Beneficial Effects on Skeletal Muscle of the Angiotensin II Type 1 Receptor Blocker Irbesartan in Experimental Heart Failure

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Background—In congestive heart failure (CHF), skeletal muscle shows increased expression of fast myosin heavy chains (MHC) and fibers, muscle atrophy, increased fatigability, and decreased endurance. Atrophy is secondary to myocyte apoptosis, which is probably triggered by tumor necrosis factor-α (TNFα). Angiotensin II receptors are thought to play a role in controlling apoptosis. We tested the hypothesis that angiotensin II receptor blockade could prevent skeletal muscle apoptosis in rats with CHF.

Methods and Results—CHF was induced by injecting 36 rats with 30 mg/kg monocrotaline. Ten additional animals were injected with saline and acted as controls. After 2 weeks, 18 of the 36 rats with CHF were treated with 7 mg · kg⁻¹ · d⁻¹ irbesartan through osmotic minipumps, and 10 of the 36 rats were treated with 2 mg · kg⁻¹ · d⁻¹ nifedipine in drinking water. After 2 additional weeks, rats were killed. Tibialis anterior cross-sectional area, MHC composition, myocyte apoptosis, Bcl-2, pro-caspase 3, and activated caspases 3 and 9 were determined, as were plasma levels of TNFα and angiotensin II. Myocyte apoptosis and muscle atrophy were significantly decreased with irbesartan compared with untreated CHF rats. Irbesartan-treated rats had fewer cells labeled positively with terminal deoxynucleotidal transferase–mediated dUTP nick-end labeling and fewer caspases; however, they also had increased Bcl-2 levels and muscle fiber cross-sectional areas. The MHC pattern in irbesartan-treated animals was similar to that in controls. Nifedipine animals behaved like the untreated CHF animals. Angiotensin II was increased 3- to 4-fold in all CHF rats (treated and untreated). TNFα levels were decreased in irbesartan-treated rats but not in nifedipine-treated rats.

Conclusions—Angiotensin II receptor blockade can protect from the development of apoptosis-dependent atrophy and from changes in MHCs. The reduction of TNFα may play a role in this process. (Circulation. 2001;103:2195-2200.)

Key Words heart failure ■ irbesartan ■ muscles ■ apoptosis ■ tumor necrosis factor

Heart failure is characterized by a decreased exercise capacity with early occurrence of fatigue and dyspnea. These symptoms are partially due to a skeletal muscle myopathy, with atrophy and a shift from the “slow” fatigue resistant type I fibers to the more fatigable “fast” type II fibers. This is evident in humans and in animal models of congestive heart failure (CHF), in which we also found a correlation between the severity of the syndrome and skeletal muscle myosin heavy chain (MHC) composition. The origin of this myopathy is not yet clear, although its specificity has been hypothesized. Similarly, the link between exercise capacity and endurance, muscle atrophy, and changes in fiber type are still ill-defined.

We recently showed in rats with CHF that the degree of muscle atrophy correlates with the magnitude of apoptosis and the severity of CHF and is accompanied by elevated plasma levels of tumor necrosis factor-α (TNFα). In humans, muscle bulk loss, one of the most important determinants of exercise capacity, is equal to secondary to myocyte apoptosis. Apoptosis is involved in myocyte loss both in CHF and in cardiomyopathies. It has been suggested that in the heart, apoptosis can be triggered by angiotensin II (AngII) or cytokines, such as TNFα. However, it is not clear whether AngII determines apoptosis via type 1 (AT1) or type 2 (AT2) receptors. Many authors have suggested that AT1 receptor stimulation brings about apoptosis. In contrast, losartan, an AT1 receptor blocker, and captopril, an ACE inhibitor, can block apoptosis both in vitro (isolated stretched myocytes) and in vivo (spontaneously hypertensive rats with CHF). All these results are intriguing and, in this article, we tested the hypothesis that AT1 blockade with irbesartan can prevent apoptosis and skeletal muscle atrophy.
Methods

Experimental Model

We studied 36 male Sprague-Dawley rats weighing 80 to 100 g that had CHF induced by monocrotaline. This alkalioid produces severe pulmonary hypertension followed by right ventricular (RV) failure without itself inducing changes in skeletal muscle MHC composition and apoptosis. Monocrotaline was injected intraperitoneally in the rats at a dose of 30 mg/kg. The rats were then divided into 3 groups.

After 2 weeks, 18 of the 36 rats had 7 mg · kg⁻¹ · d⁻¹ irbesartan delivered through Alzet osmotic minipumps. Irbesartan 250 mg was solubilized in 3.75 mL of 0.19 mol/L KOH at room temperature and sonicated for 1 minute; 0.75 mL of 50 mmol/L Tris was added and neutralized at pH 8. This is the method used for intravenous preparations. The second group consisted of 10 rats (of the 36) that were treated with 2 mg · kg⁻¹ · d⁻¹ nifedipine, which was given in drinking water. Rats were kept in single cages, and the amount of water drunk was measured daily. The final 8 rats had a saline-delivering minipump, and they formed the (untreated) CHF group.

A total of 10 additional age- and diet-matched rats were injected with saline and served as controls. After 28 days, when overt heart failure had developed, animals were killed and their body weight and tibialis anterior (TA) weight were measured. Muscles were immediately frozen in liquid nitrogen and stored at −80°C. Blood was drawn for TNFα and AngII measurements.

Experiments were approved by the Biological Ethical Committee of the University of Padua and performed according to Italian law.

Assessment of RV Hypertrophy and Failure

To ensure that the monocrotaline-treated animals developed RV failure, left ventricular mass/RV mass (LVM/RVM) and the RV mass/RV volume index (RVM/RVV) were calculated with a computerized planimeter on photographic pictures of formalin-fixed transverse sections of the heart taken in the middle portion of the interventricular septum.

Electrophoretic Separation of MHCs

MHCs (MHC2a [fast oxidative] and MHC2b [fast glycolytic]) were separated using the previously described electrophoretic method.

Assessment of MHC Distribution

The percent distribution of the MHCs was determined by a densitometric scan using a Jandel Scientific system.

Single-Fiber Cross-Sectional Area

We used single-fiber cross-sectional areas (CSA) as an index of myofiber atrophy. Cross sections were taken for histological examination and stained with hematoxylin and eosin. The fiber CSA was calculated with a computerized interactive method and expressed in μm² by counting at least 400 fibers per specimen.

Assessment of Apoptosis

In Situ Terminal Deoxynucleotidyl Transferase–Mediated dUTP Nick-End Labeling

In situ terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) of fragmented DNA was performed on cryosections using the In Situ Cell Death Detection Kit POD (Boehringer Mannheim). Labeled nuclei were identified from the negative nuclei counterstained by Hoechst 33258 and were counted after being photographed. The total number of positive nuclei was determined by counting (at a magnification of 250×) all the labeled nuclei present in the whole specimen. The number of positive nuclei was then expressed as the number of TUNEL-positive nuclei per mm². TUNEL-positive myofibers and interstitial nuclei were distinguished on the basis of their location on sections stained with laminin, which selectively reacts with the basal lamina. TUNEL positive nuclei within the basal lamina were taken as myonuclei. Separate calculations were made for total TUNEL-positive nuclei and TUNEL-positive myonuclei.

TA Western Blot for Pro-Caspase 3, Activated Caspases 3 and 9, and Bcl-2

Western blotting was performed on 12.5 polyacrylamide gels as previously described. Anti–pro-caspase 3 CPP 32 (H-277, 34 kDa) and anti–Bcl-2 (29 kDa) antibodies (Santa Cruz Biototechnology) were used with anti-rabbit alkaline phosphatase (Sigma). Anti-cleaved caspase-3 (17 kDa) and anti-cleaved caspase-9 (37 kDa) (Cell Signaling Technology) were used with anti-rabbit peroxidase-conjugated antibody and revealed by chemiluminescent substrate (SuperSignal West Pico, Pierce). The absolute values of pro-caspase 3, activated caspases 3 and 9, and Bcl-2 were calculated on the blot bands with the densitometric system described above and expressed as percent of controls.

DNA Ladder

DNA was extracted from the TA muscle as previously described. The ApoAlert LM-PCR Ladder assay kit (Clontech) was used to amplify the detection of nucleosomal ladder. A total of 0.5 μg of genomic DNA was used. Reaction conditions were as follows: 1 cycle at 72°C for 8 minutes, 25 cycles at 94°C for 1 minute and at 72°C for 3 minutes, and 1 cycle at 72°C for 15 minutes. Polymerase chain reaction (PCR) products were electrophoresed on 1.8% agarose gels.

Confocal Microscopy Immunofluorescence

Frozen sections were incubated with anti-activated caspase-3 antibody diluted 1:50 overnight at 4°C. Slices were then incubated with anti-rabbit Cy3-conjugated antibody for 1 hour at room temperature and analyzed by Bio-Rad confocal microscopy.

AngII Assay

AngII was measured on serum using an enzyme-immunometric assay kit from SPI-BIO.

TNFα

TNFα was measured with a solid-phase sandwich ELISA using a monoclonal antibody specific for rat TNFα (Euroclone).

Statistical Analysis

Values are reported as mean±SD. Student’s t tests for unpaired data were used, as was ANOVA when appropriate. A 5% difference was considered statistically significant. ANOVA was also used to look at differences in TNFα, AngII, caspases, Bcl-2, and percentage of apoptotic nuclei.

Results

Occurrence of Heart Failure in the Monocrotaline-Treated Animals

At 28 days, all the rats with monocrotaline-induced CHF showed the presence of pericardial, pleural, and peritoneal effusions at post-mortem examination (Figure 1 and Table 1). LVM/RVM was 2.6±0.8 in untreated CHF rats, 3.5±0.8 in controls (P<0.02), 2.4±0.7 in irbesartan-treated rats (P=0.0005 versus controls), and 2.0±0.5 in nifedipine-treated rats (P=NS versus irbesartan and P=0.0005 versus controls). The RV cavity was markedly dilated in all the CHF rats, as reflected by the RVM/RVV index, which was 0.24±0.18 in untreated CHF rats versus 1.10±0.7 (P=0.01) in controls and 0.41±0.09 in the irbesartan-treated animals (P=0.014 versus CHF and P=0.04 versus controls). In the nifedipine-treated animals, we observed a RVM/RVV of 0.60±0.18, which was significantly higher than that of both controls and irbesartan-treated rats (P=0.02), indicating a lower degree of dilatation.
Degree of Muscle Atrophy

The degree of TA atrophy, as measured by TA weight/body weight ratio, was 1.60 ± 0.05 in untreated CHF rats, 1.78 ± 0.05 in controls, 1.69 ± 0.04 in irbesartan-treated rats, and 1.60 ± 0.12 in nifedipine-treated rats.

TA Fiber CSA

The CSA of the TA was 1340 ± 460 μm² in the untreated CHF rats, 2150 ± 350 μm² in the controls (P=0.01), 1750 ± 300 μm² in the irbesartan-treated rats (P=0.05 versus CHF), and 900 ± 250 μm² in the nifedipine-treated rats (P<0.002 versus irbesartan group and controls; Table 1).

MHC Pattern

The electrophoretic pattern of the TA in the CHF animals showed a shift toward the fast glycolytic isoform (Table 2). In fact, MHC2a decreased from 26.4 ± 1.5% to 18.0 ± 3.5% (P=0.005). MHC2b increased from 74.0 ± 1.5% to 82.0 ± 3.5% (P=0.005). In the irbesartan group, MHC2a was 25.5 ± 5.9% (P=0.04 versus CHF) and MHC2b was 74.5 ± 5.9% (P=0.04 versus CHF), whereas in the nifedipine group, MHC2a was 18.7 ± 1.7% and MHC2b was 81.2 ± 1.7% (P=NS versus CHF).

Count of TUNEL-Positive Nuclei

The count of the total TUNEL-positive nuclei in the TA of the control rats was 1.1 ± 3.9 per mm³ whereas in the CHF rats, this percentage was higher (41.9 ± 44.1/mm³, P=0.04). In the irbesartan group, we found 12.0 ± 12.9/mm³ TUNEL-positive nuclei (P=0.03 versus controls and P=0.05 versus CHF). In the nifedipine group, the total number of TUNEL-positive nuclei was 56.1 ± 43.4/mm³ (P=NS versus CHF and P<0.05 with irbesartan; Figure 2 and Table 3).

Table 1. LVM/RVM, RVM/RVW, and TA CSA in All Rats

<table>
<thead>
<tr>
<th></th>
<th>LVM/RVM</th>
<th>RVM/RVW</th>
<th>CSA, μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>3.5 ± 0.8†</td>
<td>1.10 ± 0.7‡</td>
<td>2150 ± 350‡</td>
</tr>
<tr>
<td>CHF</td>
<td>2.6 ± 0.8*</td>
<td>0.24 ± 0.18§</td>
<td>1340 ± 460#</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>2.4 ± 0.7†</td>
<td>0.41 ± 0.09¶</td>
<td>1750 ± 300§#</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>2.0 ± 0.5</td>
<td>0.60 ± 0.18§</td>
<td>900 ± 250§</td>
</tr>
</tbody>
</table>

*P=0.04, †P=0.0006, ‡P=0.019, §P=0.02, ¶P=0.014, ††P=0.004, †‡P=0.05.

Table 2. MHC Distribution

<table>
<thead>
<tr>
<th></th>
<th>MHC2a, %</th>
<th>MHC2b, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>26 ± 1.5† ‡</td>
<td>74 ± 1.5‡ ‡</td>
</tr>
<tr>
<td>CHF</td>
<td>18 ± 3.5†</td>
<td>82 ± 3.5†</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>25.5 ± 5.9*</td>
<td>74.5 ± 5.9*</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>18.7 ± 1.7‡</td>
<td>81.2 ± 1.7‡</td>
</tr>
</tbody>
</table>

*P=0.04, CHF vs Irbesartan; †P=0.005, CHF vs Control; ‡P=0.04, Nifedipine vs Control.

Figure 1. Transverse sections of heart at level of mid-interventricular septum from control (a), untreated CHF (b), irbesartan-treated (c), and nifedipine-treated (d) rats (Heidenhein stain). RV wall is extremely dilated and thinned in untreated CHF rats. Irbesartan-treated and nifedipine-treated rats show only mild dilatation with RV hypertrophy.

Figure 2. Interstitial and myocyte TUNEL-positive nuclei in TA. Left (a, b, and c), Double labeling for TUNEL and laminin. Right, (a’, b’, and c’), Double-exposure laminin and Hoechst of same fiber (magnification, 400×). A, Interstitial apoptotic nucleus; b and c, myocyte apoptotic nuclei. Bar=50 μm. Arrows in a, b, and c indicate apoptotic nuclei.
TABLE 3. TUNEL-Positive Nuclei, Pro-Caspase 3, Bcl-2, and TNFα in All Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>TUNEL+, n/mm³</th>
<th>Pro-Caspase 3, %</th>
<th>Bcl-2, %</th>
<th>TNFα, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1.1±3.9†‡#</td>
<td>100±10†‡**</td>
<td>97±12$</td>
<td>100±8††</td>
</tr>
<tr>
<td>CHF</td>
<td>41.9±44.1*¶†</td>
<td>211±87†</td>
<td>70±16§</td>
<td>139±6¶†</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>12.0±12.9‡¶‡</td>
<td>191±72‡</td>
<td>94±35</td>
<td>108±11¶</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>56.1±43.4‡¶‡</td>
<td>213±76**</td>
<td>ND</td>
<td>140±12†</td>
</tr>
</tbody>
</table>

*A=0.04, †=0.05, CHF vs Control; ‡=0.03, Irbesartan vs Control; §=0.02, ††=0.001, CHF vs Control; †‡=0.05, CHF vs Irbesartan; ¶=0.04, ¶¶=0.003, †††=0.001, Nifedipine vs Control; ††‡=0.05, Irbesartan vs Nifedipine.

The myocyte TUNEL-positive nuclei count was 0.4±0.8/mm³ in controls, 12.6±11.9/mm³ in CHF untreated animals, 4.0±4.1/mm³ in irbesartan-treated rats, and 18.0±14.3/mm³ in nifedipine-treated rats.

Activated Caspases 3 and 9

We confirmed the occurrence of apoptosis in animals with high numbers of TUNEL-positive nuclei by testing for the presence of activated caspases 9 and 3 (the mitochondrial regulator and the executioner, respectively; Figure 3). Caspase 3 is detectable during the execution phase of apoptosis, when the caspase cascade leads inevitably to programmed cell death.20 The presence of these caspases in the muscles of untreated CHF and nifedipine-treated animals, who had the highest TUNEL positivity, and their absence in controls further indicate the presence of apoptosis and suggest that mitochondria could play an important role in this process. The presence of activated caspases 3 and 9 was shown with immunoblotting (Figures 3A and 3B) and that of activated caspase 3 was also shown with immunofluorescence and confocal microscopy (Figure 3C) in the CHF and nifedipine rats. These caspases were absent in controls. Note that the activation of caspase 3 occurred in some areas of myofiber cytoplasm.

DNA Ladder

In our model of CHF, in which only a small percentage of cells are apoptotic and in which apoptosis occurs asynchronously, the genomic DNA ladder may not be visible. For this reason, we used a PCR assay to amplify specifically the nucleosomal ladder. We found a positive DNA ladder only in the untreated and nifedipine-treated animals with CHF; the ladder was negative in controls (Figure 3D). This experiment further stresses the occurrence of apoptosis in the muscles showing TUNEL-positive nuclei.

Pro-Caspase 3

Pro-caspase 3 was significantly increased in the untreated CHF rats when compared with controls (211±87% versus 100±10%, P=0.05). In irbesartan-treated rats, although the percentage of pro-caspase 3 was lower than in untreated CHF rats, it was still significantly higher than in controls (191±72%, P=0.03). In the nifedipine-treated animals, the percentage of pro-caspase 3 was similar to that of untreated CHF rats (213±76%; Table 3).

Bcl-2

Bcl-2 was 97±12% in controls, which was similar to that in irbesartan-treated animals (94±35%, P=NS). In the untreated CHF rats, Bcl-2 was 70±16% (P=0.02 versus both controls and irbesartan; Table 3).

TNFα

There was a rise in plasma TNFα levels in the CHF animals (139±6% versus 100±8% in controls, P=0.001). In the irbesartan group, the levels did not differ from controls but were lower than those in untreated CHF rats (108±11%, P=0.05). In the nifedipine-treated animals, TNFα levels were similar to those of untreated CHF rats (140±12%; Table 3).

AngII

Plasma levels of AngII in the untreated CHF, irbesartan-treated, nifedipine-treated, and control animals are shown in Table 4. We
found significant, 3- to 4-fold higher levels of AngII in the untreated CHF, irbesartan-treated, and nifedipine-treated rats when compared with controls (P<0.008 for all).

**Discussion**

In the rat, monocrotaline induces RV hypertrophy followed by RV dilatation and CHF. In these animals, we found TA atrophy secondary to myocyte apoptosis and a shift of the MHCs toward the fast glycolytic isoforms, confirming previously published data. The occurrence of apoptosis was confirmed by different techniques, including immunoblotting, immunohistochemistry, confocal microscopy, and DNA ladder. Activated caspases 3 and 9 were also present; this ensured that the caspase cascade, which inevitably leads to programmed cell death, was initiated.

Irbesartan, a drug that selectively blocks the AT1 receptor, could not prevent RV hypertrophy in this study, as shown by an LVM/RVM index that did not differ between the irbesartan and untreated CHF groups. The occurrence of RV dilatation, however, was partially prevented. In irbesartan-treated animals, RVM/RVV was, in fact, significantly lower than in controls and higher than in untreated CHF rats. This confirms that a certain degree of compensated hypertrophy with a lower degree of failure was present in the monocrotaline-treated rats that were treated with irbesartan for 2 weeks. Therefore, we can assume that irbesartan produced only a partial improvement in the hemodynamic pattern. A similar finding was observed in the nifedipine-treated animals, in which RV dilatation was prevented to an even higher degree and a greater deal of compensated RV hypertrophy was found. Nifedipine was used to obtain favorable hemodynamic changes in a model of CHF due to pulmonary hypertension, without directly interfering with AngII receptors.

Despite their similar hemodynamic effects, irbesartan greatly differed from nifedipine in terms of biological effects. This is, in fact, the first demonstration in vivo that an AT1 blocker can produce beneficial effects on the skeletal muscle of rats with experimental CHF. These effects could not be obtained with the calcium blocker. In irbesartan, the MHC pattern was similar to that in controls, with a partial improvement in the degree of TA atrophy (higher TA weight/body weight and larger myocyte CSA). In the nifedipine group, however, neither MHC composition nor indices of muscle atrophy were different from untreated CHF animals.

We can reasonably speculate that this effect of irbesartan on muscle atrophy is secondary to the lower levels of apoptosis. The absolute number of TUNEL-positive cells was lower in the irbesartan group when compared with untreated CHF rats. In the nifedipine-treated rats, in which apoptosis was detected at even higher levels than in untreated CHF rats, muscle atrophy was worse. Pro-caspase 3 and activated caspas 3 and 9, which are compulsory steps in the death receptor signaling pathway, were equally decreased in irbesartan-treated animals but remained high in nifedipine-treated rats. Bcl-2, which has a protective effect, was significantly higher in irbesartan-treated than in untreated CHF rats and was similar to that in controls.

The role of TNFα in CHF is not entirely understood. We know that in CHF, the circulating amounts of TNFα are increased both in humans and in animal models. TNFα blockade with specific antagonists (Enbrel) can improve the clinical status of CHF patients. Moreover, TNFα levels parallel the severity of CHF and the number of TUNEL-positive skeletal myocytes. Therefore, TNFα is known to worsen CHF by depressing cardiac contractility. Although still debated, TNFα has been shown to trigger apoptosis. We know that in the heart, it induces the production of sphingolipids, such as sphingosine and ceramide, which are in turn mediators of apoptosis.

In this study, TNFα was significantly increased in untreated CHF animals when compared with controls, and it remained high in nifedipine-treated rats. In irbesartan-treated rats, it was slightly decreased, reaching borderline significance. The TNFα figures, therefore, resemble those of TUNEL, pro-caspase 3, and Bcl-2 in suggesting the existence of a link between TNFα and apoptosis. We can only speculate why TNFα is decreased with irbesartan. We may hypothesize that the less compromised hemodynamics may have interfered with TNFα synthesis, which in CHF is linked to clinical status. If that was the case, even the nifedipine-treated rats, which had a similar hemodynamic improvement, should have shown a TNFα reduction. Therefore, we think that the interplay between TNFα, AngII receptors, and apoptosis is much more complicated.

From the present results, we can hypothesize that the favorable effects of irbesartan on apoptosis are likely to be secondary to a direct AT1-mediated antiapoptotic effect rather than to hemodynamic improvements. In fact, nifedipine-treated rats, which show a similar degree of compensated RV hypertrophy with an even lower dilatation of the RV, do not exhibit any skeletal muscle change.

The role of AngII receptors on apoptosis is far from clear. Several observations suggest that AT1 stimulation mediates apoptosis through extracellular signal-regulated kinase (ERK) inhibition, ceramide accumulation, activation of mitogen-activated protein kinase phosphatase 1 (MKP-1) with subsequent inhibition of mitogen-activated protein kinase, and Bcl-2 dephosphorylation. Apoptosis can be blocked by PD-123319 and PD-123177, which are specific AT2 blockers. In contrast, some authors have shown that AT1 blockade with losartan can equally protect from apoptosis. AT1 stimulation can lead to an increase in Fas, together with a fall in constitutive NO synthase and Bcl-2 levels. We know that in many tissues, skeletal muscle atrophy is secondary to the lower levels of apoptosis. The ACE inhibitor captopril reduced apoptosis in spontaneously hypertensive rats.

AngII-induced apoptosis can be blocked in postinfarcted, hypertrophied ventricular myocytes by AT1 blockers, but AT2 antagonists had no impact on these cellular events. The conflict regarding whether AT1, or AT2 receptors mediate apoptosis can be mediated by the observation that tissues that express primarily AT2 exhibit AT2-mediated apoptosis, whereas tissues that express primarily AT1 exhibit AT1-mediated apoptosis. Moreover, in many tissues, skeletal muscle included, the majority of physiological responses to AngII occurs through its accumulation, which is AT1-mediated. These observations allow us to speculate on the
mechanism by which irbesartan, a highly specific AT1 blocker, may have prevented apoptosis by acting through a receptor mechanism involving the AT2 receptor. This specific action can be brought about even in the presence of very high levels of AngII. In fact, in irbesartan-treated rats, we found a 3- to 4-fold increase in AngII, which was similar to increases in untreated CHF and nifedipine-treated rats. It may be also speculated that excessive AT2 stimulation due to AT1 blockade with irbesartan may reduce the number of apoptotic cells in the skeletal muscle of CHF rats. A recent observation in humans, however, shows that the AT2 gene is not expressed in the skeletal muscle of men with CHF. In the present study, AngII levels are in keeping with those in the literature, both for normal and AngII blocker–treated rats, which show a 4- to 5-fold increase in AngII. CHF itself and the reflex sympathetic tone are likely to be the cause of AngII elevation in nifedipine-treated animals.

That AngII receptor blockade and ACE inhibition can have a favorable effect on skeletal muscle fiber type, MHCs, and exercise capacity has been previously demonstrated. This article is a further insight into the pathophysiology of CHF myopathy, allowing speculations on the pathogenesis of this syndrome. In fact, muscle atrophy is the main “muscular” determinant of the reduced exercise capacity in CHF. This is due to the reduced muscle force and endurance and to the shift toward the fast and more fatigable fibers. Irbesartan has shown potential for reducing skeletal muscle apoptosis and atrophy and in protecting from the shift toward the fast MHCs, further contributing to a possible improvement in exercise tolerance and symptoms. The reason for the MHC shift remains to be established, as does the interplay between AT1 and AT2 receptors in the genesis and prevention of apoptosis. Thus, further experiments need to be performed. The role of TNFα and AngII receptors, which seem to be synergistic in the induction of apoptosis, must be further investigated.

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References

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