Endothelin-A Receptor Blockade Prevents Left Ventricular Hypertrophy and Dysfunction in Salt-Sensitive Experimental Hypertension

Lars Rothermund, MD; Roland Vetter, MD; Maike Dieterich, MD; Peter Kossmehl, MD; Özlem Gögebakan, MS; Chana Yagil, MD; Yoram Yagil, MD; Reinhold Kreutz, MD

Background—Salt-sensitive hypertension represents a major cause of left ventricular (LV) dysfunction. We therefore explored the potential effects of the selective endothelin-A (ETA) receptor antagonist darusentan on the development of hypertension, LV hypertrophy (LVH), and dysfunction in a genetic rat model of salt-sensitive hypertension.

Methods and Results—Animals from the salt-sensitive Sabra rat strain (SBH/y) and the salt-resistant strain (SBN/y) were treated with either normal diet (SBH/y and SBN/y) or with deoxycorticosterone-acetate (DOCA) and salt (SBN/y-DOCA and SBH/y-DOCA). Additional groups were treated with 50 mg · kg⁻¹ · d⁻¹ of darusentan (SBH/y-DOCA-DA and SBN/y-DOCA-DA). Systolic blood pressure and LV weight increased in response to DOCA only in the SBH/y strain (+75 mm Hg and +30%; P<0.05). LV end-diastolic pressure increased and −dP/dtmax decreased in SBH/y-DOCA compared with SBH/y (P<0.05). This was paralleled by a 5-fold upregulation of LV mRNA expression of atrial natriuretic factor (ANF) and a significant reduction of sarcoplasmic reticulum (SR) Ca²⁺-reuptake and the SR Ca²⁺-ATPase to phospholamban protein ratio (~30%). Whereas treatment with darusentan in SBH/y-DOCA-DA reduced the SBP increase by 50%, LVH elevation of ANF mRNA and LV dysfunction were completely prevented (P<0.05); this was associated with a normalization of SR Ca²⁺-reuptake and SR Ca²⁺-ATPase to phospholamban ratio by darusentan (P<0.05). A moderate elevation of interstitial fibrosis in SBH/y-DOCA (P<0.05) remained unaffected by darusentan treatment.

Conclusion—In the Sabra model of salt-sensitive hypertension, ETA-receptor blockade demonstrated striking effects on the prevention of LVH and LV dysfunction beyond its considerable antihypertensive effect. (Circulation. 2002;106:2305-2308.)

Key Words: hypertension ■ endothelin ■ heart failure ■ sodium
Preparation of the heart and histological evaluation of cardiac fibrosis were performed as described. Determination of atrial natriuretic factor (ANF) mRNA in the left ventricle and of sarcoplasmic reticulum (SR) Ca\(^{2+}\)-reuptake was performed as reported. Western blot analyses of SR Ca\(^{2+}\)-ATPase (SERCA2) and phospholamban were carried out as described elsewhere.

**Statistical Evaluation**

All data are expressed as mean±SEM. Statistical analysis was performed using 2-tailed Student’s t test and 2-way ANOVA, followed by Bonferroni’s adjustment. Differences were considered significant at the level of P<0.05.

**Results**

**Blood Pressure**

Salt-loading increased SBP in SBH/y-DOCA rats by 75 mm Hg compared with untreated SBH/y rats (Figure 1A; P<0.001). Animals from the salt-resistant strain clearly maintained their normal SBP values in response to DOCA. Darusentan attenuated the blood pressure increase in response to salt loading in SBH/y-DOCA-DA rats by 50% (P<0.001); thus SBP in this group was still elevated compared with SBH/y rats (P<0.0001).

**LV Weight**

Relative LV weight (LV/BW) was similar in SBN/y, SBH/y, and in salt-loaded SBN/y-DOCA rats without or with darusentan treatment (Figure 1B). In contrast, relative LV weight of the salt-loaded SBH/y-DOCA group was increased compared with the SBN/y-DOCA and SBH/y groups (+30%; P<0.05). Darusentan fully prevented this increase in SBH/y-DOCA-DA rats (P<0.05).

**Cardiac Fibrosis**

There were no significant differences in perivascular fibrosis between groups (Table 1). Interstitial fibrosis was moderately increased in response to DOCA in the SBH/y strain (P<0.05) and was not affected by darusentan treatment (Table).

**Expression of Left Ventricular ANF mRNA**

LV ANF mRNA expression was 5-fold higher in SBH/y-DOCA animals compared with SBN/y-DOCA or SBH/y animals (Figure 1C; P<0.05). Treatment with darusentan in SBH/y-DOCA-DA rats led to a complete suppression of this increase.

**Left Ventricular Function**

Although DOCA-loading led to a significant increase of left ventricular end-diastolic pressure (LVEDP) in both strains compared with normal diet (Figure 2A; P<0.05), this increase was more pronounced in the hypertensive strain (P<0.05). Darusentan treatment reduced the increased LVEDP in the SBH/y-DOCA-DA animals to levels observed in normotensive SBN/y-DOCA and SBN/y-DOCA-DA animals (Figure 2A). Although systolic dP/dt (+dP/dt/LVPmax) was somewhat lower in the SBH/y strain after DOCA treatment, there were no significant differences overall (Figure 2B). Diastolic dP/dt (−dP/dt/LVPmax) was significantly decreased (−15%) in SBH/y-DOCA rats compared with SBH/y rats (P<0.05), and diastolic dysfunction was normalized by darusentan treatment (Figure 2C).

**Cardiac SR Ca\(^{2+}\)-ATPase and Phospholamban**

DOCA resulted in a significant reduction (−28%) in SR Ca\(^{2+}\)-transport activity in SBH/y-DOCA rats compared with SBH/y rats, which was abolished by darusentan treatment (P<0.05, data not shown). Western blot analysis revealed no significant difference in SERCA2 protein and the SERCA2-to-phospholamban protein ratio level between SBH/y and SBN/y rats (Figure 2D). SBH/y-DOCA animals showed a marked decrease in SERCA2 and in the SERCA2-to-
phospholamban ratio compared with SBH/y animals (Figure 2D; \( P < 0.05 \)). Darusentan treatment in the SBH/y-DOCA-DA group led to a normalization of the SERCA2-to-phospholamban ratio (\( P < 0.05 \)). Phospholamban levels were not significantly altered in the experimental groups (data not shown).

**Discussion**

The Sabra salt-sensitive SBH/y and salt-resistant SBN/y rats constitute a unique experimental model of hypertension in which salt-susceptibility is genetically determined without the development of spontaneous hypertension.\(^7\) That is, the salt-sensitive SBH/y strain develops significant hypertension only during salt-loading but not under normal dietary conditions, whereas the reference salt-resistant SBN/y strain maintains normal blood pressure during high salt treatment. This experimental model therefore allows scientists to make the distinction between the effects of hypertension and salt-loading on target-organ damage.\(^7,8\) Here, we show for the first time that salt-sensitive hypertension in response to DOCA in the SBH/y strain leads to both LVH and LV dysfunction. The response of the hypertrophied left ventricle to salt loading was associated with a significant increase in ANF expression, a slight increase of interstitial fibrosis, and a hemodynamic deterioration characterized by increased LVEDP and impaired diastolic function. The latter can be related to a reduction of SR Ca\(^{2+}\) transport in SBH/y-DOCA rats, as downregulation of SERCA2 is closely related to LV dysfunction in pressure overload-induced LVH in the rat\(^9-11\) and has also been linked to LV dysfunction in the human heart.\(^12\) Moreover, we analyzed the relation between SERCA2 with its inhibitory protein phospholamban, because the abundance of SERCA2 and phospholamban in the SR and hence the ratio of SERCA2-to-phospholamban is critical for control of car-

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Left Ventricular Perivascular Fibrosis, %</th>
<th>Left Ventricular Intersitial Fibrosis, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBN/y</td>
<td>9.9±1.2</td>
<td>1.7±0.2*</td>
</tr>
<tr>
<td>SBN/y-DOCA</td>
<td>10.3±0.8</td>
<td>1.7±0.2†</td>
</tr>
<tr>
<td>SBN/y-DOCA-DA</td>
<td>8.0±1.2</td>
<td>1.9±0.1‡</td>
</tr>
<tr>
<td>SBH/y</td>
<td>10.7±1.5</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>SBH/y-DOCA</td>
<td>13.5±2.0</td>
<td>3.3±0.7*</td>
</tr>
<tr>
<td>SBH/y-DOCA-DA</td>
<td>11.4±2.5</td>
<td>4.2±1.1*</td>
</tr>
</tbody>
</table>

BW indicates body weight. \( n = 8–15 \) in each group.  
\(^*\) \( P < 0.05 \) vs SBH/y; † \( P < 0.05 \) vs SBH/y-DOCA; ‡ \( P < 0.05 \) vs SBH/y-DOCA-DA.

**Figure 2.** A, Left ventricular end-diastolic pressure (LVEDP). B, \( +dP/dt/LVP_{max} \). C, \( -dP/dt/LVP_{max} \). D, Sarcoplasmic Ca ATPase/phospholamban protein level (SERCA/PLB). \( n = 8 \) to 15 in each group.  
\(^*\) \( P < 0.05 \) versus SBH/y-DOCA; † \( P < 0.05 \) versus SBN.
diac contractility and relaxation.\textsuperscript{13} Indeed, LV dysfunction in SBH/y-DOCA rats was associated with a significant decline in SERCA2 abundance, resulting in a reduced SERCA2-to-phospholamban ratio. These findings might characterize the transition from compensated LVH to LV dysfunction in SBH/y-DOCA rats, although in the aortic coarctation model, reduction in SERCA2 protein levels were only found in the later phase of established LV failure, but not in the early phase of compensated LVH.\textsuperscript{10} In addition, it should not be dismissed that other phenotypic differences in myocytes, such as changes in isomyosins, could have contributed to the observed hemodynamic changes.\textsuperscript{14} Interstitial cardiac fibrosis cannot account for this functional deterioration because it remained unchanged after darusentan treatment. The latter findings are in agreement with our previous experiments in a transgenic rat model with high-renin hypertension.\textsuperscript{5} In this former study, LV diastolic dysfunction was improved by ETA blockade without affecting hypertension or LVH. Interstitial fibrosis was also not affected by ETA blockade in transgenic rats, suggesting that cardiac fibrosis in both the high-renin transgenic model and the low-renin DOCA salt-sensitive Sabra model could probably be attributed to direct cardiac effects of mineralocorticoids.\textsuperscript{14}

The impact of darusentan on blood pressure is demonstrated by the significant 50\% reduction of the SBP increase in response to DOCA salt in the SBH/y strain, which is in agreement with previous findings in other models.\textsuperscript{1} This antihypertensive effect of darusentan may be attributed to both renal effects mediated by decreased sodium reabsorption via the epithelial sodium channel\textsuperscript{15} and to inhibition of endothelin-1-mediated vasoconstriction.\textsuperscript{1} Interestingly, however, although SBP was still significantly elevated in SBH/y-DOCA rats treated with darusentan, the drug conferred full protection against the development of LVH and LV dysfunction, which is additionally supported by the normalization of LV ANF expression. Moreover, darusentan normalized the reduced SERCA2-to-phospholamban ratio observed in the hypertensive salt-sensitive animals.

Although extrapolation from experimental salt-sensitive hypertension in the Sabra model to human patients with salt-sensitive hypertension is limited, our findings are nevertheless of potential clinical interest. Hence, it has been proposed that there is a need for additional research about the efficacy of therapies in sub-groups of patients with heart failure who do not respond to angiotensin-converting enzyme inhibition.\textsuperscript{16} Patients with heart failure secondary to salt-sensitive hypertension may belong to such a sub-group that would benefit from alternative therapies.

In summary, our experimental findings demonstrate that the cardiac protection provided by darusentan goes beyond its antihypertensive effects in the Sabra model of salt-sensitive genetic hypertension.

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References

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