*Biochemical Pharmacology*, Vol. 37, No. 20, pp. 4027–4029, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00


# SHORT COMMUNICATIONS

## Effect of atrial natriuretic factor on brush border membrane transport of phosphate in phosphate-deprived rats

(*Received* 28 *December* 1987; *accepted* 2 *May* 1988)

It has been reported that atrial extracts and synthetic atrial peptides have marked natriuretic and diuretic effects [1, 2]. We recently showed that infusion of atrial natriuretic factor (ANF, 8–33) to rats fed a normal phosphate diet decreases reabsorption of $P_i$ and sodium coupled to $HCO_3$ in proximal tubules [3, 4]. We also demonstrated that *in vivo* infusion of ANF inhibits brush border membrane (BBM) $Na^+$-$P_i$ symport and $Na^+$-$H^+$ antiport in rats fed normal phosphate diet, [4]. ANF decreases the maximal rate of phosphate reabsorption when evaluated with phosphate infusions [3]. Phosphaturia was also observed in dogs in response to ANF infusion [2].

The renal adaptation to phosphate deprivation is characterized by enhanced tubular reabsorption of $P_i$ and a diminished phosphaturic effect of parathyroid hormone (PTH) and calcitonin [5, 6]. In spite of this apparent *in vivo* resistance to some phosphaturic agents during $P_i$ deprivation, PTH and calcitonin inhibit sodium-dependent $P_i$ uptake across the brush border membrane vesicles (BBMV) from these kidneys [5].

The mechanism through which ANF causes natriuresis is unclear. Efforts have been made to dissociate the natriuresis from the hemodynamic response and to attribute the increase in fractional excretion of sodium ($FE_{Na^+}$), at least in part, to tubule effects [7, 8]. To further clarify the tubular effects of ANF, we addressed the question of whether the phosphaturic effects can be dissociated from the natriuretic effects of ANF. The rate of $P_i$ transport across BBM, determined *in vitro*, correlates with and contributes to the differences in renal tubular handling of $P_i$ *in vivo* in various mammalian kidneys under normal and altered situations [4–6]. Therefore, in the present study, the combination of *in vivo* and *in vitro* experimental settings allows us to understand the effect of ANF on overall renal handling of $P_i$. The results indicate that, like PTH and calcitonin, ANF also inhibited BBM proximal tubule phosphate transport in rats fed a low phosphate diet without increasing urinary fractional excretion of $P_i$ ($FE_{P_i}$), but the natriuretic effect of ANF was intact.

### *Methods*

The basic design of our study was similar to that described earlier [4]. Briefly, male Sprague–Dawley rats weighing 250–300 g were fed a low (0.07%) phosphate diet for 4 days prior to the experiment and had free access to water. Rats were anesthetized with Inactin (100 mg/kg) and prepared for clearance experiments. Acute thyroparathyroidectomy (TPTX) was performed by surgical removal of the glands and heat cautery. Control clearances were performed 2 hr after acute TPTX. Rats were then infused with ANF (ANF, 8–33, Peninsula Laboratories, Inc., Belmont, CA) at a dose of 4 μg/kg/hr or with vehicle. After 20 min of ANF infusion, experimental clearances were performed. After the clearances, the kidneys were removed from the animal and immediately chilled and kept in ice-cold saline. BBMV was prepared from homogenates of renal cortex by $Mg^{2+}$ precipitation and differential centrifugation procedures as described earlier [9].

The uptake of $^{32}P_i$ and L-[$^3$H]proline was measured by a rapid filtration technique using aliquots from the same BBM preparations [4, 9]. The final concentrations of radiolabeled solutes were 0.1 mM [$^{32}$P]phosphate and 0.025 mM L-[$^3$H]proline [4, 9].

All transport parameters were measured in triplicate or quadruplicate, and data were analyzed by Student's *t*-test for statistical significance. The protein content in BBMV fractions and the $P_i$ concentrations in plasma and urine were determined by the colorimetric methods of Lowry *et al.* and Chen *et al.*, respectively, as described in previous studies [4, 5].

### *Results and discussion*

In the present study we evaluated the natriuretic and phosphaturic effects of ANF and the rate of BBM $P_i$ transport in rats fed a low phosphate diet. Infusion of ANF in rats fed a low phosphate diet was associated, on the one hand, with significant increases in fractional excretion of sodium ($FE_{Na^+}$) and glomerular filtration rate (GFR) when compared with the control period (first period) in the same animal and also from the control rats studied simultaneously (Table 1). On the other hand, fractional excretion of phosphate ($FE_{P_i}$) and plasma phosphate ($P_{P_i}$) did not change when ANF was infused. In the control rats infused with vehicle only, there were no changes in GFR, $FE_{Na^+}$, $FE_{P_i}$ and $P_{P_i}$.

Table 2 summarizes the $Na^+$ gradient-dependent uptakes of $^{32}P_i$ and L-[$^3$H]proline by BBMVs from ANF-infused rats and vehicle-infused control rats respectively. In spite of no increase in $FE_{P_i}$% in rats fed a low phosphate diet, the infusion of ANF decreased the concentrative $Na^+$-dependent uptake of $^{32}P_i$ both at 15 and 30 sec (Table 2). In contrast, $^{32}P_i$ uptake at the equilibrium phase (120 min) was not different between control and ANF-infused rats (Table 2). In aliquots of the same BBMV preparations, the concentrative $Na^+$ gradient-dependent uptake of L-[$^3$H] proline was not significantly different in ANF-infused rats compared to control rats and also the uptake at 120 min was identical (Table 2).

As is evident from the results (Tables 1 and 2), infusion of ANF to the rats fed a low phosphate diet caused natriuresis and increased GFR similar to the effect observed in rats fed a normal phosphate diet. However, in phosphate-deprived rats, the phosphaturic effect of ANF was abolished. Moreover, the inhibitory effect of ANF on $Na^+$ gradient-dependent uptake of $P_i$ across the BBMV was intact (Table 2). A lack of any effect of the infusion of ANF on $Na^+$-cotransport of proline indicates that the inhibition elicited by ANF was relatively specific for the BBM transport system for $P_i$. Furthermore, the observation that BBMV uptake of L-[$^3$H]proline after complete equilibration (120 min) was not different after the infusion of ANF indicates that intravesicular BBMV volume was not altered by ANF (Table 2). Therefore, the observed decrease (Table 2) in $P_i$ uptake, without changing proline transport, provides a strong argument for the notion that $P_i$ uptake across BBM derived from proximal tubules was reduced in response to ANF. Results indicate that renal handling of $P_i$, in rats fed a low phosphate diet in response to ANF infusion was similar to those of PTH and calcitonin [5, 6]. Further, the lack of ANF effect on urinary $P_i$

Table 1. Effect of infusion of ANF in rats fed a low phosphate diet: Clearance data

| | GFR (ml/min) | $FE_{Na^+}$ (%) | $FE_{P_i}$ (%) | $P_{P_i}$ (mM) |
|---|---|---|---|---|
| Control | | | | |
| Vehicle | 2.89 ± 0.56 | 0.74 ± 0.55 | 0.12 ± 0.05 | 1.59 ± 0.21 |
| Vehicle | 3.07 ± 0.90 | 0.19 ± 0.04 | 0.04 ± 0.01 | 1.70 ± 0.15 |
| ANF | | | | |
| Vehicle | 2.55 ± 0.31 | 0.23 ± 0.15 | 0.33 ± 0.01 | 2.04 ± 0.31 |
| ANF | 3.26 ± 0.32* | 2.99 ± 0.87* | 0.07 ± 0.01 | 1.71 ± 0.20 |

Each value is the mean ± SE for eight rats. ANF dose: 4 μg/kg/hr. Abbreviations: GFR, glomerular filtration rate; $FE_{Na^+}$, fractional excretion of sodium; $FE_{P_i}$, fractional excretion of phosphate; and $P_{P_i}$, plasma phosphate.

Significantly different from control period ($P < 0.05$).

Table 2. Effect of ANF infusion of $Na^+$-dependent ($(Na^+_o > Na^+_i)$ phosphate and proline uptake in BBMV of rats fed a low phosphate diet

| | Uptake (pmol/mg protein) | | | |
|---|---|---|---|---|
| | [$^{32}$P]Phosphate | | L-[$^3$H]Proline | |
| Time | Control | ANF | Control | ANF |
| 15 sec | 2040 ± 135 | 1663 ± 36* | 250 ± 7 | 250 ± 8 |
| 30 sec | 2629 ± 122 | 2219 ± 33* | | |
| 120 min | 595 ± 18 | 637 ± 25 | 36 ± 1 | 31 ± 1 |

Each value is the mean ± SE of N = 4 (two rats per experiment) for both control and ANF groups. ANF dose 4 μg/kg/hr.

* Significantly different from corresponding control values ($P < 0.05$).

excretion ($FE_{P_i}$) was dissociated from the changes in GFR and sodium excretion ($FE_{Na^+}$) induced by ANF infusion. These results suggest that ANF is not regulating the sodium and phosphate reabsorption via a common mechanism or at the same tubular site. Hence, the natriuresis and phosphaturia caused by ANF may be two separate effects. The increased delivery of phosphate from the proximal is not regulating the sodium and phosphate reabsorption via a common mechanism or at the same tubular site. Hence, the natriuresis and phosphaturia caused by ANF may be two separate effects. The increased delivery of phosphate from the proximal convoluted tubule in response to ANF may be reabsorbed in the pars recta segment of the proximal tubule. Enhanced reabsorption of phosphate by the pars recta has been demonstrated in rats fed a low phosphate diet [10].

The mechanism of natriuretic action of ANF is not clear. It has been suggested that both hemodynamic and tubular transport effects may be responsible for ANF action [11]. The possible sites of this action of ANF along the nephron include the proximal tubule and the medullary collecting duct. Our previous findings indicating that ANF decreases reabsorption of $P_i$ and of sodium reabsorption coupled to $HCO_3$ [4] are compatible with the tubular effect in the proximal tubule, and the results of the present study are in agreement with these observations. The possibility of a direct effect of ANF on tubular membrane transport is also supported by its inhibitory effect of $Na^+$-$H^+$ antiport in LLC-$PK_1$ [12], and OK cells* in culture originated from renal tubules.

In summary, we conclude from our results that ANF inhibited phosphate reabsorption in proximal tubules without causing phosphaturia in phosphate-deprived rats. This inhibition of phosphate reabsorption by ANF seems to be unrelated to the changes in GFR and sodium excretion ($FE_{Na^+}$) induced by ANF infusion. Thus, the phosphaturic and natriuretic effects of ANF can be dissociated, demonstrating unique aspects of regulation.

*Acknowledgements*—This work was supported by NIH Grants DK19715, R37-DK30759, DK07013, and HL14133, and by the Mayo and Reimann Foundations.

*Nephrology Research Unit*
*Departments of Physiology and Biophysics and of Internal Medicine*
*Mayo Clinic and Foundation*
*Rochester, MN 55905, U.S.A.*

AHAD N. K. YUSUFI†
TIMOTHY G. HAMMOND‡
THOMAS P. DOUSA
FRANKLYN G. KNOX

REFERENCES

1. DeBold AJ, Borenstein HB, Veress AT and Sonnenberg H, A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci* **28**: 89–94, 1981.
2. Burnett JC Jr, Granger JP and Opgenorth TJ, Effects of synthetic atrial natriuretic factor on renal function and renin release. *Am J Physiol* **247** (Renal Fluid Electrolyte Physiol 16): F863–F866, 1984.
3. Hammond TG, Haramati A and Knox FG, Synthetic atrial natriuretic factor decreases renal tubular phosphate reabsorption in rats. *Am J Physiol* **249** (Renal Fluid Electrolyte Physiol 18): F315–F318, 1985.
4. Hammond TG, Yusufi ANK, Knox FG and Dousa TP, Administration of atrial natriuretic factor inhibits sodium-coupled transport in proximal tubules. *J Clin Invest* **75**: 1983–1989, 1985.

* Moran A, Montrose MH and Murer H, Regulation of $Na^+$-$H^+$ exchange by cyclic nucleotides in OK kidney cells, *Proceedings of the Tenth ISN Congress*, Satellite Symposium in Structure, Function and Regulation of Membrane Transport Proteins, Stanstadd, Switzerland, 1987, p. 82, Abstr. No. 46.

† Address reprint requests to: Ahad N. K. Yusufi, Ph.D., Department of Physiology and Biophysics, Mayo Clinic and Foundation, 200 First Street SW, Rochester, MN 55905.

‡ Current address: Mount Sinai Medical Center, University of Wisconsin Clinical Campus, 950 North 12th St., Milwaukee, WI 53201.

5. Yusufi ANK, Berndt TJ, Murayama N, Knox FG and Dousa TP, Calcitonin inhibits $Na^+$ gradient-dependent phosphate uptake across renal brush border membranes. *Am J Physiol* **252** (Renal Fluid Electrolyte Physiol 21): F598–F604, 1987.
6. Steele TH, Underwood JL, Stromberg BA and Larmore CA, Renal resistance to parathyroid hormone during phosphate deprivation. *J Clin Invest* **58**: 1461–1464, 1976.
7. Pollock DM and Arendshorst WJ, Effect of atrial natriuretic factor on renal hemodynamics in the rat. *Am J Physiol* **251** (Renal Fluid Electrolyte Physiol 20): F795–F801, 1986.
8. Salazar FJ, Fiksen-Olsen MJ, Opgenorth TJ, Granger JP, Burnett JC Jr and Romero JC, Renal effects of ANP without changes in glomerular filtration rate and blood pressure. *Am J Physiol* **251** (Renal Fluid Electrolyte Physiol 20): F532–F536, 1986.
9. Szczepanska-Konkel M, Yusufi ANK, VanScoy M, Webster SK and Dousa Tp, Phosphonocarboxylic acids as specific inhibitors of $Na^+$-dependent transport of phosphate across renal border membrane. *J Biol Chem* **261**: 6375–6383, 1986.
10. Knox FG, Berndt TJ and Haas JA, Resistance to the phosphaturic effect of parthyroid hormone during phosphate conservation is due to enhanced phosphate reabsorption by the pars recta. In: *Phosphate and Mineral Homeostatis* (Eds. Massry SG, Olmer M and Ritz E), pp. 101–108. Plenum Publishing, New York 1986.
11. Sosa RE, Volpe M, Marion DN, Atlas SA, Laragh JH, Vaughan ED and Maack T, Relationship between renal hemodynamic and natriuretic effects of atrial natriuretic factor. *Am J Physiol* **250** (Renal Fluid Electrolyte Physiol 19): F520–F524, 1986.
12. Mohrmann M, Cantiello HF and Ausiello DA, Inhibition of epithelial $Na^+$ transport by atriopeptin protein kinase C, and pertussis toxin. *Am J Physiol* **253** (Renal Fluid Electrolyte Physiol 22): F372–F376, 1987.

*Biochemical Pharmacology*, Vol. 37, No. 20, pp. 4029–4032, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00


# Effect of age on the uptake of propranolol by perfused rat lung

(*Received* 9 *July* 1987; *accepted* 9 *May* 1988)

Organs other than the liver possess some ability to eliminate and/or extract drugs or chemicals from the circulation [1]. Some compounds are metabolized in the lungs, whereas others are removed from the circulation and accumulated in the tissue [1]. Extensive first-pass pulmonary elimination (i.e. uptake with high capacity) of propranolol after intravenous administration of 1–10 mg/kg to rats has been reported recently [2]. Furthermore, the first-pass pulmonary clearance and the extraction ratio of the drug tend to increase with age between weeks 3 and 7 and to decrease thereafter [3]. This unique but distinct age-dependence of the first-pass pulmonary clearance of propranolol is not primarily related to the lung blood flow but may be accompanied by possible age-related differences (i.e. from immaturity to senescence) in the uptake capacity and/or affinity [2–4]. However, neither detailed mechanisms nor kinetics of the age-dependent pulmonary elimination (or extraction) of propranolol were investigated. Our previous report, in which the *in vitro* lung perfusion method was used to recirculate, at 8 ml/min, Krebs–Ringer bicarbonate buffer solution (pH 7.4), that contained propranolol and 3% bovine serum albumin (BSA), through the isolated lung of 7-week-old rats, suggested the approximate magnitude of the pulmonary uptake capacity of the drug [4]. This *in vitro* perfusion method also enabled us to examine the effect of age on the pulmonary elimination kinetics of propranolol.

The present work, therefore, was designed to examine the effect of age (both growth and senescence) on the pulmonary clearance of propranolol in 3- to 104-week-old male Wistar rats by analyzing the perfusate drug concentration–time curves after *in vitro* lung perfusion with the drug.

## *Materials and methods*

*Materials.* *dl*-Propranolol (PPL) hydrochloride was donated by I.C.I.-Pharma, Ltd. (Osaka, Japan). Bovine serum albumin (BSA, fraction V) was purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.) All other chemicals and reagents, including *n*-heptane and isoamyl alcohol (Wako Pure Chemicals Co., Nagoya, Japan) used for the extraction of unchanged PPL from the perfusate or perfused lung tissue, were of analytical grade.

*Animals.* Male Wistar rats, 3 (55–75 g), 5 (105–135 g), 7 (215–230 g), 11 (350–380 g), 15 (385–410 g), 24 (455–490 g), 52 (605–685 g) and 104 (790–845 g) weeks old, were used throughout the experiments. All rats, purchased from the Shizuoka Laboratory Animal Farm (Hamamatsu, Japan), were housed in a specific pathogen-free room where the relative humidity was kept between 50 and 60% at 22–24° with normal light–dark cycles. The rats were fasted overnight and anesthetized with urethane (800 mg/kg, i.p.) before use. In all the rats used, a macroscopic observation of the lung preparation did not reveal any development of pulmonary infection due to long-term housing. Plasma pH, which is known to be sensitive to alveolar hypoxia caused by most pulmonary infections [1], was also normal, ranging from 7.37 to 7.39.

*Perfusion of isolated rat lung.* Preparation and perfusion of isolated rat lung were carried out by slightly modifying the previous method [5]. The anesthetized rat was tracheotomized by catheterization and then given positive-pressure ventilation (about 6 to 9 cm and 1.5 to 2 cm $H_2O$ peak inspiratory and end expiratory pressure respectively) into the trachea with warmed (37°), humidifed room air at approximately 70 breaths/min using an animal respirator. Anticoagulation of the animals, exposure of the lungs, and cannulation of the pulmonary artery and vein were carried out as reported previously [4], except that slightly tapered PE-205 tubing was used for the 3-week-old rats. Single-pass perfusion of the lung was started immediately with the Krebs–Ringer buffer solution mentioned previously being oxygenated with 95% $O_2$–5% $CO_2$ and perfused at 8 ml/min [4, 5] by a peristaltic pump. During continuous perfusion, the heart and lungs were removed *en bloc*. After ligation to restrict flow through the lungs from the artery to the vein, the buffer solution was replaced by fresh drug solution prepared at 2.5 μg/ml in the same buffer solution, and the heart lung preparation was then mounted in the warmed, humidified chamber of the perfusion apparatus at time zero, with the perfusate recirculating at the same rate as above [4, 5]. An aliquot (0.1 ml) of the perfusate was withdrawn periodically over 60 min from the upper reservoir chamber of the apparatus and used for analysis. Perfusate pH was found to be 7.38 to 7.40 immediately