Dose-dependent effect of ANG II-receptor antagonist on myocyte remodeling in rat cardiac hypertrophy

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Obayashi, Masakazu, Masafumi Yano, Michihiro Kohno, Shigeki Kobayashi, Taketo Tanigawa, Katsumi Hironaka, Tsutomu Ryouke, and Masunori Matsuzaki. Dose-dependent effect of ANG II-receptor antagonist on myocyte remodeling in rat cardiac hypertrophy. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1824–H1831, 1997.—The goal of this study was to examine the effect of an angiotensin II type 1 (AT1)-receptor antagonist (TCV-116) on left ventricular (LV) geometry and function during the development of pressure-overload LV hypertrophy. A low (LD; 0.3 mg·kg⁻¹·day⁻¹) or a high (HD; 3.0 mg·kg⁻¹·day⁻¹) dose of TCV-116 was administered to abdominal aortic-banded rats over 4 wk, and hemodynamics and morphology were then evaluated. In both LD and HD groups, peak LV pressures decreased to a similar extent compared with the vehicle-treated group but stayed at higher levels than in the sham-operated group. In the HD group, both end-diastolic wall thickness (3.08 ± 0.14 mm) and myocyte width (13.3 ± 0.1 µm) decreased compared with those in the vehicle-treated group (3.67 ± 0.19 mm and 15.3 ± 0.1 µm, respectively; both P < 0.05). In the HD group, myocyte length was further decreased (HD: 94.1 ± 2.9 µm; P < 0.05) in association with a reduction in LV midwall radius (HD: 3.36 ± 0.12, LD: 3.60 ± 0.14 mm; P < 0.05) and peak midwall fiber stress (HD: 69 ± 8, LD: 83 ± 10 × 10³ dyn/cm²; P < 0.05). There was no significant difference in cardiac output among all groups. The AT1-receptor antagonist TCV-116 induced an inhibition of the development of pressure-overload hypertrophy. Morphologically, not only the width but also the length of the hypertrophied myocyte was attenuated with TCV-116, leading to a reduction of midwall radius and hence wall stress, which in turn may contribute to a preservation of cardiac output.

METHODS

Animals and drug treatment. Male Wistar rats weighing 140–170 g were obtained from laboratories (Japan SLC). Rats were given food and water ad libitum and were kept on a 12:12-h light-dark cycle. After an acclimatization period of at least 5 days, they were randomly divided into four main groups: sham-operated rats (Sham) and three groups of rats with aortic banding (vehicle-treated [AC]; 0.3 mg/kg body wt of TCV-116 [low dose; LD]; and 3.0 mg/kg body wt of TCV-116 [high dose; HD]). TCV-116 administration (0.3 or 3.0 mg·kg⁻¹·day⁻¹ by gastric gavage) was started 1 day before the surgery and continued for 4 wk after the surgery. TCV-116 was suspended in 2% gum arabic solution. The same volume of gum arabic solution was given in vehicle-treated aortic-banded rats and vehicle-treated sham controls. TCV-116 was provided by Takeda Chemical Industries.

Abdominal aortic constriction. Pressure-overload LV hypertrophy was produced by constriction of the abdominal aorta (12). Rats were anesthetized and the aorta was exposed through a midline abdominal incision. A blunted needle 0.8 mm in diameter was placed alongside the abdominal aorta.
below the diaphragm but proximal to renal bifurcations and was tightly fixed with surgical silk. The needle was then removed, leaving the aorta constricted to an outer diameter equivalent to the diameter of the needle. In the Shum animals, the suture was not tightened.

Hemodynamic measurements. To investigate the effect of TCV-116 on the time course of development of pressure-overload LV hypertrophy, peak systolic pressure and LV weight were measured at 4 days, 10 days, and 4 wk after the surgery. Also, 2 wk after the chronic administration of TCV-116, the changes in daily blood pressure were measured. Other hemodynamic measurements including LV wall thickness and aortic flow were also performed 4 wk after the surgery.

Twenty-four hours after the last administration, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg) and hemodynamic parameters were obtained. After the rats were fully sedated, a tracheal tube was inserted, and the LV was catheterized with an ultraminiature catheter pressure transducer (PR 249, Millar Instruments) via the right common carotid artery. Peak LV pressure, LV end-diastolic pressure, and maximal value of the first derivative of LV pressure (+dP/dt) were measured. The ascending aorta was isolated, and the ultrasonic transit-time flow probe (T-106, Transonic Systems) was placed for measurement of phasic instantaneous aortic blood flow. The frequency response relations were curve fitted in the form of a spherical model applied for the LV (7)

\[ V = \frac{(4/3)\pi a^3}{V} \]

where \( V \) is cavity volume and \( a \) and \( b \) are inner and outer radii, respectively.

\[ \sigma = 3/2P(V/V_w)(bR)^3 \]

\[ E_{inc} = (1/2)a\sigma/(\Delta R/R) = (1/2)\kappa(BR)^{3/2} \]

where \( R = (a + b)/2 \) is the midwall radius and \( P \) is the LV cavity pressure. To determine \( \alpha \), the stress-radius (\( \sigma-R \)) relations were curve fitted in the form of \( \sigma = BR \), where \( B \) and \( \alpha \) are curve-fitting parameters. This study was approved by the Animal Care Committee of the School of Medicine, Yamauchi University.

Fixation and cell sizedata. Using an additional 16 rats, we obtained morphological data using the method reported by Oh et al. (23). After the heart was arrested by injection of potassium chloride (2 meq/ml), the thorax was opened, and polyethylene catheters (PE-200) were introduced into the LV via the left atrial appendage. After the right atrium was opened, the aorta was perfused with heparinized saline for 2–3 min to wash out blood. The myocardium was perfused at a pressure of 50 mmHg for 20 min retrogradely from the aorta with 95% ethanol and 1% acetic acid using an infusion pump (model 680, Harvard Apparatus). On fixation, LV pressure was maintained at 2.5 mmHg to minimize the effect of the fixation pressure on the dilatation of the LV chamber. After hardening, the heart was excised, and the atria and right ventricle were carefully dissected away. The LV was weighed and immersed into cold mixed solution containing 95% ethanol and 1% acetic acid.

The fixed LV was embedded with paraffin. Transmural myocardial sections were cut in a transverse plane perpendicular to the apex-to-base axis and in 4-μm sections. After deparaffinization and dehydration, the immunohistochemical study was performed using the DAKO LSAB kit based on the labeled streptavidin-biotin method. In brief, the sections were frequently washed in tris(hydroxymethyl)aminomethane-buffered saline (TBS, pH 7.4) buffer and then incubated with an appropriate dilution of the primary antibody (directed against connexin 43; Ref. 17) at 4°C overnight. Rinsing with TBS buffer was followed by incubation with biotinylated goat anti-rabbit immunoglobulin G (Zymed Lab, San Francisco, CA) for 2–3 h. After the sections were rinsed in TBS buffer, they were treated with peroxidase-labeled streptavidin. The staining was visualized by incubation with 3% 3-aminophenolphosphate and 0.1% 9-ethylcarbazole in N,N-dimethylformamide, followed by counterstaining with 0.1% hematoxylin for 5 min. A negative-control study was performed by replacing the primary antibodies with nonimmune serum or TBS, which resulted in negative staining.

After immunohistochemical staining of connexin 43, we obtained optimal contrast between intercalated disks and myocytes in the sections. The morphometric measurement...
was performed with a magnification of ×400. Using the method of Vliegen et al. (30) with some modifications, we selected 30 myocytes in one section that showed the proper longitudinal orientation and did not branch in the circular midwall muscle bundles of the LV free wall. Only myocytes in which intercalated disk was located on both sides and a nucleus was in the center of the myocyte were measured. Myocyte width was determined as the transnuclear width of the myocyte. Myocyte length was determined as the distance between the middle points of intercalated disks on both sides of the myocyte.

Interstitial percent fibrosis. The LV was fixed in 20% buffered formaldehyde. After 2–3 days, the preparations were dehydrated and embedded in paraffin. Sections were cut at 6 µm. Tissue sections were stained with picrosirius red staining (Sirius Red F3BA (Chroma-Gesellschaft) in aqueous picric acid), which is specific for collagen. The protocol for picrosirius red staining was adopted from Volders et al. (31).

The LV was excluded was determined with NIH Image (National Institutes of Health) image-analysis software. Tissue sections stained with picrosirius red were analyzed with polarization microscopy (×100). In each section taken from the middle portion of the LV free wall, 10–12 fields were randomly selected to measure percent fibrosis and the average of portion of the LV free wall, 10–12 fields were randomly selected to measure percent fibrosis and the average of percent fibrosis was obtained.

Statistical analysis. Statistical analysis was performed using analysis of variance (ANOVA). P values <0.05 were accepted as statistically significant. Fisher’s protected least significance difference was used to make individual comparisons between groups when a significant change was observed with ANOVA. Correlation coefficients were calculated using linear regression analysis.

RESULTS

Hemodynamics and LV geometry. Both peak systolic pressure and the LV weight-to-body weight ratio increased significantly in the AC group as early as 4 days after the operation, and this increase was maintained over 4 wk. Treatment with TCV-116 reduced the LV weight-to-body weight ratio in a dose-dependent manner, whereas peak systolic pressure was decreased to a similar extent regardless of the dose of TCV-116 (Fig. 1; Table 1). Figure 2 shows the change in daily blood pressure 2 wk after the chronic administration of TCV-116. A low dose (0.3 mg·kg⁻¹·day⁻¹) of TCV-116 significantly reduced peak systolic pressure to a similar extent as a high dose (3.0 mg·kg⁻¹·day⁻¹) of TCV-116.

| Table 1. Body weight, LV weight, and LV weight normalized to body weight 4 wk after surgery |
|---------------------------------|------------------|-----------------|---------------------|
|                                | Sham (n=7)       | AC (n=11)       | LD (n=10)           | HD (n=10)          |
| BW, g                          | 251±13           | 253±19          | 250±11              | 239±11             |
| LVW, mg                        | 411±34           | 703±40*         | 578±67†             | 487±43‡            |
| LVW/BW                         | 1.8±0.2          | 2.8±0.2a        | 2.3±0.3†            | 2.0±0.2‡           |

Values are expressed as means ± SD; n, no. of rats. LV, left ventricular; BW, body weight; LVW, LV weight; Sham, sham-operated rats; AC, untreated rats with aortic constriction; LD, rats with aortic constriction and 0.3 mg·kg⁻¹·day⁻¹ of TCV-116; HD, rats with aortic constriction and 3.0 mg·kg⁻¹·day⁻¹ of TCV-116. *P < 0.05 vs. Sham; †P < 0.05 vs. AC; ‡P < 0.05 vs. LD.
The left associated with a smaller V/Vw compared with the pressure-volume relations tended to shift toward a higher value compared with those in the AC group. In the AC group, peak wall stress remained at a high level despite the reduction in LV pressure. In the LD group, peak wall stress was normalized in the HD group. In the AC group, an increase in LV cavity volume, whereas the pressure-volume relations compared with the AC group, reflecting the elevation of chamber stiffness constant. Figure 3 shows the ex vivo pressure-volume relations, chamber stiffness constant, and myocardial stiffness constant 4 wk after surgery.

Table 2. Hemodynamics and LV geometry 4 wk after surgery

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 7)</th>
<th>AC (n = 11)</th>
<th>LD (n = 10)</th>
<th>HD (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>419 ± 16</td>
<td>402 ± 20</td>
<td>415 ± 42</td>
<td>423 ± 51</td>
</tr>
<tr>
<td>Peak LVP, mmHg</td>
<td>122 ± 7</td>
<td>165 ± 10*</td>
<td>137 ± 10†</td>
<td>137 ± 12†</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>5 ± 2</td>
<td>7 ± 2</td>
<td>5 ± 3</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Peak +dP/dt, mmHg/s</td>
<td>4,886 ± 882</td>
<td>6,857 ± 1,305*</td>
<td>5,960 ± 1,670</td>
<td>5,062 ± 892†</td>
</tr>
<tr>
<td>Wad, mm</td>
<td>2.81 ± 0.11</td>
<td>3.67 ± 0.19*</td>
<td>3.08 ± 0.14†</td>
<td>2.99 ± 0.16‡</td>
</tr>
<tr>
<td>Dst, mm</td>
<td>3.84 ± 0.30</td>
<td>3.65 ± 0.31</td>
<td>4.13 ± 0.25†</td>
<td>3.72 ± 0.32‡</td>
</tr>
<tr>
<td>Midwall radius, mm</td>
<td>3.32 ± 0.14</td>
<td>3.64 ± 0.11*</td>
<td>3.60 ± 0.14*</td>
<td>3.36 ± 0.12†</td>
</tr>
<tr>
<td>Peak ST, 10^3 dyn/cm²</td>
<td>70 ± 13</td>
<td>85 ± 8*</td>
<td>83 ± 10*</td>
<td>69 ± 8†</td>
</tr>
<tr>
<td>End-diastolic ST, 10^3 dyn/cm²</td>
<td>5 ± 3</td>
<td>4 ± 1</td>
<td>5 ± 3</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>21.8 ± 3.0</td>
<td>23.5 ± 5.5</td>
<td>21.9 ± 5.1</td>
<td>22.0 ± 5.5</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD; n, no. of rats. LVP, LV pressure; LVEDP, LV end-diastolic pressure; peak +dP/dt, maximal value of 1st derivative of LV pressure; Wad, LV end-diastolic wall thickness; Dst, LV end-diastolic internal dimension; ST, LV midwall fiber stress. *P < 0.05 vs. Sham; †P < 0.05 vs. AC; ‡P < 0.05 vs. LD.

Morphological data. Figure 4 shows the immunohistochemical staining of connexin 43. Both intercalated disks and myocytes were clearly detected in the sections. Figure 5 shows the histograms of myocyte width and length in a total of 120 myocytes from 4 rats in each group. The mean value of myocyte size was obtained from 30 myocytes in each rat, and these data are summarized in Table 4. In the AC group, the histograms of myocyte width and length were shifted toward a higher value compared with those in the Sham group. In the TCV-116-treated groups, the increases in myocyte width were significantly inhibited to at 8, 16, and 24 h after the last administration of the drug. There was no significant difference in blood pressure between low- and high-dose administration of TCV-116 at any time studied.

The parameters of hemodynamics and LV geometry 4 wk after the surgery are summarized in Table 2. Peak LV pressures were elevated in all three banded groups compared with the Sham group. Peak LV pressures in the TCV-116-treated groups were lower than in the AC group, but no significant difference was observed between the TCV-116 treated groups. Heart rate and LV end-diastolic pressure did not differ among all groups. Peak +dP/dt of LV pressure in the AC group was higher than in the HD and Sham groups. There was no significant difference in cardiac output among all groups including the Sham group.

LV end-diastolic wall thickness was increased in the AC group, whereas the increment of end-diastolic wall thickness was severely attenuated in both TCV-116 treated groups (LD and HD). In the HD group, the increment in either LV internal diameter or LV midwall radius was also suppressed compared with the LD group.

Although end-diastolic wall stress was not significantly different among all groups, peak wall stress was increased in the AC group, reflecting the elevation of peak LV pressure, whereas it was normalized in the HD group. In the LD group, peak wall stress remained at a high level despite the reduction in LV pressure.

Ex vivo LV pressure-volume relations, chamber stiffness, cavity and wall volume, and myocardial stiffness. Figure 3 shows the ex vivo pressure-volume relations, and Table 3 summarizes various parameters derived from the pressure-volume relations. In the AC group, the pressure-volume relations tended to shift toward the left associated with a smaller V/Vw compared with the Sham group, indicating concentric hypertrophy. The LD group had a rightward shift in the pressure-volume relations compared with the AC group, reflecting an increase in LV cavity volume, whereas the pressure-volume relations in the HD group became close to those in the Sham group. Although there was no difference in myocardial stiffness constant among all groups, the chamber stiffness constant was decreased in the LD group although unchanged in the HD group.

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 7)</th>
<th>AC (n = 11)</th>
<th>LD (n = 5)</th>
<th>HD (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/Vw</td>
<td>0.42 ± 0.05</td>
<td>0.25 ± 0.04*</td>
<td>0.40 ± 0.08†</td>
<td>0.34 ± 0.08†</td>
</tr>
<tr>
<td>Kc</td>
<td>2.20 ± 0.31</td>
<td>2.51 ± 0.40</td>
<td>1.86 ± 0.12†</td>
<td>2.26 ± 0.25‡</td>
</tr>
<tr>
<td>Km</td>
<td>13.32 ± 1.29</td>
<td>14.98 ± 1.89</td>
<td>13.80 ± 1.15</td>
<td>13.88 ± 0.44</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD; n, no. of rats. V, LV cavity volume at 10 mmHg; Vw, LV wall volume; Kc, LV chamber stiffness constant; Km, myocardial stiffness constant. *P < 0.05 vs. Sham; †P < 0.05 vs. AC; ‡P < 0.05 vs. LD.
similar extents. Myocyte length in the LD group was smaller than in the AC group but still larger than in the Sham group. In the HD group, myocyte length was further decreased and became close to that of the Sham group. There was no significant difference in interstitial percent fibrosis among all groups (Table 4).

Figure 6 shows the relation between LV weight and myocyte volume in aortic-banded groups based on the assumption of a cylindrical configuration 

$$\text{myocyte volume} = \pi \times \left(\frac{\text{average myocyte width}}{2}\right)^2 \times \text{average myocyte length}.$$  

There was a good correction ($r = 0.87$, $P < 0.001$) between these two parameters.

**DISCUSSION**

The major finding of this study is that chronic administration of the AT$_1$-receptor antagonist TCV-116 to rats with abdominal aortic constriction induced a decrease not only in myocyte width but also length of hypertrophied myocardium induced by pressure overload.

Treatment with a low dose (0.3 mg·kg$^{-1}$·day$^{-1}$) of TCV-116 inhibited the increase in LV wall thickness and, morphologically, myocyte size (width and length), whereas peak wall stress still remained at a high level. Interestingly, with a high dose (3.0 mg·kg$^{-1}$·day$^{-1}$) of TCV-116, additional reduction of myocyte length was observed accompanied by a decrease in both midwall radius and peak wall stress without a further reduction in blood pressure. Although LV contractility was decreased with the high dose of TCV-116, cardiac output was not significantly changed. These results suggest that in the effect of AT$_1$-receptor antagonist on the development of pressure-overload hypertrophy, the reduction of myocyte length might be an important factor of reducing LV wall stress and preventing a decrease in cardiac output.

LV hypertrophy and renin-angiotensin system. Afterload reduction might be involved in the mechanism by which LV hypertrophy is regressed by various antihypertensive drugs (3, 13). Indeed, in aortic-banded rats, we observed a decrease in LV weight in association with a significant reduction in systolic pressure after treatment with a low dose of TCV-116. However, Linz et al. (18) reported that the angiotensin-converting enzyme (ACE) inhibitor ramipril, at a dose that did not decrease blood pressure, reversed LV hypertrophy in aortic-banded rats, whereas the calcium antagonist nifedipine and the vasodilator hydralazine did not reverse LV hypertrophy despite a reduction of blood pressure. Kromer et al. (16) also reported that the ACE inhibitor quinapril induced a regression of pressure-overload LV hypertrophy despite a persistent elevation

![Fig. 4. Longitudinal orientation of myocytes in circular midwall muscle bundles of LV free wall by immunohistochemical staining of connexin 43. Note clearly visible intercalated disks (arrows). Bar, 30 µm.](http://ajpheart.physiology.org/)
of blood pressure. These data indicate that factors other than hemodynamic changes play a role in the pathogenesis of pressure overload-induced LV hypertrophy.

In this regard, the renin-angiotensin system (RAS) is now regarded as another important factor in the development of cardiac hypertrophy. ANG II has been shown to cause proliferation of cardiac myocytes (2, 29). Khairallah et al. (11) reported that ANG II induced cardiac hypertrophy without increasing blood pressure. Rockman et al. (26) reported that the AT1-receptor antagonist losartan prevented an increase in the heart weight-to-body weight ratio without a significant reduction in hemodynamic load in mice with aortic arch constriction. In the present study, we also found a further suppression of LV hypertrophy by an increase in TCV-116 from 0.3 to 3.0 mg·kg$^{-1}$·day$^{-1}$ without a further reduction in blood pressure, indicating the involvement of RAS in the development of LV hypertrophy.

Table 4. Myocyte size and percent fibrosis 4 wk after surgery

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 4)</th>
<th>AC (n = 4)</th>
<th>LD (n = 4)</th>
<th>HD (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocyte width, µm</td>
<td>12.8 ± 0.2</td>
<td>15.3 ± 0.1*</td>
<td>13.3 ± 0.1†</td>
<td>13.4 ± 0.3†</td>
</tr>
<tr>
<td>Myocyte length, µm</td>
<td>82.7 ± 1.9</td>
<td>98.0 ± 2.2*</td>
<td>94.1 ± 2.9†</td>
<td>82.6 ± 2.6†</td>
</tr>
<tr>
<td>Length-to-width ratio</td>
<td>6.44 ± 0.15</td>
<td>6.43 ± 0.11</td>
<td>7.09 ± 0.23†</td>
<td>6.20 ± 0.25†</td>
</tr>
<tr>
<td>Percent fibrosis</td>
<td>2.5 ± 0.3</td>
<td>2.7 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD; n, no. of rats. *P < 0.05 vs. Sham; †P < 0.05 vs. AC; ‡P < 0.05 vs. LD.
with the AT1-receptor antagonist, LV pump function (6). However, because cardiac output did not decrease positive inotropic effect of local ANG II in cardiac tissue, increase in peak contractility might be reduced as evidenced by a decreased in the untreated aortic-banded group, as evidenced by a decrease in LV internal diameter on chronic administration of TCV-116. Because AT1 blockade or ACE inhibition induces a regression of LV hypertrophy with a proportional decrease in cardiac tissue ANG II (20, 22), it is suggested that the mode of arrangement of myofibrils or sarcomeres (parallel or series addition, respectively; Ref. 8), which is responsible for the change in myocyte size, might be dependent on the extent of endogenous ANG II content of cardiac tissue during the development of pressure-overload hypertrophy.

Effect of TCV-116 on cardiac function. In pressure-overload LV hypertrophy, systolic function is usually preserved at a compensatory state (24). In the present study, systolic function was preserved or even enhanced in the untreated aortic-banded group, as evidenced by the increase in peak LV +dP/dt and normal cardiac output. With an AT1-receptor antagonist, LV contractility might be reduced as evidenced by a decrease in peak +dP/dt, probably due to inhibition of the positive inotropic effect of local ANG II in cardiac tissue (6). However, because cardiac output did not decrease with the AT1-receptor antagonist, LV pump function may be preserved in part because of a concomitant reduction of LV wall stress. Thus, although TCV-116 has a negative inotropic effect on the heart, RAS inhibition by TCV-116 decreases myocyte length, which directly reduces LV radius and LV wall stress followed by preservation of LV pump function.

Study limitations. First, the reliability of the measurement of myocyte size should be addressed. Previous reports using the isolation technique for cardiac myocytes indicated that transverse diameter of myocytes increases with pressure-overload hypertrophy (5, 15). In morphometric data from whole tissue sections, most of these findings were based on measurement using hematoxylin-eosin staining (14, 27), whereas in the present study we used immunohistochemical staining with connexin 43 because we needed to detect clearly intercalated disks for the measurement of myocyte length. Therefore, we also measured myocyte width using hematoxylin-eosin staining and obtained a finding compatible with immunohistochemical staining with connexin 43 (not shown). There are few reports in which hypertrophied myocyte length is measured using morphometry of whole tissue sections (1, 9, 30), because myocyte length is difficult to estimate for at least two reasons: true longitudinal sections are rare, and distances between intercalated disk vary within the same myocyte (9, 30). Immunohistochemical staining with connexin 43 allowed us to detect clearly intercalated disks so that we could select myocytes in which intercalated disk was located on both sides and a nucleus was in the center of the myocyte. Campbell et al. (5) reported that there was good agreement between changes in heart weight and average myocyte volume in aortic-constricted rats. We also obtained a good correlation between LV weight and average myocyte volume in aortic-constricted rats. We also obtained a good correlation between LV weight and average myocyte volume that was determined on the assumption of a cylindrical configuration (r = 0.87, P < 0.001), suggesting a reasonable estimation of myocyte size in our study. Moreover, by showing the histogram of the myocyte size, we confirmed the reasonable application of statistical analysis.

Second, we calculated LV internal diameter and midwall radius by applying several assumptions (see Hemodynamic measurements). Therefore, the accuracy of the calculation, particularly for LV internal diameter, should be addressed. In this regard, the LV internal volume derived from ex vivo pressure-volume relations showed a change compatible with the calculated LV internal diameter on chronic administration of TCV-116.

In conclusion, the AT1-receptor antagonist TCV-116 induced an inhibition of the development of pressure-overload hypertrophy: decrease in LV weight, wall thickness, and midwall radius. Morphologically, not only the width but also the length of myocytes was decreased with TCV-116, leading to a reduction of LV wall stress and a preservation of cardiac output.

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