/kg H ₂ O	932 \pm 265	383 \pm 58*	702 \pm 155
U _{Na} V, μ Eq/min	20.1 \pm 10.3	223.3 \pm 52.3***	51.9 \pm 12.9**
FE _{Na} , %	0.47 \pm 0.17	3.75 \pm 0.59***	1.31 \pm 0.62
FE _{Li} , %	17.7 \pm 1.8	30.4 \pm 0.8***	22.5 \pm 14.4*
	Clamped		
	Baseline	ANF (0.3 μ g/kg/min)	Recovery
MAP, mm Hg	128 \pm 8	121 \pm 7*	128 \pm 5
RBF, ml/min	182 \pm 21	173 \pm 19	149 \pm 14**
GFR, ml/min	27.4 \pm 1.9	28.0 \pm 1.5	31.2 \pm 4.0
RVR,			
mm Hg \cdot (ml/min) $^{-1}$	0.79 \pm 0.11	0.79 \pm 0.11	0.93 \pm 0.11**
FF	0.27 \pm 0.04	0.33 \pm 0.04*	0.39 \pm 0.07
V, ml/min	0.18 \pm 0.06	0.56 \pm 0.16*	0.24 \pm 0.38
U _{osmol} , mOsmol/kg H ₂ O	841 \pm 194	665 \pm 137	742 \pm 148
U _{Na} V, μ Eq/min	6.3 \pm 2.3	68.3 \pm 23.4*	12.3 \pm 4.0
FE _{Na} , %	0.15 \pm 0.04	0.90 \pm 0.30*	0.31 \pm 0.11
FE _{Li} , %	12.9 \pm 2.1	23.9 \pm 2.7**	16.7 \pm 2.6**

Values are means \pm SE. Abbreviations are: MAP, mean arterial pressure; RBF, renal blood flow; GFR, glomerular filtration rate; RVR, renal vascular resistance; FF, filtration fraction; V, urine flow; U_{osmol}, urinary osmolality; U_{Na}V, urinary sodium excretion; FE_{Na}, fractional excretion of sodium; FE_{Li}, fractional excretion of lithium. *P* values compare results with baseline: * $P < 0.05$; ** $P < 0.02$, *** $P < 0.005$.

$\pm 2.7\%$, $P < 0.02$. Increases were observed in urine flow from 0.18 ± 0.06 to 0.56 ± 0.16 ml/min ($P < 0.05$), urinary sodium excretion from 6.3 ± 2.3 to 68.3 ± 23.4 μ Eq/min, $P < 0.05$, and fractional excretion of sodium from 0.15 ± 0.04 to $0.90 \pm 0.30\%$. Urine osmolality was unchanged at 841 ± 194 mOsmol/kg H₂O. Renal blood flow and renal vascular resistance also did not change. Filtration fraction, however, increased (0.27 ± 0.04 to 0.33 ± 0.04 , $P < 0.05$), and mean arterial pressure decreased (128 ± 8 to 121 ± 7 mm Hg, $P < 0.05$).

Discussion

Intrarenal infusion of synthetic atrial natriuretic peptide resulted in a marked increase in sodium excretion in the absence of aortic clamping. This marked natriuretic response was associated with a significant increase in glomerular filtration rate. Aortic clamping prevented the predicted increase in glomerular filtration rate and attenuated the natriuretic response to atrial natriuretic peptide. Although the natriuretic response to atrial natriuretic peptide was attenuated by clamping, this attenuated natriuresis occurred in association with a decrease in whole kidney proximal tubule reabsorption, as estimated by the clearance of lithium. Thus, the present study confirms an important relationship between atrial natriuretic

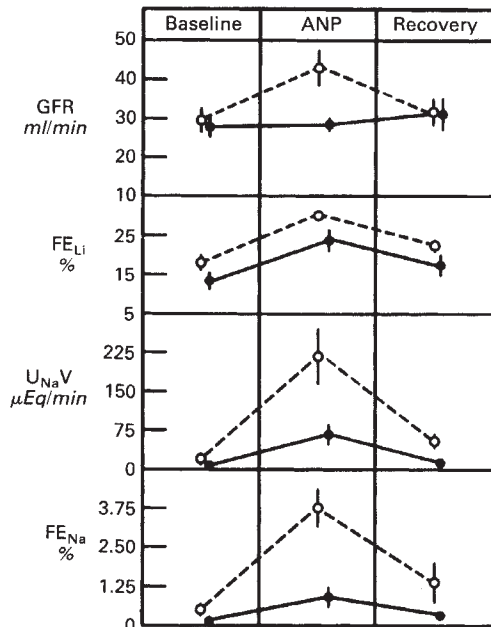


Fig. 1. The effect of atrial natriuretic peptide (ANP) on renal function in the presence and absence of control of glomerular filtration rate (GFR). Symbols are: (○) absence of control of GFR; (●) control of GFR. Abbreviations are: FE_{Li}, fractional excretion of lithium; U_{NaV}, urinary sodium excretion; FE_{Na}, fractional excretion of sodium ($N = 7$).

peptide-induced natriuresis and glomerular filtration rate, but establishes a natriuretic response independent from increases in the filtration process.

Recent studies have reported an important relationship between atrial natriuretic peptide natriuresis and glomerular filtration rate. In the isolated perfused kidney preparation, intrarenal infusion of crude atrial extract resulted in a significant increase in both glomerular filtration rate and sodium excretion [12]. Beasley and Malvin extended earlier studies and reported that continuous infusion of crude atrial extract in rats resulted in significant increases in both glomerular filtration rate and sodium excretion, in contrast to ventricular extracts which failed to augment glomerular filtration rate or sodium excretion [3]. Recent purification, sequencing, and synthesis of atrial natriuretic factor have resulted in the availability of synthetic peptide fragments with pure biological activity. Synthetic atrial peptides have permitted further elucidation of the precise action of this peptide system on renal function. Several studies have now consistently demonstrated an important association between urinary sodium excretion and glomerular filtration rate during infusion of synthetic atrial peptide fragments [1, 2, 13]. The present studies importantly extend previous studies employing synthetic atrial natriuretic peptide, and establish an important relationship between atrial natriuretic peptide-induced natriuresis and glomerular filtration rate.

The mechanism by which atrial natriuretic peptide increases glomerular filtration rate remains to be defined. Based on the significant increase in filtration fraction with no significant increase in renal blood flow, we reported in previous studies that the increase in glomerular filtration rate may be mediated by a balanced action on segmental renal vascular resistances, in which preglomerular resistance is decreased and postglomeru-

lar resistance increased [1]. Preliminary micropuncture studies in the rat by Ichikawa et al are in agreement with such an interpretation [14]. A separate mechanism may involve a direct action of atrial natriuretic peptide to increase the coefficient of ultrafiltration, K_f , recognizing the demonstration of atrial natriuretic peptide-induced generation of cGMP within the glomerulus [15].

The present studies are similar to previous investigations which employed aortic clamping to define the mechanism of glucagon-induced natriuresis [6, 7]. Such previous studies definitively demonstrated by the maneuver used in the present study that the natriuretic response to the peptide glucagon is dependent on an increase in glomerular filtration rate with no evidence for a tubular effect. In contrast to the glucagon studies, the present studies demonstrate that despite the abolition of atrial natriuretic peptide-induced increase in glomerular filtration rate, a proximal tubular action of atrial natriuretic peptide as estimated by the clearance of lithium may contribute to the attenuated natriuresis. The fractional delivery of sodium from the proximal tubule as estimated by the increase in fractional lithium excretion occurred in both the presence and absence of control of glomerular filtration rate. The decrease in whole kidney proximal tubule reabsorption, despite a blunted increase in glomerular filtration rate during aortic clamping, would support the conclusion that the decrease in proximal tubule reabsorption of superficial and/or deep nephrons in response to synthetic atrial natriuretic peptide is not dependent on an increased filtered load of this ion. Such a conclusion is supported by the recent report from Hammond and colleagues that sodium co-transport is inhibited in proximal tubular brush border vesicles harvested from rats undergoing atrial natriuretic peptide-induced natriuresis [16]. The present studies demonstrate a dissociation between glomerular filtration rate and whole kidney proximal tubule reabsorption as estimated by the clearance of lithium, such that whole kidney proximal tubule reabsorption decreases in response to atrial natriuretic peptide during aortic clamping, despite the absence of an increase in the filtration process. Thus, the present findings support the conclusion of a proximal tubular action of atrial natriuretic peptide.

Aortic clamping blocked the decrease in urinary osmolality as well as the increase in glomerular filtration rate, suggesting another contributing mechanism to the attenuated natriuretic response to atrial natriuretic peptide during control of glomerular filtration rate. Previous studies have suggested that medullary washout, due to increased papillary plasma flow characteristic of renal vasodilators, may occur during ANP infusion [17]. The present studies suggest that aortic clamping may prevent medullary washout, and that without decreases in the medullary gradient, the natriuretic response to atrial natriuretic peptide is prevented [1, 2, 17].

In summary, the present findings suggest that reduction in renal artery perfusion pressure to the lowest levels of renal blood flow autoregulation prevents atrial natriuretic peptide-induced increases in glomerular filtration rate, and attenuate the subsequent natriuresis supporting a role for glomerular filtration rate in the natriuretic response to this peptide hormone. The observation, however, of a natriuretic response and increase in fractional excretion of lithium during aortic clamping, despite the absence of an increase in glomer-

ular filtration rate, supports a direct or indirect action of atrial natriuretic peptide on proximal tubular reabsorption.

Acknowledgments

The authors thank Denise Heublein for her technical assistance and June M. Hanke for secretarial assistance. These studies were supported by the Hearst and Rappaport Foundations and by a Grant-in-Aid (83-964) from the American Heart Association.

Reprint requests to John C. Burnett, Jr., M.D., Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Medical School, Rochester, Minnesota 55905, USA.

References

- BURNETT JC, GRANGER JP, OPGENORTH TJ: Effects of synthetic atrial natriuretic factor on renal function and renin release. *Am J Physiol* 247:F863-F866, 1984
- MAACK T, MARION DN, CAMARGO MJ, KLEINERT HD, LARAGH JH, VAUGHAN ED, ATLAS SA: Effects of auriculin-atrial natriuretic factor on blood pressure, renal function, and the renin-aldosterone system in dogs. *Am J Med* 77:1069-1075, 1984
- BEASLEY D, MALVIN RL: Atrial extract increases glomerular filtration rate in vivo. *Am J Physiol* 248:F24-F30, 1985
- SONNENBERG J, CUPPLES WA, DEBOLD AJ, VERESS AT: Intrarenal localization of the natriuretic effect of cardiac atrial extract. *Can J Physiol Pharmacol* 60:1149-1152, 1982
- PETERSEN V, HVIDT S, THOMSEN K, SCHOW M: Effect of prolonged thiazide treatment on renal lithium clearance. *Br Med J* 3:143-145, 1974
- LEVY M, STARR NL: The mechanism of glucagon-induced natriuresis in dogs. *Kidney Int* 2:76-84, 1972
- JOHANNESEN J, LIE M, KIIL F: Effect of glycine and glucagon on glomerular filtration and renal metabolic rates. *Am J Physiol* 233:F61-F66, 1977
- FÜHR J, KACHZMARCZYK J, KRÜTTGEN CD: Eine einfache colorimetrische Methode zur Insulinbestimmung für Nieren clearanceuntersuchungen bei Stoffwechselgesunden und Diabetikern. *Klin Wochenschr* 33:729-730, 1955
- HAYSLETT JP, KASHGARIAN M: A micropuncture study of the renal handling of lithium. *Pflügers Arch* 380:159-163, 1979
- THOMSEN K, HOLSTEIN-RATHLOW NH, LEYSSAC PP: Comparison of three measures of proximal tubular reabsorption: Lithium clearance, occlusion time, and micropuncture. *Am J Physiol* 241:F348-F355, 1981
- THOMSEN K, SCHOW M: Renal lithium excretion in man. *Am J Physiol* 215:823-827, 1968
- CAMARGO MJF, KLEINERT HD, ATLAS SA, SEALEY GE, LARAGH JH, MAACK T: Ca-dependent hemodynamic and natriuretic effect of atrial extract in isolated rat kidney. *Am J Physiol* 246:F447-F456, 1984
- HUANG CL, LEWICHI J, JOHNSON LK, COGAN MG: Renal mechanisms of action of rat atrial natriuretic factor. *J Clin Invest* 75:769-774, 1985
- ICHIKAWA I, DUNN BR, TROY JL, MAACK T, BRENNER BM: Influence of atrial natriuretic peptide on glomerular microcirculation in vivo. (abstract) *Clin Res* 33:487A, 1985
- TREMBLAY J, GERZER R, VINAY P, PANG SC, RELIVEAU R, HAMET P: The increase of cGMP by atrial natriuretic factor correlates with the distribution of particulate guanylate cyclase. *FEBS Lett* 181:17-22, 1985
- HAMMOND TG, YUSUFI ANK, KNOX FG, DOUSA TP: Administration of atrial natriuretic factor inhibits proximal Na-coupled solute transport. *J Clin Invest* 75:1983-1989, 1985
- BORENSTEIN HB, CUPPLES WA, SONNENBERG H, VERESS AT: The effect of a natriuretic atrial extract on renal haemodynamics and urinary excretion in anaesthetized rats. *J Physiol London* 334:133-140, 1983