Beneficial effects of GH/IGF-1 on skeletal muscle atrophy and function in experimental heart failure

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Dalla Libera, Luciano, Barbara Ravara, Maurizio Voltterrani, Valerio Gobbo, Mila Della Barbera, Annalisa Angelini, Daniela Danieli Betto, Elena Germinario, and Giorgio Vescovo. Beneficial effects of GH/IGF-1 on skeletal muscle atrophy and function in experimental heart failure. Am J Physiol Cell Physiol 286: C138–C144, 2004. First published September 17, 2003; 10.1152/ajpcell.00114.2003.—Muscle atrophy is a determinant of exercise capacity in heart failure (CHF). Myocyte apoptosis, triggered by tumor necrosis factor-α (TNF-α) or its second messenger sphingosine (SPH), is one of the causes of atrophy. Growth hormone (GH) improves hemodynamic and cardiac trophism in several experimental models of CHF, but its effect on skeletal muscle in CHF is not yet clear. We tested the hypothesis that GH can prevent skeletal muscle apoptosis in rats with CHF. GH was induced by injecting monocrotaline. After 2 wk, 2 groups of rats were treated with GH (0.2 mg·kg⁻¹·day⁻¹ and 1.0 mg·kg⁻¹·day⁻¹) subcutaneously. A third group of controls had saline. After 2 additional weeks, rats were killed. Tibialis anterior cross-sectional area, myosin heavy chain (MHC) composition, and a study on myocyte apoptosis and serum levels of TNF-α and SPH were carried out. The number of apoptotic nuclei, muscle atrophy, and serum levels of TNF-α and SPH were decreased with GH at high but not at low doses compared with CHF rats. Bel-2 was increased, whereas activated caspasases and bax were decreased. The MHC pattern in GH-treated animals was similar to that of controls. Monocrotaline slowed down both contraction and relaxation but did not affect specific tetanic force, whereas absolute force was decreased. GH treatment restored contraction and relaxation to control values and brought muscle mass and absolute twitch and tetanic tension to normal levels. These findings may provide an insight into the therapeutic strategy of GH given to patients with CHF to improve exercise capacity.

Severa attempts to prevent apoptosis by blocking the ATII receptor (9) or the caspasases cascade (for instance, by using the sprogomyelinase inhibitor L-carnitine) (30) have led to preservation of muscle mass that may have a positive reflection on patients’ exercise capacity.

Growth hormone (GH) has been proposed as a therapeutic tool for the treatment of CHF (8, 14, 15, 18, 25, 34). It has been used either with the purpose of improving hemodynamics (13, 28, 34, 35) or myocardial mass (17). The results have not been univocal, and the potential side effects have discouraged clinicians from using GH in CHF. Recently, the observation that the activation of the GH/IGF-1 (insulin-like growth factor-1) axis can mediate cells’ survival and proliferation has reawakened interest in this molecule, but with a different target: the skeletal muscle (24). The aim of the present study was to see whether in an experimental model of CHF GH may prevent the apoptosis-dependent muscle waste, therefore forming the potential for improving exercise capacity.

MATERIALS AND METHODS

Experimental Model

Five groups of male 80–100 g Sprague-Dawley rats were studied: 20 controls and 50 with CHF induced by monocrotaline (Sigma, St. Louis, MO). This alkaloid produces severe and irreversible pulmonary hypertension followed by right ventricular (RV) failure that mimics the hemodynamic and neurohormonal pattern of heart failure in humans (11, 32). Monocrotaline was injected intraperitoneally at the dose of 30 mg/kg. After 2 wk, the 50 monocrotaline-treated animals were divided into 3 groups. One group of 20 rats had recombinant human GH (Pharmacia, Uppsala, Sweden) 0.2 mg·kg⁻¹·day⁻¹ subcutaneously in the scruff of the neck, and a second group of 20 had 1.0 mg·kg⁻¹·day⁻¹ subcutaneously. The third group of controls had saline. After 28 days, when, in the monocrotaline-treated animals, CHF has developed, rats were killed. Muscles were immediately frozen in liquid nitrogen. Hearts were stored in 10% formaldehyde.

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U. S. National Institutes of

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SKELETAL MUSCLE CONtributes to the origin of symptoms in exercise capacity in heart failure (CHF) (6, 11, 20, 22). An exaggerated hyperventilation due to altered ergoreflex sensitivity has been proposed as the cause of dyspnoea (6, 27). Fatigue may be the result of skeletal muscle bulk loss and changes in fiber type with preferential synthesis of fast anaerobic myosin heavy chains (MHCs) (9, 29, 30, 31, 32). Skeletal muscle atrophy, which strongly correlates with exercise capacity (22, 33), seems to be the consequence of an inflammatory status with production of proapoptotic cytokines such as tumor necrosis factor-α (TNF-α) (5, 9, 10, 11, 30, 32).

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Health. Experiments were approved by the University of Padua Biological Ethical Committee.

Right Ventricle Hypertrophy and Failure

Right ventricle mass (RVM), left ventricle mass (LVM), and right ventricular mass/right volume (RVM/vol) were measured as previously reported (32). A computerized planimeter was used on photographic pictures of formaline-fixed transverse sections of the heart taken in the middle portion of the interventricular septum. We used the ratio between RVM and RVM/vol as an index of RV dilatation, which we have shown correlates with the severity of heart decompen-sation. We included in the CHF group only animals that showed at post mortem clear signs of right heart failure, such as pleural, pericardial, and peritoneal effusions, together with marked RV dilatation, expressed by a RVM/vol <2.

MHC Distribution

The percentage of distribution of the MHCs was separated electrophoretically on a 7% polyacrylamide slab with 37.5% vol/vol glyc- erol. Gels were stained with Coomassie brilliant blue (11). The percent of distribution of the three different isoforms was determined by densitometric scan (Sigma Gel, SPSS, Chicago, IL). A linear response in terms of electrophoretic band area was attained on densitometry when 0.1–2 μg of individual MHC were analyzed.

Single-Fiber Cross-Sectional Area

Fiber cross-sectional area (CSA) was taken as an index of muscle atrophy (32). Cross cryosections were taken for histological exami-nation and stained with hematoxylin-eosin. The fiber CSA was cal-culated with a computerized interactive method (Kontron IBAS) and expressed in μm² by counting at least 400 fibers/specimen.

Apoptosis

In situ DNA nick-end labeling. In situ nick-end labeling (TUNEL) of fragmented DNA was performed on cryosections (In Situ Cell Death Detection Kit, POD Roche, Boehringer Mannheim). The total number of labeled nuclei present in the whole specimen was counted and expressed as the number of TUNEL positive nuclei/mm² (31).

Mitochondria.

A gram of tissue was minced in 10 ml of homog-enizing buffer [250 mM sucrose, 0.1 mM EGTA, 10 mM Tris-HCl, pH 7.4, and 1 tablet per 50 ml of the broad spectrum Complete inhibitors cocktail (Roche, Mannheim, Germany)]. The first pellet obtained at 600 g for 10 min represents the nuclear fraction. Supernatant was then centrifuged at 15,000 g for 10 min to obtain the mitochondrial fraction. The microsomal fraction was, pelleted by centrifuging at 100,000 g for 60 min to obtain cytosolic fraction.

Tibialis anterior Western blot for activated caspases 9, Bcl-2, Bax, and cytochrome c.

Western blot was performed with total tissue homogenates in SDS buffer on 12.5% polyacrylamide gels (9). The following antibodies were used: anti-Bcl-2 (1:500) (Oncogene, Darmstadt, Germany), anti-cleaved caspase-9 (1:1,000) (Cell Signaling Technology, Beverly, MA), anti-Bax (1:500), and monoclonal anti-cytochrome c (1:1,000) (Alexis, San Diego, CA). For caspases, Bax, and Bcl-2, 50 μg of protein were loaded; for cytochrome c, 5 μg were loaded. All blots were revealed by chemiluminescent substrate (SuperSignal West Pico, Pierce, IL). All the blots were run under conditions to yield a linear relationship between protein and signal.

ELISA for apoptosis. DNA ladder assay was performed as previ-ously described (30). Briefly, 10 muscle cryosections 20 μm thick were solubilized in 200 μl of lysis buffer (0.1% Triton X-100, 5 mM Tris-HCl, 20 mM EGTA, and 20 mM EDTA). Polyethylene glycol 8000 and NaCl were then added to a final concentration of 2.5% and 1 M, respectively. Samples were centrifuged and protein concentra-tion was adjusted by the method of Bradford to 0.01 μg/μl. Cell death enzyme-linked immunoabsorbent assay (ELISA) analysis was per-formed according to the manufacturers instructions with the Cell Death Detection ELISA PLUS kit (Roche Mannheim, Germany).

Caspase-3 assay. Caspase-3 activity was measured with the CaspACE assay system (Promega, Madison, WI).

TNF-α

TNF-α was measured with a solid-phase sandwich ELISA using a monoclonal antibody specific for rat TNF-α (Euroclone, UK).

ANG II

ANG II was measured by using an enzyme-immunometric assay kit (SPI-BIO, Massy Cedex, France). We used ANG II as an index of the CHF neurohormonal activation. In this model of CHF, decompensated rats have significantly increased levels of this peptide (9).

IGF-1

IGF-1 was measured by using an enzyme-immunometric assay kit (R&D Systems Minneapolis, MN).

SPH

SPH was determined with high-performance liquid chromatogra-phy (HPLC) with the method previously described by Dalla Libera et al. (10). Briefly, sphingolipids were extracted with butanol and de-rivatized with ophthaldialdehyde. Samples were separated on a Bio-Rad hi-Pore reversed phase column on a Bio-Rad HPLC system with a Perkin-Elmer fluorescence detector (excitation 340 nm, emission 455 nm).

Plasma Glucose

Plasma glucose was determined with a colorimetric method.

Mechanical Studies

In a separate set of six control, two monocrotaline, and six mono-crotaline animals with CHF treated with high doses of GH, the isometric contractile properties of isolated soleus were investigated. Contractility of soleus muscle was studied in vitro at 30 ± 1°C. Muscle bathing, stimulation conditions, and tension recording were performed as previously described (23). Twitches were obtained by applying single supramaximal stimuli (0.5-ms duration). The follow-ing parameters were measured: contraction time (CT), half-relaxation time (HRTT), and twitch tension (T). The frequency-tension curves were constructed from recordings of the force exerted by soleus muscles during 1-s stimulation at 15, 30, 60, 90, and 120 Hz. From the 120-Hz tetani, the following parameters were obtained: half-relaxation time of the tetanus (HRTT) and maximum tetanic tension (P0). Absolute twitch tension and tetanus tension were normalized to wet weight of the muscles to obtain the specific tension (N g⁻¹).

Muscle responses were recorded via an AT-MIO 16 analog-to-digital card and data were analyzed by a LabView-based computer program (National Instruments).

Statistical Analysis

Means ± SD are reported. Student t-test for unpaired data was used. P < 0.05 was considered statistically significant. ANOVA was used for comparing skeletal muscle, apoptotic cascade measurements, and contractile parameters measurements.

RESULTS

Body Weight

As shown in Table 1, body weight (BW) reached 237 ± 10 g in the control rats at the end of the study period. Both low
Table 1. Body weight and cardiac morphometry

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>LVM, mm²</th>
<th>RVM, mm²</th>
<th>RVM/Vol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>237 ± 10^a,b,c,d</td>
<td>43.5 ± 5.4^h,i,k</td>
<td>10.8 ± 3.4^m,a,p</td>
<td>2.6 ± 0.2^t,u,s,l,h</td>
</tr>
<tr>
<td>GH low</td>
<td>260 ± 2^e</td>
<td>55.0 ± 6.4^b</td>
<td>19.2 ± 1.0^p</td>
<td>2.2 ± 0.4^q</td>
</tr>
<tr>
<td>GH high</td>
<td>272 ± 10^o</td>
<td>50.5 ± 2.8^g</td>
<td>25.1 ± 0.2^j</td>
<td>2.7 ± 0.2^f</td>
</tr>
<tr>
<td>CHF</td>
<td>210 ± 20^e,f</td>
<td>31.5 ± 6.1^b,j</td>
<td>16.3 ± 4.0^n,o</td>
<td>1.7 ± 0.2^f</td>
</tr>
<tr>
<td>CHF+GH (low dose)</td>
<td>206 ± 20^e</td>
<td>47.9 ± 6.2</td>
<td>19.9 ± 4.4</td>
<td>1.4 ± 0.2^d</td>
</tr>
<tr>
<td>CHF+GH (high dose)</td>
<td>241 ± 31^f</td>
<td>48.2 ± 7.2^j</td>
<td>20.2 ± 6.3^m,o</td>
<td>2.0 ± 0.6^d</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.01; bP < 0.00002; cP < 0.003; dP = NS; eP = NS; fP < 0.003; gP = NS; hP < 0.0003; iP = NS; jP < 0.0001; kP = NS; lP < 0.01; mP < 0.001; nP < 0.0002; oP < 0.000002; pP = NS; qP < 0.0006; rP = NS; sP = NS; tP = NS; uP < 0.008; vP < 0.00001; wP < 0.002.

(L-GH) and high (H-GH) doses of GH led to a significant increase in BW (P < 0.01).

Animals with overt heart failure and no treatment showed a significantly lower BW compared with controls (P < 0.0002).
L-GH given to CHF rats was unable to produce any change in BW, whereas CHF rats treated with H-GH showed a BW identical to that of controls, indicating that high doses of GH were able to produce a restoration of body weight in the monocrotaline-treated rats.

Occurrence of Heart Failure in the Monocrotaline-Treated Animals

After 4 wk, all the CHF rats showed a significantly higher RV mass. The right ventricular cavity was markedly dilated as reflected by the RVM/vol index, which was 1.7 ± 0.2 in the CHF rats vs. 2.6 ± 0.2 in the control group.

In control rats, H-GH and L-GH were able to increase both RV and LV mass compared with controls. In CHF animals, H-GH and L-GH showed an increase in mass of both ventricles of similar magnitude compared with control CHF rats, which indicates a probable trophic effect of GH on the myocardium.

ANG II Levels

As shown in Table 2, ANG II levels in control rats were the same as those of the GH rats. The CHF rats showed a significant (P < 0.02) increase in ANG II, whereas H-GH failed to produce in the monocrotaline-treated rats a significant decrease in ANG II levels, indicating that there were no effects on the neurohormonal activation produced by the RV decompensation.

IGF-1

Control rats treated with H-GH showed a significant increase of IGF-1 (P < 0.02). CHF rats showed the same IGF-1 levels of controls. In CHF rats treated with H-GH, IGF-1 was significantly increased compared with control CHF animals (P < 0.02). There was also a further 30% increase compared with GH-treated controls.

Blood Glucose

We did not find significant alterations in blood glucose in any group of rats: the values were in the range 105–132 mg/dl.

Tibialis Anterior MHC Pattern

As shown in Table 3, we found in the CHF animals a shift from the fast oxidative (MHC2a) to the fast glycolytic isoform (MHC2b), which was 27 ± 1.4% and 73 ± 1.4% in the control vs. 18 ± 3% (P < 0.000001) and 82 ± 3% in the CHF, respectively. H-GH produced a reshift toward the MHC2a (26 ± 2.7; P < 0.009), whereas MHC2b was 74 ± 2.7%. L-GH gave no reshift in that the values of MHCs were similar to that of CHF. We therefore achieved a complete restoration of the control MHCs pattern only after high GH doses.

Tibialis Anterior CSA

The degree of muscle atrophy as measured by fiber CSA was 1,969 ± 257 μm² in the control vs. 1,310 ± 267 in the CHF rats (P < 0.00007). The H-GH produced an increase in CSA (1,788 ± 264), which reached statistical significance (P < 0.001) compared with CHF. By contrast, L-GH had no effect. Only high GH doses were therefore able to restore the TA muscle mass nearly to the same degree of the control rats.

TUNEL-Positive Nuclei

As shown in Table 4, the CHF rats showed an enormous increase in the number of TUNEL-positive nuclei, which was 6,266 nuclei/mm³, as opposed to 7 of the control and GH control animals (P < 0.006). The treatment with H-GH produced in the monocrotaline-treated animals a significant reduction in the number of TUNEL-positive nuclei (30 ± 17; P < 0.00001 vs. CHF and P = not significant vs. control). The number of apoptotic nuclei was brought to levels similar to those of the control rats. L-GH did not influence the number of

Table 2. Serum measurements

<table>
<thead>
<tr>
<th></th>
<th>ANG II, pg/ml</th>
<th>IGF-1, % of control</th>
<th>Glucose, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28 ± 3^b,h</td>
<td>100 ± 10^e</td>
<td>132 ± 21^b</td>
</tr>
<tr>
<td>GH</td>
<td>26 ± 4</td>
<td>165 ± 25^f</td>
<td>105 ± 9^g</td>
</tr>
<tr>
<td>CHF</td>
<td>50 ± 13^e,k</td>
<td>95 ± 21^d,l</td>
<td>128 ± 19^h</td>
</tr>
<tr>
<td>CHF+GH (low dose)</td>
<td>53 ± 20^e</td>
<td>195 ± 30^j</td>
<td>114 ± 28^l</td>
</tr>
<tr>
<td>CHF+GH (high dose)</td>
<td>241 ± 31^f</td>
<td>48.2 ± 7.2^j</td>
<td>20.2 ± 6.3^m,o</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.02; bP < 0.05; *P = NS; dP = NS; eP < 0.02; fP = NS; gP = NS; hP < 0.02; iP = NS; jP = NS; kP = NS; lP = NS.

Table 3. Skeletal muscle measurements

<table>
<thead>
<tr>
<th></th>
<th>MHC IIA, % of total</th>
<th>MHC IIB, % of total</th>
<th>CSA, μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27 ± 1.4^h,i,k</td>
<td>73 ± 1.4</td>
<td>1969 ± 257^g,h</td>
</tr>
<tr>
<td>GH</td>
<td>25.3 ± 2.3</td>
<td>74.6 ± 2.3</td>
<td>1735 ± 400</td>
</tr>
<tr>
<td>CHF</td>
<td>18 ± 3^b,c</td>
<td>82 ± 3</td>
<td>1310 ± 267^h</td>
</tr>
<tr>
<td>CHF+GH (low dose)</td>
<td>15.6 ± 2.1^d,h</td>
<td>84.3 ± 6.1</td>
<td>1370 ± 300^d</td>
</tr>
<tr>
<td>CHF+GH (high dose)</td>
<td>26 ± 2.7^d</td>
<td>74 ± 2.7</td>
<td>1788 ± 264^h</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.00001; bP < 0.008; cP = NS; dP = NS; eP < 0.009; fP < 0.0007; gP < 0.0004; hP = NS; iP = NS; jP < 0.001.
Table 4. Apoptotic cascade in skeletal muscle and serum

<table>
<thead>
<tr>
<th></th>
<th>TUNEL+, nuclei/mm²</th>
<th>TNFα, pg/ml</th>
<th>SPH, pmoles/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7 ± 3a,b,c</td>
<td>114 ± 12e-h</td>
<td>850 ± 70k,l,m</td>
</tr>
<tr>
<td>GH</td>
<td>5 ± 2</td>
<td>118 ± 20</td>
<td>900 ± 80</td>
</tr>
<tr>
<td>CHF</td>
<td>168 ± 66d-e</td>
<td>282 ± 100f-i</td>
<td>1300 ± 210k,n,o</td>
</tr>
<tr>
<td>CHF+GH (low dose)</td>
<td>140 ± 38h,i</td>
<td>272 ± 85p,q</td>
<td>1250 ± 170q</td>
</tr>
<tr>
<td>CHF+GH (high dose)</td>
<td>30 ± 17c,e</td>
<td>175 ± 30p,</td>
<td>710 ± 110p,q</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.006; **P < 0.006; *P = NS; **P = NS; ³P < 0.0001; ⁴P < 0.04; ⁵P < 0.04; ⁶P < 0.03; ⁷P = NS; ⁸P < 0.002; ⁹P < 0.04; ¹⁰P < 0.04; ¹¹P = NS; ¹²P = NS; ¹³P < 0.0001.

TUNEL-positive nuclei, which was similar to that of CHF animals.

Activated Caspase-9, Bcl2, Bax, and Confocal Microscopy for Caspase 3

We confirmed the occurrence of apoptosis in animals with a high number of TUNEL-positive nuclei by testing for the presence of the proapoptotic Bax and activated caspases 9 and 3, which are detectable during the execution phase of apoptosis and inevitably lead to programmed cell death. Bax (Fig. 1F) and caspase 9 (Fig. 1E) were shown with immunoblotting. Activated caspase 3 was shown with immunofluorescence (Fig. 1C). Higher levels of Bax and caspase 9 were demonstrated at immunoblotting (Fig. 1, lanes b and d) in CHF animals, compared with controls (Fig. 1, lanes a). The GH-treated rats (Fig. 1, lanes c–e) showed a reduced expression of these two proapoptotic molecules compared with CHF.

The antiapoptotic Bcl-2 behaved in the opposite manner. It was decreased in CHF (Fig. 1C and lanes b) compared with control (Fig. 1, lanes a). The GH animals showed Bcl-2 expression similar to that of controls (Fig. 1, lanes c–e). These data parallel and therefore confirm those previously reported for the count of apoptotic nuclei.

Cytochrome c

As shown in Fig. 2, cytochrome c, together with Apaf-1 and caspase-9, has been reported to cause caspase-3 activation. Normally, cytochrome c is located between inner and outer mitochondrial membranes and is released into the cytosol upon stimulation to bind Apaf-1 and activate caspase-3. In CHF animals, we have shown that the concentration of cytochrome c in the cytosol is increased. In contrast, GH-treated rats showed no cytochrome c in the cytosolic fraction.
Fig. 3. Evidence of apoptosis by enzyme-linked immunoabsorbent assay (ELISA) in TA muscle. ELISA was used for detection of fragmented DNA. Data indicate spectrophotometric absorption at 405-nm wavelength and are expressed as percent of the control. ELISA demonstrates significant increase of DNA fragmentation in muscles from CHF animals compared with controls. GH-treated animals did not show any change.

ELISA for Apoptosis

As shown in Fig. 3, apoptosis in the CHF was also confirmed by detection of fragmented DNA by ELISA. The GH animals showed values similar to those of controls.

Caspase-3 Activity

As shown in Fig. 4, caspase-3 was found to be activated in CHF animals. The GH animals showed values very similar to those of controls, indicating that GH may prevent caspases activation.

TNF-α

As shown in Table 4, Serum levels of TNF-α in the CHF animals were raised compared with controls (282 ± 114 pg/ml; P < 0.04). H-GH treatment produced a slight decrease in TNF-α levels compared with CHF (P < 0.002). L-GH was unable to modify levels of TNF-α that were the same of the CHF rats.

Table 5. Soleus muscle mechanical properties

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CHF</th>
<th>CHF + GH (high dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle weight, mg</td>
<td>128±3</td>
<td>83±1</td>
<td>141±5</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>Twitch</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute P₀ (N)</td>
<td>0.170±0.03</td>
<td>0.133±0.024</td>
<td>0.161±0.025</td>
</tr>
<tr>
<td>Specific P₀ (N g⁻¹)</td>
<td>1.38±0.19</td>
<td>1.42±0.20</td>
<td>1.21±0.14</td>
</tr>
<tr>
<td>CT, ms</td>
<td>45.1±5.1</td>
<td>47.7±2.8b</td>
<td>43.3±2.1b</td>
</tr>
<tr>
<td>HRT, ms</td>
<td>52.5±3.7c</td>
<td>61.6±2.9d</td>
<td>51.1±4.5d</td>
</tr>
<tr>
<td><strong>Tetanus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute P₀ (N)</td>
<td>0.99±0.19a</td>
<td>0.66±0.12cd</td>
<td>1.08±0.05f</td>
</tr>
<tr>
<td>Specific P₀ (N g⁻¹)</td>
<td>7.97±0.97</td>
<td>8.02±1.26</td>
<td>7.77±0.88</td>
</tr>
<tr>
<td>HRTT</td>
<td>99.0±14.0</td>
<td>109.0±8.5f</td>
<td>86.2±9.5f</td>
</tr>
</tbody>
</table>

Values are means ± SD; n is the number of muscles examined. P₀, maximal twitch tension; CT, contraction time; HRT, half-relaxation time; P₀, maximal tetanic tension; HRTT, half relaxation tetanic tension. *P < 0.0005; †P < 0.0001; ‡P < 0.001; §P < 0.01; ¶P < 0.04.

SPH

SPH increased in CHF (1,300 ± 210 vs. 850 ± 70 pmol/ml in control; P < 0.04). H-GH produced a decrease in SPH levels (710 ± 110) that were significantly different from CHF and similar to control rats. As for TNF-α, L-GH did not change SPH levels.

Soleus Muscle Isometric Contractile Properties

As shown in Table 5, monocrotaline treatment reduced body weight and soleus weight compared with controls (83 ± 1 vs. 128 ± 3, P < 0.0005). After high doses of GH, both body weight and soleus weight in the monocrotaline-treated rats increased to values even higher than controls (141 ± 5, P < 0.05). Monocrotaline treatment significantly increased twitch time of soleus muscle (47.7 ± 2.8 vs. 45.1 ± 5.1) while not affecting specific tension. This slowing of the soleus muscle is confirmed by the significant shift to the left of the frequency-tension curve compared with controls (P < 0.05) (Fig. 5). GH treatment produced a normalization of the contraction (42.3 ± 2.1 vs. controls 45.1 ± 5.1) and half-relaxation times (51.1 ± 4.5 vs. controls 52.5 ± 3.7). Also, the half-relaxation time of the tetanus was increased in CHF rats (109 ± 8.5 vs. 98.6 ± 14.0) and returned to control values after GH treatment (86.2 ± 9.5, P < 0.05 vs. CHF). In tetanus, absolute but not specific tension significantly decreased in CHF rats as a consequence of the reduced muscle mass (0.66 ± 0.12 vs. 0.99 ± 0.19, P < 0.005). Specific and absolute tension of the GH-treated animals were not different from the control values. Differences seen in absolute force are therefore related to the muscle mass, which is decreased in CHF rats. GH-treated animals have an absolute force identical to that of controls because they have similar muscle mass.

DISCUSSION

In this paper, we describe for the first time the effect of GH administration on skeletal muscle of rats with right-sided heart failure. At the cardiac level, both doses of GH produced only a slight degree of hypertrophy in both ventricles, as previously reported (13). By contrast, at the skeletal muscle level, the
The favorable effects of GH on skeletal muscle were not obtained at the expenses of serious side effects such as diabetes mellitus because glucose plasma levels were unchanged after GH treatment. On the contrary, positive metabolic effects were achieved at the muscle level because after GH, the MHCs expression was similar to that of controls, indicating an improved oxidative metabolism. This effect, though not entirely clear, has been previously seen with ATII blockers, where the antiapoptotic effect was paralleled by a reduction of circulating TNF-α and SPH and by a reshift of the isomyosin pattern (9, 30). It is possible that IGF-1 mediates the reshift in fiber type. In fact, the signaling pathways involved in the regulation of fiber type and size are different (26): calcineurin regulates fiber type through Ras-MAPK, whereas mTOR regulates fiber type through PI3K and PKB. Calcineurin production is stimulated by IGF-1 (24), and it may well be that the activation of the GH/IGF-1 axis may have led to calcineurin stimulation and, therefore, to changes in fiber type. The observed differences between L-GH and H-GH in terms of efficacy on the skeletal muscle may be explained by GH resistance in CHF (2).

In conclusion, the present paper focuses on a new aspect of the potential treatment of CHF with GH. GH beyond the possible improvement in hemodynamics or in cardiac mass is acting on the skeletal muscle, preserving its bulk and fiber composition. Muscle bulk is by itself a strong predictor of exercise capacity, whereas fiber type composition is a determinant of muscle fatigability and speed of muscle shortening and relaxation.

GH could therefore be envisaged as a potential treatment for preventing the apoptosis-induced muscle atrophy and for restoring the oxidative fiber pattern, with a consequent improvement of exercise capacity and symptoms in CHF. The present data suggest that future investigation of GH in CHF needs also to include an evaluation of possible changes in skeletal muscle.

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