Long-Term Hypotensive and Renal Effects of Atrial Natriuretic Peptide

JOEY P. GRANGER, TERRY J. OPGENORTH, JAVIER SALAZAR, J. CARLOS ROMERO, AND JOHN C. BURNETT, JR.

SUMMARY The present study was designed to examine the short-term and long-term effects of increased plasma levels of atrial natriuretic peptide on the glomerular filtration rate, sodium excretion, and arterial pressure. Intravenous infusion of synthetic atrial natriuretic peptide (2 μg/kg bolus, 50 ng/kg/min continuous infusion) for 45 minutes in six conscious dogs increased plasma levels of immunoreactive atrial natriuretic peptide from 69 ± 10 to 233 ± 14 pg/ml. Short-term increases in plasma levels of atrial natriuretic peptide increased the glomerular filtration rate from 53 ± 15 to 82 ± 16 ml/min and increased sodium excretion from 74.4 ± 32.6 to 146.9 ± 38.1 μEq/min. Mean arterial pressure decreased slightly, from 88 ± 3 to 83 ± 3 mm Hg, whereas no changes occurred in plasma renin activity (2.0 ± 0.6 to 1.6 ± 0.8 ng of angiotensin I per milliliter per hour) or plasma aldosterone concentration (6.9 ± 2.3 to 8.1 ± 3.9 ng/dl). To determine whether the short-term effects of atrial natriuretic peptide on the glomerular filtration rate and sodium excretion lead to a sustained reduction in mean arterial pressure, atrial natriuretic peptide (50 ng/kg/min) was infused intravenously for 5 days in six conscious dogs. Long-term infusion increased plasma levels of immunoreactive atrial natriuretic peptide from 27 ± 5 to 292 ± 31 pg/ml. The infusion caused only a transient increase in sodium excretion and had no significant long-term effect on the glomerular filtration rate. Mean arterial pressure decreased from 90 ± 3 to 74 ± 3 and 75 ± 4 mm Hg by Days 4 and 5 of the infusion. Plasma renin activity and aldosterone were unchanged during the infusion, despite the large decrease in arterial pressure. These results demonstrate that short-term increases in plasma levels of atrial natriuretic peptide result in marked increases in the glomerular filtration rate and sodium excretion but only a slight decrease in mean arterial pressure. Long-term elevation of plasma atrial natriuretic peptide, however, results in a greater reduction in arterial pressure without having significant effects on the glomerular filtration rate or sodium excretion. (Hypertension 8 [Suppl II]: II-112-II-116, 1986)

KEY WORDS • arterial pressure • glomerular filtration rate • sodium excretion • renin-angiotensin-aldosterone system

Atrial natriuretic peptide (ANP) has been thought to play an important part in the regulation of extracellular fluid volume and arterial pressure. In support of this concept, numerous studies have demonstrated that short-term administration of ANP in dogs has marked effects on renal hemodynamics, sodium excretion, and arterial pressure. In addition, ANP has been shown to suppress important blood pressure regulatory systems, such as the renin-angiotensin system and aldosterone.

Although these potent short-term effects have been demonstrated in several studies, the long-term effects of ANP on the glomerular filtration rate, sodium excretion, and arterial pressure in dogs have not been documented. Therefore, the objective of our study was to determine the long-term effects of ANP on renal function and arterial pressure regulation in conscious dogs. In addition, we examined the effects of long-term intravenous infusion of ANP on the renin-angiotensin system and aldosterone. The short-term effects of ANP infusion in conscious dogs were examined at comparable rates of intravenous administration.

From the Department of Physiology and Biophysics, Mayo Medical School, Rochester, Minnesota.
Supported by National Institutes of Health Grant HL16496, American Heart Association Grant 83-964, Minnesota Heart Association Grant MHA 61, and the Hearst and Mayo Foundations. Dr. Granger is the recipient of New Investigator Award HL33947 from the National Heart, Lung, and Blood Institute.
Address for reprints: Joey P. Granger, Ph.D., Department of Physiology and Biophysics, Mayo Medical School, Rochester, MN 55905.
LONG-TERM EFFECTS OF ATRIAL NATRIURETIC FACTOR/Granger et al.

Methods

Animal Preparation

The short-term and long-term effects of ANP infusion were examined in conscious, chronically instrumented female dogs (average weight, 16.5 ± 0.5 kg). Tygon catheters were implanted in the femoral artery and vein under aseptic conditions with pentobarbital anesthesia (30 mg/kg i.v.). The tips of the femoral artery and vein catheters were placed in the aorta, below both kidneys, and in the vena cava, respectively. The catheters were tunneled subcutaneously and exited near the neck of each animal. The dogs were allowed to recover from surgery for at least 2 weeks before any experiments were performed.

Short-Term Infusion of Atrial Natriuretic Peptide

The short-term effects of ANP were examined in six conscious dogs. The dogs were maintained on a normal dog chow diet (Nutrina Dog Food, 3.9 mg sodium and 5.24 mg potassium per gram; Cargill, St. Paul, MN, USA) and allowed free access to tap water. Eighteen hours before the experiment, food was withdrawn. The dogs were suspended in a sling for all experimental procedures.

Once the dogs had been placed in the sling, the venous catheter was connected to a syringe infusion pump for delivery of saline and 5% inulin at a rate of 1.0 ml/min. The saline-inulin solution also served as a vehicle for ANP. The arterial catheter was connected to a pressure transducer for continuous monitoring of arterial pressure.

Urinary samples were collected through a self-retaining bladder catheter (Foley 16; American Latex, Sullivan, IN, USA). Blood samples for measurement of plasma inulin, sodium, potassium, ANP, and aldosterone concentrations and plasma renin activity were obtained at the midpoint of each clearance period.

After two 15- to 20-minute control clearances, a bolus injection of synthetic rat ANP (Arg Arg-Atriopetin III, Peninsula Laboratories, Belmont, CA, USA), 2 μg/kg, was administered, followed by a continuous infusion at a rate of 50 ng/kg/min for a period of 45 minutes. After a 15-minute equilibration period, two 15-minute clearances were obtained. Two 15-minute recovery clearances were obtained approximately 30 minutes after the ANP infusion.

Long-Term Effects of Atrial Natriuretic Peptide

The long-term effects of intravenous infusion of ANP were examined in six conscious dogs. The dogs were housed in individual metabolic cages and fitted with harnesses that contained blood pressure transducers mounted at heart level and connected to a graph recorder. Twenty-four-hour urine samples, infusion volume, and water intake were measured between 0730 and 0900 each day. These measurements were used to assess daily electrolyte and water balances. Arterial pressures were monitored continuously, 24 hours a day, on a graph recorder. Mean arterial pressure signals from the recorder were delivered to an analog-digital converter and analyzed with an IBM personal computer. The analog signal from the graph recorder was sampled each minute and digitized to provide 60 samples per hour. The pressure data for each dog were subsequently averaged over the 24-hour period.

The femoral vein catheter was connected to a roller pump (Sage Instruments, Model 375A) that was used to infuse various solutions throughout the study. All solutions were pumped through a disposable filter (Cathivex; Millipore, Bedford, MA, USA) to prevent minute air bubbles and possible contaminants from entering the venous system. The filters were changed frequently throughout the study. The infusion tubing and transducers were protected by a flexible vacuum hose that was attached to the harness. The dogs were allowed to move freely in the cage but were unable to turn completely around.

During the control and experimental periods, all dogs were fed a sodium-deficient diet (H/D; Hill Pet Products, St. Paul, MN, USA) that provided approximately 5 to 7 mEq of sodium and 65 mEq of potassium per day. The dogs were allowed free access to tap water throughout the experiment. Isotonic saline was continuously infused intravenously at a rate of 260 ml/day to maintain the total sodium intake at approximately 45 mEq/day, including the sodium provided in the food.

After a 4-day control period, ANP was infused intravenously for 5 days at a rate of 50 ng/kg/min, 24 hours a day, in the isotonic saline vehicle solution. The ANP solutions were made fresh daily and changed between 0800 and 0900. The activity of ANP in the infusion bottles was tested in several bioassay rats (arterial pressure response) after being maintained at room temperature for more than 24 hours and was found to be the same as the activity of fresh ANP.

Daily measurements included arterial pressure; 24-hour urinary excretion of sodium, potassium, and water; urine osmolality; 24-hour water intake; glomerular filtration rate; plasma concentrations of ANP and aldosterone; plasma renin activity; and plasma sodium, potassium, and osmolality. The glomerular filtration rate was estimated on the basis of the 24-hour clearance of endogenous creatinine.

Analytical Procedures

Plasma and urinary sodium and potassium concentrations were measured with a Beckman E2A Electrolyte Analyzer (Arlington Heights, IL, USA). Creatinine was measured with a Beckman Creatinine Analyzer. Inulin was measured by the anthrone method. Plasma renin activity and plasma aldosterone concentrations were assayed by radioimmunoassay, according to methods published elsewhere. The rabbit antiserum to human α-ANP (RAS-8798) and the synthetic peptide of human α-ANP for standard and tracer were purchased from Peninsula Laboratories. Human α-ANP was labeled with sodium [125]Iodide by
the Iodogen procedure. The crude labeled peptide was purified first on a BioRad P-2 column and then by high-performance liquid chromatography on a C-18 column. The antiserum was first incubated in the presence of standard or sample for 24 hours. Then the labeled ANP was added for an additional 16 hours of incubation at 4°C. Free and bound fractions of human α-ANP were separated by precipitation with goat antirabbit second antibody (produced on our farm). The precipitation incubation period with the second antibody was 30 minutes. The mixture was centrifuged in the presence of 2% polyethylene glycol 8000. Total binding was 35%. The 50% displacement point of a standard curve was 28 pg. The sensitivity of the assay is 3 pg/ml.

Blood withdrawn from the dogs was placed in prechilled potassium ethylenediaminetetraacetate tubes and then immediately placed on ice to await centrifugation at 4°C. Plasma was separated and frozen at −20°C until assay. Before radioimmunoassay, C-18 cartridges were used to extract ANP from plasma and to remove interferences. The ANP eluted from the C-18 cartridges was dried and reconstituted for radioimmunoassay. The extraction procedure resulted in a recovery of 81 ± 2.0% (mean ± SE), as determined by the addition of synthetic ANP to plasma. Interassay and intraassay variations were 9 and 6%, respectively.

Control data were compared with experimental data in each group with Dunnett’s t test for multiple comparisons. The level of statistical significance was considered to be p<0.05. All data are expressed as means ± SE.

Results

Effects of Short-Term Infusion of Atrial Natriuretic Peptide

The effects of an intravenous bolus injection of ANP (2 μg/kg) followed by continuous infusion at a rate of 50 ng/kg/min in conscious dogs are shown in Figures 1 and 2. Plasma levels of ANP increased from 69 ± 10 to 233 ± 14 pg/ml (p<0.05). The increase in plasma levels of ANP was associated with a slight but significant decrease in mean arterial pressure (88 ± 3 to 83 ± 3 mm Hg, p<0.05). ANP markedly increased the glomerular filtration rate, from 53 ± 15 to 82 ± 16 ml/min (p<0.05). After the ANP infusion had been stopped, the glomerular filtration rate returned to a level (61 ± 15 ml/min) that was not significantly different from the control value. Urinary sodium excretion increased from 74.4 ± 32.6 to 146.9 ± 38.1 μEq/min (p<0.05) during ANP infusion and decreased to 41.3 ± 15.6 μEq/min after the infusion was stopped.

Although infusion of ANP elevated plasma levels of ANP fourfold, there were no significant changes in plasma renin activity (2.0 ± 0.6 to 1.6 ± 0.8 ng of angiotensin I (ANG I) per milliliter per hour) or plasma aldosterone concentrations (6.9 ± 2.3 to 8.1 ± 3.9 ng/dl).

![Figure 1. Effects of a 45-minute intravenous infusion of atrial natriuretic peptide (ANP, 50 ng/kg/min) on mean arterial pressure (MAP), glomerular filtration rate (GFR), and urinary sodium excretion (U钠V) in six conscious dogs. Values are means ± SE.](image)

![Figure 2. Effects of a 45-minute intravenous infusion of atrial natriuretic peptide (ANP, 50 ng/kg/min) on plasma concentration of immunoreactive ANP (P_{ANP}), plasma renin activity (PRA), and plasma aldosterone concentration (PAC) in six conscious dogs. Values are means ± SE. AI = angiotensin I.](image)
Long-Term Effects of Infusion of Atrial Natriuretic Peptide

The effects of intravenous infusion of ANP for 5 days at a rate of 50 ng/kg/min in conscious dogs are shown in Figures 3 and 4. Long-term infusion increased plasma levels of ANP from 27 ± 5 to 292 ± 31 pg/ml ($p<0.05$). Mean arterial pressure decreased from a control level of 90 ± 3 to 74 ± 3 and 75 ± 4 mm Hg ($p<0.05$) by Days 4 and 5 of the ANP infusion. The glomerular filtration rate was not significantly altered during the infusion (58 ± 10 ml/min during the control period, 45 ± 8 ml/min during the ANP infusion, and 48 ± 9 ml/min after the infusion). There was also no significant long-term effect on urinary sodium excretion, although it did tend to increase on the first day of the ANP infusion (45 ± 5 to 63 ± 10 mEq/day).

Plasma renin activity (1.0 ± 0.2 to 1.3 ± 0.3 ng ANG I/ml/hr) and plasma aldosterone concentrations (4.3 ± 1.0 to 3.7 ± 0.8 ng/dl) were also not significantly altered during long-term infusion of ANP.

Discussion

The present study demonstrates that short-term increases in the plasma level of ANP during intravenous infusion of a synthetic ANP result in marked increases in the glomerular filtration rate and sodium excretion while causing only a slight reduction in mean arterial pressure. Long-term elevation of plasma ANP levels, however, results in a greater reduction in mean arterial pressure without having significant long-term effects on the glomerular filtration rate or sodium excretion.

The most profound short-term effect of ANP was its ability to increase the glomerular filtration rate. Similar findings have been reported with short-term infusion of ANP at higher infusion rates in conscious and anesthetized dogs. The mechanism responsible for the relatively large increase in the glomerular filtration rate remains to be defined; however, it may be a result of a balanced effect on segmental renal vascular resistance in which preglomerular resistance is decreased and postglomerular resistance is increased. Although short-term elevation of plasma ANP levels markedly increased the glomerular filtration rate, long-term infusion of ANP failed to have a sustained effect on glomerular filtration. The lack of a sustained effect may be related to the large reduction in mean arterial pressure during long-term infusion of ANP. Short-term reduction of renal perfusion pressure to a level similar to that in the present study has been shown to abolish the effect of ANP on the glomerular filtration rate. In addition, various intrinsic autoregulatory mechanisms, such as tubuloglomerular feedback, may offset the direct effects of ANP during long-term infusion.

Short-term infusion of ANP markedly increased sodium excretion. The exact mechanism of this increase...
remains controversial, with some investigators suggesting a hemodynamic action, and others a tubular effect.1-3 In our study long-term increases in plasma levels of ANP caused a transient increase in sodium excretion on the first day of infusion, with normal levels on subsequent days. The lack of a sustained effect on sodium excretion does not necessarily imply that ANP does not have a long-term effect on the ability of the kidney to excrete sodium and water. On the contrary, ANP appears to increase the kidney's ability to excrete sodium and water by allowing sodium and water balance to occur at a lower renal perfusion pressure. Consistent with this suggestion are the findings of Takezawa and colleagues,14 who demonstrated a downward shift in the pressure-natriuresis curve when ANP was infused intravenously.

The larger reduction in mean arterial pressure during long-term infusion of ANP suggests that the mechanisms for the short-term and long-term effect on arterial pressure may be different. Short-term infusion of ANP is associated with a reduction in cardiac output but has no effect on total peripheral resistance.15 Whether cardiac output or total peripheral resistance or both are decreased during long-term administration of ANP is unknown. Studies in hypertensive rat models have consistently found a larger reduction in arterial pressure during long-term infusion of ANP, as compared with short-term administration.16-18 Further studies are needed to clarify the hemodynamic mechanism of this response.

Short-term infusion of ANP had no significant effect on plasma renin activity or plasma aldosterone concentrations, despite a fourfold increase in plasma levels of ANP. Consistent with these findings are data from a study by Freeman et al.1 in which short-term intravenous infusion of ANP (Sar-Ile-Arg-Arg-Atriopeptin III) at a rate of 175 and 350 ng/kg/min had no effect on plasma renin activity or plasma aldosterone concentration in normal conscious dogs. The lack of an effect may be related to the low basal levels, since intravenous and intrarenal infusions of ANP consistently decrease plasma renin activity and plasma aldosterone concentration when the renin-angiotensin-aldosterone system is activated.2,3 Long-term infusion of ANP also did not alter plasma renin activity or plasma aldosterone concentration, despite the large decrease in mean arterial pressure. One would have predicted an elevation in plasma renin activity when the mean arterial pressure fell to approximately 74 mm Hg. Farhi et al.19 demonstrated that in conscious dogs a reduction of renal perfusion pressure to levels observed in the present study increased plasma renin activity twofold to threefold. Thus, the level of plasma renin activity during long-term infusion of ANP appears to be inappropriately low for the level of mean arterial pressure, suggesting a sustained inhibitory effect of ANP on renin release. In support of this suggestion, Garcia et al.17 demonstrated that 7 days of ANP infusion in rats with two-kidney, one clip Goldblatt hypertension decreased plasma renin activity to normal levels.

Acknowledgments

The authors thank Denise Heublein, Sharon Schryver, Marcy Omsgard, and Marcia McDougall for their technical assistance, and June Hanke for typing the manuscript.

References

5. Freeman RH, Davis JO, Vare RC. Renal response to atrial natriuretic factor in conscious dogs with caval constriction. Am J Physiol 1984;246:F495-R500
Long-term hypotensive and renal effects of atrial natriuretic peptide.
J P Granger, T J Opgenorth, J Salazar, J C Romero and J C Burnett, Jr

Hypertension. 1986;8:II112
doi: 10.1161/01.HYP.8.6_Pt_2.II112

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/8/6_Pt_2/II112

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/