PATHOLOGICAL VENTRICULAR VOLUME OVERLOADING is caused by a variety of structural and metabolic abnormalities. Experimental models of pathological left ventricular (LV) volume overload have shown that, as in humans, if not initially lethal it may be tolerated for an extended period of time, but left heart failure develops eventually if a sustained volume overload is severe. In mitral regurgitation, this is based on muscle dysfunction at the level of the ventricle and of its constituent cardiocytes (29), which in turn appears to be caused by myofibrillar lysis (29), abnormalities of the force-frequency relationship, and calcium metabolism (2, 18).

Right ventricular (RV) volume overloading appears to be better tolerated than LV volume overloading. We have found, for instance, that experimental animals with severe RV volume overloading caused by atrial septal defects exhibit normal RV muscle function and energetics for at least 6 mo (8). Furthermore, humans with similarly severe RV volume overloading caused by atrial septal defects exhibit normal RV systolic function indefinitely if pulmonary hypertension with consequent RV pressure overloading does not supervene (16). However, because the geometry of the RV makes separation of loading conditions from intrinsic contractility difficult and because the calculations of wall stress often used to estimate RV loading are extremely complex and remain largely unvalidated, intrinsic RV contractility has been difficult to assess (20, 23).

Over the past decade, we have shown that, for both normal and abnormal myocardium, the contractile function of isolated cardiocytes assessed in vitro mirrors the intrinsic contractile function in vivo of the ventricle from which the cardiocytes were isolated (15, 29). We therefore used this principle here to test the hypothesis that intrinsic contractile function is normal in long-term, severe RV volume overloading.

METHODS

Animal models. Mongrel dogs of random sex weighing 25–35 kg were the subjects of this study. For the creation of tricuspid regurgitation, anesthesia in dogs sedated with morphine was induced with thiopental sodium (15 ml iv). After intubation, we maintained anesthesia by inhalation of halothane (5% initially, followed by 0.2–0.5%). All procedures and the care of the dogs were in accordance with institutional guidelines, which meet or exceed those of the American Physiological Society and the American Association for Ac-
right atrium in end diastole; the left anterior oblique position. 15 ml/s, and the images were taken in contrast material was injected at a rate of 0.5 mg

**Table 1. Hemodynamic profiles**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>TR</th>
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<tbody>
<tr>
<td>Ascites, peripheral edema</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Right atrial pressure (a wave), mmHg</td>
<td>4.5 ± 0.3</td>
<td>4.1 ± 0.8</td>
</tr>
<tr>
<td>Right atrial pressure (v wave), mmHg</td>
<td>3.6 ± 0.4</td>
<td>17.8 ± 2.5</td>
</tr>
<tr>
<td>RV systolic pressure, mmHg</td>
<td>267 ± 18.3</td>
<td>247 ± 1.7</td>
</tr>
<tr>
<td>RV end-diastolic pressure, mmHg</td>
<td>4.1 ± 0.7</td>
<td>15.4 ± 0.5</td>
</tr>
<tr>
<td>Aortic systolic pressure, mmHg</td>
<td>117.7 ± 5.3</td>
<td>113.3 ± 4.2</td>
</tr>
</tbody>
</table>

Values are mean ± SE; n = 7 control dogs and 5 tricuspid regurgitation (TR) dogs. a, peak a wave pressure; v, peak v wave pressure; RV, right ventricular. *P < 0.001 for a difference from control value by Student’s unpaired t-test.

Cardiocyte isolation. Immediately after the in vivo evaluation of cardiac function by one subset of investigators, and after the dogs were more deeply anesthetized, another subset of investigators, who were not informed of the results of this evaluation, isolated and evaluated cardiocytes from these same RVs as follows. A right lateral thoracotomy was performed, the pericardium was excised, and the right coronary artery was isolated. The heart was removed and placed in cold, nominally calcium-free buffer of the following composition (in mM): 13.0 NaCl, 4.8 KCl, 1.2 MgSO4, 1.2 NaH2PO4, 2.5 NaHCO3, 12.0 HEPES buffer, and 12.5 glucose; 6 mg/l insulin was also added. The right coronary artery was cannulated immediately, and perfusion with the same buffer was initiated. The RV free wall was removed from the rest of the heart, and the specimen was cleared of blood by brief perfusion with the same buffer warmed to 37°C and gassed with 100% O2. The method then used to dissociate cardiocytes from the canine RV was modified from that previously reported by us for the canine LV (29); perfusion was continued for 25 min at 37°C by recirculating the buffer, now supplemented with 155 U/ml type II collagenase and 10 µM Ca2+; mean pressure (~90 mmHg) and pH (~7.40) were kept within the physiological range. Any undissociated tissue was discarded and that remaining was placed in a chamber containing the above buffer supplemented with 0.3 mM Ca2+, 2.5% bovine serum albumin, and 62 U/ml of the same collagenase used for perfusion. After mincing the tissue into small pieces with scissors, we gently agitated the tissue for 5 min at 37°C in the same buffer while gassing the tissue with 100% oxygen. Dissociated cardiocytes were harvested by passing the supernatant through a 210-µm nylon mesh and collecting used before in evaluating our model of surgically created mitral regurgitation (3, 29).

**Table 2. Dimensions of RV cardiocytes**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, µm</td>
<td>156 ± 3</td>
<td>187 ± 4*</td>
</tr>
<tr>
<td>Width, µm</td>
<td>34 ± 1</td>
<td>40 ± 1*</td>
</tr>
</tbody>
</table>

Values are mean ± SE; n = 44 control dogs and 63 TR dogs. *P < 0.001 for a difference from control value by Student’s unpaired t-test.

**Fig. 1.** Right ventriculography of a dog with tricuspid regurgitation. The contrast material was injected at a rate of 15 ml/s, and the images were taken in the left anterior oblique position. Left: heart at end diastole; right: heart at end systole. The short arrows indicate the right atrium, and the long arrows indicate the right ventricle (RV). Note the denser opacification of the right atrium than of the RV in left and incomplete ejection of contrast material from the right atrium in right.
them by gravity. The Ca\(^{2+}\) concentration was gradually increased to 2.5 mM, after which the cells were kept for 1 h at 37°C in collagenase-free buffer before defining contractile function.

Cardiocyte mechanics. Our methods for laser diffraction measurement of sarcomere motion in isolated cardiocytes have been described before (15, 29). Cells were added to 37°C, 2.5 mM Ca\(^{2+}\) buffer in a well affixed to a glass slide. Only single, rod-shaped cells with a resting sarcomere length \(\geq 1.85\ \mu m\) and that contracted with each stimulus and were quiescent between stimuli were analyzed. Ten steady-state contractions during 0.25-Hz electrical stimulation were sampled and averaged to yield a final profile of sarcomere length and velocity versus time during contraction. Changes in sarcomere length were measured at a sampling rate of 1 kHz from movement of the first-order diffraction pattern cast by light from a substage helium-neon laser passing through the sarcomeres.

Cardiocyte morphology. Two aliquots of isolated cardiocytes from each specimen were plated in separate dishes, and randomly selected cell images were captured on an inverted microscope at a magnification of \(\times 400\) by a frame grabber. The images were printed at a final magnification of \(\times 1,100\), and cell length and width were measured manually.

Statistical analysis. The mean value and the standard error of the mean are shown for each group of data. Differences in selected measures were evaluated via an unpaired Student’s t-test, with a significant difference said to exist at the level specified in each case.

RESULTS

Characteristics of experimental model. All of the dogs with tricuspid regurgitation had, on physical examination, a grade III–IV/VI pansystolic murmur at the lower left sternal border, with a readily palpable parasternal thrill. Right heart failure, as evidenced by marked peripheral edema and ascites, was prominent in all dogs. As shown in Table 1, RV systolic pressure was normal, as was aortic systolic pressure. Right ventriculography, shown in Fig. 1, demonstrated in each dog with tricuspid regurgitation complete and rapid opacification of the right atrium at end systole, and there was incomplete ejection of contrast material from the right atrium at end diastole. Thus isolated, severe RV volume overloading causing overt right heart failure was present in each of these dogs.

Cardiocyte morphology. Cardiocyte dimensions were evaluated in terms of cellular length and width. As

Fig. 2. Photomicrographs of freshly isolated RV cardiocytes from a control dog (top) and a dog with tricuspid regurgitation (bottom). The scale bar represents 25 \(\mu m\).

Fig. 3. Display of sarcomere motion sampled at a frequency of 1 kHz during contractions of single cardiocytes. A: RV cells from a control dog (left) and from a dog with tricuspid regurgitation for 3.5 yr (right). B: left ventricular (LV) cells from a control dog (left) and from a dog with mitral regurgitation for 3 mo (right). Sarcomere mechanics were averaged for 10 contractions of each cardiocyte studied at 37°C, 2.5 mM Ca\(^{2+}\), and 0.25-Hz stimulation frequency. The data for the LV cells, taken from our previous study (see Ref. 29), were gathered with the same device under the same conditions.
given numerically in Table 2 and illustrated in Fig. 2, RV cardiocytes from the dogs with tricuspid regurgitation were significantly longer and wider than RV cardiocytes from control dogs. Both the numerical values from and the appearance of volume-overloaded hypertrophied RV cells here are strikingly similar to those found in our previous study (see Ref. 29; Table 4 and Fig. 3) of volume-overloaded hypertrophied LV cells from dogs with isolated, severe mitral regurgitation.

Cardiocyte mechanics. Representative illustrations of sarcomere mechanics in cardiocytes from control and tricuspid regurgitation RVs are shown in Fig. 3. Because our intent in this study was to compare intrinsic myocardial contractile function in RV volume overloading caused by tricuspid regurgitation with that in LV volume overloading caused by mitral regurgitation, illustrative sarcomere mechanics from our study (29) of cardiocytes from control and mitral regurgitation LVs are also shown in Fig. 3. Summary data in Fig. 4 show that the extent and velocity of sarcomere shortening in RV cardiocytes from dogs with tricuspid regurgitation were only slightly decreased when compared with corresponding values for cells from control dogs, with no statistical difference; there was no significant difference between control versus tricuspid regurgitation cardiocytes in terms of initial sarcomere length (1.941 ± 0.011 vs. 1.918 ± 0.009 μm), time to peak shortening (214 ± 2 vs. 215 ± 2 ms), peak relaxation velocity (3.90 ± 0.14 vs. 3.63 ± 0.17 μm/s), or time to peak relaxation velocity (39 ± 2 vs. 42 ± 1 ms). However, as shown for comparison purposes in Fig. 4, LV cardiocytes from dogs with severe mitral regurgitation (29), albeit at a different temporal but at a similar pathophysiological point in the progression of heart failure, showed significant decrements both in the extent and in the velocity of sarcomere shortening.

DISCUSSION

The principal finding of this study is that the contractile function intrinsic to the RV of dogs with longstanding, severe RV volume overloading is normal. This stands in distinct contrast to our earlier studies of severe LV volume overloading, wherein intrinsic contractile function was depressed (19, 27, 29). This contrast is all the more striking when one considers that the dogs with severe mitral regurgitation were studied at 3–6 mo after the lesion was produced, whereas the dogs with severe tricuspid regurgitation were studied at 3.5–4.0 yr after the lesion was produced. Indeed, we would have preferred to have more dogs available here for final study, but the fact that contractile function was normal in tricuspid regurgitation gives us confidence in the validity of our principal finding. That is, experimental artifacts exaggerated by a low sample number might well have led to a false finding of abnor-
normal function, but artifactual contributions to a finding of normal function are much less likely.

The distinction made evident through the striking difference between the present study of RV volume overloading by tricuspid regurgitation and the previous studies (19, 27, 29) of LV volume overloading by mitral regurgitation is that between muscle failure versus pump failure as the root cause of the congestive heart failure syndrome. In the present study of tricuspid regurgitation, all of the dogs had severe, clinically overt right-sided congestive heart failure, with ascites and loss of appetite. Indeed, two of the original operative cohort experienced sudden death, which triggered the final study of the remaining dogs. While it is likely that these dogs experienced a lethal arrhythmia, because they were not being monitored electrocardiographically this cannot be known with certainty. But most significantly, the root cause of congestive heart failure in these dogs is shown here to be pump rather than muscle failure. In the previous study of mitral regurgitation, all of the dogs by 6 mo after the operation had clinically overt, rapidly progressive left-sided congestive heart failure that precluded longer-term study. But in contrast to the situation in the present study, the root cause of congestive heart failure in mitral regurgitation was shown to be muscle rather than pump failure.

The principal value of this study, therefore, is the evidence provided that even with the prolonged and extreme degree of hemodynamic overloading imposed here, the cardiac hypertrophy that results is not necessarily maladaptive, in that normal cellular contractile function may be retained. That is, it is not solely the magnitude but equally the type of hemodynamic overloading that determines whether the cardiac hypertrophic response to any such challenge remains compensatory. Nor can the difference between the RV and LV functional response to volume overloading be attributed to intrinsic differences between RV and LV cardiocytes, because we have shown that the functional responses of cardiocytes from the two chambers to biventricular volume overloading is identical (28). Rather, the differing myocardial responses to these two volume loads may well relate to the established role of hemodynamic load in regulating cardiac mass (6, 7). That is, load in the form of systolic afterload immediately after major atrioventricular valvular incompetence is imposed may be relatively better preserved in the lower pressure RV with tricuspid regurgitation than it is in the higher pressure LV with mitral regurgitation. The consequence of this loading difference in terms of stimulating cardiac hypertrophy may be aggravated by the fact that there is a diminished LV hypertrophic load response during mitral regurgitation to a given amount of stroke work input (5). Furthermore, because by Laplace’s law ventricular wall stress is related to the product of intraventricular pressure and ventricular radius and because RV chamber compliance is very high, an increase in ventricular radius and a subsequent increase in wall stress may induce a more effective hypertrophic response in tricuspid than in mitral regurgitation. Thus the attenuated LV hypertrophic response to load in mitral regurgitation may lead by default to an early, sustained reliance on increases in heart rate and adrenergic tone, which we have shown to be clearly deleterious to intrinsic myocardial properties in mitral regurgitation (19, 27) and to intrinsic cardiac cellular properties in normal isolated cardiocytes (17).

This difference between RV and LV volume overloading may also be related, at least speculatively, to the following additional factors. First, the initial hemodynamic burden caused by sudden and severe atrioventricular valvular incompetence may be greater when imposed on the high-systolic-pressure LV than it is when imposed on the low-systolic-pressure RV. Second, in the present study of tricuspid regurgitation, LV function was normal, such that there may have been less neurohumoral stimulation (12). The renin-angiotensin system and sympathetic nervous system have a clear role in the development of LV failure after systemic hemodynamic overloads (14), but these systems could be less important in isolated tricuspid regurgitation in the absence of LV dysfunction. Third, because LV contraction contributes to the generation of RV stroke work via the interventricular septum (13, 23), the normal interventricular septum in tricuspid regurgitation may contribute to the maintenance of overall RV function.

Clinical experience demonstrates that RV volume overloading is well tolerated in the absence of pressure overloading superimposed by pulmonary hypertension (16, 20). Furthermore, experimental animal models of arterio-venous fistula (24, 28), atrio-ventricular blockade (21), and atrial septal defect (8) also demonstrate normal intrinsic RV function in RV volume overloading. In contrast, whereas chronic LV volume overloading in humans may be tolerated for years, LV dysfunction and heart failure often supervene eventually (4, 9, 11, 25, 26), quite possibly related to our finding of depressed intrinsic LV contractile function in this entity (29). Thus, whereas both RV and LV volume overloading may cause overt heart failure, the preserved intrinsic RV contractile function shown in this and in our earlier study (8) of RV volume overloading, and the exhausted intrinsic LV contractile function shown in our studies (3, 5, 19, 27, 29) of LV volume overloading, suggest that the etiology of this heart failure may be predominantly nonmyocardial in the former case and myocardial in the latter case.

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REFERENCES


