Rat Atrial Natriuretic Factor (ANP-III) Inhibits Phosphate Transport in Brush Border Membrane from Superficial and Juxtamedullary Cortex[1](42833)

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Abstract. **Previous studies have shown that administration of synthetic atrial natriuretic factor (ANF, 101-126) decreases sodium-dependent phosphate transport across renal brush border membrane vesicles (BBMV) in rats fed a normal or low phosphate diet. In the present study, infusion of rat ANF (atriopeptin III (ANP-III), 103–126 rat ANF) to rats fed a normal phosphate diet caused natriuretic and phosphaturic effects similar to those of ANF (101–126), but unlike ANF (101–126) did not increase the glomerular filtration rate. The effect of ANP-III infusion on sodium-dependent transport of phosphate was also determined in BBM vesicles isolated from the superficial cortex (BBMV-SC) and juxtamedullary cortex (BBMV-JM). The results indicate that ANP-III decreases phosphate transport across BBMV-SC and BBMV-JM similarly (20–24%). However, it had no effect on sodium-dependent transport of proline in these vesicles.**
The infusion of ANP-III to rats fed a normal phosphate diet inhibits phosphate uptake both in BBMV-SC and BBMV-JM and causes phosphaturia without increments in glomerular filtration rate. [P.S.E.B.M. 1989, Vol 190]

Recent studies showed that infusion of atrial natriuretic factor (ANF, 101–126) caused marked natriuretic and phosphaturic effects in rats (1, 2) and dogs (3). It has been further shown that ANF infused *in vivo* decreases brush border membrane (BBM) sodium-dependent transport of phosphate and Na^+-H^+ antiport in rats fed a normal phosphate diet (NPD) (1).

Several hormonal and nonhormonal stimuli change the rate of phosphate transport across the renal BBM (4). It has been observed that BBM isolated from different areas of kidney parenchyma which contain proximal tubules differ in the transport properties (5–8). Perhaps even more interesting, we observed that co-transport systems, in particular phosphate transport in BBM isolated from distinct renal tissue zones, are modulated by hormones or by metabolic stimuli in a different direction and extent (5, 7, 8). We reported that hormones such as thyroid hormones, parathyroid hormone, and calcitonin modulate BBM transport of phosphate to a greater extent in vesicles isolated from the juxtamedullary cortex (BBMV-JM) or deep nephrons compared with the BBM vesicles isolated from the superficial cortex (BBMV-SC) (7, 8).

This study was undertaken to determine the contribution of changes in glomerular filtration rate (GFR) and tubular phosphate reabsorption to the phosphaturic effect of ANF by studying the effect of atriopeptin III (ANP-III, 103–126 rat ANF), an analogue that does not alter GFR, and to define the localization of ANP-III action on phosphate transport in BBM vesicles isolated from superficial (BBMV-SC) and juxtamedullary cortex (BBMV-JM) of rat kidneys. The infusion of ANP-III induced a natriuresis in rats fed NPD, but without a significant increment in GFR. This peptide was similarly phosphaturic to ANF in our previous studies (1) and inhibited phosphate transport both in BBMV-SC and BBMV-JM. The results further emphasized that

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the phosphaturic effect of ANP-III is independent of GFR.

Methods

Experiments were performed with an experimental design similar to that described previously (1). Briefly, male Sprague-Dawley rats weighing 250–300 g were fed a normal P_i diet (NPD, 0.7%) for 4 days prior to the experiment and had free access to water. On the day of the experiment the rats were anesthetized with Inactin (100 mg/kg body wt). All rats were acutely thyroparathyroidectomized by heart cautery and a tracheostomy was performed. Catheters were inserted into jugular veins for infusions and into the carotid artery for blood sampling and monitoring blood pressure. A catheter was placed in the bladder for urine collection. Body temperature was maintained between 36 and 38°C by use of a heated table. Rats were infused with 1% inulin in 0.9% saline at a rate of 8 ml/hr throughout the course of the experiment for the measurement of GFR. Each animal received an infusion of 0.9% saline equivalent to 4% body wt. Control clearances were begun 2 hr after acute thyroparathyroidectomy; one urine collection (20 min) and an arterial blood sample were taken. Rats were then infused with ANP-III (Peninsula Laboratories, Inc., Belmont, CA) with a priming dose of 8 μg/kg and a sustaining dose of 4 μg/kg/hr in isotonic NaCl. Time control rats were infused with isotonic saline continuously to allow comparison of the effects of ANP-III or vehicle on clearance parameters and BBMV transport. After 20 min to obtain equilibrium of infused ANP-III, an experimental clearance was performed.

After the clearance measurements, the kidneys were removed from the animal and immediately chilled and kept in ice-cold saline. The BBMV fractions were prepared from homogenates of renal tissue using the Mg^{2+} precipitation method (9, 10). After decapsulation, transverse sectional slices of the kidney were cut with a razor blade in the middle of the cortical thickness between the kidney surface and corticomedullary junction (7, 8). The superficial cortex was carefully separated from the juxtamedullary cortex. Since straight portions of proximal tubules dip beyond the corticomedullary junction (11), the outermost layer of adjacent medulla (outer stripe, i.e., "red" medulla) was included with juxtamedullary cortical tissue (7, 8). BBMV from superficial cortex (BBMV-SC) and BBMV from juxtamedullary cortex with an outer stripe of red medulla of the same kidneys (BBMV-JM) were prepared simultaneously using an identical technique (8). In order to obtain sufficient tissue from both the superficial and juxtamedullary cortical zones, BBMV were prepared by pooling the tissue from two rats, both in control and experimental groups. As in previous studies, the quality of the BBMV was evaluated by enrichment of BBM enzymes (12). There were no differences in specific activities of alkaline phosphatase between BBMV prepared from control or ANP-III-treated rats.

All transport parameters were measured, as a rule, in triplicate or quadruplicate and the mean ± SEM from each BBMV preparation was entered as $n = 1$. The protein content in BBMV fractions was measured using the method of Lowry *et al.* (13) with bovine serum albumin as the standard as in our previous study (8). Phosphate concentrations in plasma and urine were determined according to the method of Chen *et al.* (14). GFR was determined by clearance of inulin. Inulin concentration in urine and plasma was measured by the microfluorometric method of Vurek and Pegram (15).

All values are expressed as means ± SE. Comparisons were made by using either Student's t test or analysis of variance using Duncan's new multiple range test (16).

Results

We studied the effects of ANP-III, another equally potent fragment of atrial natriuretic peptides, both on the renal handling of phosphate and sodium as well as on the uptake of phosphate in BBMV isolated from two cortical zones. Infusion of ANP-III (103–126) increased significantly the fractional excretion of both sodium ($FE_{Na\%}$) and phosphate ($FE_{pi\%}$) (Table I) and decreased mean arterial pressure (Table II). Mean arterial pressure in the vehicle-infused rats was not influenced. However, glomerular filtration rate and plasma phosphate were not changed by ANP-III infusion (Table II). We further examined the effect of ANP-III on phosphate transport in BBMV isolated from superficial and juxtamedullary cortices and the results are summarized in Table III. We observed that ANP-III also inhibits the sodium gradient-dependent transport of phosphate in BBMV similar to the effect of ANF (101–126) infusion in rats fed NPD in our previous study (1). Infusion of ANP-III resulted in a decrease of phosphate uptake to a similar degree (20–24%) both in BBMV-SC and BBMV-JM both at 5 and 30 sec (Table IIIA). In the same aliquot of BBMVs, ANP-III infusion had no effect on sodium-dependent proline uptake (Table IIIB).

Discussion

Numerous atrial peptides have been synthesized and are being used for their different biologic activities

Table I. Effect of ANP-III on Sodium and Phosphate Excretion in Rats Fed a Normal Phosphate Diet

	Control	ANP-III
ΔFENa (%)	0.18 ± 0.38[a]	2.66 ± 0.35[b]
ΔFEP_i (%)	0.68 ± 0.63	3.27 ± 0.95[b]

[a] The values are compared between experimental and respective time control periods and are expressed as mean ± SE for 10 rats. The basal FE_{P_i} values for control and ANP-III were 2.53 ± 0.94% and 2.58 ± 1.15%, respectively.
[b] Significantly different from corresponding control values (group or paired t test, $P < 0.05$ or higher degrees of significance).

Table II. Effect of Infusion of ANP-III in Thyroparathyroidectomized Rats Fed a Normal Phosphate Diet

	Control		ANP-III	
	Saline	Saline	Saline	ANP-III
GFR (ml/min)	3.6 ± 0.3	3.3 ± 0.4[a]	3.8 ± 0.4	4.3 ± 0.6[a]
P_{P_i} (m*M*)	1.86 ± 0.07	1.93 ± 0.08	1.98 ± 0.08	1.97 ± 0.08
Mean arterial pressure (mm Hg)	151 ± 3	149 ± 5	150 ± 5	131 ± 6[b]

[a] Values are mean ± SE for five experiments (10 rats).
[b] Significantly different from corresponding control values (group or paired *t* test, $P < 0.05$ or higher degree of significance).

Table III. Effect of Infusion of ANP-III on Sodium-Dependent Phosphate and Proline Transport in BBMV-SC and BBMV-JM Isolated from Rats Fed a Normal Phosphate Diet

	A: [^{32}P]Phosphate uptake (pmole/mg protein)	
	Control	ANP-III
BBMV-SC		
5 sec	584 ± 72[a]	451 ± 49[b]
30 sec	1703 ± 210	1362 ± 158[c]
120 min	292 ± 15	288 ± 11
BBMV-JM		
5 sec	501 ± 56	380 ± 45[b]
30 sec	1529 ± 110	1223 ± 98[c]
120 min	248 ± 14	245 ± 17
	B: L-[^{3}H]Proline uptake (pmole/mg protein)	
BBMV-SC		
15 sec	247 ± 18[a]	227 ± 33
120 min	52 ± 4	55 ± 9
BBMV-JM		
15 sec	433 ± 27	413 ± 30
120 min	37 ± 3	42 ± 6

[a] Values are mean ± SE for five experiments.
[b] Significantly different from corresponding control values (analysis of variance).
[c] Significantly different from corresponding control values (paired *t* test, $P < 0.05$ or higher degree of significance).

(17, 18). In previous studies (1), we used 26-amino acid synthetic form of ANF (101–126), one of the first derivatives, to examine its natriuretic and phosphaturic properties. The present studies were performed with the use of an atrial peptide (ANP-III, 103–126) which is another circulating form of ANF in rats and shares similar biologic activities. Furthermore, this ANP-III has been shown to be a useful natriuretic (19) and devoid of any effect on GFR.

The infusion of rat ANP-III induces natriuresis in rats fed NPD but without a significant increment in glomerular filtration rate. However, the phosphaturic response to this peptide was similar (Table I) to the phosphaturic response to synthetic ANF reported in our previous studies (1). The absence of an effect of ANP-III on glomerular filtration rate in rats, also observed in another study (19), was in the presence of decreased mean arterial pressure. The mechanism of natriuretic and diuretic effects of ANF is largely unknown. It has been suggested that both hemodynamic and direct tubular effects may be responsible for ANF action (20). The mechanism of ANF action through changes in glomerular filtration rate remains controversial (3, 21–25). Whereas several studies found evidence for a direct action on tubule transport by ANF (3, 22, 25), Cogan (26) reported that ANF acts in the kidney predominantly by raising GFR. Nevertheless, Harris *et al.* (27) reaffirmed the findings that ANF acts within the kidney to decrease proximal reabsorption by inhibition of angiotensin-stimulated sodium and water transport. It is apparent from this and our previous studies (1, 28) that the natriuretic or phosphaturic effects of ANF or ANP-III are not necessarily dependent on the changes in glomerular fitration rate. Results summarized in Table III show that ANP-III decreases sodium-dependent phosphate transport both in BBMV-SC and BBMV-JM to a similar extent. This suggests that the inhibition of phosphate reabsorption by ANF may not be localized to a specific nephron or specific zone of renal proximal tubules. We recently reported that hormones, such as parathyroid hormone, calcitonin, and thyroid hormones regulate BBM transport of phosphate to a greater extent in vesicles isolated from juxtamedullary cortex or deep nephrons (7, 8). In contrast, the effect of dietary phosphate derivation in rats and dogs on phosphate transport was more profound in BBMV-SC compared with BBMV-JM (6–8). This effect of ANP-III, therefore, dissociates clearly from other hormones. It has been recently demonstrated that ANF inhibits sodium reabsorption from proximal tubules of mainly juxtamedullary nephrons (29, 30). However, the results of the present study on BBM phosphate transport do not show such a preferential effect of ANP-III. Thus, it is possible that the regulatory mechanism and the site of ANP-III effects on sodium and phosphate reabsorption in proximal tubules are different and, therefore, these might be two separate effects of ANF. This is also supported by the observation that ANF produces natriuresis without phosphaturia in phosphate-deprived rats (28).

Our previous findings that ANF decreases reabsorption of phosphate and of sodium reabsorption coupled to HCO_3 and the inhibition of Na^+-H^+ antiport by ANF in BBMV (1) and in LLC-PK_1 and OK cells in culture (31, 32) suggest a direct but not yet identified tubular effect. Taken together, the results of the present study and those of previous studies (1, 2) indicate that ANP-III (i) can cause natriuresis and phosphaturia

without the increment in glomerular filtration rate and (ii) the inhibitory effect of ANF on sodium reabsorption can be dissociated from its effect on phosphate reabsorption. Hence, on the basis of our studies, we conclude that atrial natriuretic peptides might have distinct hemodynamic and tubular effects. We further conclude that rat species-specific ANP-III inhibits sodium gradient-dependent P_i transport both in BBMV-SC and BBMV-JM to a similar extent.

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