Effects of Atrial Natriuretic Peptide on Angiotensin II-Induced Antinatriuresis in Anesthetized Dogs

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Inhibitory effects of atrial natriuretic peptide (ANP) on angiotensin II (ANG II)-induced renal responses were examined in anesthetized dogs. ANG II (5 ng/kg per min) was infused intravenously and changes in renal hemodynamics and urine formation were compared between the ANP (10 ng/kg per min)-infused kidney and the contralateral vehicle-infused (control) kidney. ANG II reduced absolute and fractional urinary sodium excretion in both the ANP-infused kidney and the control kidney. ANG II also reduced glomerular filtration rate in the control kidney but not in the ANP-infused kidney. The ANG II-induced reduction in urinary sodium excretion in the ANP-infused kidney was smaller than the response in the control kidney, whereas ANP did not affect the reduction in fractional sodium excretion. These results suggest that ANP prevents hypofiltration and thereby attenuates the antinatriuresis induced by ANG II.

Key words atrial natriuretic peptide (ANP); angiotensin II (ANG II); kidney; hemodynamics; sodium excretion

It is well known that atrial natriuretic peptide (ANP) modulates the renin-angiotensin system. ANP suppresses renin release4–3 and angiotensin II (ANG II)-induced vasoconstriction.4,5 In addition, ANP has been suggested to interact with renal actions of ANG II. The natriuretic effect of ANP can be enhanced in the presence of exogenous ANG II6 and suppressed during inhibition of the converting enzyme.7 Addition of ANP reverses ANG II-induced reduction of sodium and water excretion.7,8 ANP therefore seems to counteract the antinatriuretic property of ANG II. On the other hand, there are also some reports implying that ANG II blunts the ANP-induced natriuresis.9–11 Thus the relationship between the renal effects of ANP and ANG II has been controversial. In most of these studies, however, ANP substantially reduced systemic blood pressure or increased glomerular filtration rate (GFR), which would complicate the interpretation of results.

We had previously reported that ANP, given at a low dose which causes natriuresis without affecting GFR, attenuated a decrease in blood flow induced by exogenous ANG II in the dog kidney in vivo.12 In the present study, we examined whether pretreatment of ANP at the low dose affects the ANG II-induced antinatriuresis in anesthetized dogs.

MATERIALS AND METHODS

Animal Preparation Six mongrel dogs of either sex weighing 14 to 19 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.), then intubated and artificially ventilated with room air. Decamethonium bromide (0.25 mg/kg i.v.) was given to prevent spontaneous active respiratory movement. Anesthesia was maintained by continuous i.v. infusion of sodium pentobarbital at a rate of 5 mg/kg per h throughout the experiments. Inulin, dissolved in 0.45% sodium chloride and 2.5% dextrose, was given i.v. at a priming dose of 50 mg/kg and at a maintenance dose of 1 mg/kg per min. The right brachial artery was cannulated to measure systemic blood pressure with a pressure transducer (Nihon Kohden Co., Tokyo, Japan, TP-200T). A catheter connected to another pressure transducer was inserted into the right femoral artery and the tip of this catheter was positioned near the origin of the renal arteries to measure renal perfusion pressure (RPP). The left and right kidneys were exposed by retroperitoneal flank incisions. An adjustable clamp was placed on the aorta above the origin of the right renal artery to control bilateral RPP. All visible renal nerves were dissected away from the renal vessels and cut after ligation. Catheters for urine collection were inserted into both left and right ureters. Electromagnetic flow probes (2.5—3.5 mm in diameter, Nihon Kohden) were attached to the renal arteries to measure renal blood flow (RBF) with a square-wave flow meter (Nihon Kohden, MF-27). Curved 25-gauge needles connected to polyethylene tubes were inserted into the left and right renal arteries for drug infusion. Mean arterial pressure, RPP and RBF were recorded with a polygraph system (Nihon Kohden). After completion of surgery, 60 to 90 min were allowed for stabilization.

Experimental Protocol Urine samples were collected from both kidneys over a 10-min period, and arterial blood was withdrawn at the midpoint of the urine collection. After sampling to determine basal values, ANP (Human 1-28; Peptide Institute Inc., Osaka, Japan) was infused into the renal artery (left, n = 3; right, n = 3) at 10 ng/kg per min (0.1 ml/min). Vehicle (0.9% saline) was infused simultaneously into the renal artery on the other side. Urine and blood samples were collected 10 min after the start of the ANP infusion. Then, intravenous infusion of ANG II (Peptide Institute; 5 ng/kg per min) was started during ANP infusion. RPP was controlled manually by suprarenal aortic occlusion with the adjustable clamp to maintain its level in the face of a rise in systemic blood pressure during ANG II infusion. Beginning 5 min after the start of ANG II infusion, urine and blood sampling were again carried out.

Measurements Blood samples were transferred to tubes containing EDTA 2NaH (1 ng/ml of blood) and then centrifuged to obtain plasma samples. GFR was determined by inulin clearance. Inulin concentration, © 1995 Pharmaceutical Society of Japan
Table 1. Effects of ANG II on Renal Hemodynamics and Urine Formation in the Presence or Absence of ANP

<table>
<thead>
<tr>
<th></th>
<th>Control kidney</th>
<th>ANP-Infused kidney</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Vehicle</td>
</tr>
<tr>
<td>RBF</td>
<td>160 ± 21</td>
<td>144 ± 17†</td>
</tr>
<tr>
<td>GFR</td>
<td>27.7 ± 2.2</td>
<td>31.8 ± 5.0</td>
</tr>
<tr>
<td>FF</td>
<td>36.4 ± 3.8</td>
<td>45.6 ± 9.5</td>
</tr>
<tr>
<td>UV</td>
<td>0.18 ± 0.04</td>
<td>0.22 ± 0.09</td>
</tr>
<tr>
<td>UNaV</td>
<td>37.5 ± 8.0</td>
<td>49.2 ± 11.5</td>
</tr>
<tr>
<td>FENA</td>
<td>0.95 ± 0.20</td>
<td>1.11 ± 0.25</td>
</tr>
<tr>
<td>UOsm</td>
<td>1161 ± 123</td>
<td>1074 ± 114</td>
</tr>
<tr>
<td>COsm</td>
<td>0.72 ± 0.09</td>
<td>0.81 ± 0.10</td>
</tr>
<tr>
<td>T^H_2O</td>
<td>0.54 ± 0.05</td>
<td>0.59 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± S.E. n=6. RBF, renal blood flow (ml/min); GFR, glomerular filtration rate (ml/min); FF, filtration fraction (%); UV, urine flow rate (ml/min); UNaV, urinary sodium excretion (meq/min); FENA, fractional excretion of sodium (%); UOsm, urinary osmolality (mOsm/kg H2O); COsm, osmolar clearance (ml/min); T^H_2O, free water reabsorption (ml/min). ANG II (5 ng/kg per min) was infused intravenously during intrarenal arterial infusion of ANP (10 ng/kg per min). † p<0.05 compared with the values before ANP or vehicle infusion (basal); †† p<0.05, ††† p<0.01 compared with the values before ANG II infusion (vehicle or ANP).

sodium concentration and the osmolality of plasma and urine samples were measured by the anthrone method, flame photometry and the freezing point depression method, respectively.

Data Analysis All values are expressed as means ± S.E. Data were analyzed by analysis of variance for single factor repeated measures plus Schéffe's test (differences within the kidney) or analysis of variance for multifactor repeated measures plus the simple main effects (differences between the control and ANP-infused kidneys). Differences at a p value of less than 0.05 were considered to be statistically significant.

RESULTS

Intrarenal arterial infusion of ANP increased basal urine flow rate (UV), absolute urinary sodium excretion (UNaV) and fractional excretion of sodium (FENA) but not RBF, GFR or filtration fraction (FF) in the ipsilateral kidney (Table 1). ANP increased osmolar clearance (COsm) despite a reduction in urine osmolality (UOsm). ANP also increased free water reabsorption (T^H_2O), but the change was smaller than the change in COsm: the ratio of T^H_2O to COsm decreased during ANP infusion (from 0.74 ± 0.04 to 0.51 ± 0.05, p<0.05). RBF decreased and then stabilized during ANP infusion in the contralateral control (vehicle-infused) kidney. ANP (escaping from the infused kidney to systemic circulation, probably) tended to increase UV and UNaV in the control kidney, but these changes were not statistically significant (Table 1).

Intravenous infusion of ANG II reduced GFR, UV, UNaV and FENA in the control kidney (Table 1). In the ANP-infused kidney, ANG II also caused antinatriuresis, but GFR remained unchanged. Whereas ANG II did not affect FF in the control kidney, it tended to elevate FF in the ANP-infused kidney. ANG II reduced COsm and T^H_2O without affecting their ratio in the control kidney (0.74 ± 0.02 and 0.71 ± 0.05, before and during ANG II infusion, respectively) and in the ANP-infused kidney (0.51 ± 0.05 and 0.52 ± 0.04, before and during ANG II infusion, respectively).

Figures 1 and 2 show the percentage changes from the levels before ANG II infusion. The ANG II-induced changes in GFR and UNaV in the ANP-infused kidney were smaller than those in the control kidney (Fig. 1). ANP did not affect the change in UV or FENA.

RBF decreased immediately after the start of ANG II infusion both in the control and ANP-infused kidneys,
but the initial decrease in RBF (within 2 min after the start of ANG II infusion) was blunted in the ANP-infused kidney (Fig. 2). RBF then gradually increased in the control kidney but not in the ANP-infused kidney; accordingly there was no difference between the RBF response in the control kidney and the response in the ANP-infused kidney more than 5 min of ANG II infusion.

The ANP infusion slightly lowered systemic arterial pressure from $137 \pm 7$ to $129 \pm 6$ mmHg and RPP from $133 \pm 6$ to $128 \pm 5$ mmHg. Whereas ANG II elevated systemic arterial pressure (to $144 \pm 6$ mm Hg, RPP was maintained near the pre-infusion level ($128 \pm 6$ mmHg) by the suprarenal aortic occlusion.

**DISCUSSION**

In the present study the renal hemodynamics and urinary responses to ANG II were compared in the ANP-infused and contralateral control kidneys of anesthetized dogs.

Intrarenal arterial infusion of ANP at 10 ng/kg per min increased UV, UNaV and FENA with no increase in RBF or GFR. Abe et al.\textsuperscript{13} reported that the same dose of ANP did not affect intrarenal distribution of blood flow in dogs. The ANP-induced natriuresis observed in the present study, therefore, can be ascribed predominantly to inhibition of tubular sodium reabsorption. ANP preferentially increased COSm rather than $\text{T}^3\text{H}_2\text{O}$ (indicated by the decreased ratio of $\text{T}^3\text{H}_2\text{O}$ to COSm). Thus ANP, in addition to its action on the proximal tubules,\textsuperscript{14} may inhibit renal concentration probably by acting on the distal site of the nephron as suggested by Yukimura et al.\textsuperscript{15}

Intravenous infusion of ANG II reduced UV, UNaV and GFR in the contralateral control kidney. A micropuncture study showed that ANG II enhanced fluid reabsorption at the proximal tubule of the rat kidney.\textsuperscript{16} In our study ANG II also reduced FENA as reported in previous studies,\textsuperscript{10,11,12} suggesting that ANG II enhances tubular sodium reabsorption in the *in vivo* dog kidney. Since the change in UNaV (by about 70% from the level before ANG II infusion) was greater than the change in FENA (by about 50%), both reduced glomerular filtration and enhanced tubular reabsorption may contribute to the ANG II-induced antinatriuresis observed in the present study.

ANG II infusion reduced RBF both in the ANP-infused and control kidneys. The initial decrease in RBF was blunted in the ANP-infused kidney, compared with the response in the control kidney. This confirms our previous results obtained by bolus intraarterial injection of ANG II.\textsuperscript{12} ANP may be able to antagonize the ANG II-induced renal vasoconstriction. The reduced RBF gradually recovered during ANG II infusion in the control kidney but not in the ANP-infused kidney. The recovery of RBF, despite continuous infusion of ANG II, is known to result from stimulation of renal prostaglandin (PG) synthesis. ANP inhibits ANG II-induced PGE$_2$ production in cultured rat mesangial cells\textsuperscript{17} and reduces basal release of PGE$_2$ from the dog kidney *in vivo*.\textsuperscript{18} It is, therefore, possible that ANP prevents the recovery of RBF by inhibiting production of vasodilator PGs.

Showalter et al.\textsuperscript{11} reported that ANP increased GFR in the control kidney but not in the ANG II-infused kidney in anesthetized dogs. Thus, ANG II may inhibit ANP-induced hyperfiltration. However, it is still unclear whether ANP interferes with ANG II-induced hypofiltration. In our study ANG II failed to reduce GFR in the ANP-infused kidney, implying that ANP, even at a dose that does not induce hyperfiltration, can inhibit ANG II-induced hypofiltration. ANG II elevated FF in the ANP-infused kidney since the decreased RBF was persistent. This indicates that ANG II contracts postglomerular vessels more potently than preglomerular ones in the presence of ANP. A study using videomicroscopy has demonstrated that ANP preferentially inhibited contraction of the afferent arteriole induced by noradrenaline in the perfused rat hydropnephrotic kidney.\textsuperscript{19} ANP may suppress the ANG II-induced preglomerular vasoconstriction, thereby preventing glomerular hypofiltration in the dog kidney.

The ANG II-induced decrease in UNaV (evaluated by the percentage change) in the ANP-infused kidney was smaller than the response in the control kidney. Since the ANG II-induced decrease in FENA remained unaffected, the attenuated UNaV response may be related to the inhibitory effect of ANP on the ANG II-induced decrease in GFR. ANP does not seem to affect the tubular reabsorption enhanced by ANG II in the dog kidney, as suggested in a micropuncture study of the rat kidney.\textsuperscript{20} However, ANP has been reported to inhibit the proximal tubular transport evoked by ANG II\textsuperscript{21} and ANG II-induced activation of proximal tubular Na$^+$,K$^+$-ATPase.\textsuperscript{22} It should be noted that in the present study ANG II elevated FF in the presence of ANP, although the change was not statistically significant. The elevated FF could increase peritubular oncotic pressure and thereby enhance tubular fluid reabsorption. Therefore, we cannot rule out the possibility that the altered glomerular function counteracts the inhibitory effect of ANP on the direct tubular action of ANG II. In any event, the present results suggest that pretreatment of ANP interferes with the ANG II-induced antinatriuresis by preventing glomerular hypofiltration in the dog kidney.

Despite the attenuated UNaV response, ANP did not affect the ANG II-induced decrease in UV. ANG II might preferentially enhance water reabsorption compared with sodium reabsorption. However, ANG II did not elevate urinary osmolality or the ratio of $\text{T}^3\text{H}_2\text{O}$ to COSm, indicating that ANG II did not affect urinary concentration. Although we have no direct evidence, ANG II may enhance reabsorption of water and other osmolar substances such as urea during ANP-induced natriuresis.

Although the present study demonstrates a possible interaction between the renal actions of ANG II and ANP *in vivo*, Kuhlen et al.;\textsuperscript{23} reported that ANP given at a physiological dose (5 ng/kg per min, i.v.) does not affect the renal responses induced by concomitantly administered ANG II in conscious dogs. The physiological significance of our present observation, therefore, remains to be elucidated.

In summary, the present study suggests that in the dog kidney 1) ANG II causes antinatriuresis through a
reduction of GFR and enhancement of tubular sodium reabsorption and 2) ANP inhibits the reduction of GFR and thereby attenuates the ANG II-induced antinatriuresis.

REFERENCES