

Growth hormone-releasing peptide can improve left ventricular dysfunction and attenuate dilation in dilated cardiomyopathic hamsters

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Abstract

Objective: The mammalian heart contains specific growth hormone-releasing peptide (GHRP) binding sites whose physiological significance is unknown. We sought to compare the effects of GHRP and GH on progressive left ventricular (LV) dysfunction in the TO-2 hamster model of dilated cardiomyopathy. **Methods:** TO-2 hamsters (8 weeks old) were injected with GHRP-6 (100 µg/kg day), GH (2 mg/kg day), or saline for 4 weeks; F1B hamsters served as controls. LV functional and structural changes were evaluated by echocardiography and pathology. **Results:** The increase in body weight of GH-treated TO-2 hamsters was greater than that of animals in the other two groups. Plasma GH and insulin-like growth factor-1 (IGF-1) concentrations were not increased by GHRP-6. LV fractional shortening (LVFS) decreased from $42.0 \pm 2.6\%$ to $25.4 \pm 1.8\%$ and the LV end-diastolic dimension (LVDd) increased from 4.0 ± 0.1 to 5.0 ± 0.1 mm in untreated TO-2 hamsters between 8 and 12 weeks. LVFS was substantially improved by treatment with GHRP-6 ($33.4 \pm 2.0\%$) or GH ($32.0 \pm 2.1\%$). The LVDd was significantly smaller in animals treated with GHRP-6 than in those treated with GH. The cross-sectional LV myocyte area and the amount of atrial natriuretic peptide mRNA in the LV were increased by GH but not by GHRP-6. Treatment with GH at a lower dose (0.2 mg/(kg day)) exerted minimal cardiac and systematic growth effects without improving LV function. **Conclusion:** GHRP can ameliorate the development of progressive LV dysfunction independently of the GH-IGF-1 axis, suggesting a potential new approach to the heart failure.

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Keywords: Growth hormone-releasing peptide; Growth hormone; Cardiomyopathic hamsters; LV dysfunction

1. Introduction

Dilated cardiomyopathy (DCM) is a common cause of heart failure and of the need for heart transplantation. DCM is a multifactorial disease, resulting from myocarditis, ischemia-induced injury, or mitochondrial or genetic abnormalities [1]. About 30% of DCM cases are thought to be familial, with several genes, including those for dystrophin, actin, desmin, lamin A or C, and δ -sarcoglycan, having been implicated in this disorder [2].

The TO-2 hamster has been studied as a model of human DCM. Like the human disease, this model of DCM is characterized by progressive left ventricular (LV) dilation, LV wall thinning, LV systolic dysfunction, and a reduced life span. The TO-2 hamster is deficient in δ -sarcoglycan [3], and abnormal coronary artery constrictions have been suggested to initiate the development of cardiomyopathy in this model [4]. In addition, some cases of DCM in humans were also reported to result from δ -sarcoglycan deficiency [5].

The available treatments for DCM are palliative, and the prognosis of affected individuals remains poor. Fazio et al. [6] treated DCM patients with recombinant human growth hormone (GH) and observed substantial improve-

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ment in both LV function and exercise capacity. In a randomized, double-blind, placebo-controlled study, Osterziel et al. [7] subsequently failed to detect improvement in DCM patients treated with recombinant human GH. Recent epidemiological studies have suggested that the GH and insulin-like growth factor-1 (IGF-1) axis may be an important determinant of cancer incidence [8]. The overall clinical benefits of GH therapy are thus not as promising as initially expected on the basis of animal studies [9–12]. Furthermore, some investigators have failed to demonstrate positive cardiac effects of GH treatment in animals [13–14].

An alternative approach to increasing systemic levels of GH involves administration of a GH-releasing peptide (GHRP) or peptidomimetic agonist that stimulates GH secretion through direct action on the pituitary gland [15]. Specific GHRP binding sites are also present in the mammalian heart [16]. In addition to its stimulatory effect on GH secretion, hexarelin, a hexapeptide member of the GHRP family, protected against postischemic dysfunction in perfused hearts isolated from senescent rats [17]. Furthermore, treatment with a GH secretagogue was beneficial in a model of developing heart failure [18]. It has remained unclear, however, whether the cardioprotective effects of GHRP are mediated, at least in part, by a mechanism independent of the GH-IGF-1 axis.

We have now examined the effects of GHRP-6 on progressive LV dysfunction in TO-2 hamsters. We also compared the effects of GHRP-6 with those of GH on systemic growth, myocardial cell growth, and LV function. We selected a relatively low dose of GHRP-6 that did not promote systemic growth, as judged from body weight gain in our preliminary observations, in order to eliminate the confounding effects of induced GH secretion. We also evaluated the effects of treatment with GH at a low dose to examine whether or not cardiac beneficial effects of GHRP-6 might be due to small amounts of GH secretion induced by GHRP-6.

2. Methods

2.1. Experimental animals and study protocols

Male cardiomyopathic Syrian hamsters (BIO TO-2, 7 weeks old, $n=30$) and male control hamsters (BIO F1B, 7 weeks old, $n=7$) were obtained from Bio Breeders (Fitchburg, MA). Hamsters were maintained under constant environmental conditions, with a 12-h-light–12-h-dark cycle (light on from 08:00 to 20:00 h), and had free access to food and water. Animal care was in accordance with institutional guidelines, and the experimental protocol was approved by the Committee on Laboratory Animal Utilization of Nagoya University. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH

Publication no. 85-23, revised 1996). TO-2 hamsters (8 weeks old) were injected subcutaneously with GHRP-6 (100 $\mu\text{g/kg}$ body weight per day, $n=8$) (Bachem, Bubendorf, Switzerland), recombinant human GH [2 mg/kg day (high dose: $n=8$) or 0.2 mg/kg day (low dose: $n=5$)] (Pharmacia Upjohn, Bridgewater, NJ), or saline ($n=9$) for 4 weeks. Animals were weighed every 2 weeks.

2.2. Echocardiography

Transthoracic echocardiography was performed with a 13-MHz transducer (Acuson Sequoia 512) immediately before random assignment of hamsters to treatment groups and at the completion of treatment. Hamsters were anesthetized by intraperitoneal injection of sodium pentobarbital (TO-2, 50 mg/kg; F1B, 65 mg/kg), according to the method by Ryoke et al. [9]. LV end-systolic (LVDs) and end-diastolic (LVDd) dimensions, interventricular septum thickness (IVST), and LV posterior wall thickness (LVPWT) were measured. LV fractional shortening (LVFS) was calculated. Mean LV wall thickness (LVWT) was defined as the average of IVST and LVPWT. The ratio of LVDd and mean LVWT (LVDd/mean LVWT) was also calculated for the assessment of LV remodeling process.

2.3. Plasma GH and IGF-1

Blood (2 ml) was collected from anesthetized hamsters at 12 weeks of age. Plasma was isolated and then stored at -80°C until determination of the concentrations of GH and IGF-1 with double-antibody radioimmunoassay kits for rat GH (Harbor-UCLA Research and Education Institute, Torrance, CA) and human IGF-1 (Nichols Institute Diagnostics, San Juan Capistrano, CA).

2.4. Tissue preparation

Hearts were excised, rinsed with saline, and blotted dry. The atria were removed, and both the right ventricular free wall and the LV free wall plus IVS were separated and weighed. For pathology, specimens ($n=3$ per group) were fixed by immersion in 20% formaldehyde. The remaining specimens were frozen rapidly in liquid nitrogen and stored at -80°C until analysis by reverse transcription (RT) and the polymerase chain reaction (PCR).

2.5. Pathology

Fixed tissue was dehydrated, embedded in paraffin, sectioned at a thickness of 4 μm , and stained with hematoxylin–eosin. The areas occupied by the cytoplasm and the nucleus of myocytes were measured with NIH Image software. Cells in which the nucleus was located in the center of the cytoplasm were selected for measurement. LV myocyte cross-sectional area was defined as the area of the cytoplasm plus that of the nucleus. A total of 100 cells were

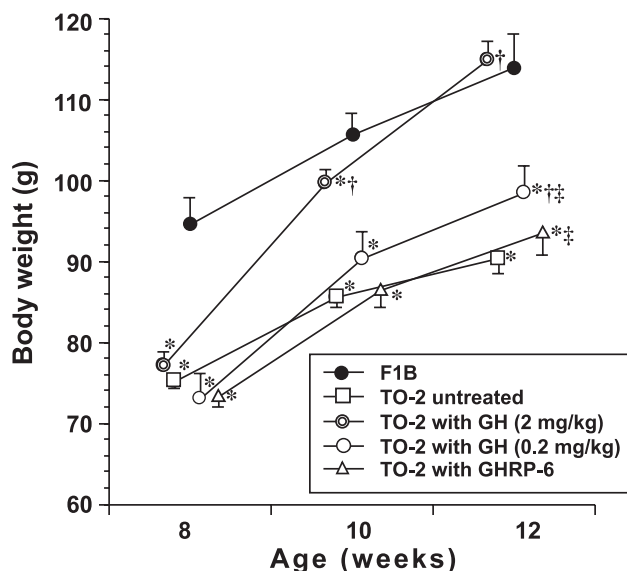


Fig. 1. Changes in body weight of hamsters in the various treatment groups. Data are means \pm S.E.M. * $P < 0.05$ vs. F1B hamsters of the same age, † $P < 0.05$ vs. untreated TO-2 hamsters of the same age, and ‡ $P < 0.05$ vs. high-dose GH-treated TO-2 hamsters of the same age.

examined randomly in each specimen, and data were averaged for these 100 cells.

2.6. RT-PCR

Specific primers and TaqMan probes for hamster atrial natriuretic peptide (ANP) and δ -sarcoglycan and mouse SERCA2a were designed with the use of Perkin-Elmer software and cDNA sequences available in GenBank (K02781, AB001508, and AF039893, respectively): ANP forward primer, 5'-AGGCCATATTGGAGCAAATCCT-3'; ANP reverse primer, 5'-TGCTTCCTCAGTCTGCTCACTC-3'; ANP probe, 5'-FAM-ATTCAAGAACCCTGTAGACCACCTGG-TAMRA-3'; δ -sarcoglycan forward primer, 5'-ATTGATGGAATGGGAAGCTTAAGAA-3'; δ -sarcoglycan reverse primer, 5'-AGACTGTGCCTTCAGCTCCTAAGACT-3'; δ -sarcoglycan probe, 5'-FAM-TCTGGAAAATTGCTCTTTCTGCGGATG-TAMRA-3'; SERCA2a forward primer, 5'-CTGGAGTTAATACTGAGATCGGCA-3'; SERCA2a reverse primer, 5'-TCCA-GACTGCAATGCAAATGAG-3'; and SERCA2a probe, 5'-FAM-CAACAGAACAGGAGAGAACACCCCTACAGC-TAMRA-3'. TaqMan rodent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control reagents (Perkin-Elmer) were used for fluorogenic detection of GAPDH mRNA as an internal standard. The identity of all amplified products was confirmed by DNA sequencing.

Isolation of RNA and RT-PCR were performed as described [19]. In brief, cDNA was synthesized from DNase-treated total RNA (2 μ g) from the left ventricle with oligo(dT)_{12–18} primers and SuperScript II reverse transcriptase (Invitrogen). The abundance of each target mRNA was determined by a fluorogenic 5'-nuclease PCR

assay with an ABI Prism 7700 sequence detector (Perkin-Elmer).

2.7. Blood pressure measurement

Separate groups of six F1B hamsters, six untreated TO-2 hamsters, and five TO-2 hamsters with GHRP-6 were used for this part of the study. After animals were anesthetized with intraperitoneal injection of sodium pentobarbital with the same dose used in the echocardiographic study, the blood pressure (BP) was measured noninvasively at the left brachial artery by a noninvasive modified tail-cuff method (BP Monitor for Rats and mice Model MK-2000, Muro-machi Kikai, Tokyo, Japan), as previously described [20].

2.8. Statistical analysis

Data are expressed as means \pm S.E.M. Differences between two groups were analyzed by Student's unpaired *t*-test. Paired comparisons were analyzed by the paired *t*-test. Differences in body weight were assessed by two-way analysis of variance followed by Scheffe's test. A *P*-value of < 0.05 was considered statistically significant.

3. Results

3.1. Systemic and heart growth

At 8 weeks of age, the body weight of F1B control hamsters was significantly greater than that of TO-2 hamsters (Fig. 1). At 12 weeks, the body weight of TO-2 hamsters treated with GH at a high dose (2 mg/(kg day)) had increased to a significantly greater extent ($+37.7 \pm 1.3$ g) than had that either of those treated with GHRP-6 ($+20.2 \pm 1.9$ g) or of untreated TO-2 hamsters ($+15.3 \pm 1.4$ g); at this time, the body weight of GH-treated TO-2 hamsters was similar to that of F1B hamsters (Table 1). The LV weights of GHRP-6-treated and untreated TO-2 hamsters were significantly smaller than that of TO-2 hamsters treated with GH at a high dose, which was similar to that of F1B hamsters. TO-2

Table 1
Body and LV weights (wt.) of hamsters at 12 weeks of age

Group	Body wt. (g)	LV wt. (mg)	LV wt. (mg)/body wt. (g)
F1B	116 \pm 4	258 \pm 11	2.23 \pm 0.05
TO-2 untreated	92 \pm 2 ^a	204 \pm 7a	2.21 \pm 0.05
TO-2 with GH (0.2 mg/(kg day))	99 \pm 4 ^{a,b,c}	207 \pm 6 ^{a,c}	2.09 \pm 0.05
TO-2 with GH (2.0 mg/(kg day))	115 \pm 2 ^b	249 \pm 6 ^b	2.18 \pm 0.04
TO-2 with GHRP-6	96 \pm 3 ^{a,c}	198 \pm 8 ^{a,c}	2.06 \pm 0.06

Data are means \pm S.E.M. LV weight indicates the LV free wall plus IVS.

^a $P < 0.05$ vs. F1B.

^b $P < 0.05$ vs. TO-2 untreated.

^c $P < 0.05$ vs. TO-2 treated with GH (2.0 mg/kg day).

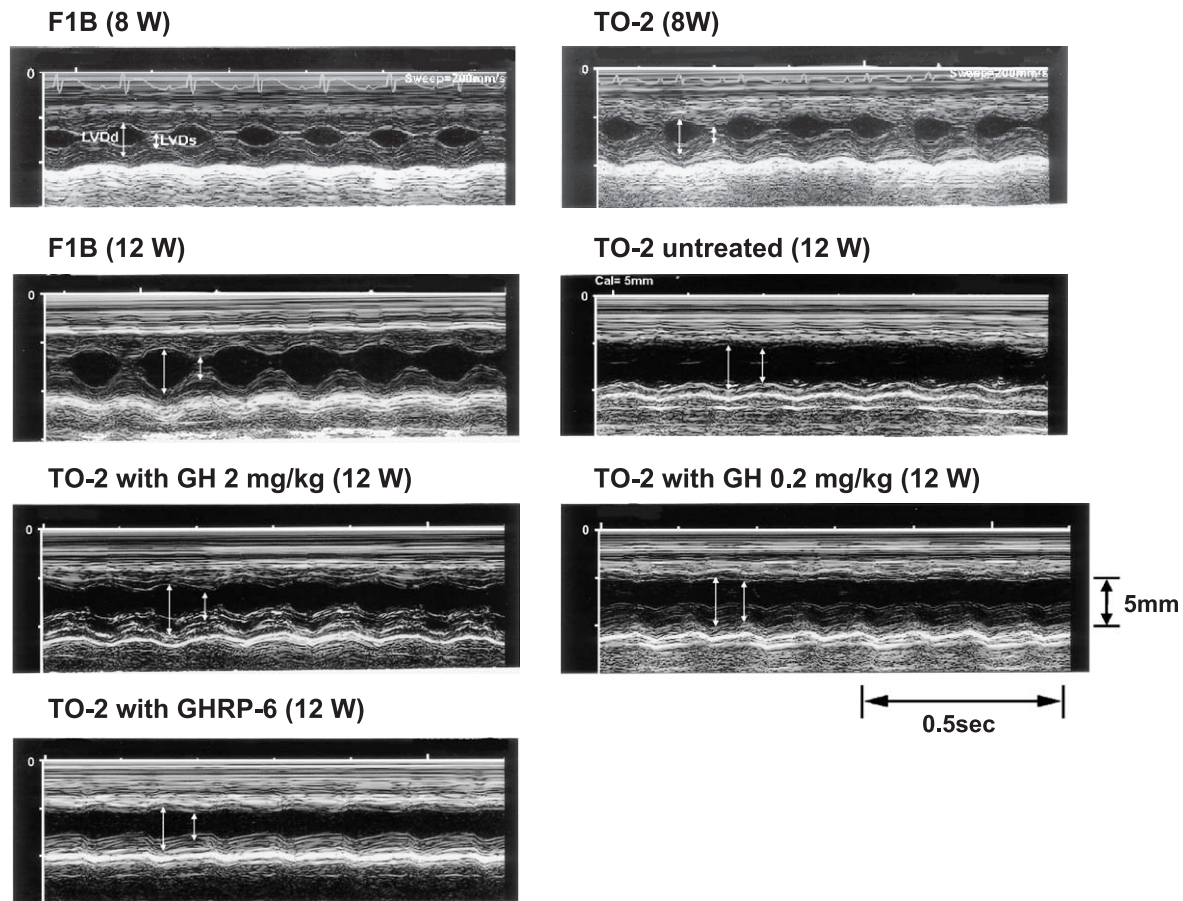


Fig. 2. Representative LV M-mode echocardiograms for F1B and TO-2 hamsters at 8 weeks of age, F1B and untreated TO-2 hamsters at 12 weeks of age, and TO-2 hamsters treated with either GH (0.2 or 2 mg/(kg day)) or GHRP-6 at 12 weeks of age.

hamsters treated with GH at a low dose (0.2 mg/kg day) exhibited a smaller increase ($+25.4 \pm 0.9$ g) in body weight compared with that apparent at the higher dose. The LV weight/body weight ratio was similar in all groups of animals at 12 weeks of age.

3.2. Physiology

Representative echocardiograms are shown in Fig. 2, and physiological data are summarized in Table 2 and Fig. 3.

Heart rates were similar in all five groups of animals at both 8 and 12 weeks of age. At 8 weeks, LVFS, LVPWT, and IVST in TO-2 hamsters were already significantly smaller than those in controls, although LVDd was similar in the two lines. LV function decreased markedly in untreated TO-2 hamsters between 8 and 12 weeks; LVDd decreased significantly ($P < 0.01$) from 4.0 ± 0.1 to 5.0 ± 0.1 mm (Fig. 3A) and LVFS decreased significantly ($P < 0.001$) from $42.0 \pm 2.6\%$ to $25.4 \pm 1.8\%$ (Fig. 3B). LV function in F1B controls remained unchanged during the observation

Table 2
Heart rate and echocardiographic data for hamsters at 8 and 12 weeks of age

Group	Heart rate (bpm)		LVDs (mm)		IVS thickness (mm)		LVPW thickness (mm)		LVDd/Mean LVWT	
	8 weeks	12 weeks	8 weeks	12 weeks	8 weeks	12 weeks	8 weeks	12 weeks	8 weeks	12 weeks
F1B	388 ± 10	358 ± 13	1.9 ± 0.1	2.1 ± 0.1	0.93 ± 0.02	1.09 ± 0.04	0.94 ± 0.02	1.07 ± 0.05	4.1 ± 0.2	3.9 ± 0.2
TO-2 untreated	351 ± 19	349 ± 16	2.3 ± 0.1^a	3.7 ± 0.2^a	0.80 ± 0.03^a	0.70 ± 0.04^a	0.77 ± 0.03^a	0.71 ± 0.02^a	5.2 ± 0.2^a	7.1 ± 0.3^a
TO-2 with GH (0.2 mg/kg day)	378 ± 25	374 ± 16	2.4 ± 0.1^a	3.7 ± 0.3^a	0.79 ± 0.04^a	0.79 ± 0.04^a	0.78 ± 0.03^a	0.80 ± 0.04^a	5.2 ± 0.4^a	6.3 ± 0.4^a
TO-2 with GH (2.0 mg/kg day)	360 ± 8	355 ± 15	2.4 ± 0.2^a	3.6 ± 0.2^a	0.75 ± 0.02^a	$0.84 \pm 0.04^{a,b}$	0.78 ± 0.03^a	$0.85 \pm 0.03^{a,b}$	5.2 ± 0.2^a	6.4 ± 0.4^a
TO-2 with GHRP-6	374 ± 10	349 ± 18	2.3 ± 0.1^a	$3.1 \pm 0.1^{a,b}$	0.79 ± 0.01^a	0.75 ± 0.02^a	0.79 ± 0.01^a	0.75 ± 0.02^a	5.1 ± 0.2^a	$6.3 \pm 0.2^{a,b}$

Data are means \pm S.E.M. Mean LVWT (mean LV wall thickness) = (IVST + LVPWT)/2.

^a $P < 0.05$ vs. F1B.

^b $P < 0.05$ vs. TO-2 untreated.

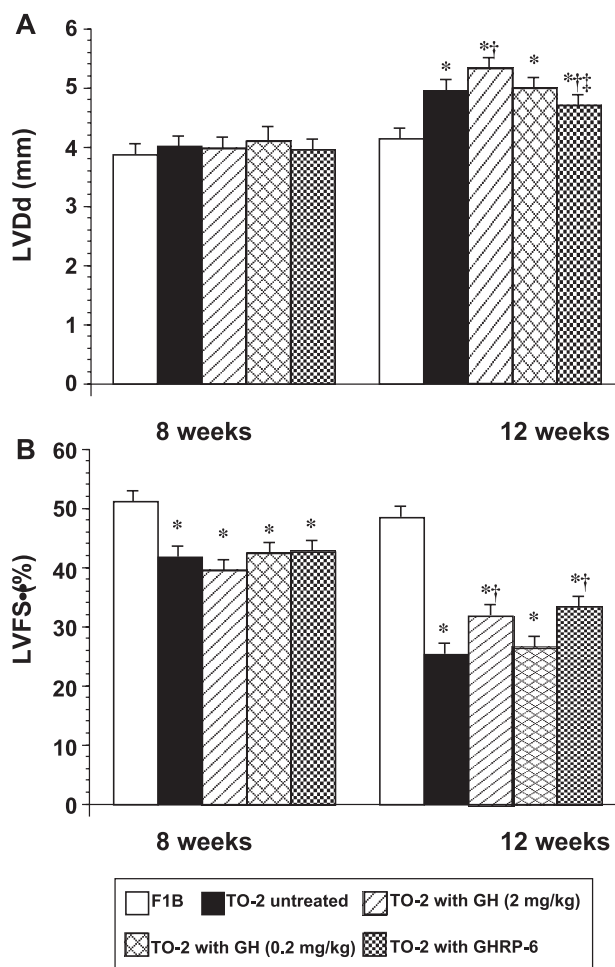


Fig. 3. Changes in LVDD (A) and LVFS (B) in animals of the various treatment groups. Data are means \pm S.E.M. * $P < 0.05$ vs. F1B hamsters of the same age, † $P < 0.05$ vs. untreated TO-2 hamsters of the same age, and ‡ $P < 0.05$ vs. high-dose GH-treated TO-2 hamsters of the same age.

period. LVFS in TO-2 hamsters treated with either GHRP-6 ($33.4 \pm 2.0\%$) or GH at a high dose ($32.0 \pm 2.1\%$) was greater than that in untreated TO-2 hamsters at 12 weeks of age. LVPWT and IVST in TO-2 hamsters treated with GH at a high dose were significantly greater than those in untreated or in GHRP-6-treated animals (Table 2). LVDD was significantly smaller in TO-2 hamsters treated with GHRP-6 (4.7 ± 0.1 mm) than in those treated with GH at a high dose (5.3 ± 0.1 mm). Although GH at a low dose induced a small increase in LV wall thickness, there was no improvement in LV function. The ratio of LVDD to mean LV wall thickness was remarkably increased in untreated TO-2 hamsters, while it was unchanged in F1B hamsters. The ratio of LVDD to mean LV thickness in TO-2 hamsters treated with GHRP-6 was significantly ($P < 0.05$) less than that in untreated hamsters. These ratios in TO-2 hamsters

treated with GH at both high and low doses also tended to be less than that in untreated TO-2 hamsters, but these changes were not significant.

3.3. Plasma GH and IGF-1

The plasma GH concentration in untreated TO-2 hamsters was significantly smaller than that in F1B controls (1.14 ± 0.12 vs. 2.95 ± 0.60 ng/ml, $P < 0.05$) but was similar to that in TO-2 hamsters treated with GHRP-6 (0.88 ± 0.33 ng/ml) at 12 weeks. The plasma IGF-1 concentration in untreated TO-2 hamsters was significantly smaller than that in controls (76 ± 14 vs. 291 ± 38 ng/ml, $P < 0.01$) but was similar to that in TO-2 hamsters treated with GHRP-6 (98 ± 26 ng/ml). The plasma IGF-1 level in TO-2 hamsters treated with GH at a high dose (186 ± 11 ng/ml) was significantly ($P < 0.05$) greater than that in untreated TO-2 hamsters.

3.4. Pathology

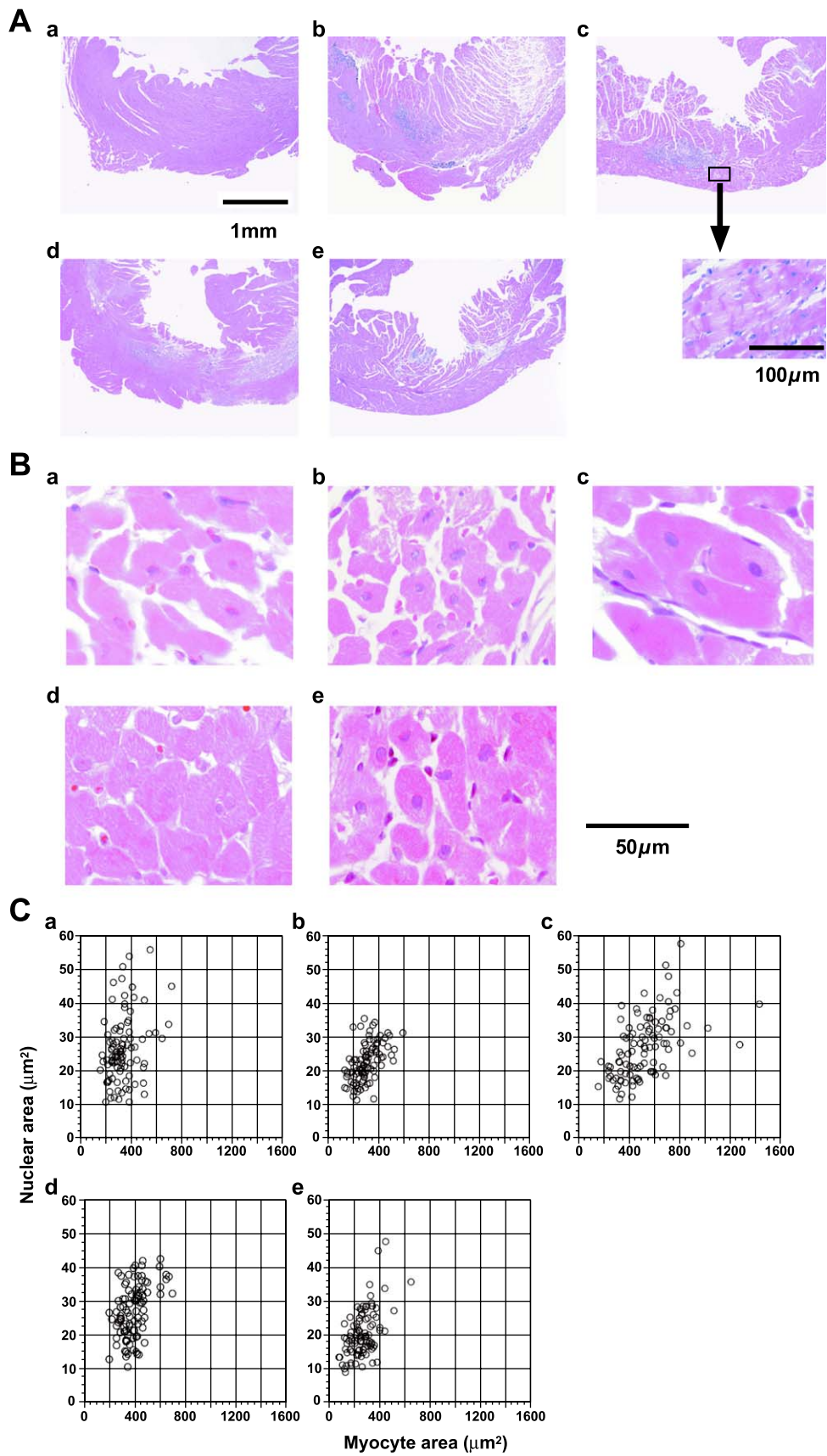
Multifocal areas of mononuclear cell infiltrates, calcification, and fibrosis were apparent in the hearts of all TO-2 hamsters, regardless of treatment, at 12 weeks of age (Fig. 4A).

These lesions were detected throughout both left and right ventricles. A contraction band was also detected in the myocardium in untreated, GH-treated (both high and low doses), and GHRP-6-treated TO-2 hamsters. However, contraction band necrosis and coagulation necrosis plus scarring were less prominent in GHRP-6-treated TO-2 hamsters compared with those in the other groups of TO-2 hamsters. The extents of contraction band necrosis and coagulation necrosis plus scarring in either high or low dose of GH-treated TO-2 hamsters were similar to those in untreated TO-2 hamsters. The LV myocyte cross-sectional area in TO-2 hamsters treated with GH at a high dose ($517.4 \pm 20.7 \mu\text{m}^2$) was significantly greater ($P < 0.05$) than those in F1B hamsters ($342.3 \pm 10.9 \mu\text{m}^2$), untreated TO-2 hamsters ($313.2 \pm 9.8 \mu\text{m}^2$), and TO-2 hamsters treated with GHRP-6 ($270.2 \pm 9.6 \mu\text{m}^2$) (Fig. 4B and C). GH at a low dose also induced a significant but small increase in the LV myocyte cross-sectional area ($402.9 \pm 10.1 \mu\text{m}^2$).

3.5. Abundance of δ -sarcoglycan, ANP, and SERCA2a mRNAs

The abundance of δ -sarcoglycan mRNA in the left ventricle of TO-2 hamsters at 12 weeks of age was $< 10\%$ of that of F1B hamsters of the same age, with no significant effect of treatment with GH or GHRP-6 being apparent (Fig. 5A). In contrast, the amount of ANP mRNA in

Fig. 4. Representative sections of LV myocardium at low magnification (A), representative sections of LV myocardium at high magnification (B) and scatter graphs of nuclear area vs. myocyte area (C) at 12 weeks of age. Sections in (A) are from an F1B hamster (a) and TO-2 hamsters that were either untreated (b), treated with GH at 2 mg/(kg day) (c) or 0.2 mg/(kg day) (d), or treated with GHRP-6 (e).



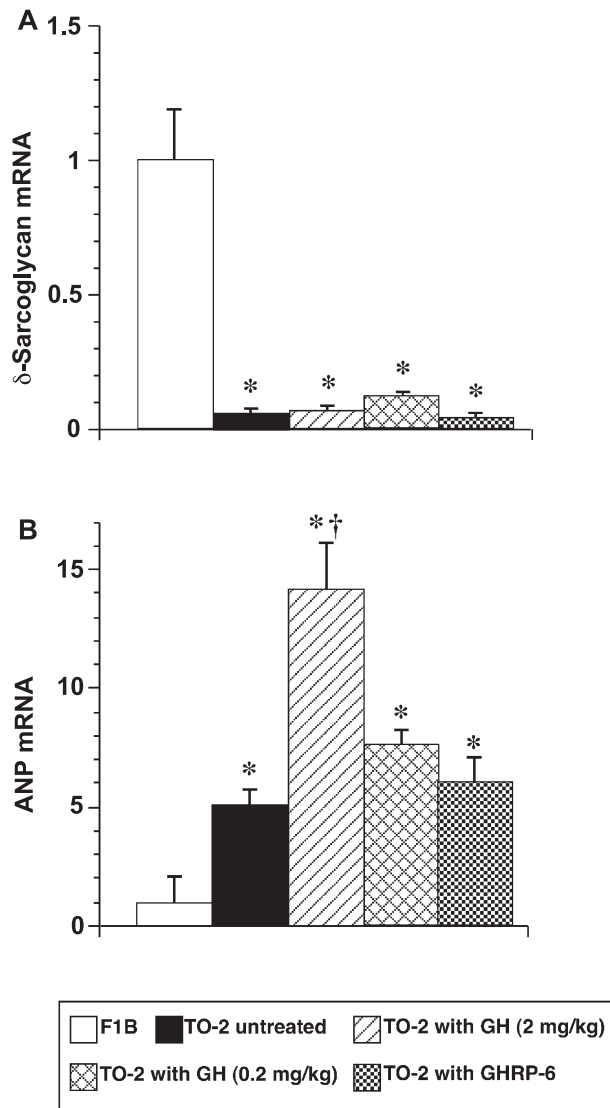


Fig. 5. Abundance of δ -sarcoglycan (A) and ANP (B) mRNAs in the left ventricle of hamsters in the various treatment groups at 12 weeks of age. Transcript abundance was corrected for the amount of GAPDH mRNA and is expressed relative to the values for F1B hamsters. Data are means \pm S.E.M. * $P < 0.05$ vs. F1B hamsters and † $P < 0.05$ vs. untreated TO-2 hamsters.

untreated TO-2 hamsters was about five times that apparent in F1B hamsters (Fig. 5B). Furthermore, the abundance of ANP mRNA in TO-2 hamsters treated with high-dose GH was ~3.1 times greater than that in untreated TO-2 hamsters. Treatment with GHRP-6 or low-dose GH did not increase the amount of ANP mRNA in TO-2 hamsters. The abundance of SERCA2a mRNA in untreated TO-2 hamsters ($76.0 \pm 27.8\%$) was similar to that in F1B controls (100%). Neither GHRP-6 nor GH significantly affected the amount of SERCA2a mRNA in TO-2 hamsters.

3.6. Blood pressure measurement

Systolic blood pressure in TO-2 hamsters treated with GHRP-6 (103 ± 11 mm Hg) was similar to that in F1B

controls (106 ± 7 mm Hg) and tended to be higher than that in untreated TO-2 hamsters (91 ± 9 mm Hg), but the difference was not significant.

4. Discussion

GHRPs, a family of small synthetic peptides, and their nonpeptide derivatives induce the release of GH both in vitro and in vivo through a direct action on the pituitary gland. GHRP-6 is a hexapeptide that stimulates GH release in a dose-dependent manner [21–22]. It has remained unclear, however, whether GHRPs exert cardioprotective effects in progressive LV dysfunction. We have now shown that GHRP-6 improved LV systolic performance and attenuated LV dilation in TO-2 hamsters. These beneficial effects of GHRP-6 were not accompanied by systemic or myocardial growth, both of which are characteristic of activation of the GH-IGF-1 axis.

4.1. Systemic and myocardial growth

Systemic growth and the plasma concentration of GH were significantly reduced in TO-2 hamsters during development of LV dysfunction compared with F1B controls. Treatment with GH at a high dose normalized the body weight of TO-2 hamsters, suggesting that the impaired systemic growth in these animals might result from GH deficiency. GH treatment at a high dose also increased the LV weight of TO-2 hamsters to a value similar to that of controls. The LV myocyte cross-sectional area of TO-2 hamsters treated with GH at a high dose was significantly greater than that of controls. Moreover, GH treatment markedly increased the amount of ANP mRNA in the LV myocardium. The atrial concentration of ANP mRNA is increased in GH transgenic mice [23]. The importance of ventricular tissue, relative to that of atrial tissue, as a source of ANP, has been thought to increase with the severity of heart failure. Therefore, the marked increase in the amount of ANP mRNA in the left ventricle of GH-treated TO-2 hamsters is likely due to a combination of the direct action of GH on myocytes and the effect of LV myocyte stretch during the progression of LV dysfunction. In contrast, GHRP-6 at the dose in the current study had no effect on systemic or myocardial growth in TO-2 hamsters. Furthermore, plasma GH and IGF-1 levels in TO-2 hamsters were not affected by GHRP-6. Although a low dose of GH had a smaller effect on systemic growth than did the higher dose, it did not exhibit favorable effects on LV function despite an associated increase in LV myocardial growth as assessed by the LV myocyte cross-sectional area and LV wall thickness. GH was shown to induce hypertrophy directly, in the absence of IGF-1, in isolated rat neonatal cardiomyocytes expressing GH receptors [24]. Together, these observations indicate that the beneficial cardiac effects of GHRP-6 are independent of the GH-IGF-1 axis.

4.2. Cardioprotective effects of GHRP-6

Treatment with GHRP-6 improved LV pump function and attenuated LV dilation in TO-2 hamsters during the period of rapid development of LV dysfunction. Both GHRP-6 and GH at a high dose preserved LV systolic performance to similar extents. The ratio of LVDd/mean LV wall thickness as a parameter of LV remodeling was also decreased similarly in TO-2 hamsters with GHRP-6 and GH at a high dose, compared with that in untreated TO-2 hamsters. However, LV dimensions in TO-2 hamsters treated with GH at a high dose were significantly greater than those in TO-2 hamsters treated with GHRP-6. On the other hand, LV wall thickness in TO-2 hamsters treated with GH at a high dose was significantly greater than that in TO-2 hamsters treated with GHRP-6. These results strongly suggested that these drug treatments should have different underlying mechanisms to improve LV function and remodeling.

GH improves contractility and increases the abundance of SERCA2 mRNA in rats with postinfarction heart failure [10]. However, in the present study, the abundance of SERCA2a mRNA in TO-2 hamsters during the progression of LV dysfunction was similar to that in controls. Similarly, the density of Ca^{2+} -releasing channels in the sarcoplasmic reticulum was previously shown to be decreased in cardiomyopathic hamsters only after the development of heart failure [11]. Changes in Ca^{2+} uptake or pumping activity in the sarcoplasmic reticulum of cardiac myocytes might thus not play an important role in the initial development of LV dysfunction, but rather may contribute to the progression to heart failure.

Evidence suggests that GHRPs act directly on the myocardium [13–17]. GHRP receptors have thus been detected in cardiac membranes of various mammalian species including hamsters [13]. A GH secretagogue was also shown to improve LV pump function in a porcine pacing-induced model of heart failure [17]; treatment resulted in an increase in the plasma concentration of IGF-1 and LV myocyte cross-sectional areas. The difference between their results and ours regarding systemic and myocardial growth may be species difference and/or the dosages used. Nonetheless, the progressive LV dilation normally apparent in the porcine model was also substantially reduced by treatment with the GH secretagogue, suggesting that both GH-dependent and -independent actions contributed to the cardioprotective effects of this drug.

Hexarelin, a GHRP derivative, preserves cardiac performance after ischemia-reperfusion injury in rats [16,25]. Given that coronary vascular dysfunction may play a major role in progressive cardiac myolysis in animals deficient in δ -sarcoglycan [4], a beneficial effect of GHRP-6 on the coronary vasculature might contribute to the preservation of LV function. Consistent with this notion, treatment with GHRP-6 reduced the size of calcified, myolytic, and contraction band areas in the myocardium of TO-2 hamsters.

Hexarelin was recently shown to bind to H9c2 cardiomyocytes and to stimulate thymidine incorporation by these cells in a dose-dependent and specific manner [26]. Furthermore, hexarelin and des-acyl ghrelin, which is devoid of GH-releasing activity, may act as survival factors in the cardiovascular system by binding to an unidentified receptor that is distinct from GHSR-1a present in the pituitary [27]. Although evidence suggests that synthetic GHRPs exert GH-independent effects in the cardiovascular system, the mechanism of action of these potential cardiovascular therapeutic agents remains to be characterized. Interestingly, ghrelin, another GHRP, exhibited cardioprotective effects and reduced LV afterload in patients with chronic heart failure [28]. However, systolic blood pressure in TO-2 hamsters treated with GHRP-6 was not different from that in untreated TO-2 hamsters. The beneficial cardiac effects of GHRP-6 might thus be attributable not to LV afterload reduction but to other mechanisms such as protection against ischemia or improvement of myocardial contractility per se.

4.3. Clinical significance

In addition to the unclear clinical efficacy of GH treatment for heart failure, GH is potentially diabetogenic, may promote neoplastic growth, and is limited with regard to routes of administration. In contrast, GHRP family of agonists can be administered orally and that they exert a direct action on the myocardium without substantial systemic growth effects. Since mutations of δ -sarcoglycan genes have been reported to be only marginally implicated in a large number of patients with familial and sporadic DCM [29], our results should be reconfirmed in other DCM animal models as well. Nonetheless, these drugs may offer a promising alternative for treatment of heart failure.

5. Conclusion

Treatment with GHRP-6 can improve LV systolic performance and attenuate LV dilation during the progressive development of LV dysfunction. These beneficial effects appear independent of the GH-IGF-1 axis and of systemic or myocardial growth. Administration of GHRPs is thus a potential new approach to the treatment of heart failure.

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