Modulation of Exogenous and Endogenous Atrial Natriuretic Peptide by a Receptor Inhibitor

Tracy L. Stevens, Chi-Ming Wei, Lawrence L. Aahrus, Denise M. Heublein, Masahiko Kinoshita, Yuzuru Matsuda, John C. Burnett, Jr

Abstract Atrial natriuretic peptide is an important peptide hormone of cardiac origin that functions to regulate cardiac preload via the regulation of sodium excretion. This natriuretic action occurs through activation of the particulate guanylyl cyclase–linked natriuretic peptide-A receptor. HS-142-1 is a newly discovered antagonist of the natriuretic peptide-A receptor that permits insight into the functional role of atrial natriuretic peptide in cardiorenal homeostasis. The first objective of this study was to define for the first time the intrarenal action of HS-142-1 on exogenous atrial natriuretic peptide–mediated natriuresis in anesthetized normal dogs. In group 1 (n=6), which received intravenous atrial natriuretic peptide at 100 ng/kg per minute, intrarenal HS-142-1 (0.5 mg/kg bolus) attenuated atrial natriuretic peptide–induced increases in glomerular filtration rate, urine flow, sodium excretion, and renal cyclic GMP generation and decreases in distal tubular sodium reabsorption. The second objective was to determine whether endogenous atrial natriuretic peptide participates in the regulation of basal sodium excretion. In group 2 (n=6), intrarenal HS-142-1 alone decreased both absolute and fractional sodium excretion and renal cyclic GMP generation and increased distal tubular sodium reabsorption. These studies demonstrate that HS-142-1 markedly attenuates exogenous atrial natriuretic peptide–mediated natriuresis via enhancement of distal tubular reabsorption and blunting of increases in glomerular filtration rate. Second, the current studies support a functional role for endogenous atrial natriuretic peptide in the regulation of basal sodium excretion. (Hypertension. 1994;23:613–618.)

Key Words • atrial natriuretic peptide • sodium • guanosine cyclic monophosphate • receptor, atrial natriuretic peptide • antagonist

Atrial natriuretic peptide (ANP) is a peptide hormone of cardiac origin1,2 that activates a particulate guanylyl cyclase–linked receptor, the natriuretic peptide-A receptor (NPR-A).3,4 ANP mediates its actions through generation of cyclic GMP (cGMP) and results in natriuresis secondary to increases in glomerular filtration rate (GFR) and decreases in distal tubular sodium reabsorption.5,7 Such actions are consistent with localization of the NPR-A receptor to glomeruli and inner medullary collecting duct cells.5,6 Although the pharmacological action of ANP is well characterized, the role of endogenous ANP in the control of basal sodium excretion has yet to be established.

Further definition of the pharmacological and physiological roles of ANP has been prohibited by the lack of a receptor antagonist. HS-142-1 was recently discovered from the fungus culture broth of Aureobasidium sp.8,9 Studies have established that HS-142-1 inhibits binding selectively to the particulate guanylyl cyclase receptor for ANP and when administered systemically attenuates ANP-induced hypotension, diuresis, natriuresis, and increases in urinary cGMP.10–13 Thus, HS-142-1 may permit further insight into the renal mechanisms of ANP-mediated natriuresis and the role of ANP in sodium homeostasis.

The objective of the present study was twofold. The first objective was to assess and define for the first time the intrarenal action of HS-142-1 on exogenous ANP–mediated natriuresis. The second objective was to evaluate the role of endogenous ANP in the control of basal sodium excretion in the presence of intrarenal HS-142-1. We hypothesized that HS-142-1 would attenuate exogenous ANP–mediated natriuresis by antagonizing ANP-induced increases in GFR and decreases in distal tubular sodium reabsorption. Second, we hypothesized that endogenous ANP participates in the control of basal sodium excretion and therefore intrarenal HS-142-1 alone would decrease sodium excretion in the normal dog.

Methods

Surgical Preparation

Experiments were performed on two groups of normal male mongrel dogs (weight, 18 to 23 kg) in accordance with the Animal Welfare Act. The dogs were given lithium 300 mg orally the day before the study. All dogs were fasted overnight before the experiment, with water allowed ad libitum. Dogs were anesthetized the day of the experiment with sodium pentobarbital (30 mg/kg IV) and supplemented during the experiment as necessary. Ventilation was performed after endotracheal intubation (9.5-mm endotracheal tube) using a Harvard respirator and supplemental oxygen at 4 L/min. The
right femoral artery and both femoral veins were cannulated and polyethylene catheters inserted for mean arterial pressure monitoring, plasma sampling, supplemental anesthesia, and infusion of inulin and ANP. The left kidney was exposed through a retroperitoneal flank dissection. The ipsilateral ureter was cannulated, and a polyethylene catheter was inserted for timed urine collection. A calibrated electromagnetic flow probe (Carolina Medical Electronics, Inc) was placed on the left renal artery, and renal blood flow was recorded via a connected flowmeter (Carolina Medical Electronics, Inc, model FM501). A curved 22-gauge needle was inserted into the left renal artery and connected via polyethylene tubing to a syringe pump (Sage Instruments, model 341-A). Patency of the renal artery was maintained through the infusion of normal saline at 0.5 mL/min. Throughout the experiment the dogs were supported in a position that simulated their normal upright posture.

### Experimental Protocol

After surgical preparation, a 1-hour equilibration period was allowed. Inulin was administered by initial bolus, and a subsequent infusion was maintained at 1 mL/min via the femoral vein to achieve a plasma concentration of approximately 50 mg/dL. At the completion of the equilibration period, a 15-minute baseline clearance period (C1) was collected. Data obtained from this and each subsequent 15-minute clearance period included hemodynamic parameters, arterial blood (20 mL) for electrolytes and hormonal analysis, and urine for volume, electrolytes, and cGMP. After the baseline period, the study protocol differed between the two groups. In group 1 (n=6), after the baseline period, a-human-atrial natriuretic peptide (28 amino acid, Peninsula Laboratories, Kyowa Hakko Kogyo Co) was then injected as a bolus of 0.5 mg/kg into the left renal artery at the beginning of the third clearance (C3). Three subsequent 15-minute clearances were then collected after HS-142-1. In group 2 (n=6), a similar protocol was followed, but in the absence of ANP administration. Specifically, a 15-minute baseline clearance was followed by four 15-minute clearances obtained after intrarenal HS-142-1.

### Analyses

Plasma collected for electrolytes was placed in heparinized tubes, and plasma collected for hormonal assay was placed in EDTA tubes. All were placed on ice, centrifuged at 2500 rpm and 3°C, decanted and refrigerated (electrolytes) or stored (hormonal assay) at −20°C until analysis. Urine collected for electrolytes was refrigerated until analysis. Urine for hormonal assay was stored at −20°C; urine undergoing analysis for cGMP was heated to more than 90°C before storage. Plasma and urinary sodium concentrations were measured using ion-selective electrodes (Beckman Instruments). Plasma and urine inulin concentrations were measured by the anthrone method. Plasma and urine lithium concentrations were measured by flame-emission spectrophotometry (Instrumentation Laboratories, model 357) and used for the measurement and calculation of proximal and distal tubular sodium reabsorption. Plasma renin was measured by radioimmunoassay as previously documented. Inter assay and intra-assay coefficients of variation for ANP were 6% and 9%, for cGMP 4.2% and 2.5%, and for renin 7.6% and 5.6%, respectively.

### Calculations

The clearance of lithium (CIL) (mL/min) was calculated as [Urine Lithium × Urine Volume (UV)]/Plasma Lithium. These clearance data were then used to indirectly calculate the proximal fractional sodium reabsorption (PFN,R) and distal fractional sodium reabsorption (DFN,R).

### Table 1. Group 1 (Atrial Natriuretic Peptide + HS-142-1) Hemodynamic, Hormonal, and Renal Parameters

<table>
<thead>
<tr>
<th>Clearance Periods</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>133±11</td>
<td>119±7*</td>
<td>130±7†</td>
<td>126±9</td>
</tr>
<tr>
<td>ANP, pg/mL</td>
<td>50±6</td>
<td>1028±242*</td>
<td>1063±270*</td>
<td>979±286*</td>
</tr>
<tr>
<td>cGMP, pmol/mL</td>
<td>5.3±1</td>
<td>33.9±7*</td>
<td>23.6±5†</td>
<td>31.6±1*</td>
</tr>
<tr>
<td>PRA, (ng/mL)/h</td>
<td>2.8±0.89</td>
<td>1.9±0.79*</td>
<td>1.4±0.34</td>
<td>0.9±0.44</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td>21±3</td>
<td>30±4*</td>
<td>25±6</td>
<td>28±3</td>
</tr>
<tr>
<td>RBF, mL/min</td>
<td>237±36</td>
<td>212±32</td>
<td>222±35</td>
<td>184±16</td>
</tr>
<tr>
<td>UV, mL/min</td>
<td>0.24±0.04</td>
<td>1.23±0.22*</td>
<td>0.63±0.19†</td>
<td>0.92±0.23</td>
</tr>
<tr>
<td>UN,V, μEq/min</td>
<td>40.3±9</td>
<td>230±32*</td>
<td>87.6±16†</td>
<td>149±14*</td>
</tr>
<tr>
<td>FmR, %</td>
<td>14±0.42</td>
<td>5.6±0.97*</td>
<td>2.5±0.24†</td>
<td>3.7±0.27*</td>
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<tr>
<td>PFmR, %</td>
<td>69±4</td>
<td>60±8</td>
<td>66±9</td>
<td>62±6</td>
</tr>
<tr>
<td>DFmR, %</td>
<td>94.8±2.2</td>
<td>84.5±2.3*</td>
<td>88.5±4.7</td>
<td>89.1±1.7*</td>
</tr>
<tr>
<td>cGMP, pmol/min</td>
<td>941±235</td>
<td>4383±786*</td>
<td>3165±785†</td>
<td>3302±488†</td>
</tr>
</tbody>
</table>

C1 indicates baseline; C2, atrial natriuretic peptide (ANP) infusion, 100 ng/kg per minute; C3, ANP infusion and 15 minutes after HS-142-1, 0.5 mg/kg intrarenal bolus; C4, ANP infusion and 60 minutes after HS-142-1, recovery; MAP, mean arterial pressure; cGMP, cyclic GMP; PRA, plasma renin activity; GFR, glomerular filtration rate; RBF, renal blood flow; UV, urine volume; UN,V, urine sodium excretion; PfR, fractional sodium excretion; PfR, proximal fractional sodium reabsorption; DfR, distal fractional sodium reabsorption; and cGMP gen, renal cGMP generation. Lithium clearances were used for the indirect calculations of PfR and DfR. Values are expressed as mean±SEM.

*P<.05 vs C1. †P<.05 vs C2.
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A. 615

300

C1 C2 C3 C4 C5

C2 C3 C4 C1 C2 C3 C4 C5

T.1 C2 C3 C4

FIG 1. Bar graphs show urinary sodium excretion (UNaV) (A), renal cyclic GMP (cGMP) generation (B), and distal fractional sodium reabsorption (DFNaR) (C), calculated from lithium and sodium clearances, in group 1. Clearance periods are as follows: C1, baseline; C2, atrial natriuretic peptide (ANP) infusion, 100 ng/kg per minute; C3, ANP infusion and 15 minutes after HS-142-1, 0.5 mg/kg intrarenal bolus; and C4, ANP infusion and 60 minutes after HS-142-1, recovery. Experiments were conducted in anesthetized normal dogs. Values are expressed as mean ± SEM. *P < .05 vs C1; †P < .05 vs C2.

Fractional sodium reabsorption (DFNaR). Calculations were used as follows: PFNaR (%) = [1 - (Cl(Na)/GFR)] x 100; DFNaR (%) = (Cl(Na) - Clearance Sodium)/Cl(Na) x 100; and Renal cGMP Generation (pmol/min) = (Urine cGMP x UV) - (Plasma cGMP x GFR).

Statistical Analysis
For each experimental group, data from all clearance periods were measured and expressed as mean ± SEM. All data were assessed by Student’s paired t tests for comparisons of absolute changes within each group and by ANOVA for repeated measures. Statistical significance was accepted for P < .05.

Results
The baseline values in both groups were similar and not significantly different.

Group 1 (Atrial Natriuretic Peptide + HS-142-1)
Table 1 displays group 1 hemodynamic, hormonal, and renal parameters. Mean arterial pressure was significantly lowered with infusion of ANP and returned to baseline with administration of HS-142-1. Both plasma ANP and cGMP concentrations increased with ANP infusion. Whereas ANP concentrations did not change with the HS-142-1 bolus, plasma cGMP significantly decreased. Plasma renin activity decreased with ANP administration, and this trend did not change after administration of HS-142-1. No change was noted in hematocrit.

GFR was significantly increased with ANP and decreased to a level not significantly different from baseline during the clearances after HS-142-1. Renal blood flow did not change during the experiment. ANP markedly increased urine flow, which was significantly decreased by HS-142-1. Both absolute (Fig 1A) and

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fractional sodium excretions increased with ANP and decreased after HS-142-1, although they remained above baseline. Renal cGMP generation followed a trend similar to that of plasma cGMP, with attenuation persisting into the recovery period (Fig 1B). Whereas proximal fractional sodium reabsorption showed no significant trend with ANP or HS-142-1, distal fractional sodium reabsorption significantly decreased during ANP infusion, and HS-142-1 increased distal fractional sodium reabsorption to a level not significantly different from baseline (Fig 1C).

**Group 2 (HS-142-1 Alone)**

Table 2 displays group 2 hemodynamic, hormonal, and renal parameters. Mean arterial pressure did not change. Plasma cGMP decreased during the third clearance period with no change in plasma ANP. Additionally, a significant increase in plasma renin from baseline was also noted. Hematocrit did not change.

No significant changes in GFR or renal blood flow were observed. In this group urinary sodium excretion significantly decreased after the intrarenal HS-142-1 bolus (Fig 2A) and returned toward baseline during the final clearance of 60 minutes after HS-142-1. Renal cGMP generation decreased after the HS-142-1 bolus and gradually returned to baseline (Fig 2B). Fractional excretion of sodium followed a trend similar to that of urinary sodium excretion. Proximal fractional sodium reabsorption was unchanged until the last clearance period, when a significant increase was observed. Distal fractional sodium reabsorption progressively increased, peaking significantly during the fourth clearance period (Fig 2C).

**Discussion**

The present investigations report for the first time the modulating action of a unique ANP receptor antagonist, HS-142-1, when administered intrarenally on the renal actions of exogenous systemically administered ANP and endogenous circulating ANP. The present studies demonstrate that HS-142-1 reduces ANP-mediated natriuresis and renal cGMP generation in the intact anesthetized dog. Second, these studies using this inhibitor alone intrarena]ly support a role for endogenous ANP in regulating basal sodium excretion. Thus, new insight into the link between the renal NPR-A receptor and ANP in sodium homeostasis is provided through the present investigations.

HS-142-1, initially isolated from the fungus culture broth of *Aureobasidium pullulans var melanigenum*, was found to inhibit the binding of 125I-labeled rat ANP to its receptor. Further studies have demonstrated that HS-142-1 exerts its action by selectively inhibiting the particulate guanylyl cyclase receptor both in vitro and in vivo: HS-142-1 inhibited 125I-labeled rat ANP binding in bovine adrenocortical membranes, sites known to inhibit particulate guanylyl cyclase receptors. Inhibitory effects were also demonstrated in rat glomeruli, cultured bovine vascular smooth muscle cells, and LLC-PK1 cells. In the anesthetized rat, HS-142-1 selectively attenuated both diuresis and natriuresis, which was induced by ANP and brain natriuretic peptide, both of which bind the NPR-A receptor. Additionally, HS-142-1 showed inhibitory effects on C-type natriuretic peptide, which binds to the NPR-B receptor. In contrast, HS-142-1 had no inhibitory effect with radiolabeled rat ANP on membranes known to contain the clearance or soluble guanylyl cyclase receptor, such as the bovine lung membrane. Furthermore, pretreatment with HS-142-1 of aortic rings and in the anesthetized rat had no effect on vasorelaxation induced by sodium nitroprusside, also known to activate the soluble guanylyl cyclase receptor. Results of the current study support HS-142-1 as a particulate guanylyl cyclase re-
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The role of the natriuretic peptides in the control of basal sodium homeostasis in the setting of normal cardiac function has been further defined. Prior studies have shown that infusion of ANP to approximate physiological levels increased sodium excretion.2 Right atrial appendage removal in the rat attenuated ANP elevation and subsequent natriuresis during volume expansion.24 Furthermore, pretreatment with ANP antibodies decreased natriuresis after saline infusion.25 The current investigation demonstrates that HS-142-1 has an attenuating effect on basal urine volume, sodium excretion, and cGMP generation. This again appears to occur through modulation of distal tubular reabsorption and furthermore supports the hypothesis that ANP plays a significant role in basal sodium regulation.

In the present study and prior studies, exogenous ANP-mediated decreases in mean arterial pressure are reversed by HS-142-1.10 This may be best explained by antagonism of ANP-mediated reductions in cardiac output23 or arterial vasorelaxation, as suggested in studies that have reported that HS-142-1 inhibited ANP- and brain natriuretic peptide-induced vasorelaxation in rabbit aortic rings.11 In the present investigation, HS-142-1 reversed the ANP-induced decrease in arterial pressure, whereas no change was observed with physiological levels of ANP. Furthermore, in both conditions significant decreases in plasma cGMP were observed, thereby suggesting that physiological levels of endogenous ANP are more important in the regulation of basal sodium excretion than blood pressure.

In the present study pharmacological levels of ANP significantly decreased plasma renin. Plasma renin activity remained suppressed after HS-142-1, supporting the potent renin-inhibitory action of pharmacological concentrations of ANP. In contrast, in the setting of physiological concentrations of ANP, plasma renin significantly increased after HS-142-1. Previous studies have reported that ANP inhibits renin secretion. This mechanism is thought to be mediated through a macula densa mechanism and/or through a direct NPR-A receptor-mediated action.5 Thus, the importance of endogenous ANP in the control of basal renal function is underscored by the actions of intrarenal HS-142-1 to both decrease sodium excretion and increase plasma renin activity. It should be noted that the present study was conducted in anesthetized dogs and that anesthesia may enhance the renal response to physiological concentrations of circulating ANP, as reported by Goetz et al.27

In summary, HS-142-1 attenuated urine volume, sodium excretion, and renal cGMP generation through modification of GFR and distal fractional sodium reabsorption via inhibition of the particulate guanylyl cyclase receptor in the presence of exogenous and endogenous ANP. Our results with this inhibitor not only provide further insight into the renal actions of exogenous ANP but suggest for the first time that endogenous ANP plays a significant role in the mediation of basal sodium homeostasis and the renin-angiotensin system in the setting of normal cardiac function.

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