Cardiac contractile function is enhanced in isolated ventricular myocytes from growth hormone transgenic mice

P B Colligan, H M Brown-Borg, J Duan, B H Ren and J Ren

Department of Pharmacology, Physiology and Therapeutics, University of North Dakota School of Medicine, 501 N. Columbia Road, Grand Forks, North Dakota 58203, USA

(Requests for offprints should be addressed to Dr J Ren; Email: jren@medicine.nodak.edu)

Abstract

Growth hormone (GH) plays a key role in cardiac growth and function. However, excessive levels of GH often result in cardiac dysfunction, which is the major cause of death in acromegalic patients. Transgenic mice with GH overexpression serve as useful models for acromegaly and exhibit impaired cardiac functions using echocardiography, similar to those of human acromegaly. However, the mechanism underscoring the impaired ventricular function has not been well defined. This study was designed to evaluate the cardiac excitation–contraction coupling in GH over-expressing transgenic mice at the single ventricular myocyte level. Myocytes were isolated from GH and age-matched wild-type mouse hearts. Mechanical properties were evaluated using an IonOptix MyoCam system. The contractile properties analyzed included peak shortening (PS), time-to-peak shortening (TPS) and time-to-90% relengthening (TR90), and maximal velocities of shortening/relengthening (± dL/dt).

Intracellular Ca2+ properties were evaluated by fura-2. GH transgenic mice exhibited significantly increased body weights and enlarged heart and myocyte size. Myocytes from GH transgenic mice displayed significantly enhanced PS and ± dL/dt associated with similar TPS and TR90 compared with the wild-type littermates. Myocytes from GH transgenic mice displayed a similar resting intracellular Ca2+ level and Ca2+ removal rate but exhibited an elevated peak intracellular Ca2+ level compared with the wild-type group. Myocytes from both groups were equally responsive to increases in extracellular Ca2+ concentration and stimulating frequency. These results suggest that GH over-expression is associated with enhanced contractile function in isolated myocytes and that the impaired cardiac function observed in whole hearts may not be due to defects at the myocyte level.


Introduction

Growth hormone (GH) is the most abundant hormone released by the pituitary gland and primarily controls postnatal somatic growth. Ample evidence suggests an important role for GH and its local effector, insulin-like growth factor I (IGF-I), in the maintenance of normal cardiac growth and function (Lombardi et al. 1997, Isgaard et al. 1999, Ren et al. 1999, Ross 1999, Tajima et al. 1999). Compromised ventricular function and increased cardiovascular risk have been observed in subjects with GH deficiency (Amato et al. 1993, Merola et al. 1993). Recent evidence has indicated that exogenous GH evokes a hypertrophic response and increases left ventricular function in both normal and failing hearts (Fazio et al. 1996, Cittadini et al. 1997, Isgaard et al. 1999), suggesting the therapeutic potential of short-term GH on heart function. Paradoxically, excessive levels of GH may also lead to increased cardiovascular dysfunction and increased morbidity as well as mortality (Bollano et al. 2000).

Cardiovascular disease has been proven to be the major cause of death in acromegaly and a concept of acromegalic cardiomyopathy has been proposed (Fazio et al. 1994, Bollano et al. 2000). However, most of these studies were limited to exogenously administered GH at the levels of whole hearts and multicellular preparations, and were conducted mainly in the acute experimental setting due to the pharmacokinetics of the hormone.

Recent advances in transgenic techniques have made it possible to integrate the GH gene with the assistance of an independent inducible system into the mouse genome, resulting in several lines of GH over-expressing transgenic mice. These mice express high levels of GH and have been widely used in the pathophysiological characterization of the hormone (Kopchick et al. 1999). However, the GH transgenic mice have been used in only a few studies of cardiovascular disease (Bollano et al. 2000, Bohlooly-Y et al. 2001). Compromised systolic cardiac function with eccentric left ventricular hypertrophy has been observed in these GH transgenic mice using echocardiography...
(Sandstedt et al. 1994, Bollano et al. 2000). However, whether the depressed ventricular function is due to the direct cardiac or the indirect hemodynamic effect of GH is essentially unknown. In addition, the effect of GH on ventricular contraction may be further complicated by the presence of heterogeneity of cells such as fibroblasts, endothelial cells, myocytes and nerve terminals. Therefore, the purpose of the present study was to evaluate the impact of prolonged GH over-exposure on excitation–contraction (E–C) coupling properties at the single ventricular myocyte level. The GH transgenic animals were derived from a single male founder (strain B6 SJL) produced by microinjection of the phosphoenolpyruvate carboxykinase (PEPCK) promoter region (300 bp)/bovine GH hybrid gene into the male pronucleus of single-cell embryos (McGrane et al. 1988, Steger et al. 1994).

Materials and Methods

Animals

All animal procedures were approved by the University of North Dakota School of Medicine Institutional Animal Care and Use Committee. Ten-month-old male GH transgenic and age-matched wild-type siblings were kindly provided by Dr Andrzej Bartke at Southern Illinois University and were maintained at the University of North Dakota vivarium facilities under controlled conditions of photoperiod (12 h light:12 h darkness) and temperature (22 ± 1 °C) with food and water available ad libitum. The production and initial characterization of transgenic animals (transgenic males crossed to C57Bl/6J × C3H/F1 females) have been described in detail previously (McGrane et al. 1988, Steger et al. 1994). The genotypes of mice used in these studies were determined by differences in body size (typically ~100% increase) and body proportions. Plasma GH levels are extremely high, ranging from several 100 to over 2000 ng/ml, providing a valid model of elevated GH exposure, a condition comparable to gigantism and acromegaly in the human.

Cell isolation procedures

Ventricular myocytes were isolated from mouse hearts using the method described by Ren (2000) with modifications. In brief, after ketamine/xylazine (5:3 vol/vol, 1:32 mg/kg body weight) sedation, the mouse hearts were rapidly removed and perfused at (37 °C) with Krebs-Henseleit bicarbonate (KHB) buffer containing (in mM): 118 NaCl, 4·7 KCl, 1·2 MgSO4, 1·2 KH2PO4, 25 NaHCO3, 10 N-[2-hydro-ethyl]-piperazine-Ν’-Ν’-ethanesulfonic acid (HEPES) and 11·1 glucose, with 5%CO2-95% O2. Hearts were subsequently perfused with the same KHB buffer containing 223 U/ml collagenase D (Boehringer Mannheim, Indianapolis, IN, USA) for 20 min. After perfusion, ventricles were removed and minced, under sterile conditions, before being filtered through a nylon mesh (300 μm). Extracellular Ca2+ was incrementally added back to 1·25 mM over a span of 30 min to avoid Ca2+ paradox. Myocytes with obvious sarcomemal blebs or spontaneous contractions were not used. Only rod-shaped myocytes with clear edges were selected for mechanical and intracellular Ca2+ transient study.

Cell shortening/relengthening

Mechanical properties of ventricular myocytes were assessed using an IonOptix MyoCam system (IonOptix Corp., Milton, MA, USA). Cells were placed in a chamber mounted on the stage of an inverted microscope (Model: Olympus, IX-70, Leeds Precision Ltd, Minneapolis, MN, USA) and superfused (25 °C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl2, 1 MgCl2, 10 glucose, 10 HEPES, at pH 7·4. The cells were field stimulated with suprathreshold voltage at a frequency of 0·5 Hz and 3-ms duration, using a pair of platinum wires placed on opposite sides of the chamber connected to an isolated pulsar stimulator (FHC Inc., Bowdoinham, ME, USA). The myocyte being studied was displayed on the computer monitor using an IonOptix MyoCam camera, which rapidly scans the image area every 8·3 ms such that the amplitude and velocity of shortening/relengthening is recorded with good fidelity. A soft-edge software (IonOptix) was used to capture changes in cell length during shortening and relengthening (Ren 2000). Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time-to-90% PS (TPS), time-to-90% relengthening (TR90) and maximal velocities of shortening (+dL/dt) and relengthening (−dL/dt) respectively.

Intracellular fluorescence measurement

Myocytes were loaded with fura-2/AM (0·5 μM) for 15 min at 25 °C and fluorescence measurements were recorded with a dual-excitation-single-emission fluorescence photomultiplier system as described (Ren 2000). Myocytes were placed on an Olympus IX-70 inverted microscope and imaged through a Fluor 40 × oil objective. Cells were exposed to light emitted by a 75 W lamp and passed through either a 360 or a 380 nm filter while being stimulated to contract at 0·5 Hz. Fluorescence emissions were detected between 480–520 nm after first illuminating the cells at 360 nm for 0·5 s then at 380 nm for the duration of the recording protocol. The 360 nm excitation scan was repeated at the end of the protocol and qualitative changes in intracellular Ca2+ concentration ([Ca2+]i) were inferred from the ratio of the fluorescence intensity at two
Statistical analyses

Data are presented as means ± S.E.M. Differences between and within groups were evaluated by two-way analysis of variance (ANOVA) with repeated measures (SYSTAT). A Tukey test was used as a follow-up for the multiple comparisons. To determine significant differences in the repeated measures factor (Ca²⁺ concentration or stimulatory frequency), the ‘within subjects’ MSerror and dferror terms from the parent ANOVA were used. To determine significant differences between the wild-type and GH transgenic group, the ‘between subjects’ MSerror and dferror terms from the parent ANOVA were used. Statistical significance was considered to be P<0.05. Approximately an even number of myocytes from 4 wild-type or 4 GH transgenic mice were used for each experiment.

Results

General features of wild-type and GH transgenic mice

Table 1 shows that 10-month-old GH-over-expressing mice exhibited significantly higher body and organ (heart, liver and kidney) weights compared with the age-matched wild-type siblings (four mice per group). The size of liver, although not heart and kidney (normalized to body weight), was significantly increased in GH mice compared with the wild-type littermates.

Cell shortening and relengthening from wild-type and GH transgenic mice myocytes

The average resting cell length was similar in myocytes from the wild-type (129 ± 3 µm, n=102 cells from 4 mice) and the GH (129 ± 3 µm, n=102 cells from 4 mice) groups. However, GH over-expression significantly increased the cross-sectional area of the myocytes (wild-type: 2067 ± 302 µm² vs GH: 2993 ± 318 µm², n=102/group, P<0.05). Myocytes from GH mice exhibited significantly enhanced PS amplitude associated with normal TPS and TR₉₀, compared with those from the wild-type siblings (Fig. 1). Consistent with the enhanced PS, the maximal velocities of shortening (+dL/dt) and relengthening (−dL/dt) were significantly elevated in myocytes from GH mice compared with those from the wild-type littermates (Fig. 2).

Intracellular Ca²⁺ transients

The fura-2 fluorescence measurements revealed that the resting Ca²⁺ level and intracellular Ca²⁺ transients decay rate (tau) were similar in myocytes from both animal groups. The time course of the fluorescence signal decay was described by a single exponential equation, and tau was used as a measure of cytoplasmic Ca²⁺ removal rate. Consistent with the enhanced myocyte shortening data (PS), the increase of intracellular Ca²⁺ in response to electrical stimuli (Δ360/380 ratio) was also significantly elevated in myocytes from the GH mice compared with those of wild-type (Fig. 3), indicating an increased intracellular Ca²⁺ recruiting ability by GH over-expression.

Effect of extracellular Ca²⁺ on myocyte shortening

Figure 4 shows that elevating extracellular Ca²⁺ concentration from 0.5 mM to 3 mM increased peak shortening concentration dose-dependently, in myocytes from both wild-type and GH mice. The myocytes from both groups were equally sensitive to the increase in extracellular Ca²⁺, suggesting that the myofilament Ca²⁺ sensitivity may not be affected by GH over-expression.

Effect of stimulation frequency on myocyte shortening

Rodent hearts normally contract at very high frequencies, whereas our mechanical evaluation was conducted at 0.5 Hz. To evaluate the impact of GH over-expression on cardiac E–C coupling under higher frequencies, we increased the stimulating frequency up to 5.0 Hz (300 beats/min) and recorded the steady-state peak shortening. Cells were initially stimulated to contract at 0.5 Hz for 5 min to ensure steady-state before commencing the frequency study. All the recordings were normalized to PS.
at 0·1 Hz of the same myocyte. Figure 5 shows an identical negative staircase in PS with increasing stimulating frequency in both wild-type and GH groups, suggesting that intracellular Ca\(^{2+}\) storage and release are unlikely to be affected by GH over-expression.

Discussion

The current study demonstrates that GH over-expression may lead to enhanced cell contraction, maximal velocity of contraction and relaxation, as well as an increased intracellular Ca\(^{2+}\) recruiting ability in individual ventricular myocytes. Our results indicated that GH over-expression does not affect the duration of contraction and relaxation, the resting intracellular Ca\(^{2+}\) levels and intracellular Ca\(^{2+}\) clearing rate. GH transgenic mice exhibited a higher body weight and organ weight, although no change in heart size (normalized to body weight) was observed. Cardiac myocytes are terminally differentiated; therefore cardiac growth is mainly conveyed through hypertropy, which is supported by our observation of increased myocyte cross-sectional area in the GH group.

There have been somewhat inconsistent findings regarding the impact of GH on cardiac function. Clinical and experimental evidence has demonstrated that short-term GH therapy has beneficial cardiovascular effects such as increased left ventricular mass, cardiac contractility, exercise performance and reduced total peripheral resistance (Fazio et al. 1996, Lombardi et al. 1997, Isgaard et al. 1999). Administration of GH, GH secretagogues and IGF-I, alone or in combination, improves cardiac function in normal and failing hearts (Isgaard et al. 1999, Ross 1999, Tivesten et al. 2000). However, lack of any cardiac effect of GH was also reported after experimental myocardial infarction (Shen et al. 1996). The beneficial effect of GH on cardiac function is consistent with the fact that GH deficiency is accompanied by severely compromised ventricular function (Amato et al. 1993, Merola et al. 1993). Conversely, GH may exert paradoxical cardiac effects by prompting the development of acromegalic cardiomyopathy characterized mainly as impaired cardiac function.

Figure 1 Contractile properties of cardiac ventricular myocytes isolated from GH and wild-type mouse hearts. Graphs depicted are representative cell shortening traces (upper left), peak shortening amplitude (PS, upper right), time-to-peak shortening (TPS, lower left) and time-to-90% relengthening (TR\(_{90}\), lower right). Myocyte shortening and relengthening were recorded at 25 °C. Cells were stimulated to contract at 0·5 Hz. Results are means ± S.E.M., \(n=102\) cells/group; \(*P<0·05\) vs wild-type group. An average of 24–26 cells per mouse were obtained from 4 wild-type and 4 GH transgenic mice.
Growth hormone and myocyte function · P B COLLIGAN and others

with myocardial hypertropy and interstitial fibrosis being responsible for early stage diastolic filling defects, and ventricular dilation being responsible for the global decrease of cardiac performance and cardiac output (Saccà et al. 1994). However, it should be mentioned that a hyperkinetic phenomenon with increased cardiac performance, cardiac output and reduced peripheral resistance characterizes the early phases of the disease (Prysor-Jones & Jenkins 1980), suggesting a short-term cardiac stimulatory property of GH consistent with the beneficial effect of GH (Lombardi et al. 1997, Isgaard et al. 1999). This is supported by the finding of preserved cardiac performance in acromegalic patients with a short duration of disease (Lombardi et al. 2000).

Using a very similar transgenic model over-expressing bovine GH, Isgaard and colleagues reported depressed echocardiographic systolic indices such as shortening fraction, ejection fraction and mean velocity of circumferential shortening associated with cardiac hypertrophy (Bollano et al. 2000). This is consistent with an earlier observation of impaired cardiac function in GH transgenic mice (Sandstedt et al. 1994). Although cardiac hypertrophy persisted in GH transgenic mice in our current study, the elevated PS, ± dL/dt and Δ360/380 ratio, indicative of an enhanced myocyte function, are in contrast to the echocardiographic or whole heart observations. There are several mechanisms that may have contributed to the discrepancy. First, severe cardiac interstitial fibrosis and lympho-mononuclear infiltration are commonly seen in acromegaly or GH excess (Lie 1980, Lombardi et al. 2000), and may have been the major cause of impaired ventricular performance in the in vivo or whole heart settings. The enhanced cardiac myocyte contractile function may essentially be a compensatory response to interstitial fibrosis. Secondly, GH is known to elicit both direct and indirect cardiac effects through regulation of peripheral vascular resistance (Cittadini et al. 1999). Short-term GH exposure or treatment produces an increased cardiac index and a decreased peripheral vascular resistance (Fazio et al. 2000). However, increased mean arterial blood pressure and structural narrowing of the resistance vasculature were recently reported in bovine GH transgenic mice with prolonged GH exposure (Bohlooly-Y et al. 2001). This finding suggests that the depressed cardiac performance in the GH transgenic mice may be a secondary response to the increased afterload (i.e. peripheral vascular resistance). The contractile function was evaluated in unloaded (isotonic) ventricular myocytes in our current study. Therefore, caution is needed when comparing cardiac contractile function using isolated myocytes and whole heart since the isolated myocytes do not have the surrounding non-myocyte components and peripheral vascular load.

The present study revealed elevated myocyte shortening, maximal velocity of shortening and relengthening and intracellular Ca2+ recruiting ability associated with similar

Figure 2 Maximal velocities of cell shortening (+dL/dt) and relengthening (−dL/dt) in ventricular myocytes isolated from GH and wild-type mouse hearts. Results are means ± S.E.M., n=102 cells/group; *p<0.05 vs wild-type group. An average of 24–26 cells per mouse were used from 4 wild-type and 4 GH transgenic mice.

Performance. Heart failure with dysfunction in both ventricles is commonly seen in late stage acromegaly (Fazio et al. 1994, Lombardi et al. 1996, Bollano et al. 2000). Excessive GH or IGF-I may contribute to an acromegalic or subacromegalic condition with increased risk of diabetes, hypertension, hyperlipidemia and hyperinsulinemia, often making it difficult to examine the uncomplicated acromegalic cardiomyopathy. The GH transgenic mice used in the current study are insulin resistant and hyper-insulinemic (Balbis et al. 1996, Dominici et al. 1998) which may have added to the complexity of GH over-expression. Nevertheless, deterioration of cardiac structure and function may also develop under acromegaly in the absence of hypertension, diabetes and other predisposing factors (Fazio et al. 1994). The mechanisms responsible for the transition from GH excess, cardiac hypertropy, to an eventual deterioration of the cardiac function remain to be determined. It appears that acromegalic cardiomyopathy progresses through different pathophysiological phases.
duration of shortening/relengthening, resting intracellular Ca\textsuperscript{2+} and rate of intracellular Ca\textsuperscript{2+} decay in GH transgenic mice. These findings suggest that the effect of GH on cardiac contractile function may be specific with regard to certain contractile proteins. Myocardium exposed to high plasma GH (due to a GH secreting tumor) displays significantly enhanced maximum Ca\textsuperscript{2+}-activated force per cross-sectional area, suggesting that GH may directly increase the myofilament Ca\textsuperscript{2+} sensitivity (Mayoux et al. 1993). Results from our current study showed a similar response to an increase in extracellular Ca\textsuperscript{2+} levels and a parallel increase in cell shortening and intracellular Ca\textsuperscript{2+} ($\Delta$360/380 ratio), suggesting that the myofilament Ca\textsuperscript{2+} sensitivity is unlikely to be responsible for the enhanced cardiac contraction in these GH transgenic mice. The lack of difference in frequency–PS responses between the GH and wild-type myocytes (Fig. 5) indicates that the ability of sarcoplasmic reticulum (SR) to replenish Ca\textsuperscript{2+} is most probably not dependent on GH. It appears that the elevated cardiac contractile protein abundance due to the hypertrophic effect of GH (Isgaard et al. 1999) may play a major role in the enhanced PS and $\Delta$L/dt. The comparable TPS and TR$_{90}$ between the two groups suggest that SR Ca\textsuperscript{2+} release and resequestration are not affected by GH over-expression. The indifferent resting intracellular Ca\textsuperscript{2+} level and intracellular Ca\textsuperscript{2+} decay rate between the GH and wild-type groups indicates a normal intracellular Ca\textsuperscript{2+} homeostasis in the GH transgenic mouse hearts. It is also worth mentioning that the cardiac effects of GH may be mediated through IGF-I, as this peptide is known to be a critical ‘surviving factor’ for heart function (Tanaka et al. 1998, Ren et al. 1999).

In summary, our study showed that elevation of intrinsic GH levels is associated with enhanced cardiac contractile function at the single myocyte level. While this observation is not consistent with an in vivo study (Bollano et al. 2000), it suggests a potential role for the non-myocyte components such as interstitial fibrosis or peripheral vasculature under acromegaly or GH excess. On the other hand, our results support the beneficial effects of GH or

Figure 3 Intracellular Ca\textsuperscript{2+} properties in ventricular myocytes isolated from GH and wild-type mouse hearts. Upper left: typical experiments showing intracellular Ca\textsuperscript{2+} transients from a GH and a wild-type mouse heart (wild-type: tau=255 ms; GH: tau=273 ms). Upper right: baseline intracellular Ca\textsuperscript{2+} levels. Lower left: increase in intracellular Ca\textsuperscript{2+} levels in response to electrical stimuli. Lower right: intracellular Ca\textsuperscript{2+} transient decay rate (tau) in myocytes from GH transgenic and wild-type mouse hearts. Results are means ± s.e.m., $n=37$ cells/group; *$P<0.05$ vs wild-type group. An average of 8–10 cells per mouse were used from 4 wild-type and 4 GH transgenic mice.
GH secretagog therapy in heart failure. The larger amount of novel information on the cardiovascular effects of GH have surfaced which will undoubtedly be the focus of future research.

Acknowledgements

The authors wish to thank Sharlene Rakocy for technical assistance. This work was supported in part by the American Diabetes Association (to J R), the National Institute of Health and the American Federation of Aging Research (to H M B-B).

References


Balbi A, Bartke A & Turyn D 1996 Overexpression of bovine growth hormone in transgenic mice is associated with changes in hepatic insulin receptors and in their kinase activity. *Life Science* 59 1369–1371.


American Diabetes Association (to J R), the National Institute of Health and the American Federation of Aging Research (to H M B-B).

Figure 4 Effect of increase in extracellular Ca2+ concentration (0.5 mM–3.0 mM) on peak cell shortening (PS) in ventricular myocytes isolated from GH and wild-type mouse hearts. Results are means ± S.E.M., n=16 cells/group. Four cells per mouse were used from 4 wild-type and 4 GH transgenic mice.

Figure 5 Peak cell shortening (PS) of ventricular myocytes isolated from GH and wild-type mouse hearts at different stimulus frequencies (0.1–5.0 Hz). Each point represents PS normalized to that of 0.1 Hz. The baseline PS values at 0.1 Hz are 7.06 ± 1.00% and 6.75 ± 0.69% in wild-type (27 cells) and GH (25 cells) groups respectively (means ± S.E.M.). An average of 6–8 cells per mouse were used from 4 wild-type and 4 GH transgenic mice.


Received 21 January 2002
Accepted 5 February 2002