EARLY EFFECTS OF RIGHT VENTRICULAR VOLUME OVERLOAD ON VENTRICULAR PERFORMANCE AND β-ADRENERGIC SIGNALING

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Objective: Right ventricular dysfunction is a poorly understood but persistent clinical problem. This study was undertaken to evaluate ventricular performance and β-adrenergic receptor signaling in a tricuspid regurgitation model of right ventricular overload.

Methods: Seventeen dogs were chronically instrumented with epicardial dimension transducers. By means of the shell-subtraction model, right ventricular pressure-volume relationships were evaluated in normal and right ventricular overload states. Right ventricular chamber performance was quantified by the stroke work at an end-diastolic volume relationship.

Results: Right ventricular volume overload caused a 28% ± 11% and 31% ± 9% decline in chamber performance acutely and at 1 week, respectively, whereas end-diastolic volume increased from 45 ± 21 to 60 ± 30 mL (P = .019). β-Adrenergic receptor signaling in myocardial samples was assessed, examining adenylyl cyclase and G-protein–coupled receptor kinase activity. Stimulated adenylyl cyclase activity significantly decreased, and G-protein–coupled receptor kinase activity significantly increased in both left and right ventricular samples caused by increased levels of β-adrenergic receptor kinase 1. No change in β-adrenergic receptor density was seen at 1 week.

Conclusions: Early right ventricular overload is associated with impaired right ventricular chamber contractility, dilation, and, importantly, a biventricular alteration of β-adrenergic receptor signaling. (J Thorac Cardiovasc Surg 2000;119:342-9)

A complete experimental and clinical understanding of right ventricular (RV) function remains elusive because of difficulty assessing RV volume and performance.1 Previous observations and studies support the notion that the right ventricle tolerates volume overload well and pressure overload poorly.1 In the face of preserved ejection fraction, RV volume overload in a number of models leads to depressed left ventricular (LV) function, septal shifting, and alterations of LV loading.2 Although load-insensitive measures of contractility have been extensively applied to the left ventricle, few studies have examined RV performance in volume overload. Maughan and colleagues3 established the validity of RV pressure-volume relationships. Twenty years later, Karunanithi and colleagues4 examined a number of load-insensitive indices of ventricular function in the normal right ventricle and elegantly demonstrated the linearity of the preload recruitable stroke work (PRSW) relationship in the normal canine right ventricle, confirming its utility as a measure of RV contractility.

Alterations of β-adrenergic receptor (β-AR) signaling have also been characterized in clinical and experimental heart failure.5–7 LV and biventricular failure is associated with downregulation of β-ARs, depression of adenylyl cyclase (AC) activity, and enhanced G-protein

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receptor kinase (GRK) activity mediated through increased expression of the β-AR kinase (βARK1). Similarly, studies of right heart failure have reported a decline in biventricular AC activity,8 and one report demonstrated that in experimental RV failure there is a specific decline in RV but not LV β-AR density.9 The present study was developed to assess intrinsic RV performance independently of confounding load changes and to examine global biventricular β-AR signaling abnormalities in RV dysfunction because of isolated RV volume overload.

Methods

All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Science and published by the National Academy of Sciences (Department of Health and Human Services publication No. 85-23, revised 1985).

Seventeen healthy adult dogs (22-30 kg) were anesthetized and intubated with pentobarbital (50 mg/kg), fentanyl (200 µg), and succinylcholine (INN: suxamethonium; 1 mg/kg). In addition, cefazolin (1 g) and iron dextran (250 mg) were administered preoperatively. A left lateral thoracotomy was performed, and pneumatic vena caval occluders were placed around the superior and inferior venae cavae. The heart was suspended in a pericardial cradle, and pulse transit waveform as a running 5-point polyorthogonal transformation. The cardiac cycle was then defined by means of dP/dt, measurable dP/dt.

Experimental protocol. Dimension transducers were placed across the base-apex major axis and the anteroposterior minor axis diameters of the left ventricle. In addition, transducers were placed across the septal free wall minor axis diameters of both the left and right ventricles. The septal crystal (1.5 mm outer diameter) was placed through the tract of a 16-gauge needle that was introduced into the septum. All other crystals were sutured to the epicardium (Fig 1). Two silicone rubber pleural catheters were then placed, and the pericardium was left open. All hardware was tunneled though a Teflon skin button dorsal and caudal to the incision. All animals were allowed to recover for 7 to 10 days before the study. Animals were studied in the conscious state with morphine sedation and to examine global biventricular β-AR signaling abnormalities in RV dysfunction because of isolated RV volume overload.

Tissue analysis. To determine whether this model of RV dysfunction leads to global β-AR signaling abnormalities, we used samples from the LV and RV free walls from 9 animals with severe TR to perform biochemical analysis. Eight animals underwent sham thoracotomy and instrumentation and composed the control group.

β-AR density and AC activity. AC activity and β-AR binding were performed from myocardial sarcolemmal membranes, as described previously.3,13 For AC activity, membranes (30-40 µg of protein) were incubated for 15 minutes at 37°C with α-phosphorous 32 adenosine triphosphate under basal conditions or isoproterenol (INN: isoprenaline; 10^-4 mol/L), and cyclic adenosine monophosphate was quantified. Ligand-binding assays were done in triplicate. Total β-AR...
density was determined by incubating 25 µg of sarcolemmal membranes with a saturating concentration of iodine 125 cyanopindolol (300 pmol/L) and 20 µmol/L alprenolol to define nonspecific binding.

**GRK activity assays.** Concentrated cytosolic extracts were incubated with rhodopsin-enriched rod outer segment membranes in 75 µL of GRK lysis buffer, as described previously. Phosphorylated rhodopsin was visualized by autoradiography of dried gels, and GRK-mediated phosphate incorporation was quantified by means of a PhosphorImager system (Molecular Dynamics, Inc, Sunnyvale, Calif).

**βARK1 immunodetection.** Immunodetection of myocardial levels of βARK1 was performed on cardiac cytosolic protein extracts after immunoprecipitation, as previously described. βARK1 was immunoprecipitated by using 1:2000 of a monoclonal anti-βARK1 antibody and 35 µL of 50% slurry of protein A-agarose conjugate agitated for 1 hour at 4°C. The 80-kd βARK1 protein was visualized by means of standard chemiluminescence (ECL, Amersham Corp, Arlington Heights, Ill). Quantification of immunoreactive products was done by scanning the final autoradiography films and using the ImageQuant software (Molecular Dynamics).

**Neuropeptide Y assay.** For neuropeptide Y determination, tissue samples of myocardium were homogenized in radioimmunoassay buffer (0.1 mol/L sodium phosphate, pH 7.4, containing 0.1% β-mercaptoethanol and 0.1% bovine serum albumin). Neuropeptide Y was then determined with a commercially available radioimmunoassay (Peninsula Labs, San Carlos, Calif).

**Statistical analysis.** Except where noted, all summary data are expressed as a mean ± SD. A paired Student t test was used for all comparison of in vivo hemodynamic data. One-way analysis of variance was used for all other comparisons.

**Results**

**In vivo studies.** The shell-subtraction model reflected alterations of RV volume, with predictable increases in RV volume caused by volume overload (Fig 2). Acute volume overload caused significant increases in end-diastolic pressure (EDP) and EDV in the right ventricle (Table I). Subacute volume overload was characterized by persistent elevations of EDP and EDV. RV volume overload caused a depression of dP/dt and systolic pressure acutely, with reversal at 1 week (Table I). No animal in this study showed clinical signs of RV failure, such as ascites or peripheral edema.

To evaluate RV performance, we performed a linear regression analysis on the SW/EDV relationship (n = 9). SW-EDVc decreased after acute TR from 657.5 ± 192 to 474.0 ± 221 kiloergs (kergs) (P = .03). One week of TR showed a persistent depression of ventricular function at 451.6 ± 254 kergs (P = .0003, Fig 3). The volume-intercept of this relationship is unchanged acutely but appears to shift to the right after 1 week (P = .11, Table I). The PRSW relationship remained highly linear, with mean regression coefficients of 0.92, 0.92, and 0.88 for baseline and acute and subacute volume overload, respectively.

Subacute TR was not associated with significant increases in RV free wall volume compared with that found in sham-operated control animals (38.1 ± 4 vs 35.9 ± 9 mL, P = .47). Thus, RV volume overload was not accompanied by myocardial hypertrophy at 1 week.

**β-AR signaling.** Total β-AR density was unchanged between TR and control animals in both ventricles (Fig 4). However, significant depression of AC activity was seen both under basal and isoproterenol-stimulated conditions, consistent with functional uncoupling and...
desensitization of β-ARs (Table II). Importantly, this phenomenon was seen in both left and right ventricles. To further examine this apparent β-AR desensitization, we evaluated myocardial GRK activity and found an approximate 2-fold increase in GRK activity (Fig 5) in cytosolic fractions isolated from both ventricles ($P = .02$, Fig 5). This increase in GRK activity correlated with a significant increase in the myocardial content of βARK1 (Fig 6).

To examine sympathetic activation, we examined neuropeptide Y concentrations in samples of myocardium from both ventricles in the two groups ($n = 3$ each). Decreases in myocardial neuropeptide Y concentrations reflect an increase in sympathetic activation as

**Table I. Summary of RV hemodynamic parameters**

<table>
<thead>
<tr>
<th></th>
<th>EDV (mL)</th>
<th>EDP (mm Hg)</th>
<th>SBP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>$dP/dt_{max}$ (mm Hg/s)</th>
<th>$M_w$ (kerg/mL)</th>
<th>$V_w$ (mL)</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td>44.7 ± 20.9</td>
<td>3.8 ± 2.2</td>
<td>29.2 ± 4.0</td>
<td>126.6 ± 11.5</td>
<td>691.0 ± 49.8</td>
<td>19.1 ± 5.1</td>
<td>24.6 ± 10.9</td>
</tr>
<tr>
<td>Acute</td>
<td>60.4 ± 30.2*</td>
<td>8.1 ± 3.2‡</td>
<td>22.5 ± 2.1§</td>
<td>122 ± 15.7</td>
<td>462.6 ± 12¶</td>
<td>12.6 ± 3.7‡</td>
<td>21.9 ± 15.0</td>
</tr>
<tr>
<td>One week</td>
<td>59.8 ± 28.9†</td>
<td>7.88 ± 3.6</td>
<td>27.6 ± 3.8</td>
<td>125.7 ± 22.3</td>
<td>584.1 ± 113</td>
<td>15.6 ± 4.4*</td>
<td>30.0 ± 16.5#</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; HR, heart rate; $M_w$ and $V_w$, slope and x-intercept, respectively, of the SW/EDV relationship.

* $P = .02$ versus baseline.
† $P = .03$ versus baseline.
‡ $P = .021$ versus baseline.
§ $P = .012$ versus baseline.
¶ $P = .016$ versus baseline.
# $P = .11$ versus baseline.

**Table II. AC values at baseline and after $10^{-4}$ mol/L isoproterenol expressed as mean ± SEM as a percentage of sodium fluoride (NaF) (10 mmol/L) values for control animals ($n = 8$) and after 1 week of TR ($n = 9$)**

<table>
<thead>
<tr>
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<th>LV</th>
<th>RV</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Isoproterenol ($10^{-4}$ mol/L)</td>
</tr>
<tr>
<td>Control</td>
<td>30.0 ± 2.7</td>
<td>35.0 ± 1.4</td>
</tr>
<tr>
<td>One week of TR</td>
<td>19.9 ± 1.6*</td>
<td>27.2 ± 1.6†</td>
</tr>
</tbody>
</table>

* $P = .02$ versus control.
† $P = .008$ versus control.
§ $P = .006$ versus control.
¶ $P = .046$ versus control.

![Fig 3](image-url). RV chamber performance was assessed by using the SW-EDVc. RV volume overload resulted in a sustained depression of RV chamber performance. *$P = .03$ versus baseline.

![Fig 4](image-url). Total β-AR density (mean ± SEM) in animals with TR at 1 week ($n = 9$) versus control animals ($n = 8$). No statistically significant difference was observed between each group and chamber ($P = .5$).
neuropeptide Y is coreleased from sympathetic nerves. A significant biventricular decrease in myocardial neuropeptide Y concentration was seen in TR animals compared with control animals (15.6 ± 0.88 pg/100 µL [TR right ventricle] and 11 ± 2.5 pg/100 µL [TR left ventricle] vs 25.3 ± 1.8 [control right ventricle] and 25.3 ± 1.4 [control left ventricle]; P = .009).

**Discussion**

**In vivo function.** The current study presents novel findings on the mechanical and molecular nature of early RV performance with an imposed volume load. The development and validation of the shell subtraction model has allowed practical in vivo measurement of RV volumes and the extension of load-insensitive measures of contractility to the study of RV function and interdependence with LV function. Alternately, the explosion in molecular cardiology has expanded our understanding of ventricular function. The SW versus EDV relationship is a well validated measure of contractile performance in the left ventricle, and work over the last 15 years has demonstrated its relative heart rate and afterload insensitivity.11 Karunanithi and colleagues4 and others14,15 rigorously confirmed that the PRSW relation could be applied to the right ventricle and was superior to a number of other indices of contractile function. The present study applied the PRSW analysis to a model of RV dysfunction to minimize confounding effects of RV preload and afterload inherent in TR.

Experimental work in RV dysfunction has centered on models of ischemia, decompensated failure, and pulmonary stenosis.2 Isolated TR appears to be a well-tolerated state in canines. In long-term studies in dogs with TR induced by severing chordae over 1 to 3 years, overt right heart failure was only seen in animals with 3 years of TR, and animals at 1 year showed no significant alterations LV function.16

Clinical studies of patients with RV volume overload have presented a mixed picture. Several studies report preserved RV function in the face of long-standing TR or Ebstein anomaly.17-19 All of these studies, however, rely on load-dependent methods to determine function, particularly ejection fraction, and no study has examined the early effects of pathologic RV volume loading with autonomic blockade.

The current study demonstrated that acute and subacute volume overload caused a depression in contractile performance with dilatation of the right ventricle and elevations of EDP. The trend toward a rightward shift of the volume intercept suggests a measure of sarcomere rearrangement in response to the imposed volume load.20

Interestingly, experimental studies of LV volume overload have failed to show significant deterioration of chamber performance with early mitral regurgitation21 or aortic regurgitation in canines.10 Autonomic activity appears to have significant role in compensation for LV failure and may account for some of the discrepancy in previous studies. Autonomic blockade was therefore used in this study to better characterize intrin-
sic RV performance in the face of possible autonomic compensation. This study is among the first reports to observe that early RV and LV volume overload may differ in that RV volume overload is associated with an immediate and persistent decline in chamber function, whereas LV chamber function is preserved.

LV function was not directly evaluated in this particular study. As noted above, previous studies of TR in canines failed to demonstrate a significant reduction in LV performance at 1 year. Similarly, studies that directly address the issue of interdependence have demonstrated an enhancement of LV contractile function with acute elevations of RV afterload and decline in function because of RV volume overload.14,17 No previous study has examined LV function by using load-insensitive indices, and most studies, including this one, were performed with the pericardium open, which decreases direct ventricular interaction. Although severe TR in this model may depress cardiac output of the left ventricle, impairment of intrinsic LV performance is likely to be small.

**β-AR alterations.** A significant global depression of β-AR signaling was seen in animals with severe TR at 1 week compared with control animals. Previous studies in human heart failure have described a downregulation of β-AR density and functional uncoupling from second messenger systems.5 More recently, GRKs, in particular βARK1, have been found to mediate this uncoupling in human heart failure.22,23 Unique to the present study is that a pattern of biventricular uncoupling was seen in a model of compensated RV dysfunction but not overt failure. Importantly, we saw a significant increase in cytosolic GRK activity in TR animals, which can be attributed to the increased myocardial protein content of βARK1.24 This data suggests that GRK-mediated (ie, βARK1) desensitization is the mechanism for the functional β-AR uncoupling seen in this model of RV dysfunction. Thus, GRK-mediated β-AR desensitization appears to be critically important in cardiovascular disease and consistent with our data in which β-AR–mediated AC activity was significantly depressed without loss of β-AR density. Although we did not measure β-AR subpopulations or G-protein subtypes, 1 week after the creation of RV volume overload is probably too early to see β-AR density changes, which is a late feature of chronic heart failure. Some of the alteration in AC activity may be accounted for by changes in G-protein subtypes. More important, however, 1 week was the time frame during which we uncovered changes in βARK1 and its effects on β-AR signaling. Our β-AR density data does not look at individual subtypes (β1 vs β2), which may have changed in a reciprocal manner, but our signaling data suggests that other mechanisms besides receptor number are responsible for attenuated β-AR function.

Thus despite clinical compensation, there appears to be global β-AR dysfunction with early RV volume overload. The exact mechanism of this molecular interdependence between the right and left ventricles is not elucidated in this study. However, central neurohormonal activation may account for biventricular desensitization. Larson and colleagues25 demonstrated elevations of plasma epinephrine and norepinephrine levels with 30 days of RV volume overload. Alternately, Bristow and associates26 postulated that local tissue factors and ventricular hypertrophy may play a role. We found a biventricular depression of myocardial concentrations of neuropeptide Y and, in the absence of significant hypertrophy, suggests the role of a central catecholamine trigger in this model.

This global depression of β-AR signaling has multiple implications. First, it suggests that βARK1 and GRK activity are early molecular mediators during right heart dysfunction. The role of βARK1 and GRK activity in myocardial dysfunction has been previously demonstrated in mice where global βARK1 levels increased after chronic isoproterenol stimulation27 and alterations of myocardial βARK1 significantly affected in vivo LV contractile function.22,28 Second, sustained abnormalities may predict and mediate progressive dysfunction and inotropic insensitivity. The present study provides novel and appealing targets for genetic-based treatment strategies, such as the inhibition of receptor desensitization with a βARK1 inhibitor.22,29 Future studies will help elucidate the duration and progression of β-AR abnormalities in right heart failure and dysfunction.

The present study is dependant on the shell-subtraction model to accurately estimate RV volumes and, although this has been validated in the normal canine myocardium, may not reflect alterations of chamber geometry caused by volume overload and chordae transection. Recent work by Waldman and colleagues30 demonstrated that longitudinal strains throughout the right ventricle vary with loading conditions. Preliminary sonomicrometric RV volume determinations with severe TR have correlated with volumes determined by 3-dimensional echocardiography.31 Few alternative methods exist to practically determine dynamic pressure-volume relationships in conscious animals.

The current study is among the first to apply load-insensitive indices of RV function in a chronic animal model of RV volume overload and to demonstrate concomitant global alterations of β-AR signaling. By using
the shell subtraction model for RV volume determination, a significant reduction in chamber contractility was demonstrated in RV volume overload. This study confirmed previously reported biventricular β-AR signaling abnormalities with isolated RV dysfunction.8,9 However, our changes occurred earlier than previously reported and were found for the first time to be associated with enhanced GRK activity mediated by βARK1. This provides further evidence of potential targets for cardiac gene therapy. These findings demonstrate fundamental differences of RV behavior with respect to the left ventricle and provide new insight into mechanisms of early RV dysfunction.

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