The Effects of Atrial Natriuretic Peptide on Renal Function and the Renin-Aldosterone System in Anesthetized Rabbits

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NUSHIRO, N., ABE, K., SEINO, M., ITOH, S. and YOSHINAGA, K. The Effects of Atrial Natriuretic Peptide on Renal Function and the Renin-Aldosterone System in Anesthetized Rabbits. Tohoku J. exp. Med., 1987, 152 (3), 301-310 —— To determine the effects of α-human atrial natriuretic peptide (ANP) on renal function and the renin-aldosterone system in anesthetized rabbits, ANP (0.05 µg/kg/min) or 5% dextrose solution in vehicle control was infused intravenously. The infusion of ANP resulted in a significant decrease in mean arterial pressure with an increase in renal blood flow (RBF). ANP also produced significant increases in urine volume and urinary sodium excretion. ANP tended to increase glomerular filtration rate, filtered sodium load and net tubular reabsorption of sodium. However, there were no significant differences in these parameters compared with control group. Fractional sodium excretion was increased significantly by ANP. Plasma renin activity (PRA) was suppressed only at 30 min after the infusion of ANP, while a significant fall in plasma aldosterone concentration (PAC) lasted even in the recovery period. These results indicate that ANP produces a diuresis and natriuresis through the increased RBF in anesthetized rabbits. It is also suggested that ANP suppresses PAC independent of the inhibition of PRA. —— atrial natriuretic peptide; renal hemodynamics; natriuresis; renin-angiotensin-aldosterone system

It has been demonstrated that atrial extract, atrial natriuretic factor or atrial natriuretic peptide possess vasodilating, natriuretic and diuretic properties. (de Bold et al. 1981; Briggs et al. 1982; Keeler and Azzarolo 1983; Pollock and Banks 1983; Camargo et al. 1984; Burnett et al. 1984, 1986; Maack et al. 1984; Wakitani et al. 1985; Kleinert et al. 1986). But the mechanism by which atrial extract or atrial natriuretic peptide produces a natriuresis and diuresis is still unclear. Some studies have suggested that contribution of an increase in renal blood flow (Keeler and Azzarolo 1983; Wakitani et al. 1985) or glomerular filtration rate (Camargo et al. 1984; Maack et al. 1984; Beasley and Malvin 1985; Huang et al. 1985) to the natriuresis and diuresis, and others have indicated direct

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tubular effects (Briggs et al. 1982; Sonnenberg et al. 1982; Pollock and Banks 1983; Burnett et al. 1984, 1986; Hammond et al. 1985).

These studies were done by using rats and dogs. There was little study in literature using rabbits (Baum and Toto 1986). Therefore, one of our objectives in the present experiments was to examine the effect of constant intravenous infusion of \(\alpha\)-human atrial natriuretic peptide (ANP) on renal function in rabbits.

On the other hand, atrial extract or atrial natriuretic peptide has been reported to suppress both renin secretion in dogs (Burnett et al. 1984; Maack et al. 1984) and aldosterone production in vitro (Atarashi et al. 1984, 1985; Chartier et al. 1984; Campbell et al. 1985). Therefore, another objective in the present study was to examine the effect of constant infusion of ANP on the renin-aldosterone system in anesthetized rabbits.

**MATERIALS AND METHODS**

Experiments were performed on eleven female albino rabbits weighing 3.0-3.2 kg. The animals were raised on standard rabbit chow which provided 9.1 mEq sodium/100 g and 63.4 mEq potassium/100 g, and were allowed free access to water. The rabbits were fasted overnight (20 hr) and water was allowed ad libitum before beginning the experiment. The rabbits were anesthetized with urethan (450 mg/kg) and \(\alpha\)-chloralose (45 mg/kg). Tracheotomy was then performed and the trachea was intubated for spontaneous breathing. The left jugular vein was cannulated with polyethylene tubing (PE50) and a catheter (PE60) was inserted into the inferior vena cava through the left femoral vein for infusion of a 5% dextrose solution or ANP. A third polyethylene catheter (PE60) was placed into the abdominal aorta adjacent to the renal arteries through the left femoral artery for monitoring arterial pressure and collecting arterial blood sample. The left renal artery was then exposed through a left peritoneal flank incision and gently exposed. A noncannulating electromagnetic flow probe (2.0 mm, Nihon Kohden, Tokyo), connected to an electromagnetic flow meter (MF-27, Nihon Kohden), was placed around the renal artery for measuring renal blood flow (RBF). Mean arterial pressure (MAP) was monitored with a pressure transducer and amplifier (Biophysiograph, 180 system, San-ei, Tokyo), and recorded on a pen oscillograph together with RBF. The left ureter was then cannulated with tubing for collection of urine.

A solution of 5% dextrose was intravenously infused to the extent of 2% of body weight as priming infusion and then was followed by sustaining infusion at a rate of 0.57 ml/min during the whole experimental period. \(\alpha\)-Human atrial natriuretic peptide (ANP; Protein Research Foundation, Osaka) was dissolved in distilled water to the appropriate concentration for intravenous administration. After completion of the surgery, at least 60 min were allowed for stabilization of arterial blood pressure and renal blood flow. Then a priming dose of 50 mg/kg of creatinine in 4 ml of a 5% dextrose solution was given through the left jugular vein catheter and was followed by prolonged infusion of 0.5 mg/kg/min in 0.28 ml/min of a 5% dextrose solution throughout the experiment to estimate glomerular filtration rate. Twenty min after the priming injection of creatinine, the experimental protocol was started.

The rabbits (n = 11) were divided into two groups of 1) control (n = 5) and 2) ANP (n = 6). In ANP group, the same control period (30 min) was taken as in control group. During and at the end of control, urine sample and 5 ml of arterial blood sample were taken respectively, to determine urine volume (UV), urinary sodium excretion (\(U_{Na}\)V), urinary potassium excretion (\(U_{K}\)V), urinary excretion of creatinine, systemic arterial hematocrit, plasma concentrations of sodium and creatinine, plasma renin activity (PRA) and plasma
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aldosterone concentration (PAC). Then ANP was infused intravenously at a rate of 0.05 μg/kg/min in 0.57 ml/min of a 5% dextrose solution for 60 min through the left femoral vein catheter. After starting the infusion, six consecutive 10 min periods were taken, and the infusion of ANP was stopped. Then, a 30 min recovery period was followed. During each period, urine sample was collected. Arterial blood samples were also drawn 30 and 60 min after the start of ANP infusion and at the end of the recovery period. After each of these blood sample collections, the same amount of fresh arterial blood drawn from a donor rabbit was supplemented. In the control group, the same procedures as in the ANP group were performed. In this group, instead of ANP infusion, a 5% dextrose solution at a rate of 0.57 ml/min was infused for 60 min through the left femoral vein catheter.

Plasma and urinary concentrations of creatinine were determined by cleanlizer (VS-700s, Nihon Denshi, Tokyo). Plasma and urinary concentrations of sodium were determined by an autoanalyzer (STAT/ION-II, Nihon Technicon, Tokyo). PRA and PAC were measured by the radioimmunoassay described elsewhere (Abe et al. 1978). Renal vascular resistance (RVR) was calculated by dividing mean arterial pressure by renal blood flow and was expressed as mmHg/ml/min. Net tubular reabsorption of sodium (RNa) were calculated by subtracting urinary sodium excretion from filtered sodium load (FNa, glomerular filtration rate times plasma concentration of sodium) and was expressed as μEq/min. UV, UNaV, and UKV obtained in the 30 min control and recovery periods were expressed as values excreted for 10 min.

The group differences were assessed using the two way analysis of variance. All data in the text, figures and tables were expressed as means±s.e.

RESULTS

Absolute values for various parameters in each period in the control and ANP groups are summarized in Table 1. Although there were significant differences in MAP and fractional excretion of sodium (FENa) between the two groups in the control, no significant differences in heart rate, RBF, RVR, UV, UNaV, GFR, FNa

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<th>Table 1. Absolute values for various parameters in control period in vehicle control and ANP infusion group</th>
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<td>Parameter</td>
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Values are means±s.e.; HR, heart rate; other abbreviations are the same as those in the text. *p < 0.05 compared with ANP infusion group.
and \( R_{Na} \) were observed. \( U_{NaV} \) appeared to be slightly greater excretion in the control group, but this difference was not significant.

Fig. 1 illustrates the effects of intravenous infusion of ANP on MAP, RBF and RVR. The infusion of ANP resulted in a slight, but significant, decrease in MAP and an increase in RBF. This increase in RBF was sustained for 50 min

![Fig. 1](image1)

Fig. 1. Changes in mean arterial pressure (MAP), renal blood flow (RBF) and renal vascular resistance (RVR) in control (○---○, \( n = 5 \)) and atrial natriuretic peptide (●----●, \( n = 6 \)) infusion group.

Values are expressed as delta change from each control level.

\* \( p < 0.05 \), \* \* \( p < 0.01 \), compared with vehicle control in each period.

Fig. 2. Changes in urine volume (UV), urinary sodium excretion (\( U_{NaV} \)) and urinary potassium excretion (\( U_{KV} \)) in control (□, \( n = 5 \)) and atrial natriuretic peptide (●, \( n = 6 \)) infusion group.

Values are expressed as delta change from each control level.

\* \( p < 0.05 \) compared with vehicle control.
after the start of ANP infusion, whereas the significant fall in MAP lasted till the recovery period. The calculated RVR was decreased significantly for 60 min after ANP infusion, and returned to the control level in the recovery period. Heart rate did not change significantly in both control and ANP groups.

Fig. 2 shows the effects of ANP on UV, \( U_{NaV} \) and \( U_{KV} \). Intravenous infusion of ANP elicited a gradual increase in UV in the first 30 min (peak at the period of 30 min, 4.5±0.9 vs. 0.5±1.1 ml/10 min; \( p < 0.05 \)), but this diuretic effect of ANP was not sustained for 60 min.

ANP also produced a natriuresis and kaliuresis (217±63 vs. 6.1±2.6 µEq/10 min at the period of 30 min; \( p < 0.05 \) and 34±7.4 vs. 4.3±7.4 µEq/10 min at the period of 30 min; \( p < 0.05 \), respectively). In the recovery period, \( U_{NaV} \) and \( U_{KV} \) returned to their control levels.

Fig. 3 shows the effects of ANP infusion on GFR, \( F_{Na} \) and \( R_{Na} \). ANP tended to increase GFR but the change did not reach statistical significance compared with the control group. ANP also tended to elevate \( F_{Na} \) in the same manner as in GFR, but the values also did not reach statistical significance. The change in \( R_{Na} \) showed a similar pattern to that in \( F_{Na} \).

Fig. 4 illustrates the effect of ANP on \( FENa \). ANP significantly increased \( FENa \) at 30 and 60 min after the infusion of the peptide (+2.7±0.9 vs. −0.5±0.4%; \( p < 0.01 \) and +1.0±0.4 vs. −0.5±0.5%; \( p < 0.05 \), respectively). In the recovery period, \( FENa \) returned to the control level.

PRA and PAC did not change significantly, in the control group throughout the experiment. In the ANP group, the PRA and PAC in the control period were

![Graph](image)

Fig. 3. Changes in glomerular filtration rate (GFR), filtered sodium load (\( F_{Na} \)) and net tubular reabsorption of sodium (\( R_{Na} \)) in control (○−○, \( n = 5 \)) and atrial natriuretic peptide (●−●, \( n = 6 \)) infusion group. Values are expressed as delta change from each control level. There is no significant difference between two groups in each period.
29.1±5.7 ng/ml/hr and 96.6±6.7 ng/100 ml, respectively. Fig. 5 shows the effects of ANP on PRA and PAC. ANP produced a significant decrease in PRA at 30 min (−8.4±2.4 vs. +5.7±5.4 ng/ml/hr; p <0.05), and the change in PRA tended to return the control level in the recovery period. ANP elicited a significant fall in PAC in the experimental period (−10.1±4.9 vs. +17.6±4.8 ng/100 ml at 30 min; p <0.05 and −22.2±3.5 vs. 17.1±6.5 ng/100 ml at 60 min; p <0.01), and the change in PAC returned toward control level in the recovery period.

Fig. 4. Changes in fractional excretion of sodium (FE\textsubscript{Na}) in control (□) and atrial natriuretic peptide (●) infusion group.
Values are expressed as delta change from each control level.
*p <0.05; **p <0.01 compared with vehicle control.

Fig. 5. Changes in plasma renin activity (PRA) and plasma aldosterone concentration (PAC) in control (○---○, n = 5) and atrial natriuretic peptide (●---●, n = 6) infusion group.
Values are expressed as delta change from each control level.
*p <0.05; **p <0.01, compared with vehicle control in each period.
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period, although this change was still significant compared with that in the control group.

**Discussion**

In our previous study in anesthetized rabbits, intravenous bolus injection of ANP at a dose of 5 μg produced a significant decrease in MAP and an increase in RBF with a marked natriuresis and diuresis, whereas these hemodynamic alterations were not accompanied by the change in GFR as estimated by creatinine clearance at 30 min after the injection of ANP (Nushiro et al. 1987). But the intravenous injection of ANP might have resulted in a transient increase in GFR that could not have been detected in a 30 min period. Therefore, in the present experiment, the effect of ANP on renal function was examined with continuous infusion of the peptide for 60 min. Intravenous infusion of ANP at a rate of 0.05 μg/kg/min resulted in a significant decrease in MAP and an increase in RBF. These findings were compatible with other reports (de Bold et al. 1981; Wakitani et al. 1985). However, the reduction in MAP by ANP was sustained even in the recovery period, whereas the increase in RBF produced by ANP did not last till the end of ANP infusion. The failure in RBF to last till the end of the ANP infusion could have been due to the acute diuresis and natriuresis produced by ANP in the first 30 min. The intravenous infusion of ANP also produced a marked diuresis, natriuresis, and kaliuresis, which were consistent with previous results using atrial extracts or atrial natriuretic factors (Pollock and Banks 1983; Burnett et al. 1984; Maack et al. 1984). But, these diuretic, natriuretic, and kaliuretic effects of ANP declined at the periods of 30-60 min despite the sustained infusion of ANP. Maack et al. (1984) reported that intravenous infusion of auriculin resulted in a sustained natriuresis throughout the infusion in anesthetized dogs. The differences in volume status of animals and infusion rate of saline or 5% dextrose solution, tachyphylaxis to the peptide, or species difference might have been involved in the duration of natriuresis produced by the peptides.

No significant difference was observed in heart rate throughout the experiment between the control and ANP groups. This was compatible with the observation by Hirata et al. (1985) and Kleinert et al. (1986).

The intravenous infusion of ANP tended to increase GFR, FNa and RNa, but these differences did not reach statistical significance compared with control group. In previous reports in which atrial natriuretic peptides or atrial natriuretic factors were infused into the renal artery or intravenously to anesthetized animals, a significant increase in GFR was observed during infusion of the peptides (Maack et al. 1984; Beasley and Malvin 1985; Huang et al. 1985). It is not clear why intravenous infusion of ANP failed to increase GFR significantly in the present experiment, but ANP used in the present study was a small dose. Therefore, one of the reasons could be due to the dose.

ANP elicited a significant increase in FENa, suggesting that direct inhibition
of tubular sodium reabsorption might, in part, contribute to the natriuretic effect of ANP in the present experiment. Whether atrial natriuretic peptides or atrial natriuretic factors have direct tubular effect or not is controversial. Hammond et al. (1985) reported that administration of atrial natriuretic factor inhibits sodium-coupled transport in proximal tubules. Burnett et al. (1986) also observed that whole kidney fractional delivery of sodium from the proximal tubule as estimated by the fractional excretion of lithium was increased. However, Baum and Toto (1986) demonstrated that atrial natriuretic factor did not affect transport directly in the proximal tubule, by using in vitro microperfusion method. On the other hand, Briggs et al. (1982), Pollock and Banks (1983), and Koseki et al. (1986) indicated a direct effect of atrial extract or atrial natriuretic peptide on distal tubules. The reason why these inconsistent results have been demonstrated remains unclear.

Intravenous infusion of ANP resulted in significant decrease in PRA only at 30 min after the start of ANP infusion, while a significant decrease in PAC lasted even in the recovery period, which is consistent with previous studies using atrial extracts or atrial natriuretic factors (Burnett et al. 1984; Chartier et al. 1984; Maack et al. 1984; Atarashi et al. 1984, 1985; Campbell et al. 1985). Our present results suggest that ANP suppresses PAC independent of inhibition of PRA. However, in the present experiment, it is not clear whether the increase in sodium load to macula densa or direct inhibition on juxta glomerular cell contributed to the significant decrease in PRA by ANP. Opgenorth et al. (1986) reported that in the nonfiltering kidney, atrial natriuretic peptide has no inhibitory effect on renin secretion, suggesting the involvement of macula densa mechanism in suppression of renin secretion by atrial natriuretic peptide. Vallarreal et al. (1986) showed that suppression of renin secretion by atrial natriuretic factor is mediated through its interaction with the two intrarenal receptor mechanisms, the renal vascular receptor and the macula densa. In the present experiment, the decrease in PRA was only significant at 30 min after the start of ANP infusion, at which the increase in U_{Na,V} produced by ANP was maximum. Therefore, it is likely that macula densa mechanism contributes to suppression of PRA by ANP in the present study.

In conclusion, the present results suggest that intravenous infusion of ANP produced a diuresis and natriuresis through the increased RBF. It is also indicated that ANP suppresses the renin-aldosterone system in anesthetized rabbits.

Acknowledgments

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