Stress Increases Renal Nerve Activity and Decreases Sodium Excretion in Dahl Rats

JOHN P. KOEPE, SUSAN JONES, AND GERALD F. DIBONA

SUMMARY The effects of a stressful environmental stimulus (air stress) on mean arterial pressure, renal sympathetic nerve activity, and renal function were examined in conscious Dahl salt-sensitive (DS) and Dahl salt-resistant rats (DR) on low (0.4%) and high (8%) NaCl diets. Air stress increased renal sympathetic nerve activity and decreased urine flow rate and urinary sodium excretion in conscious Dahl rats on a high sodium diet, but it had no effect in rats on a low sodium diet. Mean arterial pressure did not change during air stress in any group. Renal denervation prevented the antidiuretic and antinatriuretic responses to air stress in DS and DR on a high NaCl diet. An increased renal tubular reabsorption of sodium and water appeared to mediate the antinatriuretic and antidiuretic responses to air stress, since glomerular filtration rate and renal plasma flow were unchanged. Thus, environmental stress increases renal sympathetic nerve activity and decreases urinary sodium excretion more in Dahl rats on a high NaCl diet than on a low NaCl diet. On a high NaCl diet, these responses are greater in DS than in DR. (Hypertension 11: 334-338, 1988)

KEY WORDS • hypertension • salt sensitivity • kidney function

An interaction of genetic predisposition to hypertension with environmental stress and dietary sodium intake has been described for the neural control of renal function in conscious rats.1 In conscious spontaneously hypertensive rats (SHR), a genetic-dependent model of hypertension, environmental stress (air stress) increases renal sympathetic nerve activity and decreases urinary sodium excretion to a greater degree than in conscious normotensive Wistar-Kyoto rats (WKY).2,3 High dietary sodium intake (0.9% NaCl to drink for 15 days) enhances the increased renal sympathetic nerve activity and antinatriuretic responses to air stress in conscious SHR but has no additional effects in WKY.3 Similarly, in conscious deoxycorticosterone acetate (DOCA)–NaCl rats, a sodium-dependent model of hypertension, air stress increases renal sympathetic nerve activity and decreases urinary sodium excretion but has no effect in conscious sham-treated DOCA-NaCl rats.4 The importance of the renal sympathetic nerves is indicated by the finding that surgical renal denervation prevents the antinatriuretic response to air stress in conscious SHR on either a normal or high sodium intake and in DOCA-NaCl hypertensive rats.2,4 Thus, in both a genetic-dependent and a sodium-dependent model of hypertension, environmental stress causes increased renal sympathetic nerve activity with renal sodium retention.

Hypertension in the Dahl salt-sensitive rat (DS) is dependent on a combination of genetic and environmental factors. The DS is genetically predisposed to become hypertensive, and the Dahl salt-resistant rat (DR) is genetically predisposed to resist hypertension when exposed to a high sodium diet.5 In addition, the sympathetic nervous system contributes to the pathogenesis of hypertension in DS.5 Thus, the Dahl rat provides a unique model of hypertension to further characterize the interaction of genetic predisposition to hypertension with environmental stress and dietary sodium intake in the neural control of renal function.

Materials and Methods

DS and DR were obtained from Brookhaven National Laboratories (Upton, NY, USA). At 4 weeks of age, rats were placed on either a high or a low NaCl diet (8% or 0.4% NaCl; ICN Nutritional Biochemicals,
Cleveland, OH, USA). All rats were allowed water ad libitum for drinking. The care and use of animals were within the guiding principles established by the National Institutes of Health.

Renal denervation was performed in 10 DS and seven DR 7 to 10 days before experimentation by surgically stripping the renal arteries and veins of adventitia, cutting all visible nerve bundles under a dissecting microscope (x 25), and coating the vessels with a solution of 10% phenol in 95% ethanol, as previously described. This denervation procedure prevents the antinatriuretic response to stress in SHR and DOC-NaCl rats, prevents the vasoconstrictor response to suprarenal lumbar sympathetic nerve stimulation, reduces renal catecholamine histofluorescence to nondetectable levels, and reduces renal norepinephrine concentration to less than 10% of control.

At 9 to 10 weeks of age, all rats were anesthetized with methohexital sodium (Brevital, 30 mg/kg i.p., supplemented by 10 mg/kg i.v. as needed; Eli Lilly) and implanted with catheters in the jugular vein, carotid artery, and bladder. Twenty rats were also implanted with an electrode to record renal sympathetic nerve activity. The left kidney was exposed through a flank incision, and a renal nerve bundle from the aortorenal ganglion was dissected and placed on a bipolar silver wire (Cooner Wire, Chatsworth, CA, USA) electrode. Renal sympathetic nerve activity was amplified (10,000–50,000 times) and filtered (low = 30 Hz, high = 3000 Hz) using a Grass P511 bandpass amplifier (Quincy, MA, USA) and a high impedance probe (Model HIP511, Grass Instruments). The filtered and amplified signal was channeled to a Tektronix 5113 oscilloscope (Beaverton, OR, USA) and Grass Model 7DA polygraph for visual evaluation, to an audio amplifier-loudspeaker (Grass Model AM8 audiometric) for auditory evaluation, and to a rectifying voltage integrator (Grass Model 7P10). The integrated voltage and neurogram signals were displayed on the Grass polygraph. The quality of the sympathetic nerve activity signal was assessed intraoperatively by examining the magnitude of decrease in recorded sympathetic nerve activity during sinoaortic baroreceptor loading with an intravenous injection of norepinephrine (3–4 μg). When an optimal sympathetic nerve activity signal was observed, the recording electrode was fixed to the renal nerve branch with a silicone adhesive (Wacker Sil-Gel 604, Wacker-Chemie, Munich, FRG). The electrode cable was tunneled to the back of the neck and exteriorized, and the flank incision was closed in layers.

Rats were then placed in Lucite cylinders, and a 5% dextrose solution was infused (30 μl/min i.v.) for 30 minutes. Three to 5 hours after surgical preparation, an isotonic saline infusion was begun (60 μl/min), the arterial catheter was flushed and attached to a pressure transducer (Model P23Db, Statham, Oxnard, CA, USA), and a 3-cm polyethylene catheter was attached to the urinary bladder catheter and led to a collection beaker. Inulin (30 mg/100g body wt/hr) and p-aminohippurate (PAH; 3–4 mg/100g body wt/hr) were added to the isotonic saline infusion to measure inulin and PAH clearances. The renal sympathetic nerve activity-recording electrode cable was connected to the high impedance probe that in turn was connected to the bandpass amplifier. The quality of the renal nerve activity recording was tested with an intravenous injection of norepinephrine (3–4 μg) as already described to ensure the absence of noise due to mechanical movement, respiration, or heart rate. If the quality of the sympathetic nerve activity recording was similar to that observed when the electrode was implanted, then the experiment commenced.

After 60 minutes of equilibration to the infusion, two consecutive 10-minute control periods were obtained followed by a 10-minute environmental stress (air stress) period and then by two 10-minute recovery periods. Five minutes was allowed after the onset of the stress before the collection period began; similarly, 5 minutes was allowed after the offset of stress before the recovery collections began. Environmental stress consisted of a continuous (10-minute) air jet delivered from 4 to 5 cm in front of the rat; because the air jet was delivered in the same manner among groups, the intensity of the air jet was assumed to be the same among groups. Ten-minute urine collections were made for each period, and venous blood samples (150 μl) were taken just before the control periods, before and after the air stress period, and after the recovery periods. At the end of each experiment, the quality of the sympathetic nerve activity recording was again assessed with an intravenous injection of norepinephrine (3–4 μg). Finally, the rats were killed with an overdose of methohexital sodium and postmortem renal nerve activity was recorded as a measure of background noise; these values (<0.5 integrator reset/min) were subtracted from all experimental values of renal sympathetic nerve activity.

Urine volume was determined gravimetrically. Urine and plasma sodium concentrations were measured by flame photometry (Model 143, Instrumentation Laboratories, Lexington, MA, USA). Urine and plasma inulin and PAH concentrations were determined by the anthrone and ethylenediamine methods, respectively. Glomerular filtration rate (inulin clearance), renal plasma flow (PAH clearance), and fractional excretions of sodium and water were calculated as previously described. Mean arterial pressure and heart rate were calculated from five 2-minute averages for each 10-minute collection period. Statistical analyses were performed with repeated-measures analyses of variance for main effects and interactions and Tukey's Honestly Significant Difference test for pairwise comparisons among means. Data are presented as means ± SE. Statistical significance was defined as a p level below 0.05.

Results

Body weights were similar in DS on low or high NaCl intake (216 ± 10 vs 206 ± 8 g) and in DR on low or high NaCl intake (214 ± 10 vs 192 ± 12 g). Kidney weights, however, were higher (p<0.05) in high...
NaCl diet than low NaCl diet rats (DS = 2.27 ± 0.05 vs 1.76 ± 0.06 g; DR = 2.03 ± 0.03 vs 1.78 ± 0.08 g). High NaCl intake increased baseline mean arterial pressure in DS (Figure 1) rats but not in DR (Figure 2). High NaCl intake increased baseline urine flow rate and urinary sodium excretion in both DS (see Figure 1) and DR (see Figure 2), but it had no effect on baseline glomerular filtration rate or renal plasma flow (Table 1).

Air stress had no effect on mean arterial pressure in DS (see Figure 1) or DR (see Figure 2) on low or high NaCl intake. In DS (see Figure 1) and DR (see Figure 2) on high NaCl intake, air stress decreased urine flow rate and urinary sodium excretion and increased renal sympathetic nerve activity. The decreases in urinary sodium excretion and increases in renal sympathetic nerve activity were greater (p < 0.05) in DS than in DR. Surgical renal denervation abolished the antidiuretic and antinatriuretic responses to air stress in DS (see Figure 1) and DR (see Figure 2) on high NaCl intake. In low NaCl diet DS (see Figure 1) or DR (see Figure 2), air stress had no effect on urine flow rate, urinary sodium excretion, or renal sympathetic nerve activity. Air stress had no effect on glomerular filtration rate or renal plasma flow in any group (see Table 1). Fractional water excretion and fractional sodium excretion decreased during air stress in DS and DR rats on high NaCl intake (see Table 1). These decreases were greater (p < 0.05) in DS than in DR.

**Discussion**

The main finding of this study is that air stress increases renal sympathetic nerve activity and decreases urine flow rate and urinary sodium excretion in conscious DS on a high NaCl diet, but not on a low NaCl diet. Similarly, in DR on a high NaCl diet, air stress increases renal sympathetic nerve activity and decreases urine flow rate and urinary sodium excretion. However, the responses to air stress are greater in DS than in DR. Renal denervation prevents the antidiuretic and antinatriuretic responses to air stress in DS and DR, indicating that the renal sympathetic nerves...
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**TABLE 1. Effects of Air Stress on Renal Function in Conscious DS and DR on Low or High Sodium Diets**

<table>
<thead>
<tr>
<th>Variable</th>
<th>DS</th>
<th>DR</th>
<th>HNa-DNX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Renal plasma flow (ml/min/g KW)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.80 ±0.59</td>
<td>3.34 ±0.27</td>
<td>3.28 ±0.25</td>
</tr>
<tr>
<td>A</td>
<td>3.26 ±0.83</td>
<td>3.67 ±0.36</td>
<td>3.55 ±0.85</td>
</tr>
<tr>
<td>R</td>
<td>2.94 ±0.17</td>
<td>3.39 ±0.28</td>
<td>3.55 ±0.85</td>
</tr>
<tr>
<td>Fractional water excretion (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.34 ±0.49</td>
<td>3.32 ±0.69</td>
<td>1.65 ±0.45</td>
</tr>
<tr>
<td>A</td>
<td>1.32 ±0.37</td>
<td>1.64 ±0.40*</td>
<td>1.31 ±0.36</td>
</tr>
<tr>
<td>R</td>
<td>1.69 ±0.67</td>
<td>4.01 ±1.26</td>
<td>1.35 ±0.45</td>
</tr>
<tr>
<td>Fractional sodium excretion (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.33 ±0.09</td>
<td>3.49 ±0.60</td>
<td>0.99 ±0.30</td>
</tr>
<tr>
<td>A</td>
<td>0.46 ±0.12</td>
<td>3.79 ±0.77</td>
<td>0.86 ±0.24</td>
</tr>
<tr>
<td>R</td>
<td>0.39 ±0.20</td>
<td>4.22 ±0.93</td>
<td>0.85 ±0.21</td>
</tr>
</tbody>
</table>

Data are means ± SE. DN = renal denervation; LNa = low sodium diet; HNa = high sodium diet; KW = kidney weight; C = control; A = air stress; R = recovery.

*p < 0.05, compared with control values.

are responsible for the renal responses. These results point to an important interaction among environmental factors (stress, dietary sodium) and genetic factors in the neural control of renal function. A similar interaction has been described for the SHR. The genetic factor does not appear to be required, however, since the high NaCl diet in DR results in increased renal sympathetic nerve activity, antidiuresis, and antinatriuresis. Similarly, in the DOCA-NaCl rat (a sodium-dependent model of hypertension), which also has no strong genetic predisposition to become hypertensive, air stress increases renal sympathetic nerve activity and decreases urine flow rate and urinary sodium excretion. Yet, this study and another indicate that the interaction among environmental stress, sodium diet, and genetic factors results in stronger neural control of renal function in conscious rats than does the interaction between stress and dietary NaCl intake.

The renal mechanism of the antidiuretic and antinatriuretic responses to air stress in conscious DS and DR on a high NaCl diet appears to be increased renal tubular reabsorption of water and sodium. This conclusion is based on the finding that glomerular filtration rate and renal plasma flow are not affected by air stress in any group. Moreover, mean arterial pressure is not affected by air stress and therefore does not account for the antidiuretic or antinatriuretic responses to air stress.

The central nervous system mechanisms of the antidiuretic and antinatriuretic responses to air stress in conscious DS and DR on a high NaCl diet are not known. However, electrical stimulation of the ventromedial hypothalamus results in greater increases in arterial pressure and abdominal sympathetic nerve activity in DS than in DR on either a low or a high NaCl diet. Moreover, lesion of the anteromedial hypothalamus prevents hypertension in DS on a high NaCl diet. Similarly, the sensitivity of baroreceptor reflex control of heart rate and splanchnic sympathetic nerve activity is impaired in DS. High NaCl intake decreases hypothalamic norepinephrine concentration and increases the sensitivity of central nervous system α2-adrenergic receptors in SHR, suggesting that high NaCl intake may contribute to hypertension by reducing the steady state level of stimulation of central sympathetic inhibitory α2-adrenergic receptors. High NaCl intake in DS elevates renal α2-adrenergic receptor density, but it is not known whether high NaCl diet increases central nervous system α2-adrenergic receptors in Dahl rats. The results of the present study indicate that a main locus of action of high NaCl diet is the central nervous system. If Dahl rats are affected by high NaCl intake as are SHR, reduced steady state stimulation of the central sympathetic inhibitory α2-adrenergic receptors may lead to the enhanced responsiveness of renal sympathetic nerve activity to environmental stress and, consequently, renal sodium retention.

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References
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