JCI The Journal of Clinical Investigation

Atriopeptin III. A potent natriuretic, diuretic, and hypotensive agent in rats with chronic renal failure.

B R Cole, … , M A Kuhnline, P Needleman

J Clin Invest. 1985;76(6):2413-2415. https://doi.org/10.1172/JCI112254.

Research Article

Chronic renal failure is frequently associated with volume overload, resulting in hypertension and, in some cases, congestive heart failure. Atriopeptin III (AP III), a 24-amino acid atrial peptide, is a potent vasodilator and natriuretic/diuretic agent in normal rats. An infusion of AP III at 0.2 microgram/kg per min for 60 min produced dramatic responses in animals with chronic renal failure (5/6 nephrectomy 4 wk before study). Systemic blood pressure fell 20% by the end of infusion. A pronounced rise in glomerular filtration rate (24%) was maintained during the infusion period when urine flow rate was stable (35-60 min), even though renal blood flow was unchanged from base line. Urinary volume increased 4.4-fold and sodium excretion increased 9 to 12-fold during the infusion. Fractional excretion of sodium ranged between 9 and 15% in those animals whose initial GFR values were lower than 0.5 ml/min. We conclude that AP III is a potent natriuretic/diuretic agent in rats with reduced renal mass, presumably exerting that effect predominantly through increases in GFR. This agent may well be useful in the treatment of volume overload in patients with chronic renal failure.

Atriopeptin III

A Potent Natriuretic, Diuretic, and Hypotensive Agent in Rats with Chronic Renal Failure

Barbara R. Cole, Mary A. Kuhnline, and Philip Needleman
Departments of Pediatrics and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110

Abstract

Chronic renal failure is frequently associated with volume overload, resulting in hypertension and, in some cases, congestive heart failure. Atriopeptin III (AP III), a 24-amino acid atrial peptide, is a potent vasodilator and natriuretic/diuretic agent in normal rats. An infusion of AP III at 0.2 μg/kg per min for 60 min produced dramatic responses in animals with chronic renal failure (5/6 nephrectomy 4 wk before study). Systemic blood pressure fell 20% by the end of infusion. A pronounced rise in glomerular filtration rate (24%) was maintained during the infusion period when urine flow rate was stable (35–60 min), even though renal blood flow was unchanged from base line. Urinary volume increased 4.4-fold and sodium excretion increased 9 to 12-fold during the infusion. Fractional excretion of sodium ranged between 9 and 15% in those animals whose initial GFR values were lower than 0.5 ml/min. We conclude that AP III is a potent natriuretic/diuretic agent in rats with reduced renal mass, presumably exerting that effect predominantly through increases in GFR. This agent may well be useful in the treatment of volume overload in patients with chronic renal failure.

Introduction

Atrial peptides have been shown to have vasodilatory and natriuretic-diuretic effects when injected into test animals (1–3). Atriopeptin III (AP III)[1] is a 24-amino acid peptide having potent vasodepressor (4) and natriuretic actions (5) in the rat. Concomitant with its natriuretic effect, AP III produces an increase in glomerular filtration rate (6), which is likely to be a significant factor in the production of the natriuresis (7).

Rats with reduced renal mass demonstrate compensatory changes, one of which is increased glomerular filtration rate per residual nephron (8). The purpose of the present study was to determine whether AP III could produce an elevation in total glomerular filtration rate in rats with reduced renal mass and thus an already increased filtration rate per glomerulus, and to ascertain the sensitivity of these animals to the natriuretic and diuretic effect of AP III.

Methods

Male Sprague-Dawley rats weighing 250–300 g were anesthetized with ether, and, through a midline abdominal incision, two of three left renal artery branches were ligated as previously described (9). 4 d later, the contralateral kidney was removed through a flank incision, again under ether anesthesia. The animals were then provided 0.45% NaCl as their drinking solution and were allowed to stabilize for 4 wk.

At the time of study, the rats were anesthetized intraperitoneally with Dial-urethane, 0.1 ml/100 g rat, which maintained stable anesthesia throughout the clearance study. The composition of Dial-urethane was 100 g diallylbarbituric acid, 400 g urethane, 500 mg ethylenediamine tetracetic acid, and 400 g monoethyl urea made up to a volume of 1 liter with distilled water. Polyethylene (PE 10 and PE 50; Clay Adams Div., Becton-Dickinson & Co., Parsippany, NJ) catheters were placed in both femoral arteries for infusion of AP III and for blood collection, as well as in a carotid artery for continuous blood pressure determination (P23ID; Gould/Statham pressure transducer, Gilson Duograph Model ICT-2H). A tail vein was catheterized via a 25-gauge butterfly needle for constant infusion of a maintenance solution of 0.45% NaCl containing 0.7% inulin (Taylor Chemicals, Inc., Sparks, MD) and trace [^{3}H]*p*-aminohippurate (New England Nuclear, Boston, MA; specific activity, 368 mCi/g), at a rate of 0.072 ml/min. The urinary bladder was catheterized through a suprapubic incision of a silastic catheter (C. W. Alban and Co., St. Louis, MO) for collection of urine into tared tubes.

After equilibration with the infusion solution for 1 h, one 20-min control urine collection was made, with blood drawn midway. Upon completion of the base-line clearance period, AP III was infused continuously at a rate of 0.06 or 0.2 μg/kg per min for 60 min. 5-min urine collections were made throughout, with blood drawn midway during each of the first four urine collections and then midway in subsequent alternate collections. After discontinuation of the AP III infusion, three 10-min recovery urine samples and midperiod blood samples were obtained. During periods when urine volume exceeded that of the combined infusions, the difference in volume was administered intravenously as a bolus at the completion of the period.

Urine volume was quantitated gravimetrically. Urine and plasma were analyzed for sodium and potassium by flame photometry (IL Model 43), for inulin by the anthrone method (10), and for [^{3}H]*p*-aminohippurate by liquid scintillation counting (Aquasol-2, New England Nuclear; Packard Tri-Carb Liquid Scintillation Spectrometer Model 3375). Calculations for glomerular filtration rate (GFR), total renal plasma flow, and absolute and fractional urinary excretions of sodium and potassium were by standard methods.

The AP III utilized was the synthetic 24-amino acid peptide found to have identical potency to the naturally occurring peptide. The AP III was prepared by solid-phase peptide synthesis by the Monsanto Molecular Biology Laboratories, St. Louis, MO.

As has been previously demonstrated (11), the extent to which total GFR was reduced after 5/6 nephrectomy varied among the animals,

Address reprint requests to Dr. Cole, Department of Pediatrics, Washington University School of Medicine, P.O. Box 14871, St. Louis, MO 63178.

Received for publication 3 September 1985.

1. *Abbreviations used in this paper:* AP III, atriopeptin III; FE_K, fractional excretion of potassium; FE_{Na}, fractional excretion of sodium; GFR, glomerular filtration rate; SNGFR, single nephron glomerular filtration rate.

J. Clin. Invest.

0021-9738/85/12/2413/03 $1.00
Volume 76, December 1985, 2413–2415

presumably because of variation in distribution of the ligated artery branches. Therefore, the data from the animals were divided into two groups, that from animals with base-line GFR values less than 0.5 ml/min (mean, 0.27±0.03 SEM; $n = 6$), termed "severe," and from animals whose base-line GFRs ranged from 0.5 to 1.0 ml/min (mean, 0.74±0.04 SEM; $n = 8$), termed "moderate." In those normal rats drinking 0.45% NaCl, base-line GFR values averaged 2.00±0.34 SEM ($n = 5$). Data between groups were compared by the unpaired t test; when time periods were compared within groups, the paired t test was used (12). $P < 0.05$ was considered significant.

Results

AP III, even at very low doses, proved to be a potent diuretic and natriuretic agent in these animals with reduced renal mass. Fig. 1 shows the responses to two doses of AP III in animals with "moderately" reduced GFR, showing the time interval necessary for maximum diuresis and natriuresis to occur. Urinary flow rate and sodium excretion were maximal and sustained by 25 min with the 0.2 μg/kg per min dose, falling when the AP III infusion was stopped. AP III infused at 0.06 μg/kg per min elicited a maximal diuresis that was about half that prompted by the higher dose and occurred after 40 min of infusion.

Fig. 2 compares the renal function and blood pressure responses to the 0.2 μg/kg per min AP III infusion in the groups with "moderately" and "severely" reduced GFR. In both groups of animals, GFR elevation was maximal during the period 15–30 min of infusion, although the extent of the elevation may be overestimated by the increasing urine flow rate during this period (13). From 35–60 min, when urine flow was constant, GFR in both groups remained significantly greater than base-line ($P < 0.01$).

Renal plasma flow also rose during the period of increasing flow rate (15–30 min) but fell to base-line levels during the period 35–60 min at a time of diminishing systemic blood pressure (Fig. 2). Thus, elevated GFRs were maintained during this latter period in the absence of elevated renal plasma flow, as depicted by the gradual increases in filtration fraction throughout AP III infusion.

Preinfusion blood pressure measurements ranged 80–130 mmHg in the anesthetized animals with "moderately" decreased GFR, and 110–170 in those with "severe" reductions. Blood pressure values fell an average of 13% by 30 min of infusion and 21% by 60 min, with no significant difference between groups (Fig. 2). Upon cessation of AP III infusion, the blood pressure began to climb toward base-line values.

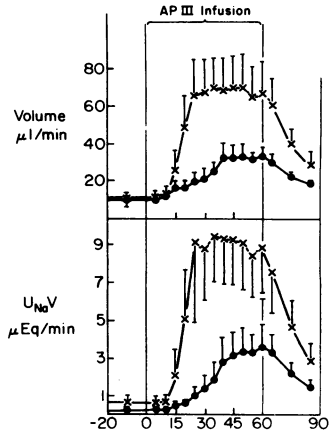

Figure 1. The effects of two doses of AP III in rats with initial GFR values between 0.5 and 1.0 ml/min. After a control period, AP III was infused at 0.06 μg/kg per min (•) ($n = 5$) or 0.2 μg/kg per min (×) ($n = 8$). A 30-min recovery period completed the study.

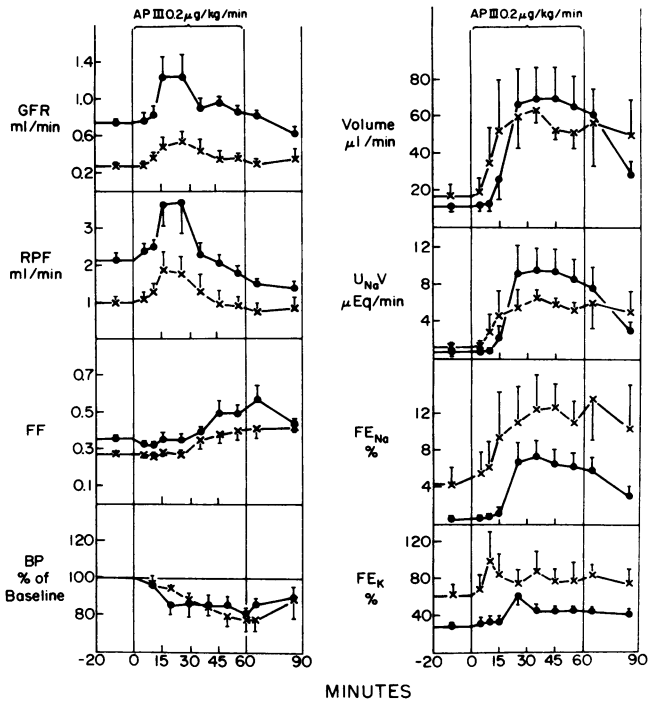

Figure 2. The effects of AP III, 0.2 μg/kg per min, in two groups of rats with reduced renal mass. One group was identified as having control GFR values between 0.5 and 1.0 ml/min ("moderate," $n = 8$), and the other had GFR values less than 0.5 ml/min ("severe," $n = 6$). After a 20-min control period, AP III was infused for 60 min, followed by a 30-min recovery period. BP, blood pressure (systemic); FF, filtration fraction; RPF, renal plasma flow; $U_{Na}V$, urinary sodium excretion.

Initial urine flow rates were similar in the two groups of animals and rose 3.5-fold to 6-fold by 25 min of infusion. Urinary sodium excretion rose 5-fold in the "severe" rats but 15-fold in the "moderate" ones. Fractional excretion of sodium (FE_{Na}) was greater during the base-line period in those animals with lower GFR, rising to a mean of 12±0.8% (SEM) in the "severe" animals compared with 6.6±0.7% (SEM) in the "moderate" animals (Fig. 2).

Fractional excretion of potassium was also higher in the "severe" than the "moderate" animals during control and infusion periods. Fractional excretion of potassium (FE_K) values remained elevated through the period of stable natriuresis and diuresis.

Discussion

Two striking observations arise from this study. First, even though animals with reduced renal mass have elevated single nephron glomerular filtration rates (SNGFR) (8), AP III produces an increase in total GFR that is sustained throughout administration of the peptide. Either the compensatory increase in SNGFR is not maximal in these azotemic animals and can thus rise during AP III infusion, or there is recruitment of perfused but nonfiltering glomeruli as found in previous studies of animals with reduced renal mass (14). This elevation was demonstrated during the period in which urine flow rates were stable, and at a time when renal plasma flow was not elevated and systemic blood pressure had fallen. In rats with normal renal function, Huang et al. (7) have shown that atrial peptides elevate SNGFR significantly and in concert with rises in total GFR. These authors

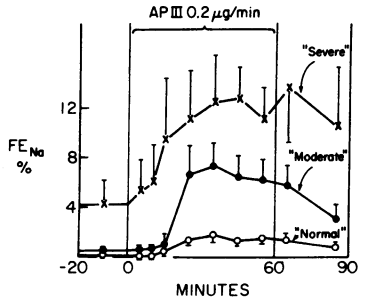

Figure 3. The effects of AP III, 0.2 μg/kg per min, on fractional excretion of sodium. See Fig. 2 legend for definition of groups with reduced renal mass. "Normal" animals drank 0.45% NaCl for 5 d before study.

calculated that the stimulated excretion of sodium was appropriate for the observed increments in GFR. Note that our experimental animals with reduced renal mass, presumably already having increased SNGFR, still demonstrated an increased total GFR.

Second, the natriuresis and diuresis stimulated by the AP III infusion at 0.2 μg/kg per min were striking, with FE_{Na} values increased 12-fold in the animals with moderately reduced GFR and tripled (but with absolute FE_{Na} values ranging from 9 to 15%) in the animals with greater reduction in GFR. The comparison of FE_{Na} values in the animals with reduced mass and the normal but salt-loaded rats is seen in Fig. 3. Assuming that the reduction in total GFR accurately reflects the lesser number of functioning nephrons in the chronic renal failure animals, the maximal amount of sodium excreted per nephron in the animals with severe failure during AP III infusion exceeds that of the normal animals by 10–15 times. It seems likely that the elevated GFR has a major role in this natriuretic and diuretic response. Since the animals with lower GFR values had initial FE_{Na} values averaging 4%, it would be anticipated that influences for tubular sodium reabsorption, such as aldosterone, would have been minimal. The data do not, however, exclude an additional tubular effect of atriopeptin that may be magnified in the animals with reduced renal mass. In support of this theory is the observation that those animals infused with 0.06 μg/kg per min AP III exhibited natriuresis and diuresis during the period from 45 to 60 min, a time when urine flow was stable and total GFR was not statistically different from base-line levels (0.81±0.12 SEM, compared with base line of 0.71±0.09 SEM, $n = 5$).

These data suggest that AP III may be useful in the treatment of volume overload in patients with chronic renal failure. Both the hypotensive and the natriuretic actions of the peptides would be helpful in treating these patients during sodium-retaining periods.

References

1. Currie, M. G., D. M. Geller, B. R. Cole, J. G. Boylan, W. YuSheng, S. W. Holmberg, and P. Needleman. 1983. Bioactive cardiac substances: potent vasorelaxant activity in mammalian atria. *Science (Wash. DC).* 221:71–73.

2. Currie, M. G., D. M. Geller, B. R. Cole, N. R. Siegel, K. F. Fok, S. P. Adams, S. R. Eubanks, G. R. Galluppi, and P. Needleman. 1984. Purification and sequence analysis of bioactive atrial peptides (atriopeptins). *Science (Wash. DC).* 223:67–69.

3. Geller, D. M., M. G. Currie, K. Wakitani, B. R. Cole, S. P. Adams, K. F. Fok, N. R. Siegel, S. R. Eubanks, G. R. Galluppi, and P. Needleman. 1984. Atriopeptins: a family of potent biologically active peptides derived from mammalian atria. *Biochem. Biophys. Res. Commun.* 120:333–338.

4. Wakitani, K., T. Oshima, A. D. Loewy, S. W. Holmberg, B. R. Cole, S. P. Adams, K. F. Fok, M. G. Currie, and P. Needleman. 1985. Comparative vascular pharmacology of the atriopeptins. *Circ. Res.* 56: 621–627.

5. Thibault, G., R. Garcia, F. Carrier, N. G. Seidah, C. Lazure, M. Chrétien, M. Cantin, and J. Genest. 1984. Structure-activity relationships of atrial natriuretic factor (ANF). I. Natriuretic activity and relaxation of intestinal smooth muscle. *Biochem. Biophys. Res. Commun.* 125: 938–946.

6. Wakitani, K., B. R. Cole, D. M. Geller, M. G. Currie, K. F. Fok, and P. Needleman. 1985. Atriopeptins: correlation between renal vasodilation and natriuresis. *Am. J. Physiol.* 18:F49–F53.

7. Huang, C.-L., J. Lewicki, L. K. Johnson, and M. G. Cogan. 1985. Renal mechanism of action in rat atrial natriuretic factor. *J. Clin. Invest.* 75:769–773.

8. Kaufman, J. M., H. J. DiMeola, N. J. Siegel, B. Lytton, M. Kashgarian, and J. P. Hayslett. 1974. Compensatory adaptation of structure and function following progressive renal ablation. *Kidney Int.* 6:10–17.

9. Shankel, S. W., A. M. Robson, and N. S. Bricker. 1967. On the mechanism of the splay in the glucose titration curve in advanced experimental renal disease in the rat. *J. Clin. Invest.* 46:164–172.

10. White, R. P., and F. E. Samson. 1954. Determination of inulin in plasma and urine by use of the anthrone method. *J. Lab. Clin. Med.* 43:475–478.

11. Robson, A. M., J. Mor, E. R. Root, B. V. Jager, S. W. Shankel, J. R. Ingelfinger, R. A. Kienstra, and N. S. Bricker. 1979. Mechanism of proteinuria in nonglomerular renal disease. *Kidney Int.* 16:416–429.

12. Alder, H. L., and E. B. Roessler. 1964. Introduction to Probability and Statistics. Chapter 10. W. H. Freeman and Company, San Francisco. 123–140.

13. Keeler, R. 1983. The effects of dead-space and urine flow changes on measurements of glomerular filtration rate by clearance methods. *Can. J. Physiol. Pharmacol.* 61:435–438.

14. Damadian, R. V., E. Shwayri, and N. S. Bricker. 1965. On the existence of nonurine forming nephrons in the diseased kidney of the dog. *J. Lab. Clin. Med.* 65:26–39.