Alterations of adenylyl cyclase and G proteins in aortocaval shunt-induced heart failure

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Wang, Xi, Emmanuelle Sentex, Donald Chapman, and Naranjan S. Dhalla. Alterations of adenylyl cyclase and G proteins in aortocaval shunt-induced heart failure. Am J Physiol Heart Circ Physiol 287: H118–H125, 2004. First published February 12, 2004; 10.1152/ajpheart.00798.2003.—Unlike most other experimental models of congestive heart failure, the volume overload model induced by aortocaval shunt (AVS) in rats was found to exhibit enhanced β-adrenergic receptor (β-AR) signaling. To study whether the adenylyl cyclase (AC)-G protein system is involved in such a change, we examined cardiac AC activity and protein content as well as Gαs and Gαi activities, protein contents, and mRNA levels in both left (LV) and right (RV) ventricles at the failing stage (16 wk after surgery). Basal and forskolin-stimulated AC activities were significantly increased in both LV and RV from the failing hearts; this change was associated with an upregulation of type V/VI AC protein. In contrast to 5′-guanylyl imidodiphosphate and NaF, the stimulatory effect of isoproterenol on AC was increased in the failing heart. Although Gαs and Gαi protein contents in the failing hearts were not altered, the mRNA level for Gαs was decreased by 20% and that for Gαi was increased by 20%. In addition, the activity of Gαo, but not Gαs, as assessed by toxin-catalyzed ADP ribosylation, was significantly decreased in the failing heart. Losartan and imidapril treatments improved cardiac function and attenuated alterations in mRNA levels for Gαs and Gαi proteins, as well as Gαo activity, without affecting changes in AC protein content or activities in heart failure due to volume overload. These data suggest that increased AC activity may contribute to the enhanced β-AR signaling in the AVS model of heart failure, whereas alterations in gene expression for G proteins may be of an adaptive nature at this stage of heart failure.

Gαi and Gαo proteins; cardiac hypertrophy; volume overload

The β-adrenergic receptor (β-AR)-coupled signal transduction system mediates the positive inotropic and lusitropic effects of neurally released and circulating catecholamines in the heart (2–4, 9, 16, 34). This system is composed of β-ARs, guanine nucleotide-binding proteins (G proteins) and adenylyl cyclase (AC). Conformational changes of β-AR on binding of catecholamines activate Gαi protein α-subunit, which stimulates AC activity and leads to increased generation of the second messenger cAMP. This increase in cAMP level then activates cAMP-dependent protein kinase (PKA), stimulates the phosphorylation of a series of Ca2+-regulating proteins, and results in an increase in intracellular concentration of Ca2+ as well as cardiac contractility. Several studies have revealed downregulation of β1-AR, increased expression of Gαi protein, upregulation of G protein-coupled receptor kinase (GRK)2, and impaired AC activities in the failing heart (2–4, 9, 16, 34). A review of the literature indicates that the majority of these studies were carried out during end-stage heart failure in humans and in animal models of heart failure induced by myocardial infarction (MI) of pressure overload. However, very little information is available regarding changes in β-AR-mediated signal transduction mechanisms in a model of heart failure induced by volume overload.

We (36) recently characterized the aortocaval shunt (AVS)-induced model of cardiac hypertrophy and heart failure in rats and found that, unlike most other experimental models of congestive heart failure, the volume overload-induced heart failure exhibited an enhanced β-AR at a time when the basal cardiac function was decreased. In addition, β1-AR density, unlike β2-AR density, was markedly increased in the failing hearts due to volume overload (36). The present study investigated the status of two important components of the β-AR pathway, namely, AC activity and G proteins in both the left ventricle (LV) and the right ventricle (RV) in the rat model of volume overload-induced heart failure. In view of the beneficial effects of angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor (AT1R) antagonists in the volume-overload model (28, 29), we examined the effects of imidapril (35), an ACE inhibitor, and losartan (29), an AT1R antagonist, on changes in AC and G proteins in the heart failure induced by AVS.

MATERIALS AND METHODS

Animal model and experimental groups. Experiments were conducted in accordance with the Guide to the Care and Use of Experimental Animals issued by the Canadian Council on Animal Care. The AVS was produced in rats as described previously (36). Sham-operated animals served as controls, and shunted rats were randomly divided into three groups: AVS, AVS treated with losartan (AVS+Los), and AVS treated with imidapril (AVS+Imp). Losartan (20 mg/kg·day−1) and imidapril (1 mg/kg·day−1) were dissolved in tap water, and treatment was started 3 days after the surgery by gastric gavage; tap water served as vehicle for the sham-treated group. Animals were used 16 wk after the surgery, when clinical signs of heart failure were readily evident. Preliminary studies showed no difference between sham and sham with treatment of losartan or imidapril with respect to the parameters studied, and thus no sham treatment groups were included.

In vivo hemodynamic assessment. Arterial systolic pressure, arterial diastolic pressure, mean arterial pressure, LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), heart rate (HR), rate of pressure development (+dP/dt), and rate of pressure decay (−dP/dt) were recorded in vivo in anesthetized animals through carotid catheterization as described previously (36).

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Myosin heavy chain isoenzyme analysis. Because a shift in myosin heavy chain (MHC) isoenzymes is considered to be a good marker for cardiac hypertrophy and heart failure (25, 35), the composition of MHC isoenzymes was determined by polyacrylamide gel electrophoresis in the presence of pyrophosphate (25). Portions of both LV and RV (∼50 mg) were cut into small pieces and extracted for 15 min by gentle agitation at 0°C with 3 vol/vol of (in mM) 40 Na₃P₂O₇ (pH 8.8), adjusted with HCl at 2°C, 1, 1,4-dithioerythritol, and 5 EGTA. After centrifugation at 2,000 g for 15 min, the supernatant was collected and diluted 1:10 (vol/vol) with ice-cold glycerol and immediately loaded on the gel. The gel contained 3.8% acrylamide and 0.12% N,N′-methylenbis-acrylamide, and the electrophoresis buffer, 10 mM was 20 mM Na₃P₂O₇ (pH 8.8)-10% glycerol (vol/vol). Electrophoresis was carried out at 2°C for ~16 h at a voltage gradient of 10 V/cm; gels were stained with Coomassie brilliant blue R250 for 2 h and were destained with 7% acetic acid by diffusion. Relative amounts of isoenzymes were estimated from densitometric tracings with a Quick Scan densitometer (Desaga, Heidelberg, Germany). MHC isoenzymes V₁, V₂, and V₃ were quantitated by measuring peak heights, and values were expressed as percentage of total isoenzymes.

Determination of AC activity. The AC activity was determined by measuring the formation of [32P]cAMP from [3-32P]ATP as described previously (31). Unless otherwise indicated, the incubation assay medium contained (in mM) 50 glycylglycine (pH 7.5), 0.5 cAMP, 0.5 MgATP, 100 NaCl, 5 MgCl₂, 0.1 EGTA, and 0.5 3-isobutyl-1-methylxanthine with 10 U/ml adenosine deaminase, [32P]ATP (1–1.5 × 10⁶ cpm), and an ATP-regenerating system comprising 2 mM creatine phosphate, 0.1 mg/ml creatine kinase, and 36 U/ml myokinase in a final volume of 200 µl. The reaction was initiated by addition of 40–60 µg of crude membranes to the reaction mixture, which had been equilibrated for 3 min at 37°C. The incubation time was 10 min at 37°C, and the reaction was terminated by addition of 0.6 ml of 120 mM zinc acetate containing 0.5 mM unlabeled cAMP. [32P]cAMP formed during the reaction was determined on co precipitation of other nucleotides with ZnCO₃ by the addition of 0.5 ml of 144 mM Na₃CO₃ and subsequent chromatography with a double-column system as described previously (31). The unlabeled cAMP served to monitor the recovery of [32P]cAMP by measuring absorbency at 259 nm. The AC activity was expressed as picomoles of cAMP per milligram of protein per 10 minutes. The AC activity was expressed as picomoles of cAMP per millgram of protein per 10 minutes.

Statistical analysis. All values are expressed as means ± SE. Western blot and Northern blot data are expressed as percentage of control. One-way ANOVA followed by unpaired Student’s t-test was used for comparisons between groups. Differences were considered statistically significant at a level of P < 0.05.

RESULTS

General and hemodynamic features. Table 1 shows the general and hemodynamic characteristics of rats 16 wk after AVS with or without imidapril or losartan treatments. Consistent with our previous findings (36), marked hypertrophy of both LV and RV was observed in the AVS group without any changes in either body weight or HR. LVSP, +dP/dt, and −dP/dt were significantly depressed, whereas LVEDP was dramatically increased, indicating both systolic and diastolic dysfunction at this stage of heart failure. All these changes in the AVS group were attenuated by treatment with imidapril or losartan. Unlike the hypertrophic and contractile function, the depressed arterial systolic pressure, arterial diastolic pressure, and mean arterial pressure in the AVS group were not reversed.

Table 1. General and hemodynamic characteristics of sham-treated and experimental rats with heart failure induced by aorticocaval shunt for 16 wk with and without imidapril or losartan treatment

<table>
<thead>
<tr>
<th></th>
<th>SH</th>
<th>AV</th>
<th>AV + Imp</th>
<th>AV + Los</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>595 ± 21</td>
<td>607 ± 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVW (mg)</td>
<td>1,001 ± 23</td>
<td>1,472 ± 66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RLV, mg</td>
<td>281 ± 11</td>
<td>472 ± 27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td>545 ± 35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>beats/min</td>
<td>350 ± 18</td>
<td>339 ± 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP, mmHg</td>
<td>118 ± 2.6</td>
<td>109 ± 6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>82 ± 4.8</td>
<td>60 ± 3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>356 ± 20</td>
<td>356 ± 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>4.5 ± 0.6</td>
<td>14.7 ± 2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>6.330 ± 206</td>
<td>4.665 ± 182</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>6.292 ± 315</td>
<td>5.297 ± 166</td>
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</table>

Data are expressed as means ± SE from 4–6 hearts/group. Imidapril (1 mg kg⁻¹ day⁻¹) and losartan (20 mg kg⁻¹ day⁻¹) were given daily by gavage. Sh, sham-treated control; AV, aorticocaval shunt; AV + Imp, AV with imidapril treatment; AV + Los, AV with losartan treatment; BW, body weight; LVW, left ventricular (LV) weight; RVW, right ventricular weight; ASP, arterial systolic pressure; ADP, arterial diastolic pressure; MAP, mean arterial pressure; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; +dP/dt, maximum rate of pressure development; −dP/dt, maximum rate of pressure decay. *P < 0.05 compared with SH group; †P < 0.05 compared with AV group.
significantly by treatment with imidapril or losartan. As shown in Fig. 1, AVS also induced MHC V$_1$ to V$_3$ shift; however, this change in MHC isoenzyme composition was attenuated by losartan but not by imidapril. It should be pointed out that the inability of imidapril to attenuate the volume overload-induced shift in myosin isoforms may not be due to the dose of this agent because the same dose of imidapril was observed to prevent the shift of myosin isoforms in heart failure due to MI (35).

**Altersations in AC.** Figure 2 shows changes of AC activities in both LV and RV in the absence or presence of different stimulants. A significant increase of AC activity was observed in the absence (basal) as well as presence of isoproterenol, Gpp(NH)p, NaF, or forskolin in the AVS group. When the data were converted to fold stimulation of the enzyme activity over the respective basal values (Table 2), only isoproterenol- and forskolin-stimulated AC activities were increased, whereas Gpp(NH)p- and NaF-stimulated AC activities were unaltered in the AVS group. Neither imidapril nor losartan reversed these changes. To examine whether the increased basal AC activity in the AVS group was associated with an increase in protein content of the enzyme, we used type V/VI AC antibody to determine the protein content of AC. Figure 3 shows a dramatic increase in AC protein content in both LV and RV from the AVS group. The treatment of AVS animals with imidapril or losartan did not reverse changes in protein content, indicating that these drug treatments were ineffective in modifying AC and G proteins in heart failure.

**Table 2. Fold stimulation of cardiac adenylyl cyclase activities due to different agents in rats with heart failure induced by aortocaval shunt for 16 wk with and without imidapril or losartan treatment**

<table>
<thead>
<tr>
<th></th>
<th>isoproterenol</th>
<th>Gpp(NH)p</th>
<th>NaF</th>
<th>Forskolin</th>
</tr>
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<tbody>
<tr>
<td>LV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>3.89±0.21</td>
<td>3.81±0.29</td>
<td>4.80±0.22</td>
<td>5.75±0.13</td>
</tr>
<tr>
<td>AV</td>
<td>5.48±0.36*</td>
<td>3.87±0.14</td>
<td>4.93±0.36</td>
<td>8.58±0.50*</td>
</tr>
<tr>
<td>AV+Imp</td>
<td>5.27±0.19</td>
<td>3.81±0.15</td>
<td>4.82±0.28</td>
<td>9.28±0.29</td>
</tr>
<tr>
<td>AV+Los</td>
<td>5.05±0.22</td>
<td>3.39±0.24</td>
<td>4.50±0.27</td>
<td>8.38±0.34</td>
</tr>
<tr>
<td>RV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>2.55±0.16</td>
<td>3.75±0.18</td>
<td>4.69±0.16</td>
<td>5.78±0.33</td>
</tr>
<tr>
<td>AV</td>
<td>3.57±0.18*</td>
<td>3.89±0.30</td>
<td>4.51±0.29</td>
<td>8.24±0.52*</td>
</tr>
<tr>
<td>AV+Imp</td>
<td>3.98±0.11</td>
<td>4.09±0.23</td>
<td>4.73±0.35</td>
<td>8.40±0.12</td>
</tr>
<tr>
<td>AV+Los</td>
<td>3.77±0.31</td>
<td>4.08±0.22</td>
<td>4.39±0.30</td>
<td>9.06±0.36</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE calculated from the data shown in Fig. 2. Imidapril (1 mg·kg$^{-1}$·day$^{-1}$) and losartan (20 mg·kg$^{-1}$·day$^{-1}$) were given daily by gavage. Doses for isoproterenol, Gpp(NH)p, NaF, and forskolin are 100 µM, 30 mM, 10 mM, and 100 µM, respectively. *Significantly ($P < 0.05$) different from respective sham-treated group.

**Fig. 1. Aortocaval shunt-induced changes in myosin heavy chain (MHC) isoenzymes and the effect of treatment of losartan or imidapril for 16 wk after surgery.** A: Coomassie blue staining of MHC isoenzymes in polyacrylamide gel in the presence of pyrophosphate. LV, left ventricle; RV, right ventricle; Sh, sham treatment; AV, aortocaval shunt; AV+Imp, AV with imidapril treatment; AV+Los, AV with losartan treatment. B: densitometric tracings of MHC isoenzymes. C: statistical data derived from 4 separate hearts in each group. Imidapril (1 mg·kg$^{-1}$·day$^{-1}$) or losartan (20 mg·kg$^{-1}$·day$^{-1}$) was given daily by gavage. V$_1$, V$_2$, and V$_3$, MHC isoenzymes. *$P < 0.05$ compared with Sh; **$P < 0.05$ compared with AV.

**Fig. 2. Adenylyl cyclase (AC) activities in the absence (basal) or presence of different stimulants in the LV (A) and the RV (B) 16 wk after aortocaval shunt.** The concentrations of isoproterenol (Iso), 5’-guanylyl imidodiphosphate (Gpp[NH]p), NaF, and forskolin (Forsk) were 100 µM, 30 µM, 10 mM, and 100 µM, respectively. Values are means ± SE from 4–6 hearts in each group. *$P < 0.05$ compared with Sh. All other conditions are the same as described in Table 2.
AC. The possibility that higher doses of imidapril or losartan were required for attenuating the increase in AC protein content was not ruled out.

Alterations in G proteins. Because cardiac AC activity is predominantly under the dual regulation of Gs and Gi proteins (2, 9), we determined changes in the G protein levels in the heart. Because G-protein subunit H9251 has ADP ribosylation sites for CT (Gs/H9251) and PT (Gi/H9251), both CT- and PT-catalyzed ADP ribosylation activities were measured in sham-treated and experimental groups. Figure 4 reveals two bands of ribosylation substrates catalyzed by CT (one at 52 kDa and the other at 45 kDa), reflecting the protein products from spliced Gα gene, whereas only one band at 40 kDa was detected as ribosylation substrate for Gα protein. A significant decrease in CT-catalyzed ADP ribosylation was observed in the AVS group in both LV and RV; imidapril or losartan treatment partially reversed this change (Fig. 4, top). On the other hand, no change was found in PT-catalyzed ADP ribosylation among all the groups (Fig. 4, bottom). Because the accuracy of this methodology depends on a number of factors such as biophysical membrane properties, posttranslational modifications of Go proteins, and several cofactors required for the ADP ribosylation reaction, the changes detected may reflect a change in one of the factors rather than a real change in the protein expression. To examine this possibility, we used the Western blot method to detect relative protein content of both Gs and Gi subunits. The results in Fig. 5 show no change in either Gs (Fig. 5, top) or Gi subunit (Fig. 5, bottom) protein content in both LV and RV from all groups. This seems to suggest that the decreased CT-catalyzed ADP ribosylation may be due to either the biophysical properties of the membrane or posttranscriptional modulation of Gs subunit rather than a decreased expression of the Gs protein content. We also measured the mRNA level of both Gs and Gi2 proteins and found a small (20%) but significant decrease for Gs protein and increase for Gi2 protein; RNA loading and typical bands for Gs and Gi proteins are shown in Fig. 6A, and the statistical data are shown in Fig. 6B.
Altering the G protein in heart failure. Compared with two other components in the β-AR system, namely, β-ARs and G proteins, relatively less information is available about changes in AC during the development of heart failure. This may be due to several reasons, including that 1) this enzyme is regulated by Gε and Gi proteins and is extremely unstable and difficult to assess under in vivo and in vitro conditions, and 2) there are no specific agonists or antagonists available at present for this enzyme. However, it is the amount of AC that may set a limit on the status of β-AR transmembrane signaling because the estimated molar proportion of β-AR-Gε-AC is 1:200:3 (24). In addition, increased cardiac β-AR or Gi protein expression does not yield a proportional increase in transmembrane signaling (11, 14, 21), whereas increased expression of type VI AC in neonatal cardiomyocytes has been shown to increase β-AR-stimulated production of cAMP proportionally. Such evidence indicates the important role of AC in mediating, regulating, and controlling the signals imposed on β-ARs. Furthermore, the activity of AC is modified in different ways, including changes in the receptor, G proteins, and catalytic unit. The activities of AC measured in the presence of different stimulants reflect the contribution of respective sites in regulating the enzyme. Whereas isoproterenol is known to activate AC through β-ARs, both Gpp(NH)p and NaF are used to activate Gε proteins. Gpp(NH)p is a less hydrolyzable analog of GTP, which binds to the Gε subunit and thus keeps the Gε protein in a constant active state. On the other hand, NaF directly interacts with the Gε subunit and activates its function. Forskolin is known to interact with the catalytic unit of AC directly and exert stimulating effects. Our results show increased basal AC activity as well as AC activity in the presence of different stimulants at 16 wk after induction of AVS in rats. This change functionally reflects an enhanced response to β-AR stimulation both in vivo and in vitro by isoproterenol (36).

Because of the upregulation of basal AC activity, the increase in AC activity observed in the presence of different stimulants may reflect amplification based on the basal values rather than an increase due to stimulation. The data calculated as the fold stimulation over the basal level indicated that isoproterenol- and forskolin-induced activation of AC in the AVS group was greater than, whereas the fold stimulation due to Gpp(NH)p or NaF in the AVS group was similar to, that in the control group. This suggests enhanced signals at the receptor and effector levels. Unpublished data in our laboratory have indicated that β₁-AR density, examined by binding experiments, and β₁-AR protein content, examined by Western blot analysis, were significantly increased. In addition, GRK activity and GRK2, -3, and -5 protein content were markedly decreased in the membrane fraction but increased in the cytosolic fraction in the AVS group. These results together confirm an upregulation of the β-AR mechanism at the receptor level in the AVS group. By using an antibody that recognizes both type V and type VI isoforms of AC, we have shown that an increase in protein content of AC was induced in both LV and RV 16 wk after AVS; this forms the basis for the increased basal and forskolin-stimulated activities in failing hearts due to volume overload.

It should be pointed out that Di Fusco et al. (10) have also studied AC activities in this model at an early stage (10 days after surgery). These investigators reported a decreased stimulation of AC activity by isoproterenol, forskolin, and NaF. Such a discrepancy in results may be due to the difference in the time at which the tissues were examined after AVS was induced. In fact, we (36) found time-dependent changes in cardiac function and responses to isoproterenol in this model. In a rat model of MI, the attenuated response to isoproterenol in the LV was associated with significantly depressed basal as well as forskolin-stimulated AC activities, whereas enhanced basal and forskolin-stimulated AC activities were observed in the hypertrophied and compensated RV (31, 32). On the other hand, forskolin-stimulated AC activity was depressed in association with or without corresponding changes in the basal AC activity in different experimental models of heart failure (1, 3, 7, 15, 19, 30, 32). Rapid pacing-induced heart failure caused a reduction in basal and forskolin-stimulated AC activities, as well as the steady-state mRNA level for both type V and type VI AC in dogs (18). Other studies using the paced-pig model of heart failure found that AC activities were depressed at a severe stage of heart failure, with decreased mRNA expression for type VI AC but not for type V AC (23). Espinasse et al. (12) also reported a reduction of type VI AC mRNA level without any change in type V AC in severe heart failure due to MI. However, because of the unavailability of antibodies for type V AC, these results were not further studied.
or type VI AC we were unable to measure the AC protein expression of different AC isoforms. At any rate, the results in this study indicate an increased expression of AC proteins in hearts failing due to volume overload. This observation, along with a dramatic increase in the basal and forskolin-stimulated AC activities, suggests an upregulation of AC in heart failure due to AVS. It is reasonable to assume that such an upregulation of AC will induce an increased activation of downstream effectors, such as PKA. In fact, Lavendero et al. (20) carried out a time course study of PKA activity in this model. In agreement with our view, they found 2.7-, 9-, and 4-fold increases of PKA activity at 2, 7, and 56 days, respectively. Thus increased activity of AC and its downstream effectors form the biochemical basis for sensitized β-AR signaling in this model of heart failure induced by volume overload.

Alterations in G proteins in AVS-induced heart failure. Because functional studies have shown an enhanced response of AVS rat hearts on β-AR stimulation both in vivo and in vitro (36), it can be argued that the changes in G proteins may participate in enhanced β-AR signaling in this model. To our surprise, we detected no changes in protein expression for both Gαs and Gαi subunits in this experimental model. On the other hand, functional studies using toxin-mediated ribosylations showed a significant reduction in CT-mediated Gαs ribosylation without any change in PT-mediated Gαi ribosylation. Furthermore, a slight but significant decrease of Gαs mRNA level and increase of Gαi mRNA level were found. These changes seem to suggest the desensitization of β-AR signals through G proteins, which is in contrast to the findings with respect to myocardial functional responses to β-AR stimulation in this model. Because the estimated molar proportions of the elements of the β-AR-Gαs-AC complex in cardiac myocytes are 1:200:3 (24), it seems that a large amount of G proteins is not coupled with the β-AR system. In fact, some studies have shown that Gαs proteins can regulate myocardial contractility in a cAMP-independent mechanism by affecting the L-type Ca²⁺ channel on the membrane (22, 37). Thus the decreased Gαs protein functionality may contribute to the decreased basal myocardial contractility observed in this experimental model. However, because the fold stimulation of AC activity by NaF or Gpp(NH)p was not altered in the AVS group, G proteins can be seen to serve as an adaptative change at this stage of heart failure. Nonetheless, the discordance of mRNA expression and protein expression may be due to the fact that we only examined the steady-state mRNA level, whereas alterations in the rates of mRNA degradation, transcriptional changes, and protein degradation influence the gene and protein expression. It is possible that the duration of the experimental period after the AVS used in this study may not be appropriate to reveal the required changes.

Modification of AC and G proteins in AVS-induced heart failure by imidapril and losartan. It has been reported that plasma renin activity and cardiac renin activity were induced shortly after the induction of AVS in rats (28) and treatment with ACE inhibitor and AT1R blocker attenuated the hemodynamic and hypertrophic responses induced by AVS (26, 27, 29). Our results show that treatments of experimental animals with imidapril or losartan produced similar effects in attenuating hemodynamic and hypertrophic responses to AVS. In addition, these agents were found to modify the β-AR signaling pathway by attenuating the increased β1-AR density and protein content as well as redistribution of GRK isoform content in the failing hearts due to volume overload (unpublished data). As discussed above, the changes in CT-mediated ADP ribosylation and mRNA levels for Gαs and Gαi proteins did not appear to contribute to the sensitized β-AR signaling in this model. Instead, they might contribute to decreased basal myocardial contractility through a cAMP-independent pathway. Although it is difficult to conclude regarding the mechanisms of the effects of treatments on Gs-related ADP ribosylation as well as mRNA levels for G proteins, one possibility is that these drugs may directly or indirectly target G proteins. Similar results were shown by Horn et al. (17) in human congestive heart failure treated by captopril and lisinopril. It should be pointed out that afterload of the heart in the drug-treated groups, as assessed by arterial systemic pressures, was not lower than that in the AVS group and thus it appears that the observed attenuation of changes in G proteins and mRNA levels for both Gs and Gi proteins is not due to reduction in the afterload. On the other hand, LVEDP in the drug-treated AVS group was reduced to half of that in the untreated shunted group, indicating a reduction in wall stress in the AVS hearts on drug treatments. Accordingly, it is possible that attenuation of the observed changes in G protein gene expression and other β1-AR signal transduction mechanisms may be a result of improved hemodynamic function of the heart in the AVS group. Although both imidapril and losartan are considered to act by reducing either the formation or the effect of angiotensin II, it may be noted that the AVS-induced shift in myosin was attenuated by losartan but not by imidapril. Differences between the mechanisms of actions of AT1R antagonists and ACE inhibitors have also been reported by others (2, 6, 29). Furthermore, increased AC activities in the absence or presence of different stimulators such as isoproterenol, Gpp(NH)p, NaF, and forskolin as well as protein content.

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for AC in AVS animals were not altered by treatments with imidapril and losartan. Although the possibility that treatments with these agents for periods longer than those used in the study may normalize the AC activity and protein content in the volume-overloaded hearts is not ruled out at this time, the data do not support the view that the observed effects of drug treatments on β-AR mechanisms are entirely due to the beneficial actions of these agents on heart function.

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