Treatment With Growth Hormone Enhances Contractile Reserve and Intracellular Calcium Transients in Myocytes From Rats With Postinfarction Heart Failure

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Background—Recombinant human growth hormone (GH) improves in vivo cardiac function in rats with postinfarction heart failure (MI). We examined the effects of growth hormone (14 days of 3.5 mg · kg⁻¹ · d⁻¹ begun 4 weeks after MI) on contractile reserve in left ventricular myocytes from rats with chronic postinfarction heart failure.

Methods and Results—Cell shortening and [Ca²⁺], were measured with the indicator fluo 3 in myocytes from MI, MI+GH, control, and normal animals treated with GH (C+GH) under stimulation at 0.5 Hz at 37°C. Cell length was similar in MI and MI+GH rats (150±5 and 157±5 μm) and was greater in these groups than in the control and C+GH groups (140±4 and 139±4 μm, P<0.05). At baseline perfusate calcium of 1.2 mmol/L, myocyte fractional shortening and [Ca²⁺] transients were similar among the 4 groups. We then assessed contractile reserve by measuring the increase in myocyte fractional shortening in the presence of high-perfusate calcium of 3.5 mmol/L. In the control and C+GH groups, myocyte fractional shortening and peak systolic [Ca²⁺], were similarly increased in the presence of high-perfusate calcium. In the presence of high-perfusate calcium, both myocyte fractional shortening and peak systolic [Ca²⁺], were depressed in the MI compared with the control groups. In contrast, myocyte fractional shortening (14.1±9% versus 11.1±9%, P<0.05) and peak systolic [Ca²⁺], (647±43 versus 509±37 mmol/L, P<0.05) were significantly higher in MI+GH than in MI rats and were comparable to controls. Left ventricular myocyte expression of sarcoplasmic reticulum Ca²⁺ ATPase 2 (SERCA-2) and left ventricular SERCA-2 protein levels were increased in MI+GH compared with MI rats.

Conclusions—Calcium-dependent contractile reserve is depressed in myocytes from rats with postinfarction heart failure. Long-term growth hormone therapy increases contractile reserve by restoring normal augmentation of systolic [Ca²⁺] in myocytes from rats with postinfarction heart failure. (Circulation. 1999;99:127-134.)

Key Words: growth hormone ▪ myocardial infarction ▪ myocytes ▪ calcium ▪ heart failure

Growth hormone administration in growth-hormone–deficient states and excess secretion in acromegaly produce a hyperkinetic state with enlargement (hypertrophy) of the heart and other organs that is associated with augmentation of circulating plasma volume, a reduction in systemic vascular resistance, elevation of cardiac output, and changes in glucose metabolism.¹ ² These effects may be mediated in part by the vasodilator peptide insulin-like growth factor-1 (IGF-1),³⁸ which promotes cardiac protein synthesis and expression of cardiac-restricted genes.⁹¹⁰ Variably, growth hormone hypersecretion in acromegalic adults can result in a transition from a hyperkinetic state to overt heart failure with the phenotype of a dilated cardiomyopathy.¹¹ The actions of growth hormone and IGF-1 on cardiac performance in normal animals simulate human acromegaly and promote changes in isolated muscle contractility.¹²⁻¹⁵

See p 15

Growth Hormone Effects in Failing Hearts

The administration of growth hormone to patients with idiopathic dilated cardiomyopathy was associated with hypertrophic remodeling of the dilated, thin-walled hearts and an increase in cardiac output relative to myocardial oxygen consumption.¹⁶ When growth hormone was administered immediately after coronary ligation in rats, 3-week treatment in comparison with no therapy was associated with an increase in body weight, concentric hypertrophy, and better preservation of cardiac output associated with a decrease in systemic vascular resistance.¹⁷ Treatment with growth hormone early after experimental coronary ligation also preserved collagen matrix and reduced aneurysm formation,¹⁸ although this favorable effect was not observed when growth
hormone was administered together with β-adrenergic blockers.\textsuperscript{18} The benefits of the induction of mild concentric hypertrophy early after experimental infarction with regard to retarding left ventricular dilatation and improving systolic function have also been clearly demonstrated by Litwin et al\textsuperscript{20} in response to a different growth-promoting agent, 2-tetradecylglycidic acid.

**Effects of Growth Hormone in Postinfarction Failure**

The effects of growth hormone and IGF-1 administration on contractile performance later after experimental infarction are controversial. Duerr et al\textsuperscript{21} administered IGF-1 plus growth hormone to rats for 4 weeks beginning 4 weeks after infarction, which stimulated body growth with minor changes in left ventricular remodeling, decreased systemic vascular resistance, and increased cardiac output compared with no treatment. Yang et al\textsuperscript{22} administered growth hormone for 15 days to rats beginning 4 weeks after coronary ligation and observed an increase in cardiac index and dP/dt and reduction in systemic vascular resistance in the absence of an increase in the ratio of left ventricular weight to body weight. Subsequently, Jin et al\textsuperscript{23} administered growth hormone plus IGF-1 for 2 weeks beginning 3 months after infarction, in the presence or absence of captopril that was begun early after coronary ligation. In this study, the increases in cardiac index and stroke volume were significantly greater in animals treated with growth hormone plus IGF-1 than in animals treated with captopril alone, which implies an effect on myocardial contractility independent of changes mediated by systemic vasodilation.

We hypothesized that growth hormone therapy may directly increase contractile function in myocytes from rats after infarction. Growth hormone was administered at a dose and a duration that were insufficient to promote an increase in left ventricular mass. To examine contractile reserve, we measured isolated cell shortening and [Ca\textsuperscript{2+}]\textsubscript{i} in response to a different growth-promoting agent, 2-tetradecylglycidic acid. We observed that growth hormone improved contractile reserve and increased the intracellular calcium transient in myocytes from rats with postinfarction heart failure.

**Methods**

**Animal Model**

Male Sprague-Dawley rats 8 weeks of age were obtained from Charles River Breeding Laboratories, Hollister, Calif. Myocardial infarction (MI) was produced by left coronary artery ligation, as described previously.\textsuperscript{25} Sham-operated control animals underwent the same procedure, except that the suture was passed under the coronary artery and then removed. Four weeks after surgery, rats of the control (C) and MI cohorts were randomized into recombinant human growth hormone (GH)-treated and nontreated groups (C+GH, control, MI+GH, and MI). Growth hormone (Genentech, Inc) was injected subcutaneously (3.5 mg kg\textsuperscript{-1} d\textsuperscript{-1} for 14 days). Six weeks after surgery, including 14 days of treatment with growth hormone or vehicle, closed-chest in vivo left ventricular pressure was measured, as described by our laboratory.\textsuperscript{24}

**Simultaneous Measurement of [Ca\textsuperscript{2+}]\textsubscript{i} and Cell Motion**

The animals were then euthanized, and left ventricular myocytes were prepared with use of collagenase perfusion by a modification of the methods of Capogrossi et al,\textsuperscript{26} as reported from our laboratory.\textsuperscript{27–29} After the collagenase perfusion and before myocyte dissociation, the well-defined scar and its colorless margin were excised from the left ventricle. Fifty microliters of 88.5 mmol/L fluo 3-AM in DMEM was added to 450 μL of fetal calf serum containing 0.05% pluronic F-127 (Molecular Probes, Inc), sonicated, and stored at −80°C. Myocytes were attached to coverslips with cell adhesive (Cell-Tak, Collaborative Research, Inc) and loaded with 1 μmol/L fluo 3\textsuperscript{30–32} (Molecular Probes, Inc) at room temperature for 20 minutes. Probenecid (0.5 mmol/L) was present in every solution to address the formal possibility of loss of fluo 3 via the anion transporter, which has been reported in measurement of [Ca\textsuperscript{2+}]\textsubscript{i} in some cell types.\textsuperscript{33} The coverslip was washed and placed in a flow-through heated (37°C) cell superfusion chamber on the stage of an inverted microscope (Nikon). The excitation source was a high-pressure mercury arc lamp. With the interference filter, the excitation wavelength was 480±20 nm. The excitation beam was chopped at 360 Hz to reduce bleaching, and the myocyte was illuminated via epifluorescent optics with a Fluor ×40 dry lens with correction collar (magnification, ×40; numerical aperture, 0.85; working distance, 0.37 mm; focal length, 4.20 mm; Nikon Inc). The emission light was collected by the objective lens filtered by 535±25 nm and transmitted to a custom-modified photomultiplier spectrophotometer system (FM-1000, Rincon Scientific Instruments). At the beginning of each experiment, myocyte autofluorescence was measured with 4 to 6 unloaded myocytes. An adjustable iris was used to restrict the optical image to only 1 myocyte of interest in each experiment to minimize fluorescence from other myocytes. The image of the beating myocyte was obtained by illumination via the 50-W standard microscope light source passed through a 640-nm band-pass filter. Myocyte motion was monitored with a solid-state camera (GP-CD60, Panasonic) and measured with a video detector system (Crescent Electronics). The analog output signals of cell motion and the fluorescent transient were monitored and recorded continuously. Myocytes were stimulated at 0.5 Hz with 3-ms pulses. Myocyte fractional shortening was stable during 20 minutes of observation (106±6% of baseline in control cells and 96±3% in cells from postinfarction hearts, n=3 in each group, P=NS). There was also no decrease in fractional cell shortening before and after fluo 3 loading in myocytes from control and postinfarction hearts (n=3 per group; data not shown). To estimate calibrated levels of the [Ca\textsuperscript{2+}]\textsubscript{i} transients, immediately after each experiment the myocyte was superfused with the same buffer supplemented with 30 mmol/L 2,3-butanediol monoxide and 10 μmol/L calcium ionophore ionomycin in the presence of 1 mmol/L calcium. Then a 1 mol/L MnCl\textsubscript{2} stock solution was added to the buffer to yield a final concentration of 10 mmol/L. The cell was abruptly superfused with Mn\textsuperscript{2+} for saturation of fluo 3. After measurement of the mean value of the autofluorescence (F\textsubscript{BKG}) and the fluorescence intensity with Mn\textsuperscript{2+} (F\textsubscript{Mn}), the values of F\textsubscript{max}, F\textsubscript{min}, and estimated [Ca\textsuperscript{2+}]\textsubscript{i} were calculated as follows with the calibration method of Kao et al,\textsuperscript{32} as described elsewhere:\textsuperscript{35}

\[
F_{\text{max}} = (F_{\text{Mn}} - F_{\text{BKG}}) / 0.2 + F_{\text{BKG}}
\]

\[
F_{\text{min}} = (F_{\text{max}} - F_{\text{BKG}}) / 40 + F_{\text{BKG}}
\]

\[
[\text{Ca}^{2+}]_i = K_d \times (F_{\text{max}} - F_{\text{min}}) / (F_{\text{BKG}} - F)
\]

where K\textsubscript{d} is the dissociation constant at 37°C for fluo 3 and is taken as 864 nmol/L.\textsuperscript{34} The fluorescence intensity of fluo 3 saturated with Mn\textsuperscript{2+} is ~20% of that saturated with Ca\textsuperscript{2+}, whereas the fluorescence intensity of Ca\textsuperscript{2+}-free fluo 3 is 1/40 that of the Ca\textsuperscript{2+}-bound form.\textsuperscript{32} With this approach, basal calibrated systolic and diastolic [Ca\textsuperscript{2+}]\textsubscript{i} levels are similar to values we have extensively reported in adult rat myocytes at stimulation rates of 0.5 Hz and at 37°C with use of the fluorescence indicator indo 1.\textsuperscript{28–29}
The yields of viable myocytes, which were defined as the percentage of rod-shaped myocytes paced at 0.5 Hz with clear striations and exclusion of trypan blue, were 60% to 70% in the control myocytes and 40% to 60% in myocytes from postinfarction hearts. One to 4 experiments were performed in sequence from separate coverslips of myocytes isolated from 1 heart (MI, n=10 hearts; C+GH, n=9 hearts; control, n=7 hearts; and C+GH, n=6 hearts).

Experimental Protocol
The myocytes from the control, C+GH, MI, and MI+GH groups were superfused with oxygenated HEPES-buffered solution of the following composition (mmol/L): NaCl 137, KCl 3.7, MgCl2 0.5, HEPES (free acid) 4.0, CaCl2 1.2, glucose 5.6, and probenecid 0.5, with a final pH of 7.40. The myocytes were paced at 0.5 Hz at 37°C. To assess calcium-dependent contractile reserve, myocyte fractional shortening was then measured in the presence of 3.5 mmol/L CaCl2. Western blot analysis of SERCA-2 protein levels

Analysis of LV Myocyte mRNA Levels
Immediately after isolated left ventricular myocytes were prepared as described above, half of the myocyte cell suspension was used for total RNA extraction with Tri Reagent (Sigma). Left ventricular myocyte RNA was extracted from control (n=6), MI (n=9), and MI+GH (n=10) hearts. The concentration of RNA in each sample was assessed spectrophotometrically. For the Northern blot analyses, 20 µg of total RNA from individual LV myocyte samples was size fractionated by electrophoresis in a 1.5% agarose-formaldehyde gel and transferred to a nitrocellulose membrane (Stratagene) by pressure transfer (Posiblot Pressure Blotter, Stratagene). The membrane was prehybridized for 10 minutes and hybridized sequentially with specific probes for 1 hour in QuikHyb hybridization solution (Stratagene) at 65°C. After hybridization, the membrane was washed in various concentrations of sodium chloride/sodium citrate buffer and SDS and exposed to Kodak MR film for 6 hours to 3 days at −80°C. The relative amounts of each mRNA were determined by densitometric analysis (Molecular Dynamics) and normalized to GAPDH. Stripping of the membrane for reuse was performed after 2-day exposure of the membrane to film. Probes used were the cDNA fragment encoding the SR calcium ATPase (SERCA-2, provided by D. MacLennan), a 20-bp oligonucleotide encoding β-mysin heavy chain, the cDNA fragment encoding rat GAPDH, and an 84-bp synthetic oligonucleotide complementary to the coding region of rat ANF. The cDNA fragments were radiolabeled with [γ-32P]ATP with T4 polynucleotide kinase.

Statistical Analysis
All values are expressed as mean±SEM. The statistical analysis of differences among the groups was done by ANOVA comparison or ANOVA for repeated measures where appropriate and Fisher’s exact test for post hoc analyses. Statistical significance was accepted at the level of \( P<0.05 \).

Results
Table 1 reports the body weight, left ventricular weight, and in vivo left ventricular pressure. The body weights at baseline and after 14 days of growth hormone administration or no treatment were similar in the 4 groups. Left ventricular weight and the ratio of left ventricular weight to body weight in MI and MI+GH rats were similar and were greater than in control rats. Infarct size (ratio of myocardial scar weight to left ventricular weight) was similar in the MI and MI+GH groups. Growth hormone treatment for 14 days had no effect on left ventricular weight or any hemodynamic parameter in the normal rats (C+GH) compared with untreated control rats. Left ventricular systolic pressure was increased in rats from the MI group, but no change was observed in MI+GH rats. LV diastolic pressure and LV systolic pressure were similar in the 4 groups. Left ventricular end-diastolic pressure was decreased in rats from the MI group but was similar in MI+GH and control rats.
sure was significantly increased in rats in the MI group but was not increased in MI+GH rats compared with the control group.

Table 2 reports the characteristics of cell motion and the [Ca\(^{2+}\)] transient in myocytes from the 4 groups under baseline perfusion with 1.2 mmol/L calcium. There were no differences in cell size or any functional parameters in myocytes from normal rats treated with growth hormone (C+GH) compared with normal untreated controls. The end-diastolic cell lengths in myocytes from MI and MI+GH rats were similar and were greater than in myocytes from the control group, which is consistent with left ventricular dilatation in postinfarction heart failure. The fractional cell shortening, peak positive and negative first derivatives of cell motion, time to peak shortening (data not shown), and time to 50% decline in [Ca\(^{2+}\)] were similar in myocytes from the 4 groups. Peak systolic [Ca\(^{2+}\)] and end-diastolic [Ca\(^{2+}\)] were similar in myocytes from the 4 groups under the baseline condition of 1.2 mmol/L calcium.

We examined contractile reserve by measuring myocyte shortening in response to the elevation of extracellular calcium from 1.2 to 3.5 mmol/L. Representative tracings from control and C+GH myocytes are shown in Figure 1. Figure 2 shows the changes in fractional cell shortening and peak systolic [Ca\(^{2+}\)] in myocytes from the control and C+GH groups at 1.2 and 3.5 mmol/L perfusate calcium. Fractional cell shortening and peak systolic [Ca\(^{2+}\)] in myocytes of the C+GH group were similar to those in the control group in response to the elevation of perfusate calcium.

Representative tracings from MI and MI+GH myocytes are shown in Figure 1. Figure 2 shows the changes in fractional cell shortening and peak systolic [Ca\(^{2+}\)] in myocytes from the MI and MI+GH groups. In response to the elevation of perfusate calcium to 3.5 mmol/L, fractional cell shortening in MI myocytes was depressed compared with that in the control group (P<0.05). In contrast, fractional cell shortening in myocytes from the MI+GH group was significantly greater than that in MI in response to the elevation of perfusate calcium (P<0.01) and was comparable to that in the control group. As shown in Figure 2, the impaired contractile reserve in myocytes from the MI group was related to depressed augmentation of peak systolic [Ca\(^{2+}\)]. In contrast, in the presence of 3.5 mmol/L perfusate calcium, peak systolic [Ca\(^{2+}\)] in myocytes from MI+GH was greater than that in MI rats (P<0.01) and was comparable to the control groups. There were no significant differences in end-diastolic [Ca\(^{2+}\)], between MI and MI+GH rats at either 1.2 or 3.5 mmol/L perfusate calcium. Under pacing conditions of 0.5 Hz, there was no effect of growth hormone on the time course of the calcium transient in control or postinfarction rats.

Figure 3 shows that the relationship between fractional cell shortening and peak systolic [Ca\(^{2+}\)] in control myocytes was similar to that in C+GH rats. In myocytes from the MI+GH rats, both peak systolic [Ca\(^{2+}\)] and cell shortening were greater than in myocytes from MI rats, without an upward and leftward shift of this relationship, which implicates the absence of a change in myofilament responsiveness to calcium within the range of intracellular calcium studied in this experiment.

The effects of postinfarction remodeling and growth hormone administration on gene expression were measured in left ventricular myocytes from MI, MI+GH, and control rats. Expression levels of all genes were analyzed as the ratio to GAPDH. Atrial natriuretic factor (ANF) message levels were similar in the MI+GH versus MI groups (2.8±0.6 versus 1.3±0.3 densitometric units, P=NS) and were not detectable in the control group. The message levels of β-myosin heavy chain were similar in both MI and MI+GH (3.5±0.9 versus 3.1±0.97) and were increased compared with control (1.0±0.1 densitometric units, P<0.05 versus MI and MI+GH). As shown in Figure 4, there was no depression of message levels of SERCA-2 in MI compared with controls (1.4±0.3 versus 1.0±0.2 densitometric units, P=NS). However, SERCA-2 message levels were increased in myocytes from MI+GH versus MI rats (2.5±0.4 versus 1.4±0.3 densitometric units, P<0.05). Left ventricular protein levels of SERCA-2 (Figure 4) were also increased in MI+GH versus MI rats (177±10% versus 105±11% of control values, P<0.001).

| TABLE 2. Characteristics (by Group) of Myocyte Function and Intracellular Ca\(^{2+}\) |
|-----------------|---|---|---|---|
|                | Control | C+GH | MI  | MI+GH |
| No.             | 20      | 20   | 22  | 22    |
| Myocyte end-diastolic length, \(\mu\)m | 134±4   | 136±4 | 146±4* | 152±4† |
| Fractional cell shortening, % | 8.7±.8 | 8.8±1.2 | 8.4±.7 | 8.0±.6 |
| Peak positive first derivative of cell motion, \(\mu\)m/s | 225±19 | 231±20 | 235±21 | 259±25 |
| Peak negative first derivative of cell motion, \(\mu\)m/s | -196±19 | -207±20 | -215±16 | -228±26 |
| Peak systolic [Ca\(^{2+}\)], nmol/L | 366±21 | 443±43 | 408±28 | 465±39 |
| End-diastolic [Ca\(^{2+}\)], nmol/L | 93±8   | 109±11 | 112±12 | 118±13 |
| Time to peak [Ca\(^{2+}\)], ms | 33±3   | 29±2   | 38±2  | 35±3  |
| Time to 50% decline in [Ca\(^{2+}\)] from peak, ms | 86±5   | 77±4   | 82±3  | 75±4  |

Data were obtained under the perfusion conditions of 37°C, low perfusate Ca\(^{2+}\) 1.2 mmol/L, and pacing rate 0.5 Hz. Values are mean±SEM.

*P<0.05, †P<0.01 vs control group and vs C+GH.
Discussion

The present study demonstrates that contractile reserve in response to calcium is depressed in left ventricular myocytes from rats with postinfarction heart failure. Long-term treatment with growth hormone at a dose and a duration that do not promote left ventricular hypertrophy has no effect on myocyte contractile function or size in normal rats. However, long-term growth hormone administration in postinfarction rats is associated with enhanced myocyte contraction and the augmentation of peak systolic $[Ca^{2+}]_i$, in response to the elevation of perfusate calcium. These observations show that long-term growth hormone therapy can improve contractile reserve independently of changes in left ventricular mass in rats with postinfarction heart failure.

Contractile Reserve in Postinfarction Myocytes

The absolute level of calibrated calcium may differ, depending on the indicator that is used and the experimental conditions. Nonetheless, these observations are entirely consistent with prior studies of abnormal intracellular calcium regulation in other models of dilated cardiomyopathy. Siri et al. and Buckelmann et al. have examined myocytes from failing guinea pig hearts and end-stage human dilated cardiomyopathy, respectively, and observed a similar reduction in peak systolic $[Ca^{2+}]_i$, which is hypothesized to directly contribute to impairment of contractile reserve.

Steady-state myofilament calcium sensitivity was not studied. However, over the range of calcium that was studied, the relationship between cell shortening and $[Ca^{2+}]_i$ was not

Figure 1. Top, Representative traces of myocytes from sham-operated control rats with (C+GH) and without (Control) growth hormone treatment in response to elevation of perfusate calcium from 1.2 to 3.5 mmol/L. Upper trace displays cell motion, and lower trace displays $[Ca^{2+}]_i$-sensitive fluorescence transient. In tracings of cell motion, systolic shortening is shown as an upward deflection. Bottom, Representative traces of myocytes from rats with postinfarction failure with (MI+GH) and without (MI) long-term growth hormone treatment.
Left, Relationship between perfusate calcium and fractional cell shortening (top) and relationships between perfusate calcium and peak systolic calcium ([Ca\textsuperscript{2+}]) (bottom) in myocytes from control and C + GH groups. Right, Same relationships from MI and MI + GH groups.

Figure 2. Left, Relationship between perfusate calcium and fractional cell shortening (top) and relationships between perfusate calcium and peak systolic calcium ([Ca\textsuperscript{2+}]) (bottom) in myocytes from control and C + GH groups. Right, Same relationships from MI and MI + GH groups.

Growth Hormone and Contractile Reserve

Our observation supports the hypothesis that growth hormone administration has direct beneficial effects on contractile function in myocytes from postinfarction rats. The underlying molecular mechanism may be related in part to changes in myocyte gene expression. Consistent with previous reports,\textsuperscript{40,41} the expression of left ventricular β-myosin heavy chain isoform and ANF was upregulated in myocytes from postinfarction rats but was not further modified by growth hormone administration. In humans with dilated cardiomyopathy and depression of peak systolic [Ca\textsuperscript{2+}], the intracellular mechanism is speculated to be related to a reduced expression of the sarcoplasmic reticulum ATPase pump (SERCA-2), resulting in impaired sarcoplasmic reticulum calcium loading and release critical for systolic cross-bridge activation.\textsuperscript{39} In rat models of heart failure, the message and protein levels of SERCA-2 appear to vary with the severity of infarction as well as the stage and duration of heart failure.\textsuperscript{36,42} In the present study, although left ventricular myocyte message levels of SERCA-2 were not depressed in left ventricular myocytes from untreated postinfarction rats, both message and left ventricular protein levels of SERCA-2 were significantly increased in myocytes from postinfarction rats treated with growth hormone.

We postulate that the augmented expression of SERCA-2 may modulate other defects in intracellular calcium regulation, which may contribute to the depression of peak systolic [Ca\textsuperscript{2+}] in failing hearts during increased work states. Recent studies\textsuperscript{43,44} suggest that abnormalities in the "gain" of sarcoplasmic reticulum calcium release in response to inward calcium current can influence the fractional release of calcium from the sarcoplasmic reticulum. In addition, nuclear resonance spectroscopy experiments by Neubauer et al\textsuperscript{45} have shown that impaired contractile reserve during high-calcium perfusion in postinfarction rat hearts is associated with the reduction of free energy release from ATP hydrolysis (ΔG\textsubscript{ATP}) during high work states. Under steady-state conditions, the calcium content of the sarcoplasmic reticulum will depend only on the thermodynamic gradient generated across the sarcoplasmic reticulum membrane and buffering characteristics within its lumen.\textsuperscript{46,47} However, as recently reviewed by Shannon and Bers,\textsuperscript{47} slight non–steady-state reductions in ΔG\textsubscript{ATP} will result in a decline in the sarcoplasmic reticulum gradient and calcium load. In this non–steady-state situation, factors that enhance calcium uptake, such as an increase in the number of pumps, may help to sustain a transport rate sufficient to bring sarcoplasmic reticulum calcium content to normal levels.

Limitations

First, intracellular calcium regulation differs in the rat compared with some other species and is characterized by a greater dependence on calcium removal by the sarcoplasmic reticulum relative to the other cellular calcium transport systems.\textsuperscript{48} Second, it is not yet known whether long-term therapy will be associated with beneficial effects or the late cardiomyopathic changes seen in acromegalic heart failure. It is also not known whether treatment with long-term growth hormone administration to stimulate contractility will be associated with the late adverse effect on mortality that has been observed in human clinical trials of agents with mixed positive inotropic and vasodilator properties.\textsuperscript{49} The present study also did not address potential interactive effects of growth hormone therapy with β-adrenergic blockers, which now play an important role in the long-term treatment of patients after infarction.\textsuperscript{19} With these limitations, the present study shows that growth hormone increases contractile reserve in myocytes of postinfarction rats and supports the
hypothesis that growth hormone treatment has a direct effect on myocyte contractile function and intracellular calcium regulation.

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