Characterization of cardiac hypertrophy and heart failure due to volume overload in the rat

XI WANG, BIN REN, SONGYAN LIU, EMMANUELLE SENTEX, PARAMJIT S. TAPPIA, AND NARANJAN S. DHALLA
Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, and Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R2H 2A6

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Wang, Xi, Bin Ren, Songyan Liu, Emmanuelle Sentex, Paramjit S. Tappia, and Naranjan S. Dhalla. Characterization of cardiac hypertrophy and heart failure due to volume overload in the rat. J Appl Physiol 94: 752–763, 2003; 10.1152/japplphysiol.00248.2002.—Alterations in general characteristics and morphology of the heart, as well as changes in hemodynamics, myosin heavy chain isoforms, and β-adrenergic receptor responsiveness, were determined in Sprague-Dawley rats at 1, 2, 4, 8, and 16 wk after aortocaval fistula (shunt) was induced by the needle technique. Three stages of cardiac hypertrophy due to volume overload were recognized during the 16-wk period. Developing hypertrophy occurred within the first 2 wk after aortocaval shunt was induced and was characterized by a rapid increase of cardiac mass in both left and right ventricles. Compensated hypertrophy occurred between 2 and 8 wk after aortocaval shunt where normal or mild depression in hemodynamic function was observed. Decompensated hypertrophy or heart failure occurred between 8 and 16 wk after aortocaval shunt and was characterized by a shift in myosin heavy chain isoform and a decrease in β-adrenoceptor density, decreased in vivo and in vitro cardiac function, and a shift in myosin heavy chain isoform expression. However, the positive inotropic effect of isoproterenol was augmented at all times during the 16-wk period. Characterization of β-adrenoceptor binding in failing hearts at 16 wk revealed a significant increase in β1-receptor density, whereas β2-receptor density was unchanged. Consistent with this, basal adenyl cyclase activity was significantly increased, and both isoproterenol- and forskolin-stimulated adenyl cyclase activities were also increased. These results indicate that upregulation of β-adrenoceptor signal transduction is a unique feature of cardiac hypertrophy and failure induced by volume overload.

aortocaval fistula; β-adrenergic mechanisms

CARDIAC HYPERTROPHY IS AN adaptive response of the heart to hemodynamic overload, during which terminally differentiated cardiomyocytes increase in size without undergoing cell division. Initially, the hypertrophic response may serve to compensate cardiac function; however, prolonged hypertrophy can become detrimental, resulting in cardiac dysfunction and heart failure (19, 23). Arteriovenous (AV) shunt or fistula (1, 13, 14, 16, 20, 21, 27, 28) has long been used as a model for inducing volume overload similar to that seen in conditions such as hyperthyroidism, anemia, and bundle branch block. However, changes in cardiac hypertrophy and hemodynamic parameters due to AV shunt are quite variable. Increase of cardiac mass has been reported to range from 20 to 100% (13, 16, 27, 28, 31), whereas cardiac contractility was increased (16), decreased (2, 34), or unchanged (27, 28). Circulatory congestion was also reported to be absent (6) or present (34). Such conflicting observations may be due to differences in the techniques utilized to induce volume overload or the stage of cardiac hypertrophy. Conventionally, AV fistula in rats is created by end-to-side anastomosis of the aorta and the vena cava (13) or by end-to-end anastomosis of the left iliolumbar vein (27). Both procedures require microvascular surgery, and the circulatory system had to be obstructed for 15–30 min (13, 27). Furthermore, because these surgical procedures require ~40 min to complete, the mortality was as high as 47–76% (13, 27). In addition, the shunt size as well as the hypertrophic and hemodynamic characteristics were inconsistent (13, 27).

Garcia and Diebold (15) described a simple, rapid, and effective method in which a needle (18 gauge) was used for making a shunt between the abdominal aorta and the inferior vena cava. The puncture site was then sealed with a drop of cyanoacrylate glue. The circulation was only occluded for 1–2 min, and the entire procedure was finished within 10 min; the mortality was under 10% (15). Subsequently, Huang et al. (22) evaluated the needle technique by using three different sizes of needle and confirmed that this technique can control the size of the shunt and provide consistent changes in hypertrophic growth and hemodynamic changes.

Several investigators have employed the volume overload model for studying different aspects of cardiac remodeling, including changes in the renin angiotensin system (41), extracellular matrix (7, 11, 32, 40), and ventricular contractility (6). Some studies have shown
increased cardiac output and cardiac index due to volume overload (22, 27, 38, 41). Most of these studies were carried during 10 wk after the surgery when the signs of overt heart failure were not obvious; however, Brower and Janicki (7) have characterized changes in the in vitro cardiac contractility at 21 wk after the surgery and reported that heart failure due to volume overload may be associated with a depression of the intrinsic cardiac function. The present study was undertaken to investigate the time course changes in both in vivo and in vitro hemodynamics during the development of cardiac hypertrophy due to volume overload induced by the needle technique in rats. Furthermore, it was planned to carry out experiments to examine biochemical markers of heart dysfunction such as myosin isozyme composition and the status of β-adrenergic system in this model of cardiac hypertrophy.

**METHODS**

**Preparation of the animal model.** Experiments were conducted in accordance with the Guide to the Care and Use of Experimental Animals issued by the Canadian Council on Animal Care. Male Sprague-Dawley rats weighing 250–300 g were kept in a temperature-controlled room with a 6:00 AM to 6:00 PM light-dark cycle. Tap water and rat chow were provided ad libitum. The aortocaval shunt was produced serving as controls were subjected to the same surgical procedure except for creation of the shunt. During the whole process, the animal was ventilated by positive-pressure inhalation of 95% O2 and 5% CO2 mixed with isofluorane, as recommended by the University Animal Care Committee.

**General characteristics.** For determination of general characteristics, rats were weighed and then anesthetized by a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). The heart was removed and immediately placed in ice-cold saline to wash out the blood. Total heart, right ventricular (RV), and left ventricular (LV) weight were measured after the removal of connective tissue; the septum was included in the LV. The lung and liver were also taken out, and their wet weight was assessed. Both lung and liver tissues (~0.5 g each) were dried at 70°C for 3 days. To make sure that constant weight was achieved, tissue was weighed daily until no further weight loss could be detected. Dry-to-wet weight ratios (dry/wet weight) for these tissues were then calculated. Gross morphology of the heart was determined at 4, 8, and 16 wk after surgery. The hearts from sham and experimental groups were taken out, washed, and fixed in formalin buffer for 1 wk. The hearts were then blotted dry and sagitally cut to show the size of the cavity and thickness of the LV and RV walls as well as that of the septum.

**Hemodynamic studies in vivo.** In vivo cardiac performance and arterial hemodynamics were measured via a carotid artery catheter at 1, 2, 4, 8, and 16 wk after the induction of aortocaval shunt. Rats were anesthetized with an intraperitoneal injection of ketamine and xylazine mixture. The right carotid artery was dissected, and an ultraminiature catheter connected to a pressure transducer (model SPR-249, Millar Instruments, Houston, TX) was inserted into the right carotid artery and then advanced into the LV. The LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), heart rate, maximum rate of pressure development (+dP/dt), and maximum rate of pressure decay (−dP/dt) were recorded and stored in a computer (Gould). The catheter was subsequently withdrawn to the aorta, and the arterial systolic pressure (ASP) and arterial diastolic pressure (ADP) were measured. The pulse pressure (PP) was then calculated as the difference between ASP and ADP; and the mean arterial pressure (MAP) was calculated as the sum of ADP and 1/3 PP.

**Myosin heavy chain isozyme analysis.** The composition of myosin heavy chain isozymes was determined by polyacrylamide gel electrophoresis in the presence of pyrophosphate (39). Portions of the LV and RV (~50 mg) were cut into small pieces and extracted for 15 min by gentle agitation at 0°C with three volumes (vol/wt) of 40 mM Na2HPO4 (pH 8.8), 1 mM EDTA, 10% glycerol, 1.25 mg/ml protease inhibitor cocktail (Roche, LK-1), and 5 mM 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,N’,N’-methylene-bis(acrylamide). Electrophoresis buffer contained 20 mM Na2HPO4 (pH 8.8) and 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 destained with 7% acetic acid by diffusion. Relative amounts of isoforms were derived from densitometric tracings using 2202 ultrasonic laser densitometer (LKB). The V1, V2, and V3 isozymes were quantitated by measuring peak heights, and the values were expressed as percentages of the total isoforms.

**Isoalted heart perfusion and contractile measurements.** In vitro contractile function was measured by using an isolated perfused heart preparation as described elsewhere (45). Briefly, at 4, 8, and 16 wk after the induction of an aortocaval shunt, the animals were anesthetized with a mixture of ketamine and xylazine. After administration of heparin (1,000 units), the thorax was opened, and the heart was removed and immediately placed into ice-cold saline. The adherent connective tissue was removed, and the heart was perfused in the Langendorff perfusion apparatus at a constant flow (10 ml/min) with Krebs-Henseleit solution containing (in mmol/l) 120 NaCl, 4.74 KCl, 1.2 KH2PO4, 1.2 MgSO4·7H2O, 25 NaHCO3, 1.25 CaCl2, and 11 glucose. This solution (pH 7.4) was continuously oxygenated with 95% O2 and 5% CO2 mixture and maintained at 37°C. It should be mentioned that the constant flow in the experimental setting used in this study generates 60–70 mmHg pressure. The atriocentral node conduction was surgically blocked, and
the heart was paced at 300 beats/min by a square wave of 1.5-ms duration at twice the threshold voltage throughout the experiments by using a Philips & Bird stimulator (Richmond, VA). The LVDP as well as +dP/dt and −dP/dt were measured by using a transducer connected to a latex balloon (Harvard Apparatus, St. Laurent, PQ), which was inserted into the LV. The balloon was connected with a plastic tube to a syringe filled with double-distilled water. The system was adjusted to make sure no air bubbles were inside the balloon and the tube, water was withdrawn from the balloon, and the deflated balloon was inserted into the LV. Water was then injected slowly into the balloon to adjust the initial LVEDP to 10 mmHg. The size of the balloon was adjusted so as to be appropriately sized for different stages of volume overload and different experimental groups to ensure that they fit into the LV cavity. It should be pointed out that the balloon itself may not contribute to the pressure measured because it was not fully inflated. All data were recorded and stored by using the Biopac Data Acquisition System (Biopac System, Goleta, CA), with recording being started after the heart was stabilized for 30 min. For studying the isotropic responses of these hearts (pH 7.5), 100 mM NaCl, 5 mM MgCl2, 0.1 mM EGTA, 0.5 mM 3-isobutyl-1-methylxanthine, 10 U/ml adenosine deaminase, (125I)ICYP (1–1.5 × 10−6), 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 the 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 the addition of 0.6 ml of 120 mM zinc acetate containing 0.5 mM unlabeled cAMP. The [32P]cAMP formed during the reaction was determined after precipitation with ZnCO3 by the addition of 0.5 ml of 144 mM Na2CO3 and subsequent chromatography by a double-column system, as described earlier (43). The unlabeled cAMP served to monitor the recovery of [32P]cAMP by measuring absorbency at 259 nM; AC activity was expressed as picomoles of cAMP per milligrams of protein per 10 min. The AC activity was linear with respect to protein concentration and time of incubation under the assay conditions used. When G protein-stimulated AC was estimated, 30 μM 5′-guanylylimidodiphosphate [Gpp(NH)p] or 10 mM NaF were added to the reaction mixture; other experiments were carried out in the presence of 100 μM isoproterenol or 100 μM forskolin.

Statistics. All values were expressed as means ± SE. One-way ANOVA followed by unpaired Student’s t-test was used for comparisons between sham control and experimental groups. Difference was considered significant at a level of P < 0.05.

RESULTS

General characteristics of the AV shunt rat model. Of 60 rats that underwent AV shunt surgery, five (8.3%) died within 24–72 h after the operation. Necropsy examination failed to reveal bleeding from the puncture site or vascular obstruction. However, the hearts were markedly dilated, with the right atria, LV, and RV being greatly enlarged, suggesting that these animals died from their inability to compensate for the acute volume overload. Although no further mortality occurred between 1 and 8 wk after the operation, three rats (5%) died between 10 and 16 wk after the surgery. Necropsy examination of these rats demonstrated hypertrophied heart, congested liver and lung, presence of ascites and pleural effusion, as well as edema of the limbs, suggesting that these animals died of congestive heart failure. In addition, rats that survived for 16 wk after AV shunt surgery were sluggish in their movements, and the hair around their face and neck area was stained with blood, most probably due to bloody sputum as a consequence of lung congestion. The AV shunt rats killed at 16 wk demonstrated ascites. The sham control group showed no mortality during 16 wk after the operation.

The time course changes in the general characteristics in rats with and without AV shunt are shown in Table 1. Although there was no significant difference in body weight between the sham and AV shunt groups at each time interval, heart weight of the experimental group increased progressively during 1–16 wk. Accordingly, heart weight-to-body weight ratio was significantly increased at all time intervals. While examining LV and RV weight separately, we found that hypertrophy of the RV was greater than that of the LV (152, 193, and 249% of control for RV vs. 112, 147 and 188% of control for LV) at 1, 2, and 16 wk, respectively, after the aortocaval shunt. However, the extent of LV hypertrophy at 4 and 8 wk was similar to that of the RV (187 and 171% of control for RV vs. 181 and 171% of control...
pertrophy in rat heart at 4, 8, and 16 wk after the aortocaval shunt

Table 1. General characteristics of rats with or without an aortocaval shunt for different time intervals

<table>
<thead>
<tr>
<th></th>
<th>BW, g</th>
<th>LVW, mg</th>
<th>RVW, mg</th>
<th>HW, mg</th>
<th>HW/BW, mg/100 g</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sh</td>
<td>294 ± 5</td>
<td>730 ± 43</td>
<td>134 ± 7</td>
<td>870 ± 40</td>
<td>296 ± 24</td>
<td>311 ± 10</td>
</tr>
<tr>
<td>AV</td>
<td>288 ± 8</td>
<td>815 ± 35</td>
<td>204 ± 9</td>
<td>1,021 ± 32</td>
<td>355 ± 14*</td>
<td>318 ± 16</td>
</tr>
<tr>
<td>2 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sh</td>
<td>346 ± 15</td>
<td>751 ± 60</td>
<td>166 ± 6</td>
<td>917 ± 70</td>
<td>265 ± 9</td>
<td>321 ± 16</td>
</tr>
<tr>
<td>AV</td>
<td>351 ± 18</td>
<td>1,100 ± 67*</td>
<td>320 ± 15*</td>
<td>1,420 ± 70*</td>
<td>404 ± 16*</td>
<td>309 ± 23</td>
</tr>
<tr>
<td>4 wk</td>
<td></td>
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<td></td>
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<tr>
<td>Sh</td>
<td>418 ± 14</td>
<td>764 ± 57</td>
<td>206 ± 10</td>
<td>970 ± 70</td>
<td>232 ± 8</td>
<td>325 ± 20</td>
</tr>
<tr>
<td>AV</td>
<td>424 ± 18</td>
<td>1,380 ± 65*</td>
<td>385 ± 31*</td>
<td>1,715 ± 62*</td>
<td>405 ± 27*</td>
<td>320 ± 18</td>
</tr>
<tr>
<td>8 wk</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sh</td>
<td>513 ± 25</td>
<td>875 ± 74</td>
<td>238 ± 10</td>
<td>1,107 ± 88</td>
<td>215 ± 10</td>
<td>316 ± 13</td>
</tr>
<tr>
<td>AV</td>
<td>530 ± 31</td>
<td>1,493 ± 82*</td>
<td>408 ± 48*</td>
<td>1,898 ± 95*</td>
<td>358 ± 34*</td>
<td>314 ± 20</td>
</tr>
<tr>
<td>16 wk</td>
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<td></td>
</tr>
<tr>
<td>Sh</td>
<td>612 ± 31</td>
<td>1,003 ± 80</td>
<td>247 ± 20</td>
<td>1,240 ± 90</td>
<td>202 ± 6</td>
<td>326 ± 15</td>
</tr>
<tr>
<td>AV</td>
<td>636 ± 28</td>
<td>1,890 ± 90*</td>
<td>615 ± 60*</td>
<td>2,500 ± 100*</td>
<td>393 ± 28*</td>
<td>333 ± 21</td>
</tr>
</tbody>
</table>

Values are means ± SE obtained from 8–12 rats in each group. BW, body weight; LVW, left ventricular weight (including septum); RVW, right ventricular weight; HW, heart weight; HW/BW, heart weight-to-body weight ratio; HR, heart rate; Sh, sham control; AV, aortocaval shunt. *P < 0.05 vs. Sh group.

Table 2. Lung and liver weights in rats with or without inducement of aortocaval shunt for different time intervals

<table>
<thead>
<tr>
<th></th>
<th>Lung Wet Wt, g</th>
<th>Lung Dry/Wet Wt</th>
<th>Liver Wet Wt, g</th>
<th>Liver Dry/Wet Wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sh</td>
<td>1.40 ± 0.04</td>
<td>19. 1.41 ± 0.48</td>
<td>14.1 ± 0.48</td>
<td>29.8 ± 0.5</td>
</tr>
<tr>
<td>AV</td>
<td>1.59 ± 0.04*</td>
<td>18.2 ± 0.7</td>
<td>15.8 ± 0.67</td>
<td>30.1 ± 0.8</td>
</tr>
<tr>
<td>2 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sh</td>
<td>1.45 ± 0.01</td>
<td>21.9 ± 0.5</td>
<td>16.5 ± 0.7</td>
<td>30.1 ± 0.7</td>
</tr>
<tr>
<td>AV</td>
<td>1.81 ± 0.14*</td>
<td>19.0 ± 0.7</td>
<td>17.1 ± 1.18</td>
<td>29.1 ± 1.2</td>
</tr>
<tr>
<td>4 wk</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Sh</td>
<td>1.48 ± 0.16</td>
<td>21.5 ± 0.6</td>
<td>17.4 ± 1.7</td>
<td>31.4 ± 0.7</td>
</tr>
<tr>
<td>AV</td>
<td>1.82 ± 0.12*</td>
<td>20.3 ± 0.8</td>
<td>18.5 ± 0.6</td>
<td>30.5 ± 0.6</td>
</tr>
<tr>
<td>8 wk</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sh</td>
<td>1.79 ± 0.08</td>
<td>21.9 ± 1.0</td>
<td>20.1 ± 0.09</td>
<td>30.7 ± 0.2</td>
</tr>
<tr>
<td>AV</td>
<td>2.04 ± 0.06*</td>
<td>20.9 ± 0.7</td>
<td>23.6 ± 0.91*</td>
<td>27.9 ± 0.5*</td>
</tr>
<tr>
<td>16 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sh</td>
<td>1.90 ± 0.08</td>
<td>20.3 ± 0.4</td>
<td>20.2 ± 1.53</td>
<td>30.2 ± 0.5</td>
</tr>
<tr>
<td>AV</td>
<td>2.59 ± 0.06*</td>
<td>21.7 ± 0.9</td>
<td>26.2 ± 1.12*</td>
<td>27.3 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE obtained from 8–12 separate organs in each group. Dry-to-wet ratios (dry/wet wt) for lung and liver were obtained as described in METHODS. *P < 0.05 vs. Sh group.

for LV, respectively). No significant difference was found in heart rate between sham and AV shunt groups throughout the observation period; this is similar to the findings of other investigators (13, 21, 27, 28, 41).

Morphological changes and circulatory congestion. The gross morphological changes in the heart are shown in Fig. 1. The left panel shows a progressive enlargement of the heart from 4 to 16 wk in the AV shunt group, whereas the right panel shows the dilation of the LV and RV cavities and increase of wall thickness in the vertically cut heart, indicating eccentric hypertrophy in this model. Our histology studies (data not shown) confirmed the previous observation of Hatt et al. (20) that, in the AV shunt group, the myocytes were thickened and nuclei were enlarged. In contrast to Hatt et al., disarray of myofibrils was also seen in some hearts at 8 and 16 wk after the AV shunt was induced.

To determine whether congestive heart failure occurred in this experimental model, we examined the circulatory congestion in rats at different time intervals by determining the dry/wet weight for lung and liver tissues. Table 2 shows that the lung wet weight in the AV shunt group was significantly increased at each time interval; however, the dry/wet weight was not altered, indicating a fluid-independent increase in lung mass after the AV surgery. Flaim et al. (13) reported that the fluid-independent increase in lung mass might be related to increased hematocrit in this model; however, in this study, no effort other than gentle surface

Fig. 1. Morphological studies showing development of eccentric hypertrophy in rat heart at 4, 8, and 16 wk after the aortocaval shunt (AV) was induced. Sh, sham control. The ruler scale is in cm.

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blotting was made to squeeze the blood from the organ. The histological examination of the lung tissue (data not shown) suggested that the increase in actual weight of the lung may be due to a progressive thickening of pulmonary interstitial tissue with dilated capillaries and fine fibrosis, indicating chronic edema in the lung after the AV shunt. Liver wet weight and dry/wet weight were not altered 4 wk after creation of the aortocaval shunt; however, the liver weight was increased and the liver dry/wet weight was decreased, indicating liver congestion. Histology of the liver tissue (data not shown) revealed distended central veins and fibrosis of the vessel wall at 8 and 16 wk of the AV shunt.

In vivo cardiac performance and arterial hemodynamics. The time course changes in cardiac performance under in vivo conditions due to volume overload are shown in Fig. 2. Although a significant elevation of LVEDP was detected throughout the 16-wk observation period (Fig. 2A), the changes were biphasic in nature. The first peak occurred at 1 wk, and this elevation was then reduced somewhat at 2 and 4 wk, whereas it started to increase at 8 wk and reached another peak at 16 wk. On the other hand, the LVSP was unaltered at 1, 2, and 4 wk but was progressively decreased at 8 and 16 wk after the AV shunt (Fig. 2B). Similarly, no changes were detected for \( \frac{dP}{dt} \) and \( \frac{dP}{dt} \) at 1, 2, and 4 wk after the surgery, but progressive depressions were seen at 8 and 16 wk (Fig. 2, C and D). The results in Fig. 3 show the time course changes in arterial hemodynamics on inducement of the AV fistula. Biphasic changes in both ASP (Fig. 3A) and MAP (B) were detected; significant depressions were evident at earlier stages (1 and 2 wk) and later stages (16 wk) without any significant alterations in between (4 and 8 wk) on inducement of the AV shunt. The PP was significantly lower in the AV shunt group compared with the sham group, although the extent of decrease was milder at 4 and 8 wk compared with other time points (Fig. 3C). The PP was significantly increased throughout the 16-wk observation period, indicating the presence of AV shunt in the experimental group (Fig. 3D).

Myosin isozyme composition. In view of the critical role played by a shift in the composition of myosin heavy chain isoforms in heart function in rodents (31), we examined the time course of changes in myosin heavy chain isozyme composition in the AV shunt group. Figure 4, top, shows the positions of \( V_1 \), \( V_2 \), and \( V_3 \) on the gel electrophoresis. The bottom panels show the time course changes in the myosin heavy chain isozyme composition in both RV and LV after the AV shunt was induced in rats. There was a slight age-dependent increase in \( V_3 \) in both LV and RV from sham controls, which is similar to that observed by Mercadier et al. (31). However, no significant shift of myosin heavy chain \( V_1 \) to \( V_3 \) was detected in LV until 8 wk and in RV until 16 wk. Table 3 shows the values of \( V_1 \), \( V_2 \), and \( V_3 \) (in percentage) in RV and LV at 4, 8, and 16 wk after the AV shunt was induced; the values at 1 and 2 wk were not shown because they were similar to those at 4 wk. No change was detected in myosin heavy chain isoform expression between the 4-wk sham and AV groups. On the other hand, a progressive decrease...
in V1 and an increase in V3 were apparent in the LV from the 8- and 16-wk AV shunt groups. In contrast to the changes in the LV, it was interesting to observe that no significant change in the composition of myosin heavy chain isozymes was evident in the RV from the 1- to 8-wk AV shunt animals (Table 3).

In vitro contractile function and response to isoproterenol. Because in vivo performance of the heart is largely influenced by a number of neurohormonal factors and peripheral hemodynamic changes, we measured the LV contractile function in the isolated perfused heart to determine whether an intrinsic cardiac dysfunction was present in the heart of AV-shunted rat. Figure 5 shows that LVDP, \(+\frac{dP}{dt}\), and \(\frac{dP}{dt}\) were significantly decreased at 4, 8, and 16 wk after the AV shunt. Compared with the in vivo hemodynamic parameters, the LV contractile dysfunction in vitro occurred earlier (4 vs. 8 wk) and was more dramatic (75, 63, and 48% of sham vs. 94, 79, and 61% of sham for \(\frac{dP}{dt}\) at 4, 8, and 16 wk, respectively), indicating the effect of compensatory mechanisms under in vivo conditions. The in vitro cardiac performance of hearts from the 1- and 2-wk AV shunt group was not different from that of the sham control group (data not shown).

In view of the commonly held belief that responses of the failing heart to catecholamines are attenuated (9, 46), we studied the responses to the infusion of \(1 \mu M\) isoproterenol in isolated perfused heart. As shown in Fig. 5, the positive inotropic responses were greatly enhanced at 4 wk in the AV shunt group but were progressively reduced to the level of the sham group at 8 and 16 wk. However, due to the decreased LV func-

### Table 3. Myosin heavy chain isoenzyme composition of rats at 4, 8, and 16 wk after the aortocaval shunt was induced

<table>
<thead>
<tr>
<th>Myosin Heavy Chain Isozymes</th>
<th>LV</th>
<th>RV</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sh</td>
<td>AV</td>
</tr>
<tr>
<td>4 wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>71.2 ± 2.3</td>
<td>72.0 ± 3.3</td>
</tr>
<tr>
<td>V2</td>
<td>18.3 ± 1.2</td>
<td>16.3 ± 2.1</td>
</tr>
<tr>
<td>V3</td>
<td>10.5 ± 1.8</td>
<td>11.7 ± 0.9</td>
</tr>
<tr>
<td>8 wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>67.4 ± 2.3</td>
<td>52.9 ± 3.0*</td>
</tr>
<tr>
<td>V2</td>
<td>19.2 ± 1.2</td>
<td>23.3 ± 1.0</td>
</tr>
<tr>
<td>V3</td>
<td>13.2 ± 2.0</td>
<td>23.8 ± 2.7*</td>
</tr>
<tr>
<td>16 wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>65.6 ± 1.4</td>
<td>32.9 ± 4.0*</td>
</tr>
<tr>
<td>V2</td>
<td>18.4 ± 1.9</td>
<td>26.3 ± 3.0</td>
</tr>
<tr>
<td>V3</td>
<td>16.1 ± 1.9</td>
<td>39.9 ± 3.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE in %, obtained from 4 separate sample preparations in each group. Myosin heavy chains were extracted from left (LV) and right ventricle (RV) and separated on gel electrophoresis as described in METHODS. *P < 0.05 vs. Sh group.

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**Fig. 3.** Changes in arterial pressures in rats after AV was induced for different time intervals. A: arterial systolic pressure. B: arterial diastolic pressure. C: mean arterial pressure. D: pulse pressure. Values are means ± SE; no. of rats used at each time point was 6–8. *P < 0.05 vs. Sh.

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**Fig. 4.** Time course changes in myosin heavy chain isozyme composition in rats after the AV was induced. RV, right ventricle.
section at these later time points, the extent of stimulation by isoproterenol over the corresponding basal level was increased at all three time points. It should also be noted that there was a progressive decrease in the positive inotropic response to isoproterenol in the sham control group attributed to aging.

Assessment of β-AR system. Because the inotropic response of isolated heart to isoproterenol stimulation was not attenuated as in other types of heart failure (9, 46), we examined this aspect under in vivo conditions at 16 wk in AV-shunted rats. As shown in Table 4, a bolus injection of isoproterenol stimulated the contractile function of sham and AV shunt groups to similar levels, despite their difference in basal levels. This indicated that the β-adrenergic system in this model is maintained or upregulated. To find out the biochemical basis for this phenomenon, we measured β-AR binding and AC activity in the LV in rats 16 wk after inducing the AV shunt. The results in Table 5 indicate an increase in the receptor density or maximum binding for β1-ARs but not β2-ARs. The receptor affinity, as reflected by Ki, was not altered for either β1- or β2-ARs, whereas the AC assay revealed a significant increase in AC enzyme activity in both the absence (basal) and presence of isoproterenol, Gpp(NH)p, NaF, and forskolin due to volume overload (Fig. 6A). When the data were expressed as stimulation with respect to the corresponding basal activities, only isoproterenol- and forskolin-stimulated AC activities were increased, whereas Gpp(NH)p- and NaF-stimulated enzyme activities were unaltered in the AV-shunted hearts (Fig. 6B).

DISCUSSION

First, it should be pointed out that the aortocaval shunt in rats, as employed in this study, is by no means a new model of volume overload, because there exists literature characterizing the aortocaval shunt model from different aspects (1, 13, 14, 16, 20, 21, 27, 28). However, the needle technique (15, 22, 41) used in this study is a breakthrough for inducing the volume overload. Previously, the fistula was produced by side-to-side or end-to-side anastomosis of vessels, which not only caused severe trauma to the animal but also induced variable shunt sizes in the surviving animals. Furthermore, flow through the fistula was constricted due to the remodeling of the vessel wall after the injury due to operation, and thus the data collected from the surviving animals did not reflect only the volume overload but also the pressure overload. Due to these limitations, the occurrence of hypertrophy and heart failure, as well as changes in hemodynamic function, were not consistent in this volume overload model (1, 13, 14, 16, 20, 21, 27, 28).

Table 4. Effect of a bolus injection of isoproterenol on in vivo hemodynamics of rats with heart failure induced by aortocaval shunt for 16 wk

<table>
<thead>
<tr>
<th></th>
<th>LVDP, mmHg</th>
<th>+dP/dt, mmHg/s</th>
<th>−dP/dt, mmHg/s</th>
<th>HR, beats/min</th>
</tr>
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<tr>
<td>Sh</td>
<td>Basal</td>
<td>133 ± 10</td>
<td>6,450 ± 250</td>
<td>6,377 ± 232</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>259 ± 18</td>
<td>13,780 ± 947</td>
<td>12,959 ± 857</td>
</tr>
<tr>
<td></td>
<td>Fold stimulation</td>
<td>1.95</td>
<td>2.13</td>
<td>2.03</td>
</tr>
<tr>
<td>AV</td>
<td>Basal</td>
<td>65 ± 7*</td>
<td>4,051 ± 206*</td>
<td>3,675 ± 155*</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>263 ± 21</td>
<td>13,997 ± 1,042</td>
<td>13,046 ± 905</td>
</tr>
<tr>
<td></td>
<td>Fold stimulation</td>
<td>4.05*</td>
<td>3.46*</td>
<td>3.54*</td>
</tr>
</tbody>
</table>

Values are means ± SE obtained from 10–12 animals for each group. LVDP, left ventricular developed pressure, +dP/dt, maximum rate of pressure development; −dP/dt, maximum rate of pressure decay. Isoproterenol was given as a bolus dose (10 μg/kg) through the femoral artery. *P < 0.05 compared with Sh group.
Binding. P

by aortocaval shunt for 16 wk binding in LV of rats with heart failure induced
we have presented several novel sizes of needles (6, 7, 8, 11, 15, 22, 40, 41). In this study,
and the shunt size can be controlled by using different
technique, the injury due to operation is minimized,
16, 20, 21, 27, 28). On the other hand, with the needle
technique, the injury due to operation is minimized, and the shunt size can be controlled by using different
sizes of needles (6, 7, 8, 11, 15, 22, 40, 41). In this study,
we have presented several novel findings, including the
time course changes in myosin heavy chain isoform
composition, enhanced responses to isoproterenol, in-
creased \( \beta_1 \)-AR density, and increased AC activity due
to volume overload. Furthermore, our data concerning
morphometric and hemodynamic characterization of
developing, compensated, and decompensated cardiac
hypertrophy can serve as a basis for future investigations
seeking to identify metabolic, subcellular, and
molecular defects in heart dysfunction due to volume
overload. In fact, the time course changes in this study
suggest that the development of cardiac hypertrophy
and heart failure in this model is consistent with three
stages of cardiac remodeling: namely, developing hy-
pertrophy, compensated hypertrophy, and decompens-
ated hypertrophy or heart failure as defined by Meer-
son (30).

Changes in cardiac mass. We have demonstrated a
progressive increase in cardiac mass; this is in agree-
ment with the observations of others who have em-
ployed the same needle technique for creating the
aortocaval shunt (6, 7, 8, 11, 22, 40, 41). It should be
mentioned that there are several features unique to
the cardiac hypertrophic growth induced by aortocaval
shunt. First, during the 16-wk period evaluated, the
increase of cardiac mass occurred in two bursts. The
initial burst occurred over the first 2 wk after the
operation (17 and 39% greater than control at 1 and 2
wk, respectively) and then slowed down at 4 wk (21%),
and by 8 wk the rate of cardiac growth was comparable
to that in the sham group. The other burst occurred
between 8 and 16 wk, during which the cardiac muscle
mass grew from 11 to 32% in the AV shunt group.
These dynamic changes in growth rate after the aorto-
caval shunt correlate with the acute-response, com-
penated, and decompensated phases of the disease.
The second feature is that the gain in the RV weight was
more than that in the LV. This may be due to the
different hemodynamic challenges that the LV and RV
face on induction of the AV shunt. By using radioac-
tive microsphere techniques, some studies have shown
dramatically increased cardiac output after inducing
an AV shunt (14, 21, 27), with as much as 75% of the
cardiac output being shunted through the fistula (22).
In addition, pulmonary hypertension has long been
recognized as a consequence of AV fistula in dogs and
humans (12, 18). Accordingly, the RV can be seen to be
under the challenge of both volume and pressure over-
load, thus exhibiting a more rapid and greater hyper-
trophic response. The more extensive hypertrophy re-
sponse of the RV compared with that of the LV has also
been observed by other investigators (27, 28, 41). By
measuring the length and width of isolated cardio-
myocytes, Liu et al. (27, 28) found that the increase in
RV cell volume at 1 wk was more prominent than that
in the LV or the intraventricular septum (27). Similar
findings were also reported at 1 and 5 mo after the AV
fistula was created (28). Ruzicka et al. (41) also re-
ported an increase of 40 and 76%, respectively, in LV
and RV weight 4 wk after inducing an AV shunt.
Finally, the extent of cardiac hypertrophy in the AV
shunt group doubled at 16 wk compared with that of
the sham control; this feature is comparable to that
occurring in human hypertrophic hearts (3, 4), whereas
the extent of cardiac hypertrophy in most other types of
experimental models in rodents does not attain this
level (10, 19, 25).

In vivo and in vitro hemodynamic changes. Our in-
vivo hemodynamic data suggest a sustained elevation
of LVEDP during the 1- to 16-wk period; however,
discrepancies exist with respect to the time course
changes. Brower et al. (6) showed a peak increase in
LVEDP at 3 wk postsurgery and gradual decline at 5
and 8 wk. In their recent study with 21-wk observation
after the aortocaval shunt, the LVEDP-LV end-dia-

<table>
<thead>
<tr>
<th>( \beta_1 )-AR</th>
<th>( \beta_2 )-AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_a ), pmol</td>
<td>( B_{max} ), fmol/mg</td>
</tr>
<tr>
<td>Sh</td>
<td>43 ± 2.8</td>
</tr>
<tr>
<td>AV</td>
<td>48 ± 4.7</td>
</tr>
</tbody>
</table>

Values are means ± SE obtained from 6 animals. \( B_{max} \), maximal binding. * \( P < 0.05 \) compared with Sh group.

Fig. 6. A: alteration of adenylyl cyclase (AC) activities in the LV of AV rats 16 wk after AV was induced. B: fold stimulation. Gpp,
5’-guanylylimidodiphosphate; Forsk, forskolin. Concentrations of
Iso, Gpp, NaF, and Forsk were 100 \( \mu \)M, 30 mM, 10 mM, and 100 \( \mu \)M, respectively. Values are means ± SE obtained from 6 LV for each
group. * \( P < 0.05 \) vs. Sh.

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stolic volume curve, an index of diastolic function, was not different among 8, 15, and 21 wk postsurgery, suggesting no further deterioration of LV diastolic function in their animals. Ruzicka et al. (41) showed a peak elevation of LVEDP in the first week and, thereafter, a gradual decrease by 4 and 7 wk. Our data are similar to the latter because we found a dramatic increase in LVEDP at 1 wk after the surgery. This change reflects the sudden increase of wall stress due to the volume overload after the AV shunt was opened, whereas the significant reduction in LVEDP from 1 to 2 wk indicates that the developing cardiac hypertrophy and dilation of the cardiac chamber tend to normalize the wall stress. In fact, Dolgievich et al. (11) have demonstrated by echocardiography that one-half of the LV dilation occurred in the first week after surgery. This structural remodeling induced by the AV shunt forms the basis for the decline in LVEDP at 2 wk. By 4 wk postsurgery, LVEDP reached the lowest level, indicating the maximum compensation at this time point. Furthermore, the increase in LVEDP at 8 and 16 wk is in contrast to that observed by Brower and Janicki (7); however, this further deterioration of the diastolic function is consistent with further LV dilation, decreased systolic function, increased myocardial stiffness, and occurrence of heart failure due to prolonged volume overload.

Several studies have shown increased cardiac output and decreased total peripheral resistance in the AV-shunted rats (22, 27, 41). In view of the unchanged heart rate after the aortocaval shunt, it appears that the maintenance of high-output status at early stages relies on the Frank-Starling reserve and the intact contractile function. In this regard, we have observed that the in vivo LV function was maintained at 4 wk and was only reduced by ~20% at 8 wk after the AV shunt was induced. However, Ruzicka et al. (41) showed a decrease in LV systolic function, despite an increase in cardiac index, and argued that the decreased total peripheral resistance might account for the large increase in stroke volume. On the other hand, Huang et al. (22) showed that the cardiac index was more than three times higher than that of control at 5 wk after aortocaval fistula. It is also possible that the maintained LV systolic function may contribute toward the dramatic elevation of cardiac output at this stage. Our data concerning changes in arterial hemodynamics during 16 wk after the AV shunt was induced reveal that the PP was increased throughout the experimental period. Our data are consistent with these previous reports in that there was an overall decrease in MAP in the AV shunt groups, although the decrease at 4 and 8 wk did not attain statistical significance. Such variations in the temporal course are due to differing methodologies for creating the shunt. It should also be pointed out that our data reflect a slight decrease in ASP but no change in LVSP at 1 and 2 wk; this discrepancy may be due to the dramatic decrease in total peripheral resistance as the blood flow is immediately shunted to the vena cava, thus causing a slight decrease in ASP.

The difference from our in vivo and in vitro hemodynamic data suggests in vivo compensation by neurohumoral mechanisms, such as the sympathetic nervous system and the renin-angiotensin system. In this regard, Communal et al. (8) have reported a decrease in catecholamine concentration in the ventricle without any change in plasma levels 4 wk after inducing an aortocaval shunt in rat. Although Ruzicka et al. (41) have reported an increase in the levels of plasma and cardiac renin activities, angiotensin II concentrations in plasma and ventricles were not determined. In view of the fact that the shunt is produced infrarenal and the blood flow to both sides of the kidney was not reduced (22) on creation of the aortocaval shunt, the activation of the renin-angiotensin system may be minimal in this experimental model until the occurrence of cardiac decompensation. Alternatively, an increase in myocardial sensitivity to in vivo circulating hormones or local neurotransmitter may occur to compensate for contractile function at early stages of cardiac hypertrophy due to volume overload.

Myosin isozyme composition. Myosin heavy chain isozyme composition has long been known as an indicator of changes in myosin Ca²⁺-ATPase and myocardial contractility (37, 42) in rodents. It should be pointed out that V₁ myosin heavy chain exhibits high Ca²⁺-ATPase activity and fast velocity of contraction, whereas V₃ myosin heavy chain has low Ca²⁺-ATPase activity and slow velocity of contraction (37, 42). A correlation of cardiac hypertrophy and myosin heavy chain isozyme shift from V₁ to V₃ has been obtained from several hemodynamic overload models (24, 31). However, our results indicate that a shift in myosin heavy chain isozyme expression occurred in the decompensated hypertrophic stage after the AV shunt when the heart weight was doubled compared with the control. Although significant hypertrophy was present at earlier stages, V₃ myosin heavy chain expression was not increased. This is consistent with the findings of Mercadier et al. (31), who found that increased expression of the V₃ myosin heavy chain isozyme was not correlated significantly with the extent of cardiac hypertrophy in rats with AV fistula, whereas a good relationship between V₃ expression and cardiac hypertrophy was observed in the aortic stenosis and aortic insufficiency models. Our results also demonstrate a difference in the time course of the V₃ shift between LV and RV. Theoretically, this shift should occur earlier in the RV because rapid and more prominent hypertrophy was observed in this ventricle compared with the LV. On the contrary, the myosin isozyme shift occurred in the LV at 8 wk, whereas such a change was only observed at 16 wk in the RV. The probability is that the delayed appearance of myosin isozyme shift in the RV...
reflects progressive LV decompensation, which then imposes even greater workload on the RV, resulting in the development of RV decompensation. Nevertheless, our results indicate that one of the molecular mechanisms for the observed contractile dysfunction may be related to the alteration of myosin heavy chain isozyme composition, because the time course of myosin heavy chain isozyme shift from V1 to V3 in the LV corresponded to the changes in LV function under in vivo conditions.

Occurrence of heart failure. Chronic heart failure is easily detected in human with an AV fistula (5, 17, 44), as well as in large animals with aortocaval shunt (1, 34). However, occurrence of heart failure in the rat aortocaval shunt model is rather controversial. For example, Liu et al. (28) demonstrated no contractile dysfunction 5 mo after inducing a large shunt by end-to-side anastomosis of the left iliolumber vein and aorta in rats. By using left ventriculography to evaluate cardiac function, Yang et al. (47) also reported that cardiac dysfunction was minimal in rat with AV shunt 12 wk after the surgery. In contrast, by studying the pressure-volume relationship, Brower et al. (6) demonstrated a decrease in intrinsic contractility at 1, 3, 5, and 8 wk after inducing the AV shunt, but the number of rats progressed to clinical overt heart failure was <3% during the 8-wk observation period. However, when these investigators (7) extended their studies for a prolonged period and used different approaches to measure the morbidity and mortality, they were able to show 80% incidence of heart failure after 21 wk of chronic volume overload. It seems that the variation in the occurrence of heart failure among different studies may be related to differences in techniques applied for producing the shunt, magnitude, and duration of the volume overload and approaches to measure the signs of heart failure. Nevertheless, our data are in agreement with those of Brower and Janicki (7), namely that heart failure occurred in rats with chronic volume overload produced by the AV shunt. In addition to dramatic changes in LV mass, diastolic and systolic dysfunction, as well as biochemical alterations, there were signs of circulatory congestion and mortality during 8–16 wk after the AV shunt. As reported by Brower and Janicki, the lung wet weight was significantly increased; however, the lung dry/wet weight was not increased because the dry weight was also increased proportionally. Histological examination indicated that the pulmonary interstitial septa were thickened in the lungs of the chronic AV shunt groups. This suggests that the increased lung wet weight may be due to both edema and organic changes, which may occur in the lung after chronic pulmonary overloading. Consistent with the development of pulmonary edema, liver wet weight was also significantly increased with a corresponding decrease in the dry/wet weight after 8–16 wk of the volume overload. Histology studies revealed distended central veins and fibrosis of the vessel wall in the liver, suggesting long-term congestion in the liver. Furthermore, there was a mortality of 5% due to heart failure in the later stage of the AV shunt; these values were comparable to those reported by Brower and Janicki (7). Taken together, we believe that congestive heart failure occurs in rats after a prolonged period of volume overload.

Upregulation of β-adrenergic system. One unique feature of this volume overload model is that, although the failing stage was achieved at 16 wk after the AV shunt was induced, the positive inotropic response of the heart to isoproterenol was not depressed. In fact, the stimulatory effect of isoproterenol was increased in the isolated heart at 4, 8, and 16 wk of inducing the AV shunt. In vivo response to isoproterenol was also maintained at 16 wk postsurgery. This observation is in contrast to several reports showing attenuated responses of hypertrophied failing hearts to catecholamines (see Refs. 9 and 46 for reviews). However, we have also observed an upregulation of β1-receptor density and increased AC activity in the AV-shunted hearts, in contrast to several studies that found down-regulation of both β-ARs after in vivo infusion of isoproterenol in rats (29, 33). Because chronic β-blockade in vivo is known to upregulate the β-ARs density (26, 36), it is possible that upregulation of β1-ARs observed in the aortocaval shunt model is related to decreased stimulation of the sympathetic nervous system. In fact, Communal et al. (8) demonstrated a decreased concentration of catecholamines in the ventricle without any change in plasma levels in rats 4 wk after inducing aortocaval shunt in rat. Thus the upregulation of β1-ARs without a change in β2-AR may be associated with a decrease in neuronally released norepinephrine from the AV-shunted hearts. In addition, our results showed increased AC activities in basal, isoproterenol-, Gpp(NH)p-, NaF-, as well as forskolin-stimulated AC in both LV and RV 16 wk after the AV shunt was induced. It is pointed out that isoproterenol is known to activate AC through the β-ARs, whereas Gpp(NH)p and NaF activate AC through G proteins and forskolin exerts its stimulatory effect by interacting with the catalytic unit of AC directly (9, 46). Because AC activity was increased under basal and all stimulatory conditions, it is hard to assess the individual contribution of β-ARs, G protein, and AC to this phenomenon. However, when the data were expressed in terms of the stimulation with respect to basal enzyme activity, the results indicated that isoproterenol and forskolin induced greater activation of AC in the AV shunt group, whereas the extent of stimulation due to Gpp(NH)p or NaF was similar between the sham and experimental groups. This pattern of enhanced signals at the receptor and effector levels is consistent with increased β1-receptor density and increased basal activity of AC in the failing hearts due to AV shunt. Furthermore, upregulation of AC in the RV and downregulation of AC in the viable LV have been reported to occur during the development of heart failure due to myocardial infarction (43).

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