Role of Atrial Natriuretic Peptide in Experimental Renal Failure

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Various aspects of atrial natriuretic peptides (ANP) have been studied since its reports by de Bold 1,2 Although ANP is known to play an important role in the volume regulation of body fluid by displaying natriuresis probably due to vasodilating mechanism, its significance in renal failure has not been clarified. The present study was carried out in order to clarify the renal physiological and systemic hemodynamic effect of exogenous ANP in animal models with deteriorated renal function. In addition, the site of action of ANP was also evaluated in the kidney.

MATERIALS AND METHODS

1. Effect of α-human ANP (α-hANP) in Dogs with Renal Failure:

Eight mongrel dogs of both sexes, weighing 10–20 Kg, were used for this experimental study. Renal failure was induced in these dogs by a two-stage surgical procedure which consisted of a left bipolar nephrectomy followed by a right total nephrectomy one week later. Sham-operated dogs were used as controls.

(1) Hemodynamic Studies:

Following recovery from nephrectomy, the dogs were anesthetized with sodium pentobarbital and ventilated artificially. Systemic blood pressure (BP) and intra-atrial pressure were monitored by two pressure transducers; one was connected to a catheter placed in the abdominal aorta and the other one in the right atrium. Cardiac output (CO) and renal blood flow (RBF) of the left kidney were measured with each electromagnetic flow meter.

After attaining a stable urinary flow rate, a priming dose of α-hANP 0.1 µg/kg (synthesized by Peptide Institute OSAKA) was intravenously injected into the left femoral vein and was subsequently followed by an intravenous drip infusion of α-hANP at 0.1 µg/kg/min for 30 min. Lactate Ringer solution was also injected intravenously at a rate of 0.1 ml/kg/min during the entire experiment.

(2) Clearance Studies:

In order to determine renal clearance, inulin and paraaminohippurate (PAH) were dissolved in Lactate Ringer solution described above and infused at concentrations of 25 mg/dl and 5 mg/dl, respectively. Urine was collected via a catheter inserted into the right ureter every 10 min for half an hour. Arterial blood was sampled at the midpoint of each period. At the onset of the study on renal clearances in renal failure dogs the electromagnetic flow meter was removed from the animals, because it could potentially cause vasoconstriction in the small- or middle-sized artery, resulting in less reproducible values in RBF.

Inulin clearance (C_{in}), PAH clearance (C_{PAH}), and fractional excretion of sodium (F_{E_{Na}}) were then calculated.

2. Effect of rat ANP (rANP) in Rat with Renal Failure:

Male Sprague-Dowley rats, weighing 140–150g, were nephrectomized by the two-stage surgical procedure consisting of the left two-thirds bipolar nephrectomy followed by right total nephrectomy 2 weeks later. After hemodynamic stabilization of 2–3 weeks, rats weighing 360–380g were anesthetized with sodium pento-
Fig. 1. Effect of α-human ANP (α-hANP) on hemodynamics in control dogs.

Fig. 2. Effect of α-human ANP (α-hANP) on hemodynamics in dogs with renal failure.

barbital and were infused with rANP (Peptide Institute OSAKA) at 0.25 µg/min.

1. Clearance Studies

C_in, C_PAH, F_Na and urinary flow rate were measured and mean femoral arterial blood pressure was recorded continuously by a strain-gauge transducer connected to a polygraph.

2. Immunohistochemical Studies

Indirect fluorescence method was performed in order to identify the site of action of rANP in the remaining kidney by means of immunohistochemical method. After the clearance study, frozen sections of each kidney, 4 µm in thickness, were made and stored.

RESULTS

1. Effect of α-human ANP (α-hANP) in Dogs with Renal Failure:

(1) Hemodynamic Studies:

In the control group, mean arterial blood pressure (MBP) decreased from 117.5 ± 3.5 mmHg to 105 ± 5.8 mmHg, CO increased from 1.1 ± 0.2 L/min to 1.3 ± 0.3 L/min and RBF from 82 ± 8.6 ml/min to 96 ± 4.6 ml/min after the administration of initial doses of α-hANP. In the nephrectomized animals MBP similarly decreased from 128.5 ± 4.5 to 111.0 ± 3.5 mmHg and CO decreased from 0.9 ± 0.1 to 0.7 ± 0.2 L/min in

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renal failure dogs (Figs. 1 and 2). MBP of the control dogs dropped to 98 mmHg and remained stable during the infusion of α-hANP, whereas CO and RBF decreased to about 1 L/min and 74 ml/min, respectively. These values were maintained during drip-infusion of α-hANP. In the nephrectomized animals, similar trends were observed in MBP and CO. Right atrial pressure and heart rate were unchanged in response to α-hANP and maintained at around 10 cmHg and 130—135 /min (not shown in Figure 10) respectively.

(2) Clearance Studies:
In the control dogs, C_in and C_PAH increased transiently but significantly, from 22.5 ml/min to 32.5 ml/min, and from 134.2 ml/min to 158.2 ml/min, respectively at the initial administration of α-hANP. During drip-infusion, they were maintained at around 25 ml/min and 115 ml/min respectively as shown in Fig. 3. Urine volume increased 6-fold from 0.25 ml/min to 1.47 ml/min, and FENA also increased 4-fold from 1.02% to 4.21%. In renal failure dogs, C_in increased to 9.6 ± 2.9 ml/min from 7.5 ± 2.1 ml/min. FENA increased from 4.5 ± 2.2 to 11.5 ± 5.3% by administration of α-hANP in addition to the increased basal level by this time. C_PAH and urine volume increased to 52.5 ± 19.5 from 33.2 ± 14.2 ml/min and to 1.25 ± 0.32 from 0.43 ± 0.23 ml/min, respectively at the initial doses of α-hANP (Fig. 4).

2. Effect of rat ANP (rANP) in Renal Failure Rat:
(1) Clearance Studies
C_in increased from 0.52 ± 0.06 to 0.91 ± 0.21 ml/min, C_PAH from 1.53 ± 0.09 to 2.14 ± 0.20 ml/min and FENA from 1.32 ± 0.06 to 5.85 ± 0.34%. The urine flow rate also increased 3 times to control period. Blood pressure decreased to 100 ± 5 mmHg from 124 ± 5 mg.
These results were observed during injection of rANP. There were statistically significant changes in BP and renal clearances before and after administration of rANP.

(2) Immunohistochemical Studies
As shown in Fig. 5, positive findings of exogenously administered rANP were observed in the proximal tubule in addition to those in the frequently stained sites in renal vessels, such as glomerular afferent and efferent arteries and medullary vascular bundle.

DISCUSSION
ANP decreased MBP and CO in the renal failure dogs as well as in the controls. On the other hand, RBF in the control dogs increased at the time of administration of initial doses and then decreased during the period of maintenance doses. In case of renal failure, RBF could not be measured due to low reproducibility which was probably caused by a potential weak point of an electromagnetic flow meter. These results are different from the reports from Yukimura and Hintze in which RBF was increased by ANP. Right atrial pressure and heart rate were unchanged in response to α-hANP. Kleinert examined the mechanism of lowering blood pressure using anesthetized or conscious dogs, and indicated that MBP is lowered in the models by a mechanism other than the reduction in total peripheral resistance, namely, in cardiac output. Cole reported that a pronounced rise in glomerular filtration rate was maintained during the infusion period, even though RBF was unchanged from base line in 5/6 nephrectomized rats.

The present studies demonstrated that all of those $C_{in}$, $C_{PAH}$, $F_{E1}$, and urine volume increased in response to ANP without mediating systemic hemodynamics despite the decreased renal mass.

Recent evidences suggest that ANP has no direct action on the proximal tubule. However, in the 5/6 nephrectomized rats of the present study, positive staining of extrinsic rANP was observed even in the proximal tubule in addition to the vasculature. There is also evidence that fractional excretion of lithium, an indicator of proximal tubular function, goes up in renal failure.

The reduction in GFR is accompanied by an increase in Na excretion per nephron if dietary Na intake is not changed. DeWardner anticipated the existence of natriuretic substance other than aldosterone, prostaglandin, as a humoral factor that influences Na transport in the tubules. Such natriuretic hormone was different from ANP. On the other hand, Smith and Brenner found that plasma ANP levels rose progressively in 5/6 nephrectomized rats with increasing Na intake. They suggested that ANP may play an important role in promoting the adaptive increase in Na excretion per nephron in renal failure.

CONCLUSION
1) ANP may be applied to the treatment of volume overload in patients with renal failure in which the number of functioning nephron is decreased.

2) An enhancement of tissue-receptor sensitivity to ANP, a stimulation of ANP secretion, and an alternation of ANP metabolic pathway may occur to adapt to the changes in body fluid in animals with renal failure.

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REFERENCES

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