Positive inotropic effect of insulin-like growth factor-1 on normal and failing cardiac myocytes

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Abstract

Objective: The acute administration of growth hormone (GH) or insulin-like growth factor-1 (IGF-1) improves cardiac performance, possibly contributing to the beneficial effects of GH therapy on heart failure (HF). GH can induce the production of IGF-1 and thus the actions of GH may be mediated through its IGF-1 induction. However, these effects have not yet been demonstrated in failing hearts and the cellular basis of GH or IGF-1-induced inotropic effects remains unknown. We examined the direct effects of GH and IGF-1 on the contractile function and intracellular calcium ([Ca^{2+}]_i) homeostasis in normal and failing myocytes. Methods: To determine whether GH and IGF-1 have a direct effect on myocardial contractility and whether the GH/IGF-1-induced effect was the result of changes in Ca^{2+} activation, cell shortening and [Ca^{2+}]_i transients were simultaneously measured in the left ventricular myocyte preparations, isolated from normal and rapid pacing-induced HF dogs. Results: Basal shortening of HF myocytes was reduced by 64% (p<0.01). In normal and HF myocytes, GH (0.4–40×10^{-3} IU/ml) had no effect on either cell shortening or [Ca^{2+}]_i transients. In normal myocytes, IGF-1 exerted a positive inotropic effect in a time- and dose-dependent manner (25–500 ng/ml), associated with a parallel increase of [Ca^{2+}]_i transient amplitude. IGF-1 increased the shortening magnitude in normal (121±5% increase from baseline, p<0.05) and HF (118±4% increase from baseline, p<0.05) myocytes. It also increased [Ca^{2+}]_i transient amplitude in normal and HF cells by 124±6 and 125±7%, respectively. The percent increase of cell shortening and [Ca^{2+}]_i transient amplitude was comparable between normal and HF myocytes. Furthermore, IGF-1 did not shift the trajectory of the relaxation phase in the phase-plane plots of cell length vs. [Ca^{2+}]_i, indicating that it did not change myofilament Ca^{2+} sensitivity. Conclusions: In both normal and HF conditions, IGF-1 exerted an acute direct positive inotropic effect in adult cardiac myocytes by increasing the availability of [Ca^{2+}]_i to the myofilaments, possibly explaining the beneficial effect of GH on HF. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Growth hormone (GH) is essential for both cardiac development and preservation of the cardiac structure and contractile performance [1]. GH can induce the production of insulin-like growth factor-1 (IGF-1) and most of the actions of GH are mediated via IGF-1 induction. Recent clinical, as well as, experimental studies have shown both GH and IGF-1 to have a beneficial effect on heart failure (HF) [2–4]. These effects of GH/IGF-1 might result, at least in part, from a direct positive inotropic action independent of a growth effect because the short term administration of GH to HF rats due to myocardial infarction exerted a functional benefit even in the absence of increased cardiac mass [5]. Furthermore, the acute administration of IGF-1 enhanced LV contractile performance in normal human subjects [6] as well as in patients with HF [7]. Cardiac myocytes have receptors for GH and

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2. Methods

2.1. Preparation of animal models

HF was induced in adult mongrel dogs (15–25 kg body weight) by rapid ventricular pacing [16–18]. Briefly, under general anesthesia, a bipolar pacing lead (1236T; Pace Setter Inc.) was introduced into the external jugular vein through the external pulse generator. The protocols were approved by the Committee on the Ethics of Animal Experiments, Kyushu University, and the investigation conformed with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Isolation of cardiac myocytes

The animals were sacrificed with a lethal dose of α-chloralose and the heart was quickly excised. Cardiac myocytes were isolated from the left ventricular (LV) free wall, as described previously [17,18]. A wedge of LV free wall was dissected free of the heart and was perfused by a branch of the left circumflex coronary artery with nominally calcium-free buffer of the following composition (mmol/l): NaCl 140.0, KCl 4.8, MgSO4 2.4, NaH2PO4 1.2, NaHCO3 2.5, HEPES 12.0, and glucose 12.5 warmed to 37°C and gassed with 100% O2. To dissociate the myocytes, the perfusate was changed to the buffer supplemented with type II collagenase (60 units/ml; Wako Pure Chemical Industries, Ltd.), and the perfusion was terminated when the heart became flaccid. The mean perfusion pressure and pH were kept at approximately 80 mmHg and 7.4, respectively. After the completion of perfusion, the LV myocardium was minced with scissors in fresh collagenase-containing buffer with 3% bovine serum albumin and 300 μmol/l Ca2+, and was gently agitated for 5 min in the same buffer. The dissociated myocytes were suspended into the supernatant, filtered through 210-μm nylon mesh, and harvested from the filtrate. Myocytes were kept in collagenase-free buffer supplemented with 1.25 mmol/l Ca2+ for 1 h at room temperature before assessing their contractile function.

2.3. Evaluation of myocyte viability and morphology

To examine the effects of GH (40×10^-3 U/ml; Genentech) and IGF-1 (150 ng/ml; AUSTRAL Biologicals Inc.) on cellular viability, freshly isolated myocytes were treated for up to 30 min with a drug and then the viability was assessed by the percentage of rod-shaped cells excluding 0.4% trypan blue dye in 10 randomly chosen 1×1-mm fields [19]. To examine the effects of GH and IGF-1 on cellular morphology, photomicrographs of the isolated normal myocytes were obtained during the treatment, and the two dimensional surface area of the myocytes was determined by digitizing the lateral edges (final magnification ×650). The maximum length and width of the myocyte were determined from these cell images [19].

2.4. Simultaneous measurement of myocyte contractility and [Ca2+]i transient

To determine whether GH and IGF-1 had direct effects on the myocyte contractility and whether the effects were the result of changes in Ca2+ activation, myocytes shortening and the [Ca2+]i transient were measured simul-
taneously in the isolated myocyte preparations. The cell length was measured during electrical field stimulation (0.25 Hz) by using the edge detector (C6294-01; Hamamatsu Photonics) and the [Ca\(^{2+}\)] transient was measured using the calcium-selective fluorescent dye indo-1 [19]. Briefly, myocytes were loaded with 2 \(\mu\)mol/l indo 1 acetoxyethyl ester (AM; Molecular Probes) in the same buffer supplemented with 1.25 mmol/l Ca\(^{2+}\), fatty acid-free 0.5% BSA, and 0.03% Pluronic F-127 (Molecular Probes) for 5 min at room temperature. The cells were subsequently washed with buffer containing 1.25 mmol/l Ca\(^{2+}\) for 30 min before being used for experiments. Indo 1-loaded myocytes were placed in a chamber on the stage of an inverted microscope (Olympus) and stimulated to contract between a pair of platinum wire electrodes. A single myocyte was then excited at 350 nm by epi-illumination, and indo 1 fluorescence emission light, split by a 455-nm dichroic mirror and selected by the use of rectangular band-pass interference filters in the wavelength ranges of 380 to 430 nm (405-nm channel) and 455 to 505 nm (480-nm channel), was directed to a pair of photomultiplier tubes. The photocurrent from each tube was integrated at a 1-ms interval, and the ratio of indo 1 emission at the two wavelengths was calculated as the index of [Ca\(^{2+}\)], by a computer.

The myocytes were simultaneously illuminated with red light (wavelength above 620 nm) through the normal bright-field illumination optics of the microscope. The bright-field images were collected by an objective lens, and first separated by a 530 nm long-pass dichroic mirror. The cell image was projected onto a photodiode array of the edge detector with 10 ms temporal resolution, and the cell length was monitored simultaneously with indo 1 fluorescence. The contractile state of the individual myocytes was assessed by measuring the magnitude of cell shortening, defined as the diastolic cell length (DCL; the longest cell length measured during diastole) minus the cell length at peak shortening during each beat, divided by DCL for that beat and then expressed as the % of DCL [20].

After the extent of cell shortening became stable, 4 to 6 successive contractions were sampled and averaged to yield a profile of cell shortening and [Ca\(^{2+}\)] transient. Both cell shortening and [Ca\(^{2+}\)] transient for each myocyte were analyzed using 4–6 myocytes for each animal and values per each myocyte were averaged to obtain the final data. The magnitude of cell shortening, diastolic and peak systolic [Ca\(^{2+}\)] and the duration of contraction and [Ca\(^{2+}\)] transient (time to 50 and 90% recovery) were measured in each myocyte. Only myocytes with the following characteristics were analyzed: single rod-shaped cells unattached to either adjacent cells or debris, that contracted with each stimulus but were quiescent between stimuli. After the baseline contractions were recorded, GH (0.4–40 \(\times\) 10\(^{-3}\) IU/ml) or IGF-1 (25–500 ng/ml) was added to the superfusate of the following composition (mmol/l): NaCl 140.0, KCl 4.8, MgSO\(_4\) 2.4, NaH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 2.5, HEPES 12.0, CaCl\(_2\) 1.25 and glucose 12.5 (pH 7.4) warmed to 37°C and gassed with 100% O\(_2\). Myocyte contraction and [Ca\(^{2+}\)] transient were sequentially recorded from a single myocyte after treatment with GH or IGF-1. The reversibility of the IGF-1-induced effects on myocyte contractility was examined by replacing the superfusate containing IGF-1 with normal buffer.

2.5. Assessment of myofibrillar sensitivity to Ca\(^{2+}\)

To determine whether IGF-1 can alter the responsiveness of myofilaments to cytoplasmic Ca\(^{2+}\), we plotted the phase-plane diagrams of the cell length vs. [Ca\(^{2+}\)] during a single contraction before and after IGF-1 treatment. Spurgeon et al. showed that the [Ca\(^{2+}\)]-cell length trajectory during the relaxation phase of the twitch contraction in single cardiac myocytes defines a quasi-equilibrium of cytosolic [Ca\(^{2+}\)], myofilament Ca\(^{2+}\) binding, and cell length. Their findings indicate that the position of this trajectory reflects the relative myofilament responsiveness to Ca\(^{2+}\) [20]. To confirm that this approach is capable of detecting changes in our system, we examined the effects of pharmacological interventions known to produce primary alterations in Ca\(^{2+}\) sensitivity on the slope and position of the [Ca\(^{2+}\)]-cell length trajectory during the relaxation phase of the twitch contraction in normal myocytes; including EMD57033 (1 \(\mu\)mol/l) to increase the sensitivity and butanedione monoxime (BDM; 5 mmol/l) to decrease it.

2.6. Statistical analysis

All data are expressed as the means±standard error of the mean (SEM). Student’s unpaired \(t\) test was used to evaluate mean differences between control and HF. For comparisons of changes in cell shortening and [Ca\(^{2+}\)] transient parameters after IGF-1 treatment, one way analysis of variance (ANOVA) was used to evaluate the mean differences based on the post-hoc \(t\) test with Bonferroni’s correction. All tests were considered statistically significant at \(p<0.05\).

3. Results

3.1. Effects of GH and IGF-1 on myocyte viability and morphology

There was no significant decline in the percentage of viable rod-shaped normal control myocytes after 20 min exposure to \(40\times10^{-3}\) IU/ml of GH (from 78±4% to 74±3%; \(n=33\) cells from 5 dogs, \(p=NS\)) and 150 ng/ml of IGF-1 (from 75±6% to 70±5%; \(n=35\) cells from 5 dogs, \(p=NS\)). Virtually all rod-shaped control myocytes
treated with IGF-1 excluded trypan blue dye, thus indicating that they were functionally intact during the period of study. The length and width of isolated cardiac myocytes were not affected by 20 min exposure to GH (cell length; from 155±9 to 156±9 μm, p=NS and cell width; from 35±4 to 34±4 μm, n=32 cells from 5 dogs, p=NS) and IGF-1 (cell length; from 170±16 to 170±17 μm, p=NS and cell width; from 27±2 μm to 27±2 μm, n=32 cells from 5 dogs, p=NS).

3.2. Effects of GH and IGF-1 on myocyte contractility and [Ca\(^{2+}\)] transient

Although diastolic cell length was not changed by IGF-1 (150 ng/ml), it exerted a positive inotropic effect in normal myocytes after 10 min of exposure (Fig. 1A), which was completely reversed after the removal of IGF-1 by replacing the superfusion medium containing IGF-1 with normal buffer (Fig. 1B). The positive inotropic response was associated with a parallel increase in [Ca\(^{2+}\)] transient amplitude without altering the resting level (Fig. 1A). IGF-1 did not affect either the duration of myocyte shortening (time to peak shortening, time to 50% decline, and time to 90% decline) or that of [Ca\(^{2+}\)] transient dynamics (Table 1). The effect of IGF-1 on the myocyte shortening was dose-dependent (25±500 ng/ml), wherein its effect peaked at 150–300 ng/ml (Fig. 2A).

Even though pre-IGF-1 basal myocyte shortening decreased in HF myocytes by 64% compared to the normal level (p<0.01), IGF-1 (500 ng/ml) increased the extent of cell shortening (Fig. 2A) not only in control myocytes (126±6% increase from baseline; n=10 cells from 6 dogs, p<0.05) but also in HF myocytes (118±26% increase from baseline; n=8 cells from 5 dogs, p<0.05). It also increased the [Ca\(^{2+}\)] transient amplitude (Fig. 2B) in normal myocytes (126±6% increase from baseline; n=10 cells from 6 dogs, p<0.05) and HF myocytes (125±7% increase from baseline; n=8 cells from 5 dogs, p<0.05). The percentage of IGF-1-induced increase in cell shortening and [Ca\(^{2+}\)] transient magnitude was comparable between the control and HF myocytes (Fig. 2).

In normal (n=6 cells from 4 dogs) and HF myocytes (n=6 cells from 4 dogs), GH (0.4–40×10\(^{-3}\) IU/ml) had no effects on myocyte shortening and [Ca\(^{2+}\)] transients (Fig. 3).

To determine whether the actions of IGF-1 were mediated through a specific cell surface receptor, the IGF-1R, the blocking effects of an anti-IGF-1R antibody (αIR3; Oncogene Science) on myocyte contraction and [Ca\(^{2+}\)] transient were examined. αIR3 (1 μg/ml) significantly attenuated the positive inotropic effects of IGF-1 in control cells. The enhancement of cell shortening with IGF-1 (150 ng/ml) was significantly attenuated from 118±6% to 101±1% of the control value (n=5 cells; p<0.01) in the presence of antibody. In the same experiment, αIR3 alone increased myocyte shortening significantly to 113±1% of the control level (n=5 cells; p<0.01).

3.3. Effects of IGF-1 on myofibrillar responsiveness to Ca\(^{2+}\)

We first confirmed that the common trajectory of [Ca\(^{2+}\)]-cell length relation at the terminal portion of these plots shifted appropriately in response to the perturbations to alter the myofilament Ca\(^{2+}\) sensitivity. The trajectory shifted leftward following the addition of myofilament Ca\(^{2+}\) sensitizing drug, EMD57033 (1 μmol/l); a rightward
shift occurred following the addition of BDM (5 mmol/l), which is known to decrease the sensitivity of myofilaments to Ca\(^{2+}\) (Fig. 4). The slope and position of the trajectory during the relaxation phase of the phase-plane diagrams of cell length vs. [Ca\(^{2+}\)] did not shift after IGF-1 in normal myocytes as is shown in Fig. 5, thus indicating that IGF-1 did not alter the myofibrillar Ca\(^{2+}\) sensitivity. Taken together, these results indicated that IGF-1 exerted a direct positive inotropic effect by increasing the availability of [Ca\(^{2+}\)] to the myofilaments in both normal and HF conditions.

### 4. Discussion

The present study demonstrated that IGF-1, but not GH, had a direct positive inotropic effect on the isolated adult cardiac myocytes. The acute enhancement of contractility observed by IGF-1 administration depends upon the increase of Ca\(^{2+}\) available to the contractile apparatus, but is not due to an augmented responsiveness of myofilaments to Ca\(^{2+}\).

Our results showing that IGF-1 exerted a direct positive inotropic effect on the fully differentiated adult cardiac myocytes are consistent with previous studies in which IGF-1 was shown to increase the contractility of neonatal cardiac myocytes by 122% at a concentration of 25 ng/ml [10] and of isolated ventricular muscle by 110–120% [11,13]. The positive inotropic effect was mediated via IGF-1 receptor since αIR3, the anti-IGF-1 receptor antibody, inhibited the positive inotropic action of IGF-1 in our study. Interestingly, αIR3 itself slightly increased the basal contraction of myocytes. This stimulation was most likely due to an IGF-1-mimic effect of αIR3, as has also been shown in previous studies [21]. Since the effect of IGF-1 on the contractile function was studied using the isolated myocyte preparations, we obviated the potential problems in intact tissue preparations of diffusion limita-

![Fig. 2](https://academic.oup.com/cardiovascres/article-abstract/43/1/157/355445)
mechanism is a prolongation of the action potential duration, which has been demonstrated in a model of GH hypersecretion [22]. However, this might not play an important role in the IGF-1-induced acute positive inotropic effects seen in this study since the sarcolemmal Ca current has been shown to be unchanged by IGF-1 using a patch-clamp technique [11]. Therefore, further studies focusing on the effects of IGF-1 on Ca uptake/release function of the sarcoplasmic reticulum are needed to clarify the mechanisms responsible for IGF-1-induced alterations in Ca homeostasis.

Based on the present observations of the [Ca\textsuperscript{2+}]\textsubscript{i}-cell mechanism and the presence of cells other than myocytes that might bind and respond to IGF-1 or metabolize this growth factor.

There are two possible mechanisms for the positive inotropic effects of IGF-1; the increased availability of Ca\textsuperscript{2+} to the myofilaments and the increase of myofilament sensitivity to cytoplasmic Ca\textsuperscript{2+}. To determine whether IGF-1-induced effects resulted from alterations of Ca\textsuperscript{2+} availability, we measured cell shortening and [Ca\textsuperscript{2+}] transient simultaneously and thus showed IGF-1 to exert a parallel increase of [Ca\textsuperscript{2+}], transient amplitude. The increase of [Ca\textsuperscript{2+}], transient amplitude was consistent with the results of Freestone et al. in adult rat cardiac myocytes [11]. However, the exact intracellular mechanism for the changes in Ca\textsuperscript{2+} homeostasis is not clear. One possible

**Fig. 3.** (A) Effects of GH on myocyte shortening. Recordings were obtained sequentially for the same cell before and after 10 min of exposure to GH (40×10\textsuperscript{-3} IU/ml). GH did not affect the myocyte shortening. (B) Dose-response relationship of GH-induced effects on myocyte shortening magnitude in the control (open circles; n=6 cells from 4 dogs) and HF myocytes (closed circles; n=6 cells from 4 dogs). Data are calculated as the percent change from the baseline values and expressed as the means±SEM.

**Fig. 4.** Representative phase-plane plots of myocyte shortening versus [Ca\textsuperscript{2+}] transient in normal myocyte before (open circles) and 10 min after the treatment (closed circles) with EMD57033 (A) and BDM (B). Note that the trajectory at the terminal portion of these plots shifts leftward following the addition of myofilament Ca sensitizing drug, EMD57033 (1 µmol/l); a rightward shift occurs following the addition of BDM (5 mmol/l), which is known to decrease the sensitivity of myofilaments to Ca\textsuperscript{2+}. The arrows indicate the direction of the trajectory during the course of a single contraction.

**Fig. 5.** Representative phase-plane plots of myocyte shortening versus [Ca\textsuperscript{2+}] transient in normal myocyte before (No IGF-1; open circles) and 10 min after IGF-1 treatment (150 ng/ml; closed circles). Note that the peak shortening and peak [Ca\textsuperscript{2+}], transient increased by the treatment of myocytes with IGF-1, but that the trajectory at the terminal portion of these plots was not shifted by IGF-1. The arrows indicate the direction of trajectory during the course of a single contraction.
length trajectory, IGF-1 did not change the myofilament Ca\(^{2+}\) sensitivity. In the present study, the sensitivity of myofilaments to Ca\(^{2+}\) was evaluated according to the methods described by Spurgeon et al. [20], in which they showed the [Ca\(^{2+}\)]\(_i\)-cell length trajectory during the relaxation phase of the twitch contraction in single cardiac myocytes to define a quasi-equilibrium of cytosolic [Ca\(^{2+}\)]\(_i\), myofilament Ca\(^{2+}\) binding, and cell length. Their findings indicate that the position of this trajectory reflects the relative myofilament response to Ca\(^{2+}\). We confirmed this method to be capable of detecting the alterations of Ca\(^{2+}\) sensitivity by demonstrating the appropriate shift of this trajectory in response to perturbations that alter Ca\(^{2+}\) sensitivity (Fig. 4). Specifically, the trajectory shifts leftward following the addition of myofilament Ca\(^{2+}\) sensitizing drug, EMD57033; while a rightward shift occurs following the addition of BDM, known to decrease the sensitivity of myofilaments to Ca\(^{2+}\).

IGF-1 did not shift the terminal portion of this trajectory, which indicated that Ca\(^{2+}\) sensitivity was not altered by IGF-1 (Fig. 5). However, previous studies have yielded conflicting results concerning the alterations of myofilament Ca\(^{2+}\) sensitivity during an increase of GH or IGF-1. Mayoux et al. showed an increase of Ca\(^{2+}\) sensitivity in chemically skinned fibers from rats bearing GH-secreting tumor [14]. In contrast, Strömmer et al. demonstrated a decrease in Ca\(^{2+}\) sensitivity in the isolated isovolumic rat heart after injecting GH and IGF-1 [12], but they also reported the acute administration of IGF-1 to sensitize the myofilaments to Ca\(^{2+}\) [13]. Even though the exact reasons responsible for these conflicting results remain unknown, the Ca\(^{2+}\) sensitive fluorescent signal measured on the epicardial surface of the multicellular tissue preparations might not accurately reflect [Ca\(^{2+}\)]\(_i\) transients within myocytes. Myofilament Ca\(^{2+}\) sensitivity of tension development measured using skinned myocyte preparation is not changed by the exogenous treatment with IGF-1 [15]. Furthermore, we observed that IGF-1 does not alter the Ca\(^{2+}\) sensitivity of tension development in skinned myocytes (unpublished data). Taken together, the present results and those by others [11,15] performed in myocytes consistently demonstrated that the positive inotropic effects of IGF-1 are probably due to an increased Ca\(^{2+}\) availability to the myofilaments rather than an increased Ca\(^{2+}\) sensitivity.

Another important finding of the present study was that IGF-1 exerted a similar positive inotropic effects in HF as in control myocytes (Fig. 2). The positive inotropic effects induced by IGF-1 in failing hearts are in clear contrast to those of β-adrenergic agonists, which are well known to be blunted in HF [16]. Based on the present observations, the receptor function and/or the signal transduction system of IGF-1 should be preserved at a normal level in HF, even though we have not examined these parameters in our model of HF. Recent studies reported a beneficial effect on cardiac performance of GH and IGF-1 therapy in both experimental and clinical HF [2–5,7]. However, the use of GH in humans is still largely experimental. The ability of this hormone to enhance systolic performance in the diseased heart may therefore be of value in defining new therapeutic strategies for the management of clinical HF.

GH did not affect myocyte contractility (Fig. 3), a finding consistent with a recent study by Cittadini et al. [13]. Even though GH receptors have been demonstrated to be present on the heart [23], their physiological role probably does not include acute modulation of myocardial contractility on the basis of the present observations and those by Cittadini et al. The receptors for GH in cardiac myocytes may mediate other functions such as protein synthesis [24]. GH can stimulate IGF-1 production in either a paracrine manner at the target tissue or through a distal endocrine mechanism in tissue such as the liver. Therefore, GH may indeed have an effect on myocyte contractility via IGF-1 in the in vivo setting.

Several potential limitations should be acknowledged in this study. First, even though the concentration range of GH used in this study overlapped with the normal daily serum GH level (1–10 ng/ml or 2.5–25×10\(^{-3}\) IU/ml), the concentration of IGF-1, which produced a significant effect on myocyte contractility (150 ng/ml), appeared to be relatively high compared to the plasma concentration of IGF-1 (15–30 ng/ml). However, IGF-1 can be produced within the heart and thus it may be possible that the accumulation of IGF-1 in an autocrine/paracrine manner might reach much higher local concentrations. Second, the acute effects of IGF-1 on myocyte contractility in vitro may differ from those in vivo since IGF-1 is present for longer periods of time under in vivo conditions. It remains to be clarified as to whether a longer treatment in this HF model with IGF-1 might be beneficial. Even though no data are available, chronic GH/IGF-1 administration is expected to be beneficial in our model because a recent preliminary report has shown GH supplementation to improve both LV and the myocyte contractile function in rapid pacing-induced HF in swine [25].

In conclusion, the present study provides compelling evidence that IGF-1 exerts a direct positive inotropic effect on cardiac myocytes by increasing Ca\(^{2+}\) availability. While the extension of acute in vitro effects at the myocyte level into the long-term in vivo effects at the organ level may not be appropriate, the present study is considered to provide a potential cellular mechanism for the beneficial effects of GH therapy in HF.

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