Increase in $G_{i\alpha}$ Protein Accompanies Progression of Post-Infarction Remodeling in Hypertensive Cardiomyopathy

Ichiro Kouchi, Oliver Zolk, Friedrich Jockenhövel, Gabi Itter, Wolfgang Linz, Bodo Cremers, Michael Böhm

Abstract—Hypertensive cardiac hypertrophy and myocardial infarction (MI) are clinically relevant risk factors for heart failure. There is no specific information addressing signaling alterations in the sequence of hypertrophy and post-MI remodeling. To investigate alterations in $\beta$-adrenergic receptor G-protein signaling in ventricular remodeling with pre-existing hypertrophy, MI was induced by coronary artery ligation in Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). Ten weeks after the induction of MI, the progression of left ventricular dysfunction and increases in plasma atrial natriuretic peptide (ANP) and cardiac ANP mRNA were more pronounced in SHR than WKY. In addition, the impaired contractile response to $\beta$-adrenergic stimulation was observed in the noninfarcted papillary muscle isolated from SHR. Immunochemical $G_{i\alpha}$ protein and $\beta$-adrenoceptor density were not significantly altered by MI in both strains. However, immunochemical $G_{i\alpha}$ was increased (1.5-fold) in the noninfarcted left ventricle of the SHR in which infarction had been induced when compared with that in SHR that underwent sham operation. This increase was observed especially in rats with a high plasma ANP level. Furthermore, there was a positive correlation between $G_{i\alpha}$ and the extent of post-MI remodeling in WKY. A similar correlation between $G_{i\alpha}$ and the extent of hypertensive hypertrophy was observed in SHR. In conclusion, the vulnerability of hypertrophied hearts to ischemic damage is greater than that of normotensive hearts. An increase in $G_{i\alpha}$ could be one mechanism involved in the transition from cardiac hypertrophy to cardiac failure when chronic pressure overload and loss of contractile mass from ischemic heart disease coexist. (Hypertension. 2000;36:42-47.)

Key Words: myocardial infarction • cardiomegaly • ventricular remodeling • G proteins • atrial natriuretic factor

Ventricular remodeling after myocardial infarction (MI) is increasingly recognized as being one of the most important causes of chronic heart failure.1 To define the pathophysiological mechanisms underlying that process, coronary artery ligation in the rat model has been well studied.2,3 This classic model is attractive because the structural and functional alterations that occur during post-MI remodeling (eg, hypertrophy of noninfarcted myocardium, an increase in collagen content, and progressive contractile dysfunction) are similar to those alterations observed in humans.3 However, in addition to ischemic damage, chronic stress that is due to residual coronary stenosis and, in particular, systemic hypertension, is often involved in the progression of human ischemic cardiomyopathy. The coronary artery ligation model in which there are no additional pathologic factors such as those cited may not be applicable to clinical settings.

Hypertensive cardiac hypertrophy is another major cause of heart failure.4 Although hypertrophy is thought of initially as being a compensatory mechanism, chronic pressure overload results in the decomposition of the hypertrophied heart.5 In addition, left ventricular (LV) hypertrophy is an independent risk factor for MI.4 Thus, the prevalence of hypertensive patients suffering MI is increasing, and their prognosis is worse than that of normotensive patients with MI. However, there is no precise report regarding the mechanisms underlying post-MI remodeling that occurs in cases of pre-existing hypertrophy. Although the mechanisms responsible for the transition from cardiac hypertrophy to cardiac failure are redundant and possibly dependent on the type of damage to the myocardium, an activation of the sympathetic nervous system with myocardial release of norepinephrine and consequent desensitization of the myocardial $\beta$-adrenergic signal transduction have been generally demonstrated in chronic heart failure,6 hypertensive cardiac hypertrophy,7 and post-MI remodeling.8,9 $\beta$-Adrenergic desensitization produces an important contribution to contractile dysfunction in these conditions. An increase of inhibitory G protein $\alpha$ subunit ($G_{i\alpha}$)10,11 and a downregulation of $\beta$-adrenergic receptors11,12 are the key alterations that have been identified to date. In this study, the spontaneously hypertensive rat (SHR), which is the widely used experimental model in the study of hypertensive hypertrophy,13 was
used to simulate post-MI remodeling with hypertensive hypertrophy, and the alteration in the β-adrenergic receptor G-protein regulated system was investigated.

**Methods**

**Postinfarction Remodeling Model**

All experiments were performed in accordance with the German animal protection law. Male Wistar-Kyoto rats (WKY) and SHR (age 16 weeks) (Harlan Winkelmann, Indianapolis, Ind) were anesthetized with an intraperitoneal injection of ketamine (35 mg/kg) plus xylazine (2 mg/kg) and were mechanically ventilated. The pericardium was opened via left thoracotomy, and the left coronary artery was ligated 2 to 3 mm beneath its origin. The sham operation consisted of the identical procedure except for coronary ligation. Ten weeks after the surgery, the rats were reanesthetized, and the right carotid artery was cannulated with a polyethylene catheter to enable the monitoring of blood pressure and heart rate. Then each heart was excised and was mounted on a Langendorff apparatus. Each isolated heart was perfused in the working-heart mode with a filling pressure of 15 mm Hg and an afterload pressure of 60 mm Hg, as described previously. After the evaluation of cardiac performance, the heart was weighed, and the LV was sectioned transversely into 4 slices. Photographs were taken from each slice, and the tissues were then snap-frozen in liquid nitrogen and were stored at −80°C. The area of scarring and the LV were determined by planimetry and were corrected for the tissue weight.

**Myocardial Membrane Preparation**

Noninfarcted LV myocardium from rats with infarcted hearts and the corresponding portion from the hearts of rats that underwent sham operation were homogenized by means of a glass Teflon homogenizer in homogenization buffer (5 mmol/L Tris/HCl, 1 mmol/L EDTA, 5 mmol/L MgCl₂, 5 mg/L leupeptin, and 5 mg/L aprotinin, pH 7.4). The homogenate was centrifuged at 500g for 10 minutes. The supernatant was incubated with an addition of 1 mol/L KCl for 10 minutes and was centrifuged at 100 000g for 30 minutes. The final pellet was resuspended in incubation buffer (25 mmol/L Tris/HCl and 20 mmol/L MgCl₂, pH 7.4) and was stored at −80°C. All preparation was performed at 4°C. Protein concentrations were determined according to the method of Bradford.

**Radioligand Binding Study**

Assays were performed in a total volume of 250 μL of incubation buffer. The incubation was performed at 37°C for 60 minutes. β-Adrenoceptors in myocardial membranes were studied with ¹²⁵I-cyanopindolol, as described previously.

**Western Blot Analysis**

G protein α subunits were studied with immunoblotting techniques. The polyclonal antisera MB1 was raised in rabbits against the carboxyl-terminal decapeptide of retinal transduction (KENLKDCCGLF) coupled to keyhole limpet hemocyanine. The MB1 recognized Gₛ and Gᵢ, but not Gₛ and Gᵢ. The membrane fractions were electrophoresed in SDS-polyacrylamide gels and were transferred to nitrocellulose filters. The filters were incubated with the first antibodies for Gₛ (MB1) or Gᵢ (RM1) and then with the second antibody (horseradish peroxidase–conjugated goat anti-rabbit IgG, Amersham). Immunoreactive signals were detected by means of the ECL kit (Amersham).

**Plasma ANP Determination**

Plasma ANP was measured with a specific radioimmunoassay kit according to the manufacturer’s instruction (Biotrend) after extraction by means of Sep-Pak C₁₈ cartridges (Bond Elut, Varian).

**Northern Blot Analysis of Cardiac ANP**

Total RNA was isolated from LV tissue and was analyzed as described previously. In brief, 10 μg of total RNA was separated by gel electrophoresis, was blotted onto nylon filters, and was fixed by ultraviolet crosslinking. The blots were hybridized with a random-primed 32P-radiolabeled cDNA probe for ANP. The signals on the autoradiographs were quantified densitometrically. GAPDH was used as an internal control to normalize for differences in loading of RNA.

**Isolated Papillary Muscle Studies**

In separate experiments, animals from SHR groups were assigned to papillary muscle studies, and isometric force of contraction was recorded as described previously. Ten weeks after the coronary ligation or sham operation, the hearts were excised, and LV noninfarcted papillary muscles were dissected free. The muscles were suspended in an organ bath and were electrically stimulated (frequency 1 Hz, impulse duration 5 ms, voltage 10% to 20% above threshold). After the baseline data had been recorded, the muscles were incubated with 30 mmol/L isoproterenol.

**Statistical Analysis**

All data are described as mean±SEM. Statistical significance was estimated with the Student t test for unpaired observations or 1-way ANOVA with Fisher’s least significant difference as the post hoc test. The slopes for linear regression lines were compared by ANCOVA. A value of P<0.05 was considered significant.

**Results**

**Structural and Functional Alterations**

The rat hearts in which MI had been induced were characterized by ventricular hypertrophy in the noninfarcted area and marked thinning of the infarcted LV free wall. Infarct size, hemodynamics, and heart weights are summarized in the Table. In the both strains, LV and right ventricular (RV) weights normalized to body weight were increased, and LV pressure and peak positive dp/dt were decreased by MI. Most of these alterations were well related to the infarct size (Figure 1). The slopes of linear regression for RV weight and LV(+ dP/dt) were significantly (P<0.01) steeper in SHR than in WKY (Figure 1B, C).

**Plasma and Cardiac ANP**

Significant increases in plasma ANP concentration (Figure 2A) that were due to MI were seen in SHR (P<0.001) and WKY (P<0.05). Sham-operated (P<0.001) or infarcted (P<0.001) SHR had a higher plasma ANP level than did respective WKY. ANP mRNA levels in the remodeled LV myocardium (Figure 2B) were upregulated in SHR (P<0.05) but not in WKY (P=0.144).

**β-Adrenergic Receptors**

There were no differences in β-adrenergic receptor density of LV membranes between WKY and SHR (¹²⁵I-cyanopindolol bound in fmol/mg protein n=8 to 11 each, WKY-sham 27±2, WKY-MI 24±4, SHR-sham 26±5, SHR-MI 31±6). No significant change during post-MI remodeling was detected in either strain. The antagonist affinities as judged on the basis of the kDa values did not differ among all groups (62 to 88 pmol/L).

**Immunoechemical Gₛ and Gᵢ**

The RM/1 recognized both 45- and 52-kD forms of Gₛ. Immunoechemical signals of the both Gₛ bands were similar between sham-operated and MI animals in either WKY and SHR (45-/52-kDa in densitometric unit n=8 to 11 each,
On the other hand, immunochemical \( G_i \) was increased by 53% (\( P, 0.01 \)) in SHR-MI compared with that in SHR-sham, but it was not altered in WKY (Figure 3).

**Contraction of Isolated Papillary Muscle**

The basal force of contraction of papillary muscles isolated from SHR was significantly decreased (\( P, 0.05 \)) in SHR-MI (0.58 ± 0.12 mN) when compared with that in SHR-sham (0.71 ± 0.35 mN). As shown in Figure 4, the isoproterenol-stimulated force of contraction was more markedly depressed (\( P<0.01 \)) in SHR-MI (0.66 ± 0.12 mN, 118 ± 8% of basal value) than in SHR-sham (1.10 ± 0.51 mN, 150 ± 6% of basal value).

**Remodeling and \( G_i \)**

Figure 5A shows a significant correlation between \( G_i \) and infarct size in WKY but not in SHR. The same data of \( G_i \) were then analyzed in a classification according to plasma ANP level; low ANP, \(<100\) pg/mL; high ANP, \(\geq 100\) pg/mL (Figure 5B). Although there was no difference in infarct size among the 3 MI groups (WKY-low ANP 21 ± 2%, SHR-low ANP 22 ± 3%, SHR-high ANP 24 ± 4%), there was a significant increase in \( G_i \) of SHR-high ANP compared with that in SHR-low ANP (\( P<0.01 \)) and WKY-low ANP (\( P<0.001 \)) groups. Finally, the relationship of LV weight to \( G_i \) was analyzed in rats that had undergone sham operation (Figure 6A) and in those in which post-MI remodeling occurred (Figure 6B). The significant linear correlation was observed in the SHR that had undergone sham operation and in WKY with post-MI remodeling.

**Discussion**

This is the first report to explore the \( \beta \)-adrenergic receptor G-protein alterations in the post-MI remodeling model of SHR. It demonstrated that hypertensive hypertrophied hearts underwent more progressive LV dysfunction because of MI compared to normotensive hearts, that \( G_i \) increased in the

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**Table: Infarct Size, Body and Heart Weights, and Hemodynamic Data**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WKY-sham (n=16)</th>
<th>WKY-MI (n=21)</th>
<th>SHR-sham (n=18)</th>
<th>SHR-MI (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS, %</td>
<td>23 ± 2</td>
<td>21 ± 2</td>
<td>23 ± 2</td>
<td></td>
</tr>
<tr>
<td>BW, g</td>
<td>457 ± 10</td>
<td>474 ± 8</td>
<td>373 ± 5†</td>
<td>382 ± 6†</td>
</tr>
<tr>
<td>LV/BW, mg/g</td>
<td>1.84 ± 0.03</td>
<td>2.11 ± 0.05*</td>
<td>2.87 ± 0.05†</td>
<td>3.16 ± 0.05†</td>
</tr>
<tr>
<td>RV/BW, mg/g</td>
<td>0.46 ± 0.02</td>
<td>0.64 ± 0.05*</td>
<td>0.60 ± 0.02†</td>
<td>0.89 ± 0.07†</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>81 ± 4</td>
<td>76 ± 4</td>
<td>118 ± 5†</td>
<td>95 ± 4†</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>347 ± 8</td>
<td>340 ± 8</td>
<td>351 ± 9</td>
<td>328 ± 10</td>
</tr>
<tr>
<td>LVP, mm Hg</td>
<td>98 ± 4</td>
<td>86 ± 3*</td>
<td>116 ± 5†</td>
<td>104 ± 4†</td>
</tr>
<tr>
<td>+dp/dt, mm Hg/s</td>
<td>3522 ± 103</td>
<td>2862 ± 117*</td>
<td>5013 ± 102†</td>
<td>3819 ± 233†</td>
</tr>
</tbody>
</table>

WKY indicates Wistar-Kyoto rat; SHR, spontaneously hypertensive rat; sham, sham-operated; MI, myocardial infarction; IS, infarct size expressed as a percentage of left ventricle; BW, body weight; LV, left ventricular weight; RV, right ventricular weight; MAP, mean arterial pressure; HR, heart rate; LVP, left ventricular pressure; and +dp/dt, left ventricular peak positive dp/dt. Values are expressed as mean ± SEM.

\*\( P<0.05 \) vs. sham.
\†\( P<0.05 \) vs. WKY.
post-MI remodeling myocardium with pre-existing hypertrophy in association with the impairment of contractile response to β-adrenergic stimulation, and that both the development of LV hypertrophy that occurred because of chronic pressure overload and that resulting from the loss of myocardium were correlated with an enhancement of \( G_{i\alpha} \).

The pathophysiological alterations after coronary artery ligation in rats resemble those in patients with MI. 1,3 Although the infarcted myocardium thins with a scar formation, the noninfarcted myocardium hypertrophies in response to the increased wall stress. Pfeffer et al2 have demonstrated that there is a good correlation between the degree of impairment of LV function and infarct size in the rat model of infarction. Two previous studies compare hypertensive and normotensive hearts with respect to functional changes after MI. Fletcher et al18 have shown that the reductions of LV pressure and peak stroke volume that developed because of post-MI remodeling were more remarkable in SHR than in normotensive rats. Nishikimi et al 19 have indicated that the increases in LV end-diastolic pressure and RV weight after MI were greater in SHR than in WKY. Similarly, in this study, the increase in RV weight and LV dysfunction in proportion to infarct size were more prominent in SHR than in WKY. RV weight reflecting the elevated LV filling pressure is a good marker for cardiac decompensation.2,19 In addition, plasma ANP and cardiac ANP mRNA levels, which are reliable indices of decompensation after infarction,20 increased more remarkably in SHR than in WKY. These mechanical and neurohumoral changes indicate that hypertrophied hearts are more susceptible to ischemic damage and demonstrate more advanced heart failure than do normotensive hearts.

The SHR is an established model of genetic hypertension in which hypertensive cardiac hypertrophy similar to that occurring in hypertensive patients develops.13 The hypertrophied myocardium is initially an adaptational response to reduce wall stress, but that hypertrophy progresses to heart failure.5 The prolonged sympathetic activation leading to β-adrenergic desensitization is a key alteration in the transition from cardiac hypertrophy to cardiac failure.7 In human end-stage heart failure, a downregulation of β-adrenoceptors and an increase in \( G_{i\alpha} \) have been well documented.10–12 Nevertheless, there are controversial reports concerning the number (a decrease, 7 no change, 21 or an increase 22 ) of β-adrenoceptors in the heart of SHR. The majority of previous reports7,23,24 have demonstrated an increase in \( G_{i\alpha} \) of SHR, with the exception of 1 report showing no change.25 In this study, neither the numbers of β-adrenoceptor nor \( G_{i\alpha} \) were significantly different in WKY-sham and SHR-sham.
The inconsistent result might be explained by several factors that could influence the advance of \( \beta \)-adrenergic desensitization. Because cardiac hypertrophy advances with age, the difference in the age of SHR could explain the inconsistency. In addition, a genetic heterogeneity among SHR provided by different suppliers could evoke a variability in the development of hypertension.\(^3\) The extent of cardiac hypertrophy and cardiac failure may vary, even when the rats are of similar age, in different rat colonies.\(^5\,13\)

The infarcted area was completely replaced by fibrous tissue and no longer contributed to LV performance at 10 weeks after MI. Therefore, the alterations in the \( \beta \)-adrenergic receptor G protein signaling that was associated with impaired LV function were studied in the residual noninfarcted myocardium. No significant change in \( \beta \)-adrenergic number was induced by MI in WKY; this is consistent with prior studies examining the noninfarcted myocardium of rats with chronic MI.\(^9\,26\) Recent reports\(^27\,28\) have demonstrated that \( G_i \) increased 4 to 16 weeks after MI in the failing heart of normotensive rats. As shown in this study (Figure 5A), an increase in \( G_i \) correlates with infarct size in normotensive hearts. Drexler et al\(^20\) demonstrated that plasma ANP increased in association with the decompensation of post-MI normotensive rat hearts only when the infarct size exceeded 30% of LV. Thus, the smaller infarct size in our model could account for the less alteration in \( G_i \) and the less overt heart failure in WKY with MI when compared with the respective results in previous studies\(^27\,28\) in which rats with a large MI (>30% of LV) were enrolled. In contrast, a significant increase in \( G_i \) that was independent of infarct size, and was in accord with a high level of plasma ANP, was induced by MI in hypertensive rat hearts. In addition, papillary muscles isolated in the post-MI remodeled heart of SHR had a reduced contractile response to \( \beta \)-adrenergic stimulation. Because \( \beta \)-adrenoceptors and \( G_i \) were unchanged, these findings suggest that the activation of \( G_i \) is functionally important for the impairment of LV function in this animal model. It is noteworthy that there is a close correlation between the level of \( G_i \) and LV hypertrophy either as a result of chronic hypertrophy (in SHR) or of post-MI remodeling (in WKY), which could suggest that \( G_i \) signals the hypertrophic responses and/or stimuli, even though the causal relation remains to be elucidated. There is a considerable variability in \( G_i \) and LV hypertrophy in SHR-sham that could account for the variable level of \( G_i \) and the heart failure that developed after similarly sized MI in SHR. In this respect, an elevation of \( G_i \) preceding MI should predict a poor outcome of post-MI remodeling in hypertensive hearts. Considered together, an increase in \( G_i \) could be involved in the transition from cardiac hypertrophy to cardiac failure in response to pressure overload and a coexisting loss of contractile mass that results from infarction.

Although it is not thoroughly understood which mechanisms are responsible for the regulation of \( G_i \) expression, it has been speculated that the mechanisms should involve chronic activation of the sympathetic nervous system. Chronic exposure of \( \beta \)-adrenoceptor agonists has been reported to increase \( G_i \) in rat hearts.\(^29\) Our results indicating good correlation between \( G_i \) and 2 different phenotypes of LV hypertrophy encourage this hypothesis, because a magnitude of LV hypertrophy should reflect the time-integrated exposure to either pressure overload or ischemic damage, both of which should be accompanied by prolonged sympathetic activation.\(^1\,7\) Recent studies\(^1\,10\) have revealed that \( \beta \)-adrenoceptor couples dually with \( G_i \) and \( G_s \), that \( \beta \)-adrenoceptor couples exclusively with \( G_i \), and that the coupling of \( \beta \)-adrenoceptor to \( G_i \) negates \( G_i \)-mediated cardiac contractility. \( \beta \)-Adrenoceptor signaling and its coupling to \( G_i \) could be relevant to the pathogenesis of cardiac hypertrophy and failure.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (M.B.) and NOVARTIS Foundation (Japan) for the Promotion of Science (I.K.).

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*Hypertension*. 2000;36:42-47
doi: 10.1161/01.HYP.36.1.42

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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