Expression of Human Angiotensinogen-Renin in Rat
Effects on Transcription and Heart Function

Sabine Bartel, Brigitte Hoch, Donathe Vetter, Ernst-Georg Krause

Abstract—In double transgenic rats (dTGR) harboring the human angiotensinogen (hAOGEN) and human renin (hREN) genes, we studied cardiac transcript levels of hypertrophy-related, Ca\(^{2+}\) regulatory, and \(\beta\)-adrenergocor–associated proteins. The contractile properties and the cellular signaling of isolated hearts exposed to (-)isoproterenol and/or angiotensin (Ang) I were evaluated. dTGR developed hypertension of 174.1±7.6 versus 109.6±2.0 mm Hg (\(P<0.05\)) in Sprague-Dawley rats and heart hypertrophy. In hearts of dTGR, the transcript levels of ANP, \(\beta\)-MHC, and \(\alpha\)-MHC were altered (percentage versus Sprague-Dawley rats, 100%) by 304%, 178%, and 78%, respectively. Transcript levels of L-type Ca\(^{2+}\) channel, Ca\(^{2+}\) release channel, SERCA2a, phospholamban, G\(_i\) and G\(_s\) proteins were unchanged. Isolated hearts of dTGR indicated higher baseline contractility versus Sprague-Dawley rats. (-)Isoproterenol-modified contractility occurred in both groups; however, the extent (predrug value, 100%) was less in hearts of dTGR versus Sprague-Dawley rats (+dP/dt, 310±42% versus 534±63%; \(P<0.05\)). Interestingly, (-)isoproterenol shortened the relaxation time by \(\approx25\%\) in both groups. This finding was reflected by a protein kinase A–related phospholamban phosphorylation. Ang I depressed the heart contractility but did not interact with the protein kinase A pathway. In conclusion, we have found that expression of the hAOGEN-hREN complex in dTGR elicited specific effects on transcripts of ANP and myofibrillar proteins. Although the \(\beta\)-adrenergically mediated relaxation was not impaired in the hypertrophied hearts, the extent of \(\beta\)-adrenergic inotropic responsiveness was reduced. (Hypertension. 2002; 39:219-223.)

Key Words: hypertrophy \(\bullet\) contraction \(\bullet\) relaxation \(\bullet\) angiotensin I \(\bullet\) adrenergic receptor agonists

Cardiac hypertrophy is an adaptation to hemodynamic overload accompanied by a switch to embryonic gene expression profiles in myocardial cells.\(^1\)\(^-\)\(^6\) These changes were assumed to cause functional adaptation/impairment of hypertrophied hearts.\(^3\)\(^,\)\(^7\) Additionally, defects in \(\beta\)-adrenergocor–modified responses occur in the hypertrophied heart.\(^6\)\(^,\)\(^8\)\(^,\)\(^9\) The renin-angiotensin system (RAS) plays a prominent role in the pathophysiology of hypertrophy.\(^10\)\(^,\)\(^11\) An interesting model of hypertrophy was established using double transgenic rats (dTGR) resulting from crossings of homozygous human renin (hREN) with homozygous human angiotensinogen (hAOGEN) rats.\(^12\) Transcripts for the hAOGEN transgene were detected in all tissues tested, whereas the hREN transgene was present in the kidney, ovary, and adrenal gland. The transgenic offspring of the cross show significantly elevated total plasma renin activity and chronic elevation of the blood pressure, resulting in cardiac hypertrophy and adrenergocor dysfunction.\(^12\)\(^,\)\(^13\) The expression of human RAS provides the possibility to study human RAS-caused hypertrophy without interaction with the endogenous RAS. Therefore, we studied in the myocardium of dTGR the expression of markers for cardiac hypertrophy (ANP, \(\alpha\)-MHC, \(\beta\)-MHC), the transcript levels of Ca\(^{2+}\) regulatory proteins (L-type Ca\(^{2+}\) channel, Ca\(^{2+}\) release channel, Ca\(^{2+}\) ATPase [SERCA2a], phospholamban [PLB]) as well as G proteins. The sarcoplasmic reticulum plays the major role in the regulation of intracellular Ca\(^{2+}\) concentration in cardiomyocytes. The Ca\(^{2+}\) pumping activity of SERCA2a is controlled by PLB, which depresses Ca\(^{2+}\) sequestration in its dephosphorylated form. The importance of cAMP-dependent protein kinase (PKA)-dependent phosphorylation of PLB in the \(\beta\)-adrenergic regulation of inotropy and lusitropy has been reported by several groups.\(^14\)\(^,\)\(^15\) The coupling between the cAMP and the angiotensin (Ang) II system in the heart remains controversial.\(^16\)\(^,\)\(^17\) But there are no data available concerning the interaction between cAMP and Ang I signaling in adult rat myocardium at the level of PKA-mediated phosphorylation of PLB. Therefore, we studied contractile parameters, the activation of PKA and the phosphorylation of PLB in isolated hearts of Sprague-Dawley rats and dTGR subjected to \(\beta\)-adrenergic stimulation and/or Ang I.

Methods

Animals
Male dTGR (transgenic rats[hREN\(\times\)hAOGEN]), age 5 to 6 weeks, were obtained from our in-house animal facility. Age-matched male Sprague-Dawley rats were from Sprague Dawley, Schönwalde, Germany. The rats were kept in a 12-hour/12-hour light/dark cycle. The
Reverse Transcription–Polymerase Chain Reaction
RNA preparation was performed as described by Hoch et al.\textsuperscript{18} Polymerase chain reactions (PCRs) were performed in the linear ranges of the amplification reactions (cycle numbers given in italics) and normalized to GAPDH.\textsuperscript{19} Primer combinations and conditions for amplification of the \( \alpha_i \) subunit of the L-type \( \text{Ca}^{2+} \) channel (24), the \( \text{Ca}^{2+} \) release channel (21), PLB (19), and GAPDH (19) are given by Hoch et al\textsuperscript{18}; for \( \alpha \)-MHC and \( \beta \)-MHC (16), by Luther et al\textsuperscript{20} PCR condition for ANP (19) is given in Hoch et al\textsuperscript{18} with the forward primer AGAGAGTGAGCGGAGACGAC and the reverse primer GCAGAGTGAGGAGGAGTAGGG. For SRECA2a (19); forward primer GGAAATCCCTAGAGATGTCGCAATGCCCTC, reverse primer CCGGATCCGTGAAACACGCAATCACGCAGCA, cycling conditions as given by Hoch et al\textsuperscript{18} were used. Specificity of amplification products was confirmed by sequencing (InViTek).

Heart Perfusion
Hearts were perfused as described\textsuperscript{15} using a perfusion pressure of 80 mm Hg. Developed left ventricular pressure (dLVP), maximal rate of left ventricular pressure development (+dP/dt), and decline (−dP/dt) and coronary flow were recorded.

Drug Administration
For \(-\)isoproterenol, hearts were exposed for 2 minutes to 5 or 50 nmol/L \(-\)isoproterenol. For Ang I, after a 10-minute preexposure with the standard medium containing 0.05% bovine serum albumin (BSA), hearts were treated for 30 minutes with 0.3 \( \mu \)mol/L Ang I. For Ang I and 5 nmol/L \(-\)isoproterenol, \(-\)isoproterenol was co-added during the last 2 minutes of Ang I infusion. Control hearts were perfused with BSA for 40 minutes.

Western Blot Analysis
Nonphosphorylated and PKA phosphorylated PLB was immunologically detected as described.\textsuperscript{15} \( \text{Go}_{\alpha} \), \( \text{Go}_{\beta_2} \), and \( \text{Go}_{\gamma} \) proteins were assayed by adapted procedures reported in Bartel et al.\textsuperscript{21} The immunoreactions were evaluated as reported by Kuschel et al.\textsuperscript{15} Results are expressed as arbitrary units of optical density.

Other Assays
The activation of PKA was detected as reported in Kuschel et al.\textsuperscript{15} The Lowry method was used to measure the protein concentration.\textsuperscript{22}

Statistics
Data are mean ± SEM. Statistical analysis was done with Student’s unpaired \( t \) test or ANOVA when appropriate. Differences were considered significant at \( P<0.05 \).

Results
Characteristics of Sprague-Dawley Rats and dTGR
Table 1 summarizes characteristics of Sprague-Dawley rats and dTGR used in this study. The blood pressure of dTGR was increased 1.6-fold (\( P<0.05 \)). Furthermore, dTGR had an increased heart weight-to-body weight index (\( P<0.05 \)).

Gene Expression in Hearts of Sprague-Dawley Rats and dTGR
Evaluation of reverse transcription (RT)-PCR derived signals (n=5) demonstrate significant increases of the ANP mRNA levels in left ventricular tissue of dTGR (304.5 ± 23.5% versus Sprague-Dawley rats, 100.0 ± 3.6%; \( P<0.05 \)) and a qualitative and quantitative switch in the expression of \( \alpha \)-MHC and \( \beta \)-MHC mRNA (Figure 1). For the \( \alpha \)-MHC mRNA level, a significant decrease (77.8 ± 3.4%) in hearts of dTGR compared with control (100.0 ± 3.6, \( P<0.05 \)) was detected, accompanied by a re-expression of the embryonic \( \beta \)-MHC mRNA (178.0 ± 17.5% versus Sprague-Dawley rats, 100.0 ± 12.0; \( P<0.05 \)). The transcript levels of various \( \text{Ca}^{2+} \)-cycling proteins did not change significantly in dTGR compared with controls (Figure 2). Western blotting data (optical density) indicate no significant altered expression of G proteins (\( \text{Go}_{\gamma} \); Sprague-Dawley rats, 0.60 ± 0.04 versus dTGR, 0.72 ± 0.05; \( \text{Go}_{\alpha} \); Sprague-Dawley rats, 3.02 ± 0.10 versus dTGR, 3.32 ± 0.12; \( \text{Go}_{\beta_2} \); Sprague-Dawley rats, 2.19 ± 0.20 versus dTGR, 2.35 ± 0.23; n = 5 to 6).

Functional Studies in Isolated Hearts of Sprague-Dawley Rats and dTGR
Baseline contractile parameters of hearts from Sprague-Dawley rats and dTGR are presented in Table 2. There was a marked
increase in dLVP (164%), +dP/dt (162%), and −dP/dt (172%) in dTGR compared with Sprague-Dawley rats (100%). In another subset of experiments, hearts of both groups were subjected to β-adrenergic intervention (Figure 3). The observed percentage alterations of dLVP, +dP/dt, and −dP/dt were significantly lower (∼56%) in dTGR. The contractile baseline values of isolated hearts of Sprague-Dawley rats and dTGR were measured to be as follows: dLVP, 71.3 ± 21.5 versus 27.1 ± 5.5 mm Hg; +dP/dt, 1620 ± 121 versus 2825 ± 134 mm Hg/s; −dP/dt, 1334 ± 11 versus −2328 ± 92 mm Hg/s. In (-)isoproterenol–exposed hearts of Sprague-Dawley rats and dTGR, the parameters were changed to the following: dLVP, 271.4 ± 27 versus 215.7 ± 14.8 mm Hg; +dP/dt, 8662 ± 101 versus 8672 ± 227 mm Hg/s; −dP/dt, 7539 ± 260 versus 7217 ± 370 mm Hg/s. The relaxation time (t½) was shortened in (-)isoproterenol–treated hearts of Sprague-Dawley rats, from 42.6 ± 0.6 to 32.4 ± 0.8 ms (P < 0.05), and of dTGR, from 42.0 ± 0.7 to 31.3 ± 1.2 ms (P < 0.05), respectively.

(-)Isoproterenol-Dependent Signaling in Hearts of Sprague-Dawley Rats and dTGR
(-)Isoproterenol activated PKA to similar degrees in the soluble and particulate heart tissue fractions in both groups (Figure 4). Next, we studied the PKA-related Ser16 phosphorylation of PLB in (-)isoproterenol–exposed hearts using a phosphorylation site-specific antibody. (-)Isoproterenol–stimulated Ser16 phosphorylation was observed in hearts of both groups. Ser16 phosphorylation in Sprague-Dawley rats was increased from a baseline value (optical density) of 0.23 ± 0.2 to 12.6 ± 1.7 and 25.4 ± 3.9 and in dTGR to 15.0 ± 0.02 and 24.9 ± 2.6, when hearts were treated with 5 or 50 nmol/L (-)isoproterenol.

Interaction of Ang I With the β-Adrenergic System in Hearts of Sprague-Dawley Rats and dTGR
Ang I attenuated the dLVP in both Sprague-Dawley rats and dTGR (Figure 5A). Contractile baseline values of hearts (n = 5) of Sprague-Dawley rats and dTGR were measured to be as follows: dLVP, 71.3 ± 4.8 versus 107.9 ± 5.9 mm Hg; +dP/dt, 1671 ± 84 versus 3017 ± 140 mm Hg/s; −dP/dt, 1381 ± 112 versus −2296 ± 148 mm Hg/s. Ang I reduced significantly (P < 0.05) these baseline values in Sprague-Dawley rats and dTGR to levels as follows: dLVP, 58.2 ± 3.2 versus 75.0 ± 3.9 mm Hg; +dP/dt, 1196 ± 29 versus 2342 ± 118 mm Hg/s; −dP/dt, 1159 ± 80 versus −1443 ± 82 mm Hg/s. The coronary flow was reduced in Ang I exposed hearts of both groups by 33.5 ± 0.9% and 29.0 ± 1.4% (P < 0.05). (-)Isoproterenol enhanced the contractility in Ang I–pretreated hearts of Sprague-Dawley rats and dTGR to levels as follows: +dP/dt, 6694 ± 305 versus 6428 ± 271 mm Hg; and −dP/dt, −5926 ± 353 versus −5640 ± 305 mm Hg/s. For comparison, we performed experiments in which the hearts were challenged with (-)isoproterenol alone. +dP/dt was changed from 1621 ± 75 to 6421 ± 280 mm Hg/s (Sprague-Dawley rats, n = 6) and from 3002 ± 161 to 6836 ± 265 mm Hg/s (dTGR, n = 8) under these conditions. −dP/dt was increased from −1324 ± 106 to −6676 ± 333 mm Hg/s in Sprague-Dawley rats and from −2122 ± 122 to −5818 ± 279 mm Hg/s in dTGR.

![Figure 3](https://hyper.ahajournals.org/content/92/5/565/s1)

**Figure 3.** β-Adrenoceptor–dependent contractile effects in hearts of Sprague-Dawley rats (SD) and dTGR. Isolated hearts from SD (n = 8) and dTGR (n = 9) were exposed to (-)isoproterenol (50 nmol/L) for 2 minutes. Data are mean ± SEM. *P < 0.05 dTGR vs SD. Predrug values were set as 100%.

![Figure 4](https://hyper.ahajournals.org/content/92/5/565/s2)

**Figure 4.** PKA activation in Sprague-Dawley rats (SD) and dTGR. PKA activation was measured in (-)isoproterenol–exposed (Iso, 50 nmol/L) hearts. Data are means ± SEM, n = 3 to 5. *P < 0.05 vs control (Ctr).

### Table 2. Baseline Contractile Parameters of Isolated Hearts of Sprague-Dawley Rats and dTGR

<table>
<thead>
<tr>
<th>Group</th>
<th>dLVP, mm Hg</th>
<th>+dP/dt, mm Hg/s</th>
<th>−dP/dt, mm Hg/s</th>
<th>Coronary Flow, mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprague-Dawley rats</td>
<td>65.5 ± 2.1</td>
<td>1864 ± 87</td>
<td>1286 ± 72</td>
<td>9.6 ± 0.7</td>
</tr>
<tr>
<td>dTGR</td>
<td>108.0 ± 2.6*</td>
<td>3020 ± 72*</td>
<td>2208 ± 54*</td>
<td>11.2 ± 0.7</td>
</tr>
</tbody>
</table>

Data are mean ± SEM resulting from Sprague-Dawley (n = 9) or dTGR (n = 11) hearts.

*P < 0.05 vs Sprague-Dawley rats.
dTGR. (-)Isoproterenol shortened \( t_{1/2} \) in hearts of Sprague-Dawley rats to \( 77.5 \pm 1.7\% \) and of dTGR to \( 75.6 \pm 1.6\% \) compared with the baseline values (100%). To analyze the potential role of PLB in modulation of lusitropy, we studied the PKA-related phosphorylation of PLB (Figure 5B). Because of the relatively low level of PLB, \( (\approx 16\% \) of total phosphorylated PLB), the variance of the PLB \( \beta \) values is higher compared with that of PLB \( \alpha \). Summarizing the densitometric data (optical density) of PLB \( \alpha \) and PLB \( \beta \), there is no difference in the (-)isoproterenol–caused PLB phosphorylation between both groups (Sprague-Dawley rats: isoproterenol, 17.8 \pm 3.3, and Ang I+ isoproterenol, 17.9 \pm 3.2; dTGR: isoproterenol, 20.8 \pm 2.2, and Ang I+ isoproterenol, 19.5 \pm 0.6) (Figure 5B). Ang I alone has not affected the phosphorylated status of PLB.

**Discussion**

**Gene Expression in Hearts of dTGR**

We have found that the expression of \( G_{\alpha} \) and \( G_{\beta} \) proteins and of \( Ca^{2+} \)-regulating proteins involved in E-C coupling and \( Ca^{2+} \) uptake into the sarcoplasmic reticulum were unchanged in hearts of dTGR. Therefore, these proteins seem to not be responsible for the diminished \( \beta \)-adrenergic responsiveness of the hearts of dTGR. Interestingly, we have recognized that there is a human RAS-related shift in the \( \alpha \)-MHC/\( \beta \)-MHC composition. Remodeling of myofibrils may play a role in myocardial dysfunction in hearts of hypertensive rats. A shift from \( \alpha \)-MHC to \( \beta \)-MHC is associated with decreased myosin ATPase activity and shortening but also an energetically more efficient contraction. Moreover, it has been demonstrated that the myosin ATPase of \( \beta \)-MHC is not a target of the cAMP-regulated pathway. Whether the change in the proportion of \( \alpha \)-MHC to \( \beta \)-MHC indicates additionally a process of accelerated aging in dTGR, dying after a mean time of 55 days remains to be established. Previous studies have shown that blocking of the Ang II type 1 receptor may prevent the myofibrillar remodeling and account for the beneficial effects of angiotensin antagonists in old SHR. These findings may also explain the alterations in the MHC status of dTGR characterized by increased plasma renin activity.

**\( \beta \)-Adrenergic Responsiveness in Hearts of dTGR**

We observed increased baseline contractility in hearts of dTGR, which may be linked to the \( \alpha \)-MHC–to–\( \beta \)-MHC shift. These data are in line with results reported for hearts of old hypertensive rats. The diminished (-)isoproterenol–mediated inotropic response could be attributed, at least in part, to the inverse shift of \( \alpha \)-MHC and \( \beta \)-MHC and their relative content. Furthermore, the decreased myocardial \( \beta \)-adrenergic responsiveness has been attributed to downregulation of \( \beta \)-adrenoceptors and to increased \( G_{\beta} \) proteins. But in our study, no altered \( G_{\alpha} \) and \( G_{\beta} \) protein profile was found in dTGR. Other groups, including ours, have demonstrated the important role of \( \beta \)-adrenergically mediated phosphorylation of PLB in regulating relaxation. (-)Isoproterenol–shortened relaxation is associated with PKA-dependent PLB phosphorylation also in the hearts of dTGR.

**Interaction Between Ang I and (-)Isoproterenol in Hearts of dTGR**

Cardiac ACE has been demonstrated to be the major enzyme for Ang II generation. However, the myocardial function of Ang II is not clear. Although Ang II exerted positive inotropic effects in heart preparations of dog and in human atrial heart, the opposite was found in neonatal and adult rat cardiomyocytes. Additionally, it has been demonstrated that Ang I/Ang II contribute to coronary vasoconstriction and impaired diastolic function of perfused rat hearts with pressure-overload–induced hypertrophy. Now, we present data showing that Ang I reduces the baseline contractility in both, isolated hearts of Sprague-Dawley rats and dTGR. Because of the high plasma renin activity and the occurrence of Ang I in the effluent of hearts of dTGR, we suggest that there must be a chronological vasoconstriction in dTGR. Recent data from Ang I or human renin–perfused hearts of rats overexpressing the hA0GEN gene support our conclusion. With regard to controversial reports concerning the interplay of cAMP- and Ang I/Ang II–mediated pathways in the cardiovascular system, our results clearly demonstrate contractile (-)isoproterenol responsiveness in the presence of Ang I. More interestingly, the effect of (-)isoproterenol on relaxation was maintained in hypertrophied hearts pre-exposed to Ang I. Additionally, a PKA-related PLB phosphorylation occurs in the presence of Ang I in hearts of dTGR. Thus, we postulate no relationship between Ang I and cAMP-induced shortening of relaxation time. In summary, hearts of dTGR are characterized by (1) increased ANP-transcript levels, (2) a MHC isoform-switch,
and (3) reduced β-adrenergic positive responsiveness but unimpaired lusitropy.

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References
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