Skeletal Muscle Arteriolar Reactivity in SS.BN13 Consomic Rats and Dahl Salt-Sensitive Rats

Ines Drenjancevic-Peric, Jefferson C. Frisbee, Julian H. Lombard

Abstract—Previous studies have demonstrated that angiotensin II is a crucial factor in maintaining normal vascular reactivity. In this study, we tested the hypothesis that altered reactivity to vasoactive stimuli in Dahl salt-sensitive (S) rats on a high salt diet could be prevented by introgression of chromosome 13 from the normotensive Brown Norway strain, which carries a normally functioning renin gene. Dahl S and consomic SS.BN13 rats were fed a low salt (0.4%) or high salt diet (4%) for 4 to 6 days or 4 weeks. Arteriolar responses to elevated superfusion solution PO2, acetylcholine, and sodium nitroprusside were assessed by videomicroscopy in the cremaster muscle. Arteriolar dilation to sodium nitroprusside was normal in both strains. Arteriolar constriction to elevated PO2 was enhanced in Dahl S and SS.BN13 rats on a high salt diet compared with responses in rats on a low salt diet. Arterioles of Dahl S rats on a high salt diet had an impaired dilation to acetylcholine, whereas dilator responses to acetylcholine were restored in SS.BN13 rats regardless of elevated salt intake. These data suggest that (1) restitution of normal renin control mechanisms by chromosomal transfer contributes to the recovery of dilator responses in SS.BN13 rats versus Dahl S rats but does not affect constrictor responses to oxygen, and (2) factors in the Dahl S genetic background contribute to an enhanced sensitivity of arterioles to elevated PO2 independent of elevated blood pressure. (Hypertension. 2003;41:1012-1015.)

Key Words: hypertension, renal microcirculation autoregulation sodium oxygen genetics hypertension, essential

Previous studies have demonstrated that arteriolar reactivity in response to a variety of vasoconstrictor and vasodilator stimuli is altered with the development of many types of hypertension.1-8 Potentially contributing to elevated vascular resistance. Recent studies have suggested that the maintenance of normal angiotensin (Ang) II levels, regulated by plasma renin levels controlled by the renin gene on chromosome 13, may be vital in maintaining normal responses of resistance arteries9 and distal arterioles10 to vasoactive stimuli. For example, skeletal muscle resistance arteries of Sprague-Dawley rats on a high salt diet have impaired relaxation to numerous vasodilator stimuli, which can be restored by infusion of a suppressor dose of Ang II to offset the ANG II suppression that occurs with elevated sodium intake.9

Dahl salt-sensitive (S) rats have low plasma renin activity (PRA)11 and impaired response to vasodilator stimuli,12 even when they are receiving a low salt diet. Recovery of normal renin control mechanisms by substitution of chromosome 13 from the normotensive Brown Norway rat onto the Dahl S genetic background (SS.BN13 consomic rats) restores the normal control of renin and prevents salt-induced hypertension that occurs in the Dahl S rat.13 In that study,11 the increase of PRA in response to salt depletion was significantly greater in SS.BN13 rats (from 0.6±0.1 ng Ang I/mL per hour on a high salt diet to 2.4±0.3 ng Ang I/mL per hour on a low salt diet; n=15) than in Dahl S rats (from 0.4±0.1 ng Ang I/mL per hour on a high salt diet to 1.0±0.2 ng Ang I/mL per hour on low salt diet; n=18).

Given the possible role of the renin-angiotensin system in maintaining normal microvessel reactivity, the goal of this study was to determine whether responses of skeletal muscle arterioles to vasoactive stimuli are altered in Dahl S rats on low and high salt diets and to determine whether substitution of chromosome 13 from normotensive Brown Norway rats, which have normal regulation of plasma renin activity,11 onto the Dahl S genetic background (SS.BN13 consomic rats) will restore normal arteriolar reactivity. Because salt-induced hypertension is eliminated in SS.BN13 rats,11 use of this unique genetic model should provide insight into the relative role of the Dahl S genetic background (versus elevated blood pressure) and/or the role of genes on chromosome 13 in contributing to any altered vascular reactivity that occurs in Dahl S rats on a high salt diet.

Methods

This study used age-matched SS.BN13 and Dahl S rats (16 to 18 weeks old) maintained by brother-sister mating in colonies housed in the Medical College of Wisconsin Animal Resource Center. The animals were fed a low salt (LS; 0.4% NaCl) or high salt (HS; 4% NaCl) diet for 4 to 6 days (short-term; ST), or 4 weeks (long term;
LT), with tap water ad libitum (Table 1). The Dahl S rats (SS/Jr/HSD/MCW or SS/MCW rats) used in this study are a substrain of a commercially available strain of Dahl S rats (SS/Jr/HSD rats) that was rederived at the Medical College of Wisconsin. The SS.BN13 consomic line used in these studies has chromosome 13 of an inbred normotensive Brown Norway rat strain (BN/MCW) substituted into the genetic background of the SS/Mcw strain of Dahl S rats and has been verified to be isogenic by a total genome scan. Rats used in the present study were housed in an AAALAC-accredited animal care facility, and all procedures received prior IACUC approval.

Individual rats were anesthetized with an injection of sodium pentobarbital (30 mg/kg IP). The trachea was cannulated to ensure a patent airway and a femoral artery and vein were cannulated to measure arterial pressure and to administer supplemental anesthetic. The cremaster muscle was prepared for videomicroscopy as described previously and was continuously superfused with warmed (35°C) physiological salt solution (PSS) equilibrated with a 0% O₂, 5% CO₂, and 95% N₂ gas mixture to ensure that O₂ delivery to the tissue was controlled entirely by the microcirculation. The ionic composition of the PSS was as follows (mmol/L): NaCl 119.0, KCl 4.7, CaCl₂ 1.6, NaH₂PO₄ 1.18, MgSO₄ 1.17, NaHCO₃ 24.0, and disodium EDTA 0.03. Arterioles selected for observation were located in the region of the muscle away from the incision, had brisk blood flow velocity, and demonstrated active tone, as indicated by a rapid dilation in response to topical addition of 10⁻⁶ mol/L adenosine. The reactivity of third-order arterioles in response to increasing concentrations of topically applied acetylcholine (ACh; 10⁻⁶ to 10⁻⁴ mol/L) and sodium nitroprusside (SNP; 10⁻⁴ to 10⁻² mol/L) and to increased O₂ content in the superfuse solution (5% O₂, 21% O₂) were measured by videomicroscopy and an on-screen videomicrometer.

Statistical Analysis

All data are presented as mean ± SEM. Vascular reactivity data were fit with linear (PO₂) or semilogarithmic (acetylcholine and sodium nitroprusside) regression equations, and slope coefficients (β) representing the change in vessel diameter for an incremental change in the independent variable (oxygen or agonist concentration) were calculated as previously described. Statistically significant differences between mean values were assessed by ANOVA with a post hoc Student-Newman-Keuls test. In all cases, a value of P<0.05 was considered to be statistically significant.

Results

The effect of a high salt diet and chromosomal transfer on arteriolar responses to elevated PO₂ are summarized in Figure 1 and Table 2. PO₂-induced constriction of arterioles was similar in Dahl S and SS.BN13 rats on the LS diet. Arterioles of Dahl S rats on STHS and LTHS diets had a significantly greater constriction in response to elevated PO₂ than Dahl S rats on the LS diet. Arterioles of SS.BN13 rats on the LTHS diet (but not the STHS diet) also constricted significantly more in response to elevated PO₂ than arterioles of SS.BN13 rats on the LS diet.

Arteriolar responses to ACh, summarized in Figure 2 and Table 2, were similar in Dahl S and SS.BN13 rats on the LS diet. Dilator responses to ACh were attenuated in arterioles of Dahl S rats on both STHS and LTHS diets versus responses in arterioles of SS.BN13 rats on the LS diet.

**TABLE 1. Baseline Data for the Different Animal Groups in the Present Study**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight, g</th>
<th>MAP, mm Hg</th>
<th>Vessel Diameter, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS.BN13-LS</td>
<td>365±8</td>
<td>124±7</td>
<td>Control: 26±2, Max: 31±2</td>
</tr>
<tr>
<td>SS.BN13 ST-HS</td>
<td>366±6</td>
<td>116±9</td>
<td>Control: 17±1, Max: 35±2</td>
</tr>
<tr>
<td>SS.BN13 LT-HS</td>
<td>386±8</td>
<td>129±8</td>
<td>Control: 17±1, Max: 28±2</td>
</tr>
<tr>
<td>Dahl S LS</td>
<td>362±9</td>
<td>122±8</td>
<td>Control: 21±1, Max: 30±2</td>
</tr>
<tr>
<td>Dahl S ST-HS</td>
<td>364±10</td>
<td>140±4</td>
<td>Control: 20±1, Max: 30±3</td>
</tr>
<tr>
<td>Dahl S LT-HS</td>
<td>354±9</td>
<td>157±5</td>
<td>Control: 21±1, Max: 32±2</td>
</tr>
</tbody>
</table>

Each group, n=6–9 rats. *P<0.05 vs all other groups.

**TABLE 2. Beta Coefficients (Slope) of Arteriolar Responses to Elevated PO₂, ACh, and SNP in Dahl S and Consomic SS.BN13 Rats on Low-Salt and High-Salt Diet**

<table>
<thead>
<tr>
<th>Groups</th>
<th>PO₂</th>
<th>ACh</th>
<th>SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS.BN13 LS</td>
<td>-0.200±0.04</td>
<td>0.594±0.09</td>
<td>0.521±0.14</td>
</tr>
<tr>
<td>SS.BN13 ST-HS</td>
<td>-0.317±0.06</td>
<td>0.669±0.12</td>
<td>0.408±0.04</td>
</tr>
<tr>
<td>SS.BN13 LT-HS</td>
<td>-0.397±0.03#</td>
<td>0.534±0.11</td>
<td>0.670±0.12</td>
</tr>
<tr>
<td>Dahl S LS</td>
<td>-0.190±0.04</td>
<td>0.532±0.06</td>
<td>0.413±0.06</td>
</tr>
<tr>
<td>Dahl S ST-HS</td>
<td>-0.394±0.02**</td>
<td>0.298±0.12*</td>
<td>0.495±0.07</td>
</tr>
<tr>
<td>Dahl S LT-HS</td>
<td>-0.475±0.04**</td>
<td>0.062±0.22*</td>
<td>0.545±0.08</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Each group, n=5–9 rats. *P<0.05 vs Dahl S LS and SS.BN13 regardless of diet. **P<0.05 vs Dahl S.
An enhanced response to different vasoconstrictor stimuli, including elevated PO$_2$, and an impaired relaxation to vasodilator stimuli have both been reported in hypertension. Several studies have demonstrated that the HS diet alone can lead to impaired vascular relaxation in the absence of an elevated blood pressure. These impaired vasodilator responses in animals on the HS diet may be mediated by the Ang II suppression occurring in response to elevated dietary salt intake, since normal vascular reactivity can be restored by chronic infusions of a suppressor dose of Ang II. An impaired relaxation of resistance vessels to vasodilator stimuli could contribute to the development of salt-sensitive forms of hypertension, since loss of normal vasodilator mechanisms could increase vascular resistance and contribute to an elevated blood pressure in cases when other factors, for example, kidney disease, predispose the individual to hypertension.

As noted earlier, an enhanced constriction of arterioles in response to elevated PO$_2$ has been reported in several models of hypertension, suggesting that local O$_2$-dependent autoregulatory mechanisms are altered in hypertension. Similar to studies of the in situ spinotrapezius muscle of hypertensive Dahl S rats, arteriolar constriction in response to elevated PO$_2$ is enhanced in cremasteric arterioles of Dahl S rats on STHS and LTHS diets. Of more interest is the finding that transfer of chromosome 13 does not prevent the enhanced vasoconstriction to elevated O$_2$ in SS.BN13 rats on the LTHS diet. The persistence of an enhanced constriction in response to elevated PO$_2$ in normotensive SS.BN13 rats contrasts with our previous observations in Sprague-Dawley rats, in which the enhanced O$_2$ sensitivity of arterioles occurred in rats with reduced renal mass hypertension but was not present in normotensive sham-operated control rats on an HS diet. These findings suggest that genetic factors in the Dahl S background may contribute to a salt-induced increase in arteriolar sensitivity to elevated PO$_2$, independent of elevated blood pressure.

There have been numerous reports of impaired responses to vasodilator stimuli in hypertensive animals and humans, whereas reduced endothelium-dependent responses associated with high salt intake alone have been documented in cerebral and skeletal muscle microvessels of normotensive rats. In the present study, responses to ACh were impaired in arterioles of Dahl S rats fed STHS or LTHS diets, whereas dilator responses to SNP were preserved. Previous studies have shown that basal release of NO, presumably from the endothelium, normally influences arteriolar tone in the skeletal muscle circulation of Dahl S rats and that this influence is suppressed in established salt-induced hypertension. Unaltered responsiveness to NO donors in arterioles of hypertensive rats, demonstrated in the present study and in many others, suggests that salt-induced hypertension is not accompanied by a reduced responsiveness of arteriolar smooth muscle to NO or a generalized defect in the ability of vessels to relax.

As discussed above, Ang II may be important in maintaining normal responses of resistance vessels to vasoactive stimuli. Previous studies have demonstrated that the impaired regulation of the renin-angiotensin system in Dahl S rats is restored in SS.BN13 rats by the transfer of chromosome 13 from normotensive Brown-Norway strain to genetic background of the salt-sensitive Dahl S rats. In the current experiments, the impaired dilation of arterioles in response to ACh in Dahl S rats on the HS diet was restored to normal in SS.BN13 rats, suggesting the importance of genes on chromosome 13 in maintaining normal responses to vasodilator stimuli. Whether this restorative effect is due to the recovery of normal vascular relaxation mechanisms because of the restoration of normal regulation of the renin-angiotensin system, whether it is due to the prevention of the arterial pressure elevation that occurs in response to an HS diet in Dahl S rats, or whether it is due to other genes on chromosome 13 remains to be determined. The observation that vasodilator responses to ACh were not significantly impaired by the HS diet in SS.BN13 consomic rats (in contrast to previous reports in Sprague-Dawley rats) suggests
that prevention of salt-induced hypertension through suppression of plasma Ang II levels was a key factor preventing the impaired vasodilator responses in SS.BN13 rats on the HS diet. Finally, the current study provides additional evidence that the consomic design can map important functional traits and supports the ability of chromosomal substitution approaches to provide important clues to identify genomic regions that may contribute to hypertension and other complex traits.11

Acknowledgments

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References

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