Pharmacodynamics of Atrial Natriuretic Peptide in Isolated Perfused Dahl Rat Kidneys

Michael E. Brier, Phenius V. Lathon, George R. Aronoff, and Friedrich C. Luft

Atrial natriuretic peptide (ANP) has been implicated in the development of hypertension in Dahl R and S rats. To test the responses of DR and DS kidneys in the absence of the influence of neural and humoral mechanisms, we investigated the pharmacokinetics and pharmacodynamics of ANP in isolated perfused DR and DS kidneys, obtained from rats given a high or low sodium diet, after a bolus injection of ANP (1μg) or after a bolus injection plus infusion of ANP to maintain the perfusate concentration at 1000 pg/ml. The elimination rate constant was not different between the groups (DR, 0.044 min⁻¹ vs. DS, 0.050 min⁻¹). Clearance of ANP was 4 times greater than the glomerular filtration rate, indicating that a receptor-mediated peritubular clearance is probably the primary route of elimination. DS kidneys excreted 50% less sodium than DR kidneys. However, ANP caused a 5-fold increase in fractional sodium excretion in both DR and DS. ANP also increased sodium excretion, creatinine clearance, and urine flow. No alteration in ANP kinetics occurred to account for the reportedly increased circulating concentrations of ANP seen in DS rats. We conclude that isolated DR and DS kidneys respond differently to ANP after bolus ANP administration to concentrations of 10,000 pg/ml. This difference in response is due to the sodium excretory defect inherent in the DS kidney and not to an alteration in the DS kidney's ANP responsiveness. (Hypertens Res 1995; 18: 219-225)

Key Words: salt, sodium, Dahl rats, isolated perfused kidneys, atrial natriuretic peptide

The Dahl rat is a genetic model of salt-sensitive hypertension in which the kidneys are directly involved in the production of hypertension (1-4). Atrial natriuretic peptide (ANP) has been proposed as an important modulator in the natriuretic response in this rat model of salt-induced hypertension. Differences in atrial ANP concentration, blood ANP concentration, and renal responses to ANP between Dahl salt-sensitive (DS) and salt-resistant (DR) rats have been reported (5-9). DS rats are reported to have greater atrial and plasma ANP concentrations than DR rats. Increased plasma concentrations may be due to decreased renal elimination or increased ANP release. Furthermore, the kidneys of DS rats are hyporesponsive to ANP in that changes in glomerular filtration rate, fractional excretion of sodium, and sodium excretion are greater in DR than in DS rats. However, not all investigators have observed these differences in renal function between DS and DR rats following ANP infusions (10, 11). Since cross-transplantation experiments show that the kidneys of Dahl rats are directly involved in the onset of hypertension, we chose to investigate the response of Dahl rat kidneys to ANP. We tested the hypotheses that the renal clearance of ANP by DS kidneys is impaired, and that DS kidneys do not excrete sodium to the same magnitude as DR kidneys in response to ANP administration.

Methods
Forty DS and 40 DR rats (Brookhaven strain) were purchased from Harlan Laboratories at 8 weeks of age and given either a low (0.05%) or high (4.0%) sodium diet. Experiments were performed when the animals reached 13 weeks of age. The rats were anesthetized with 50 mg/kg pentobarbital and a catheter was placed in the common carotid artery for blood pressure measurements. Perfusion of the kidney was performed as described previously (12). The right ureter was cannulated with PE10 tubing for urine collection. Sutures were placed around the right renal artery and superior mesenteric arteries. The animal was given heparin (1,000 U/kg) intravenously. The right kidney was cannulated with a blunt, 18 gauge steel needle, passed through the superior mesenteric artery and placed in the right renal artery. The sutures were ligated and the kidney was excised from the animal and placed above a water-jacketed glass condenser. During this process, perfusion of the kidney was maintained by gravity.
flow of the perfusion medium from a washout reservoir. Once the kidney was in place above the condenser, the flow was switched to 100 ml of recirculating perfusate, provided by a non-pulsatile pump. The perfusion circuit contained an in-line flow meter and a mercury manometer for the measurement of perfusate flow and pressure, respectively. These measures were recorded at the midpoint of the urine collection intervals. The perfusion medium was a Krebs-Henseleit buffer containing 5.5 g/100 ml fraction V bovine serum albumin, 100 mg/100 ml glucose, 10 mg/100 ml creatinine, and 20 amino acids as reported by Epstein et al. (13) to maximize renal function. The kidneys were allowed to equilibrate for 20 min before the experiments were begun.

Bolus Experiments
ANP was given as a 1-μg bolus injection. Following this dose, urine was collected in pre-weighed plastic beakers at 10-min intervals for 90 min. Perfusion pressure and flow were recorded at the midpoint of the urine collection interval. Renal vascular resistance was calculated as the perfusion pressure / perfusion flow. Comparisons were made between DR and DS based on mean values for the 90 min experiment. Perfusate samples (1.5 ml) were taken at the midpoint of the urine collection interval.

Infusion Experiments
ANP was given by bolus injection and by infusion to maintain the perfusate ANP concentration at 1,000 pg/ml. The infusion experiments were run for 50 min, with urine collection periods 1 and 2 serving as control periods. At the conclusion of urine collection period 2, a bolus injection of ANP (100 ng) was given and, concurrently, infusion of ANP (3.5 ng/min) was begun. Comparisons were made between the mean values for periods 1 and 2 and the mean values for periods 4 and 5. Period 3 served as an equilibrium period for the ANP infusion. Perfusate samples (1.5 ml) were taken at the midpoint of the urine collection interval.

Assays and Calculations
One milliliter of the perfusate sample was added to 1.5 ml 0.1 M acetic acid and prepared for ANP extraction. ANP was extracted by heat precipitation (14). Samples were heated at 85°C for 10-minutes. ANP was separated from the precipitate by centrifugation at 10,000 g for 3-minutes. The supernatant was diluted to within the range of the standard curve and the RIA assay performed as described previously (12). The standard curve ranged from 10 - 1,500 pg/ml. The extraction efficiency was 59%. Perfusion pressure and flow were determined at the midpoint of the urine collection interval. The actual perfusion pressure was determined by subtracting the pressure created by the internal resistance of the apparatus from the total perfusion pressure at the observed flow rates. Renal vascular resistance was calculated by dividing the renal perfusion pressure by the renal perfusion flow. Urine and perfusate concentrations of sodium and potassium were deter-

Fig. 1. (upper panel) Semi-logarithmic plot of the ANP concentration versus time in perfusate from DR (■) and DS (○) kidneys from rats maintained on a low (0.05%) sodium diet. Values are mean ± SD. (lower panel) Semi-logarithmic plot of the ANP concentration versus time in perfusate from DR (■) and DS (○) kidneys from rats maintained on a high (4.0%) sodium diet. Values are mean ± SD.
mined using a Beckman flame photometer. Creatinine was determined in urine and perfusate using a Beckman creatinine analyzer.

The elimination rate constant (Ke) in the bolus experiments was determined by linear regression of the natural logarithm of the ANP concentration versus time. Total ANP clearance (Cl) was calculated from the relationship Cl = dose/AUC, where AUC is the area under the concentration-time curve. AUC was calculated by dividing the y-intercept by Ke. Peritubular clearance (Clp) was calculated as Cl-GFR, where GFR is the glomerular filtration rate. The elimination half-life was calculated as ln2/ke.

Exogenous creatinine clearance was used as a measure of GFR. Since all transport mechanisms are saturated at these concentrations, the creatinine clearance is identical to the inulin clearance. Clearance of creatinine, sodium, and potassium were calculated using the following equation: Cl = urine concentration X urine volume/perfusate concentration X time. The fractional excretion of sodium and potassium was calculated by dividing the individual clearance by the GFR and expressed as a percentage.

A two-way analysis of variance (ANOVA) was used to test renal function and pharmacokinetic parameters in both the bolus and infusion experiments. The factors tested were the effect of Group (DR vs. DS) and diet (0.05% Na vs. 4.0% Na). Further analysis of the bolus renal function parameters was performed using ANOVA with repeated measures to detect the effect of time. Results are shown as the mean ± the standard deviation, except when stated otherwise. Half-life is reported as the harmonic mean.

**Results**

Mean arterial blood pressure was significantly higher in the DS group (143 ± 8 mmHg) than in the DR group (116 ± 16 mmHg) after 5 weeks of the 4% salt diet, indicating that these rats were indeed salt sensitive. ANP concentrations in the perfusion medium following bolus administration in rats fed a low salt or high salt diet are shown in the upper and lower panels of Fig. 1, respectively. Perfusate ANP concentrations declined exponentially, with no difference in rate between DS and DR. The pharmacokinetic parameters are shown in Table 1. The elimination rate constant and the resulting half-life were not different by group or by diet. ANP clearance was lower in kidneys from rats given the high, compared to the low, sodium diet (p=0.026). Peritubular clearance of ANP was the predominant route of kidney elimination, accounting for more than 75% of the total clearance in both groups.

Renal function parameters (mean ± SD for the 90 min experiment) following bolus ANP administration are shown in Table 2. Two-way ANOVA did not
show a difference in any of the measured variables except for FENa. DS had a lower FENa than did DR with a low sodium diet. This effect on FENa was absent in kidneys from rats fed a high sodium diet. The effect of bolus ANP administration on UNaV is shown in Fig. 2. DR kidneys excreted 1.5-fold more sodium than did DS kidneys \((p = 0.05)\) in response to ANP. However, there was no effect of diet on the ANP response. Repeated measures analysis of variance showed that DR had a greater FENa than did DS during the course of the experiment \((p = 0.05)\). GFR and UF increased after the administration of ANP and peaked during the third collection interval for both DR and DS. However, there were no differences between DR and DS. UF returned to baseline values by the sixth collection interval.

The pharmacokinetic results of the infusion experiments are shown in Table 3. DS did not differ from DR with respect to the calculated ANP clearance. However, as in the bolus experiments, diet did affect ANP clearance. Kidneys from rats given a

Table 3. Pharmacokinetic Parameters for the Infusion of ANP to Dahl Rat Kidneys from Rats Fed Low and High Sodium Diets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low sodium diet</th>
<th>High sodium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salt-resistant</td>
<td>Salt-sensitive</td>
</tr>
<tr>
<td>(n)</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Cl (ml/min)</td>
<td>3.75 ± 0.85</td>
<td>3.95 ± 0.36</td>
</tr>
<tr>
<td>CSS (ng/ml)</td>
<td>0.98 ± 0.19</td>
<td>0.89 ± 0.08</td>
</tr>
</tbody>
</table>

* Different from low sodium diet by two-way ANOVA.

Values are mean ± SD. Cl, clearance; CSS, steady-state concentration.

Table 4. Renal Function Parameters Following Infusion of ANP to DR and DS Kidneys from Rats Fed Low and High Sodium Diets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period</th>
<th>Low sodium diet</th>
<th>High sodium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Salt-resistant</td>
<td>Salt-sensitive</td>
</tr>
<tr>
<td>CICR (ml/min)</td>
<td>Control</td>
<td>0.32 ± 0.19</td>
<td>0.60 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>ANP</td>
<td>1.22 ± 1.41*</td>
<td>1.46 ± 2.11*</td>
</tr>
<tr>
<td>FeNa (%)</td>
<td>Control</td>
<td>1.93 ± 1.01</td>
<td>1.91 ± 1.01</td>
</tr>
<tr>
<td></td>
<td>ANP</td>
<td>3.51 ± 1.88*</td>
<td>3.22 ± 2.33*</td>
</tr>
<tr>
<td>FeK (%)</td>
<td>Control</td>
<td>15.6 ± 12.4</td>
<td>27.8 ± 28.6</td>
</tr>
<tr>
<td></td>
<td>ANP</td>
<td>30.8 ± 12.9</td>
<td>33.2 ± 33.8</td>
</tr>
<tr>
<td>RVR (mmHg/min/ml)</td>
<td>Control</td>
<td>2.38 ± 0.84</td>
<td>2.52 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>ANP</td>
<td>2.31 ± 0.71</td>
<td>2.72 ± 0.98</td>
</tr>
<tr>
<td>RPP (mmHg)</td>
<td>Control</td>
<td>63 ± 8</td>
<td>68 ± 9</td>
</tr>
<tr>
<td></td>
<td>ANP</td>
<td>62 ± 8</td>
<td>69 ± 10</td>
</tr>
<tr>
<td>RPF (ml/min)</td>
<td>Control</td>
<td>32 ± 5</td>
<td>29 ± 8</td>
</tr>
<tr>
<td></td>
<td>ANP</td>
<td>33 ± 4</td>
<td>30 ± 6</td>
</tr>
<tr>
<td>UF (ml/min)</td>
<td>Control</td>
<td>18 ± 6</td>
<td>30 ± 13</td>
</tr>
<tr>
<td></td>
<td>ANP</td>
<td>118 ± 33**</td>
<td>95 ± 37**</td>
</tr>
</tbody>
</table>

* Different from control \(p < 0.05\); ** different from control \(p < 0.01\); * Different from salt-resistant \(p < 0.05\) by two-way ANOVA. Values are mean ± SD. CICR, creatinine clearance; FeNa, fractional sodium excretion; FeK, fractional potassium excretion; RVR, renal vascular resistance; RPP, renal perfusion pressure; RPF, renal perfusion flow; UF, urine flow.
high sodium diet had a reduced ANP clearance, approximately 20% lower than that in rats given a low sodium diet. This decreased clearance of ANP resulted in a significant increase in the apparent steady-state concentration of ANP. ANP concentrations averaged 1,260 pg/ml in the high salt group and 930 pg/ml in the low salt group. The renal function parameters for the infusion experiments are shown in Table 4. Comparisons are made by group (DR vs. DS) and treatment (Control vs. ANP) for both low and high sodium diet. ANP administration had significant effects on the clearance of creatinine, fractional sodium excretion, and urine flow rate. Differences between DR and DS were only seen in FeK in kidneys from rats given a high sodium diet.

The effect of ANP infusion on UNaV for both DR and DS on a low and high sodium diet are shown in the upper and lower panels of Fig. 3, respectively. ANP caused significant increases in UNaV for both DR and DS. DS had a similar response to ANP infusion as did DR. No differences were observed between DR and DS in the control period or following ANP infusion, at either the low or high sodium diet.

Discussion

The Dahl DS rat is a model of salt-sensitive hypertension shown by some investigators to have increased concentrations of circulating ANP and an altered response to exogenously administered ANP (5-11). Increased circulating concentrations of ANP may be caused by increased peptide release or decreased renal elimination of ANP. The results of the present study show that the ANP elimination was linear following bolus administration and that the elimination rate was not different between DR and DS, with either bolus or infusion administration. Furthermore, the elimination half-life was similar to that reported for perfused Wistar rat kidneys (12) but shorter than the 25-27 minutes reported for SHR and WKY rat kidneys (15). These results suggest that the increased circulating concentrations of ANP seen in DS cannot be attributed to decreased ANP renal clearance. Rather, increased release is most likely responsible for the increased DS plasma ANP values, as suggested previously (16).

A high salt (sodium) diet does appear to have an effect on ANP clearance and resulting ANP concentrations. Kidneys from rats given a high sodium diet had a decreased ANP clearance after either bolus or infusion administration and also had increased steady-state ANP concentrations after ANP infusion. ANP has been shown to be cleared by receptors located in the vasculature and glomerular structures (17-19). We can estimate the contribution of this receptor to mediating clearance by subtracting the glomerular filtration rate from the total clearance. In kidneys from rats fed a high sodium diet, the contribution of receptor-mediated clearance to the total clearance is decreased. Therefore, a high sodium diet causes a decrease in ANP clearance due to a decrease in the number of clearance receptors. This effect is independent of the development of salt-sensitive hypertension. However, in whole animal experiments, Sprague Dawley rats fed an 8% NaCl diet showed a decrease in circulating ANP and an increase in ANP clearance, which was attributed to a rise in the clearance-receptor concentration (20, 21). The findings of Widimsky et al. (21) suggested that these changes were limited to changes in volume of distribution and not half-life, which suggests that the clearance receptor may mediate the plasma concentration of ANP by changing the volume of distribution. Our findings are not consistent with those of Widimsky et al. (21). The observation that a high sodium diet moderated a change in the pharmacokinetics of ANP by regulation of the clearance-receptor population is intriguing. It remains to be determined in which
DS kidneys have a reduced capacity for sodium excretion (22–24). We confirmed this observation in the bolus study, where DS kidneys excreted less sodium than DR kidneys. Furthermore, the sodium excretory defect was not related to decreased GFR in DS rats. We conclude from our work in the isolated kidney and that of others (22–24) that DS kidneys retain sodium, even in the absence of neuronal and hormonal control, when perfused at similar pressures. This sodium retention may be related to decreased delivery of sodium to the proximal tubules, inherent differences in the sodium transport by tubular cells, or changes in renal hemodynamics (22). However, we could find no difference in GFR or RVR between DR and DS. DS rats have been shown to be hyporesponsive to ANP (5, 6, 8, 25, 26). However, in our study both FeNa and UNaV increased in DR and DS in response to ANP administration. The 2-fold difference in FeNa between DR and DS rat kidneys seen in the first collection interval continued up to the end of the experiment. Therefore, DS kidneys did not appear to be hyporesponsive to ANP. Instead, DS kidneys responded to the same magnitude as DR kidneys. DS kidneys excreted less total sodium than DR kidneys because of intrinsic differences between DR and DS kidneys, not because of a difference in response to ANP. The differences between DR and DS with respect to baseline sodium excretion are lost in the infusion experiments as a whole. Differences in methodologies and time-related factors may account for this observation and also preclude direct comparisons between experiments.

The natriuretic response to ANP may be affected by dose. Steel and Challoner-Hue gave 6-fold greater bolus doses than used in our study and showed a difference in sodium excretion between DR and DS kidneys (23). We conclude that the effect of ANP on UNaV is dose-dependent. High doses of ANP result in a significant difference in sodium excretion between DR and DS; this effect is more pronounced in rats receiving a high sodium diet, and ANP administration appears to have a greater effect in DR kidneys (23). At lower doses, DR kidneys still excrete more sodium than DS kidneys; however, the differences were present prior to ANP treatment and may be related to a lower “set point” for DS kidneys and not to an altered renal response. Thus, it appears that the Dahl kidney response to ANP is dose and salt dependent.

The Dahl rat kidney and ANP provide a model system for the study of receptor-mediated clearance. Pharmacokinetic information gained from this system may be useful in understanding other systems that involve drug-receptor interactions. Examples include the colony stimulating factors, immunoglobins, and other such protein drugs. The present study showed that the method of administration (bolus vs. constant infusion) and the dietary sodium content affect ANP clearance. Similar factors may play a role in the clearance of other peptide drugs.

Finally, the importance of the present results to hypertension research centers around defining the proper phenotype for DS and DR rats. Molecular genetic differences between DS and DR rats have been identified, including studies examining cosegregation of blood pressure with the ANP receptor gene (27). In some instances, gene loci linked to hypertension in DS rats have been found for which the function of the gene such as the SA locus, for example, is unknown (28). Our results suggest the presence of an inherent alteration within the kidneys of DS rats, rather than a defect in the hormones that drive them. Furthermore, we have demonstrated that a decrease in renal responsiveness to ANP is unlikely. This finding decreases the probability that the ANP receptor gene plays an important role in DS hypertension (27). Such findings also emphasize the relevance of physiologic studies directed at the phenotypization of genetic models.

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