Effects of Antiserum Against α-Rat Atrial Natriuretic Peptide in Anesthetized Rats

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SUMMARY Although synthetic atrial natriuretic peptide is known to increase urinary volume and sodium excretion and to reduce arterial blood pressure, the physiological role of endogenous atrial natriuretic peptide is still unclear. We investigated the effects of specific rabbit antiserum against α-rat atrial natriuretic peptide on hemodynamics, diuresis, and natriuresis in anesthetized rats. A significant rise in mean blood pressure lasted for about 60 minutes after intravenous administration of the antiserum, with the maximal increment being approximately 7%. Similarly, a significant increase in cardiac output was obtained 20 minutes after injection at an increment of approximately 11%. Heart rate, however, remained unchanged. On the other hand, significant reductions in urine output and urinary sodium and potassium excretion lasted for about 20 minutes after administration of the antiserum, with maximal decrements being 63%, 63%, and 60%, respectively. No significant effects on hemodynamics, diuresis, and natriuresis were observed following injection of normal rabbit serum. These results indicate that endogenous atrial natriuretic peptide has an important physiological role in the regulation of hemodynamics and water-electrolyte balance.

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KEY WORDS • atrial natriuretic peptide • antiserum • blood pressure • cardiac output • natriuresis • rats

Mammalian atria secrete a recently discovered hormone, atrial natriuretic polypeptide (ANP), that may participate in the control of blood pressure and electrolyte balance. Mammalian atrial myocytes contain numerous granules referred to as atrial-specific granules. They contain a polypeptide material that has potent natriuretic and hypotensive activities. The main form of ANP in rat atrial granules is a 126-amino acid peptide called γ-rat ANP (γ-rANP). The circulating form of ANP released from the heart appears to be a 28-amino acid peptide called α-rANP, which is identical to cardionatrin I and Ser-Leu-Arg-Arg-atriopeptin III. α-rANP differs from α-human ANP (α-hANP) only by substitution of isoleucine for methionine at Position 12. α-rANP produces a marked natriuresis in normal animals, similar to that of α-hANP. We have demonstrated recently that the exogenous α-type of ANP, which is the circulating form, caused hypotension associated with a decrease in cardiac output without any change in total peripheral resistance in normotensive rats as well as in spontaneously hypertensive rats. However, the physiological role of endogenous ANP is still unclear because few studies have used a specific antibody of ANP.

In the present study, the hemodynamic, diuretic, and natriuretic effects of antiserum against α-rANP were investigated in normotensive rats to clarify the physiological importance of endogenous α-rANP.
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Materials and Methods

Preparation of Antiserum Against α-Rat ANP

α-rANP was conjugated with bovine thyroglobulin (Sigma Chemical Corp., St. Louis, MO, USA) by the carbodiimide method. The resulting antigenic conjugate solution was emulsified with an equal volume of Freund’s complete adjuvant and used to immunize New Zealand white rabbits by subcutaneous injection at multiple sites in the interscapulovertebral region. The anti-α-rANP antiserum was characterized by radioimmunoassay. This antiserum proved to be usable at a final dilution of 1:250,000 for radioimmunoassay of α-rANP, and half-maximal inhibition by α-rANP was observed at 25 pg per tube and was detectable as low as 1 pg per tube, as described previously.

The specificity of the antiserum was evaluated from its cross-reactivity with several ANP-related peptides, indicating that the antiserum mainly directs to the ring structure flanked by two cysteine residues (Positions 7 and 23) in the α-rANP molecule. The antiserum cross-reacts completely with α-rANP and 25-25, 25-27, and 5-28 fragments of α-rANP, but only slightly with α-hANP since it has a single amino acid replacement at Position 12 of methionine for isoleucine. Thus, the antiserum equally recognizes all rANPs so far isolated.

Experiments

We used normotensive Wistar-Kyoto rats, which were kindly provided by Prof. Kozo Okamoto (Kinki University School of Medicine, Osaka, Japan). The rats had been maintained on standard rat chow (CLEA CE-2; Nihon, Tokyo, Japan) containing NaCl, 0.85 g per 100 g of chow, and tap water ad libitum in our laboratory. Twenty-week-old rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). Mean blood pressure (MBP) and heart rate (HR) were monitored with a femoral artery catheter (PE-50) connected to the Statham pressure transducer (Gould, Saddle Brook, NJ, USA) and a polygraph (Model 141-6; San-El, Tokyo, Japan). A PE-10 catheter was inserted into the right atrium through the right jugular vein, and a thermosensor was inserted into the right atrium to maintain arterial temperature at 36 to 37°C.

Isotonic saline (20 μl/min) was infused intravenously for more than 60 minutes for equilibration until urine flow rate was stabilized. Urine was collected for two consecutive 20-minute control periods by means of a tube inserted in the bladder. Then, 25 μl of rabbit anti-α-rANP antiserum (n = 8) or 25 μl of normal rabbit serum (n = 7) was administered intravenously.

Urine samples were collected at 20-minute intervals for an additional 120 minutes. Cardiac output was measured before and 20, 60, and 120 minutes after administration.

Cardiac index (CI), stroke volume index (SVI), and total peripheral resistance index (TPRI) were calculated from the following formulas: CI (ml/min/100 g body weight) = cardiac output/100 g body weight; SVI (μl/min/100 g body weight) = CI/HR; TPRI (units × 100 g body weight) = MBP/CI.

Administration of Antiserum to Antagonize Response to Synthetic α-Rat ANP

After isotonic saline (20 μl/min) was infused intravenously for equilibration, 25 μl of rabbit anti-α-rANP antiserum (n = 4) or 25 μl of normal rabbit serum (n = 4) was administered. Then α-rANP, 300 ng/100 g body weight, was injected intravenously. Urine samples were collected at 20-minute intervals for control and experimental periods.

Analysis of Data

All data were expressed as means ± SEM, and differences were evaluated by unpaired t test for comparison between rats injected with anti-α-rANP antiserum and normal rabbit serum.

Results

Hemodynamic effects of anti-α-rANP antiserum and normal rabbit serum are shown in Figure 1. MBP in rats administered antiserum began to rise 20 minutes after injection and was significantly higher than that in rats given normal rabbit serum 40 minutes after (127 ± 4 vs 110 ± 5 mmHg; p < 0.05) and 60 minutes after (127 ± 3 vs 112 ± 5 mmHg; p < 0.05) injection. The rise in MBP disappeared 80 minutes after injection. However, no significant change in HR was obtained between rats injected with antiserum and normal rabbit serum. CI in rats administered antiserum was significantly higher than that of the control group 20 minutes after injection (28.4 ± 1.1 vs 24.8 ± 1.1 ml/min/100 g body weight; p < 0.05) and returned to close to the preinjection level 60 minutes after injection. There were no significant differences in SVI and TPRI between the two groups. Maximal increments of MBP and CI in rats administered antiserum were approximately 7 and 11%, respectively, compared with basal values.

Urinary output in rats given anti-α-rANP antiserum was significantly less than that in rats administered normal rabbit serum during the first 20-minute period after injection (1.67 ± 0.47 vs 3.08 ± 0.16 μl/min/100 g body weight; p < 0.05) and during the fourth 20-minute period (2.16 ± 0.80 vs 2.87 ± 0.18 μl/min/100 g body weight; p < 0.05; Figure 2). Maximal decrement of urinary output in rats administered antiserum was approximately 63% during the first period, compared with the basal value. The 25-μl injection of antiserum reduced urinary output during the first 20-minute period (from 3.87 ± 0.70 to 1.67 ± 0.47 μl/min/100 g body weight). This reduction was the same as that achieved by twofold or fourfold doses of the antiserum (from 3.63 ± 0.15 to 1.66 ± 0.15 μl/min/100 g body weight or from 3.05 ± 0.15 to 1.37 ± 0.12 μl/min/100 g body weight, respectively; data not shown).
Urinary sodium and potassium excretion is shown in Figures 3 and 4. Urinary sodium excretion in the antiserum group was significantly less than that in the control group in the first 20-minute period (25 ± 5 vs 64 ± 9 nEq/min/100 g body weight; p < 0.01), but the significant difference between the two groups disappeared during the second 20-minute period. Urinary potassium excretion in the antiserum group was significantly less than that in the control group during the first period (216 ± 57 vs 714 ± 63 nEq/min/100 g body weight; p < 0.01), and this significant difference between the two groups continued until the sixth 20-minute period. Maximal decrements of urinary sodium and potassium in rats administered antiserum were approximately 63 and 60%, respectively, during the first period, compared with basal values.

An injection of α-rANP, 300 ng/100 g body weight, produced strong diuretic effect in rats pretreated with normal rabbit serum (from 3.04 ± 0.15 to 30.59 ± 5.72 μl/min/100 g body weight). However, the 25-μl injection of antiserum almost completely blocked the diuretic response to α-rANP, 300 ng/100 g body weight (from 3.59 ± 0.58 to 5.23 ± 1.23 μl/min/100 g body weight; data not shown).

**Discussion**

Exogenous ANP is known to have potent hypotensive and natriuretic effects in rats,1 dogs,17,18 and even human subjects.19-22 However, the physiological role
of endogenous ANP is still unclear. In the present study, we investigated the physiological role of endogenous ANP by observing the hemodynamic, diuretic, and natriuretic effects caused by anti-α-rANP antiserum in normotensive rats.

We demonstrated that anti-α-rANP antiserum produced rises in both MBP and CI, while normal rabbit serum elicited no significant change. These results indirectly suggest that endogenous ANP has a hypotensive potency as well as the ability to reduce cardiac output. We have already reported that exogenous α-hANP caused hypotension associated with a decrease in CI without any change in TPRI in anesthetized, normotensive Wistar rats and that the reduction in MBP was significantly correlated with the decrease in CI. In addition, other investigators have reported that administration of atrial extract and atriopeptin II produced a fall in blood pressure and a sustained decrease in cardiac output in rats. These results and the present observations confirm that endogenous ANP plays an important role in the regulation of blood pressure and cardiac output.

Exogenous ANP has been reported to produce a marked diuresis and natriuresis in animals. It has been reported that atrial extract and synthetic α-rANP increase the glomerular filtration rate and the excretion of electrolytes and water in rats. On the other hand, it has been reported that the marked diuresis and natriuresis produced by synthetic α-hANP results from the inhibition of tubular reabsorption of electrolytes. Exogenous ANP clearly elicits diuresis and natriuresis. In the present study, anti-α-rANP antiserum produced decreases in diuresis, natriuresis, and kaliuresis of approximately 60%, while normal rabbit serum elicited no significant change in these functions. These results suggest that endogenous ANP has specific biological activities and plays an important role in the regulation of water, sodium, and potassium excretion from the kidneys.

By means of a radioimmunoassay for ANP, many investigators have reported that the release of ANP is increased by volume loading, mitral obstruction, atrial pacing, paroxysmal supraventricular tachycardia, and by elevated blood pressure following administration of arginine vasopressin. However, the physiological role of endogenous ANP remains undefined. Hirth et al. reported that monoclonal antibodies directed against atriopeptin II blocked diuresis and natriuresis induced by volume expansion in anesthetized rats, but these antibodies produced no change in diuresis and natriuresis except under conditions of loading with saline and exogenous atriopeptin II. Naruse et al. observed marked decreases of urinary output and urinary sodium excretion after an intravenous injection of antiserum against atriopeptin I in anesthetized rats, but no change in mean arterial blood pressure. Our antiserum against α-rANP produced not only reductions in urine output and urinary sodium and potassium excretion but also increases in MBP and CI in normovolemic Wistar-Kyoto rats. Moreover, the inhibitory effects of antiserum on diuresis and natriuresis were greater than those observed by Naruse et al. These differences in the efficacy of antibodies against ANP may result from the size of the dose administered, the specificity of the antibody, or the direct actions of the antibody itself.

Measurement of endogenous plasma level of rANP may reveal the efficacy of the antiserum. Unfortunately, the plasma level of rANP after the injection of the antiserum could not be measured because the presence of the anti-α-rANP antibody interfered with our direct radioimmunoassay. Therefore, we investigated whether this antiserum blocked the diuretic response to exogenous α-rANP. An injection of exogenous α-rANP produced a strong diuretic effect in rats pretreated with normal rabbit serum; however, the antiserum almost completely blocked the diuretic response to exogenous α-rANP. The diuresis caused by exogenous α-rANP was blocked by the antiserum, indicating that the antiserum must inhibit endogenous α-rANP. The dose of anti-α-rANP antiserum used in the present study seemed to block the diuretic effect of endogenous ANP maximally during the first 20-minute period, because maximal reduction in urinary output was the same as the reductions achieved by twofold or fourfold doses of the antiserum.

To summarize, we investigated the effect of passive immunization on hemodynamics, diuresis, and natriuresis in anesthetized rats using a specific antiserum against α-rANP. A significant rise in MBP and CI and decreases in urinary output and urinary sodium and potassium excretion were obtained after intravenous administration of anti-α-rANP antiserum, while no changes were achieved with normal rabbit serum. These results are the exact inverse of effects of exoge-
nous ANP reported by many investigators. We conclude that endogenous ANP may have an important physiological role in the regulation of hemodynamics and water-electrolyte balance.

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