Dissociation between regional dysfunction and β-adrenergic receptor signaling in heart failure

TOSHIHISA ANZAI, N. CHIN LAI, MEIHUA GAO, AND H. KIRK HAMMOND
Veterans Affairs Medical Center-San Diego and Department of Medicine,
University of California San Diego, La Jolla, California 92161.

Anzai, Toshihisa, N. Chin Lai, Meihua Gao, and H. Kirk Hammond. Dissociation between regional dysfunction and β-adrenergic receptor signaling in heart failure. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1267–H1273, 1998.—We have previously shown that left ventricular (LV) pacing-induced heart failure is associated with preserved wall thickening in the interventricular septum (IVS) compared with the posterolateral wall (PLW). The current study focuses on the relationship between regional myocardial function and altered β-adrenergic receptor (β-AR) signaling. We studied 15 pigs: 6 controls and 9 paced from the left ventricle (225 beats/min, 26 ± 3 days). Heart failure was documented by decreased LV fractional shortening (P < 0.0001) and increases in hemodynamic parameters, including end-diastolic dimension (EDD), end-systolic dimension (ESD), and left atrial pressure (P < 0.0001). In heart failure, despite marked differences in basal regional function (percent wall thickening: IVS, 33 ± 10% vs. PLW, 13 ± 7%; P = 0.0003), there were no differences between the two regions in β-AR responsiveness, measured by regional wall thickening in response to dobutamine infusion and any measurement of adrenergic signaling. Adenyl cyclase activity, β-AR number, and β-AR/Gs coupling were markedly reduced in failing LV without regional differences. In animals with heart failure, LV G protein receptor kinase (GRK) isoform 2 content was unchanged and GRK5, the other major GRK isoform, was increased more than threefold (IVS, 0.51 ± 0.20 vs. 0.12 ± 0.12 arbitrary densitometric units, P = 0.01; PLW, 0.47 ± 0.15 vs. 0.13 ± 0.09 arbitrary densitometric units, P = 0.03), but again, there were no regional differences. These data indicate that systemic rather than regional factors govern LV adrenergic signaling and that regional adrenergic signaling abnormalities poorly predict wall thickening in the same regions.

G protein-coupled receptor kinase; adenyl cyclase; pacing-induced heart failure; regional contraction

ADRENERGIC OVERACTIVATION in heart failure is associated with altered β-adrenergic receptor (β-AR) signaling including β-AR downregulation (2, 4, 22), uncoupling of β-AR and the stimulatory GTP-binding protein (Gs), and decreased adenyl cyclase activity (22). Brixtow et al. (3) reported that patients with primary pulmonary hypertension have abnormal β-AR signaling in failing right ventricle but not in nonfailing left ventricle, despite systemic adrenergic activation. They concluded that the regulatory process that accounts for adrenergic neuroeffector abnormalities in the failing human heart is under local rather than systemic control. However, the relationship between regional myocardial dysfunction and regional abnormalities in β-AR signaling in the left ventricle in dilated cardiomyopathy has not been previously addressed. Changes in isolated right ventricular failure may not predict those in left ventricular (LV) failure in terms of LV β-AR regulation, particularly with respect to local adrenergic nerve trafficking within the LV.

We have reported preserved function (wall thickening) in the interventricular septum (IVS) compared with the posterolateral wall (PLW) in LV pacing-induced heart failure (15). This model, a model of LV dilated systolic heart failure, provides an ideal opportunity to determine whether regional abnormalities in β-AR signaling within the LV are an important determinant of regional function in the LV. It is noteworthy that previous studies have shown heterogeneous regional wall thickening abnormalities within the LV in patients with idiopathic dilated cardiomyopathy as well (27). The current study was conducted to test the hypothesis that regional LV function and regional alterations in LV β-AR signaling would be tightly linked, suggesting that factors within the LV are more important than systemic factors in the pathogenesis of myocardial adrenergic desensitization in heart failure.

METHODS

Animals and model of heart failure. Animal use was in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services Publication No. (NIH) 85–23, Revised 1985) and institutional guidelines. Fifteen female Hampshire pigs (48 ± 6 kg) were used. Surgical procedures, instrumentation, and induction of heart failure by continuous rapid LV pacing have been previously described (22). After recovery from thoracotomy (10–14 days), animals underwent initial hemodynamic studies, and ventricular pacing was then initiated (225 beats/min) in nine animals (congestive heart failure (CHF)); the remaining six animals were not paced and served as controls. After signs of circulatory congestion developed and substantial hemodynamic abnormalities were present, six of the nine animals were killed, 28 ± 3 days after initiation of pacing, which was 40 ± 5 days after thoracotomy. The remaining three animals in the CHF group underwent studies of regional function in response to dobutamine 21 days after initiation of pacing and were killed 1–2 days after the studies. Hemodynamic measurements were obtained with pacemakers inactivated for 1 h before acquisition of data. We used six of nine animals from a previous study that measured regional myocardial blood flow and function sequentially during the development of pacing-induced heart failure (15). This previous study contained no data regarding adrenergic signaling. To establish that the animals in the current study had heart failure, it was necessary to include hemodynamic data from this subset of animals.

Echocardiographic studies. Two-dimensional and M-mode images were obtained using a Hewlett-Packard Sonos 1500 imaging system. Images were obtained from a right parasternal approach at the midpapillary muscle level and recorded on VHS tape. Measurements were made using criteria from the American Society of Echocardiography (23). All parameters, including end-diastolic dimension (EDD), end-systolic...
Dobutamine stress echocardiography. β-Adrenergic responsiveness to dobutamine infusion was assessed by echocardiography before and 21 days after the initiation of pacing in three animals. Dobutamine was infused into the pulmonary artery before and 21 days after the initiation of pacing in three animals.

Measurements was calculated as \[ \text{Percent wall thickening (\%WTh)} = \frac{\text{ESWTh} - \text{EDWTh}}{\text{EDWTh}} \times 100 \] and was measured in both the IVS and PLW. The coefficient of variation for these parameters on repeated measurements was <5%. All measurements were obtained with pacemakers inactivated.

Membrane preparation. Frozen transmural samples (-80°C) were powdered in a stainless steel mortar and pestle (also -80°C), placed in Tris buffer, and glass-glass homogenized, and contractile proteins were extracted (0.5 M KC1, 20 min, 4°C). The pellet of a 45,000 g centrifugation in our membrane preparation was resuspended in buffer and used for the studies. Protein concentration was determined by the method of Bradford (1).

β-AR binding studies. As previously described (12), β-AR were identified using the radioligand \[^{125}\text{I}\]iodocyanopindolol (ICYP). Data are presented as ICYP bound in femtomoles per milligram membrane protein. Determination of the inhibition constant for isoproterenol and the proportion of β-AR displaying high-affinity binding (an assessment of the degree to which β-AR are coupled with G_s) were performed in competition binding experiments by incubating 100 pM ICYP with 10^-2 to 10^-10 M isoproterenol as previously described (12).

Adenylyl cyclase assays. Methods for measuring adenylyl cyclase activity were modified from Salomon et al. (24) as previously reported (12). The following agents were used to stimulate cAMP production (final concentrations): isoproterenol (10 µM), 5'-guanylimidodiphosphate [Gpp(NH)p; 100 µM], and forskolin (100 µM). We have found that cAMP production under these conditions was linear with respect to time and protein concentration and that 3-isobutyl-1-methylxanthine (1.0 mM), adenosine deaminase (5 U/ml), or methylxanthine (1.0 mM), adenosine deaminase (5 U/ml), or methylxanthine (1.0 mM) could be added to the reaction mixture. The following agents were used to inhibit cAMP production: 8-bromo-cyclic AMP (10 µM), 8-bromo-cyclic GMP (10 µM), and theophylline (100 µM). Adenylyl cyclase activity was measured in the presence of various concentrations of isoproterenol and forskolin, with and without theophylline.

Statistics. Data are expressed as means ± SD. Data obtained from the analysis of hemodynamic consequences of heart failure and fractional shortening were assessed using Student's t-test. Changes in plasma catecholamine content in the two groups were assessed using repeated-measures ANOVA. All other data were compared using ANOVA (Statview 4.0, Abacus Concepts). Post hoc comparisons were performed using the Bonferroni correction. The null hypothesis was rejected when P < 0.05.

RESULTS

Hemodynamic studies. Compared with prepacing measurements, 26 ± 3 days of continuous pacing resulted in the characteristic hemodynamic and functional changes associated with dilated systolic heart failure. There were increases in basal heart rate (control, 117 ± 17 beats/min; CHF, 153 ± 13 beats/min, P = 0.0001), mean pulmonary artery pressure (control, 22 ± 4 mmHg; CHF, 42 ± 4 mmHg, P < 0.0001), and mean left atrial pressure (control, 12 ± 2 mmHg; CHF, 32 ± 6 mmHg, P < 0.0001). At necropsy, hearts were thin walled and dilated, and ascites was present. These data established that CHF was present.

Basal LV function. Fractional shortening, obtained with pacemaker inactivated, was markedly reduced in animals with heart failure (control, 38 ± 4%; CHF, 14 ± 5%, P < 0.0001). LV pacing was associated with significant deterioration in function of the lateral wall compared with the IVS (percent wall thickening: IVS, 33 ± 10% vs. PLW, 13 ± 7%; P = 0.0003; Fig. 1) as previously described (15).

Plasma and LV catecholamine content. Plasma norepinephrine concentration was similar between the two groups 10–14 days after initial thoracotomy (control,
Plasma norepinephrine concentration was increased after the induction of heart failure (control, 441 ± 168 pg/ml; CHF, 1,762 ± 729 pg/ml; P < 0.01). Myocardial norepinephrine content was decreased in both IVS and PLW, with no regional difference detected (control IVS, 680 ± 150 pg/mg; CHF IVS, 155 ± 81 pg/mg, P = 0.001; control PLW, 520 ± 76 pg/mg; CHF PLW, 165 ± 133 pg/mg; P < 0.0001).

β-AR binding studies. Figure 2 shows results of ICYP binding experiments performed on membrane homogenates of transmural LV samples obtained from the IVS and PLW in each of six control and six animals with pacing-induced heart failure. Data shown were obtained from a mean of three experiments per sample per animal, performed with triplicate points for each of eight concentrations of ICYP. β-AR number was decreased after pacing-induced heart failure in both IVS and PLW. There was no regional difference in the degree of β-AR downregulation. The dissociation constant for ICYP was invariant with pacing-induced heart failure in membranes from IVS and PLW. Mean r² values for the Scatchard analysis were 0.97 ± 0.05.

In both IVS and PLW, the proportion of β-AR showing high-affinity binding for l-isoproterenol was decreased after pacing-induced heart failure (Fig. 2). Isoproterenol competed for binding sites with a low-affinity constant that was unchanged after pacing-induced heart failure and was invariant by region (control IVS, 10 ± 13 nM; control PLW, 3 ± 4 nM; CHF IVS, 3 ± 3 nM; CHF PLW, 5 ± 1 nM). Similarly, isoproterenol competed for binding sites with a low-affinity constant that was unchanged after pacing-induced heart failure and was invariant by region (control IVS, 1 ± 1 μM; control PLW, 2 ± 2 μM; CHF IVS, 1 ± 0.5 μM; CHF PLW, 1 ± 0.4 μM).

Adenylyl cyclase assays. β-AR-dependent [isoproterenol + Gpp(NH)p] and Gs-dependent [Gpp(NH)p] stimulation of adenylyl cyclase were diminished in both IVS and PLW membranes after CHF (Fig. 3). Whether stimulated through the β-AR, through Gs, or more directly through the catalytic subunit of adenylyl cyclase (forskolin), net cAMP production was diminished. The mean reduction in cAMP production in IVS was 52% (range, 48–55%); the mean reduction in PLW was 43% (range, 34–49%).

Quantification of GRK2 and GRK5 by immunoblotting. Immunoblotting using an antibody against GRK2 showed no significant change in GRK2 protein content in either PLW or IVS membranes vs. control. In contrast, GRK5 protein content was increased in both IVS and PLW. Regional differences were not present (Table 1 and Fig. 4). We did not perform GRK activity assays but have previously reported a good correlation between increased cardiac GRK5 content and GRK activity in this model of CHF (20).
Dobutamine stress echocardiography. To determine the effects of dobutamine infusion on regional wall thickening, we studied three additional animals before and after the induction of CHF (Fig. 5). Dobutamine infusion increased wall thickening to similar degrees in both regions before the induction of CHF. After the development of CHF, dobutamine infusion increased wall thickening minimally in both the IVS and PLW. Impaired adrenergic responsiveness was similar in both regions.

**DISCUSSION**

The principal finding of this study is that, despite regional differences in wall thickening in LV pacing-induced heart failure, alterations in β-AR signaling are similar in both regions. This finding has two implications. First, the data indicate that systemic rather than regional factors are important in determining myocardial β-AR signaling in this model of heart failure. Second, regional myocardial adrenergic signaling poorly predicts basal regional wall thickening. Our data show that even when regional β-AR signaling is markedly reduced, basal wall thickening of that region can be relatively normal. To our knowledge, this is the first study that has examined regional adrenergic signaling in the LV in heart failure.

LV pacing-induced heart failure. LV pacing-induced heart failure is associated with alterations in transmembrane adrenergic signaling and pronounced alterations in cardiac function and reduced ability of the heart to respond to catecholamine stimulation (22, 29). These changes include myocardial β-AR downregulation, increased GRK5 protein content with attendant uncou-
that myocardial β-AR signaling is an accurate predictor of function and that an important mechanism for reduced function in heart failure is impaired adrenergic signaling. Instead, we found that multiple measures of β-AR signaling were indistinguishable between normal and abnormal regions. β-Adrenergic responsiveness, measured by regional wall thickening in response to dobutamine infusion, was decreased similarly in both regions, which is consistent with the alterations of β-adrenergic signaling. The site of pacemaker activation in the heart may influence regional blood flow and function (15). However, alterations in the region remote from pacemaker activation suggest that systemic rather than regional factors are important in the molecular pathogenesis of heart failure in this model, and perhaps in other examples of heart failure.

Isolated ventricular failure. Although regional adrenergic signaling has not been previously examined within the LV in heart failure, isolated right ventricular and LV failure models, including human primary pulmonary hypertension, have indicated that abnormalities in β-AR signaling are localized to the failing chamber despite increased levels of plasma norepinephrine. These studies suggest local rather than systemic regulation of myocardial β-AR signaling (3, 8, 32). Studies showing chamber-specific alteration in β-AR signaling are limited to pressure and/or volume overload of one chamber only (3, 8, 32). In these models, end-diastolic pressure is increased in one chamber, and abnormalities in β-AR signaling are limited to the affected chamber. In the current study, we did not measure β-AR in right-sided cardiac chambers. However, we showed in previous studies that right atrial β-AR signaling is altered in a manner similar to the LV anterior free wall (22), indicating that the changes in β-AR signaling are not isolated to the left heart in this model. It is noteworthy that the LV-pacing model is associated with biventricular heart failure (22).

Circulating and regional catecholamines. Heart failure is accompanied by systemic neurohumoral activation including an increase in plasma catecholamine

Table 1. LV regional G protein receptor kinase content

<table>
<thead>
<tr>
<th></th>
<th>GRK2 Pellet</th>
<th>GRK2 Supernatant</th>
<th>GRK5 Pellet</th>
<th>GRK5 Supernatant</th>
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<tbody>
<tr>
<td>PLW</td>
<td>Con 0.43 ± 0.09</td>
<td>0.46 ± 0.10</td>
<td>0.24 ± 0.06</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>CHF 0.48 ± 0.11</td>
<td>0.54 ± 0.20</td>
<td>0.47 ± 0.15</td>
<td>0.13 ± 0.09</td>
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<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>IVS</td>
<td>Con 0.48 ± 0.12</td>
<td>0.46 ± 0.07</td>
<td>0.23 ± 0.05</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>CHF 0.54 ± 0.16</td>
<td>0.55 ± 0.20</td>
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<td>0.12 ± 0.12</td>
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<tr>
<td>P value</td>
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<td>0.01</td>
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Values are means ± SD expressed as arbitrary densitometric units per 50 µg protein; n = 6 for each group. PLW, posterolateral wall; IVS, interventricular septum; Con, control; CHF, congestive heart failure. P value was derived from ANOVA post hoc test (Con vs. CHF).

Fig. 4. GRK immunoblots. Photographs show data from immunoblotting studies using antibodies directed to GRK2 (A) and GRK5 (B). In each case, LV membranes from a normal animal (CON) and an animal with heart failure (CHF) are shown with data from both pellet and supernatant (SUP) fractions. Samples from PLW (abnormal function) and IVS (normal function) are shown. No regional differences in myocardial GRK content were detected in normal animal or in animal with heart failure. Heart failure was associated with increased LV GRK5 content in both regions.
levels (28), increased activity of the renin-angiotensin system (7), and increased centrally mediated sympathetic activation (17). Downregulation of β-AR is known to occur if the cell is exposed to a high concentration of norepinephrine in vitro (18, 19, 25, 26). In vivo, Delehantry et al. (5) observed a significant negative correlation between interstitial norepinephrine and β-AR density, using a [3H]norepinephrine tracer in pacing-induced heart failure. Vatner et al. (30) failed to demonstrate myocardial β-AR downregulation after long-term norepinephrine infusion. However, β-AR signaling may be more susceptible to regulation by norepinephrine that is released from nerve terminals impinging on cardiac myocytes exogenously administered norepinephrine. Himura et al. (16) demonstrated that norepinephrine uptake is altered specifically in the failing chamber, associated with the destruction of sympathetic nerve terminals. Himura et al. (16) suggested that locally increased interstitial norepinephrine was related to an abnormality in norepinephrine uptake, which might play a role in regional alterations in β-AR signaling.

A precise understanding that integrates afferent hemodynamic signals, central processing, and efferent outflow for cardiac sympathetic activation remains to be established in heart failure. Distension of the left atrium and pulmonary veins results in a positive chronotropic response; the afferent pathway is via the vagus with the efferent response mediated by cardiac sympathetic nerves (9). This suggests that cardiac sympathetic activation can be influenced by afferent neural signals from cardiopulmonary baroreceptors. Because of the ubiquitous distribution of cardiac nerves throughout the LV, this could contribute to uniform regulation of adrenergic signaling in the LV independent of the influence of circulating catecholamines. The homogeneous myocardial norepinephrine depletion between IVS and PLW in the present study supports this possibility.

Dissociation of β-AR and regional myocardial function. The dissociation between basal regional myocardial function and abnormalities in β-AR signaling, as we describe here, is also seen in β-AR antagonist treatment of patients with clinical heart failure. Recent studies using carvedilol demonstrated improved LV function without upregulation of myocardial β-AR (10), although previous studies using metoprolol showed attenuation of myocardial β-AR downregulation (14, 31). The aortocaval fistula model of circulatory congestion (high-output heart failure) showed elevated plasma catecholamines and marked abnormalities in β-AR signaling despite normal heart function (13). These examples underscore the fact that cardiac function can be influenced by elements distal to myocyte cell surface adrenergic signaling and that adrenergic desensitization is only one of protean abnormalities in the syndrome of heart failure, one that is often a sequela of the failing heart rather than its cause.

In conclusion, in LV pacing-induced heart failure, wall thickening in the IVS was preserved compared with the PLW. Preserved regional myocardial function was not associated with preserved regional β-AR signaling. Alternations in β-AR signaling occurred uniformly in both the IVS and PLW, suggesting that β-AR signaling is under systemic rather than local regulation in this model of CHF. Regional adrenergic signaling abnormalities poorly predict basal wall thickening in the same regions.

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Address for reprint requests: H. K. Hammond, VAMC-San Diego (111-A), 3350 La Jolla Village Dr., San Diego, CA 92161.

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