Cardioprotective effects of growth hormone-releasing hormone agonist after myocardial infarction

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Contributed by Andrew V. Schally, December 7, 2009 (sent for review October 30, 2009)

Whether the growth hormone (GH)/insulin-like growth factor 1 (IGF-1) axis exerts cardioprotective effects remains controversial; and the underlying mechanism(s) for such actions are unclear. Here we tested the hypothesis that growth hormone-releasing hormone (GHRH) directly activates cellular reparative mechanisms within the injured heart, in a GH/IGF-1 independent fashion. After experimental myocardial infarction (MI), rats were randomly assigned to receive, during a 4-week period, either placebo (n = 14), rat recombinant GH (n = 8) or JI-38 (n = 8; 50 μg/kg per day), a potent GHRH agonist. JI-38 did not elevate serum levels of GH or IGF-1, but it markedly attenuated the degree of cardiac functional decline and remodeling after injury. In contrast, GH administration markedly elevated body weight, heart weight, and circulating GH and IGF-1, but it did not offset the decline in cardiac structure and function. Whereas both JI-38 and GH augmented levels of cardiac precursor cell proliferation, only JI-38 increased antiapoptotic gene expression. The receptor for GHRH was detectable on myocytes, supporting direct activation of cardiac signal transduction. Collectively, these findings demonstrate that within the heart, GHRH agonists can activate cardiac repair after MI, suggesting the existence of a potential signaling pathway based on GHRH in the heart. The phenotypic profile of the response to a potent GHRH agonist has therapeutic implications.

cardiac stem cells | apoptosis | remodeling | heart failure

Congestive heart failure remains a leading cause of morbidity and mortality in developed countries. Despite major therapeutic advances, current therapies fail to fully reverse heart failure and/or left ventricular (LV) dysfunction. One major therapeutic avenue is that of cytokine and/or hormonal signaling pathways, and in this regard, various experimental and clinical studies have suggested an important role for the growth hormone (GH)/insulin-like growth factor 1 (IGF-1) axis in the regulation of cardiac growth and function (1, 2). Moreover, several clinical studies have tested the impact of GH replacement on the failing human heart, with controversial results (3, 4).

In addition to GH itself and IGF-1, GH-releasing peptides such as ghrelin and synthetic GH secretagogues (GHS) are also suggested to have cardiac effects (5–8), and growth hormone-releasing hormone (GHRH) mRNA is detected in peripheral tissues, including the heart (9, 10), consistent with widespread biologic signaling potential beyond the hypothalamic–pituitary axis.

Recently, Granata et al. (10) reported that rat GHRH (1-44) promoted survival of cardiomyocytes in vitro and protected rat hearts from ischemia–reperfusion injury. The detection of the GHRH receptor (GHRHR) on the cardiomyocyte sarcolemmal membrane supports the view that GHRH may elicit direct signal transduction within the heart, independent of the GH/IGF-1 axis per se (10). Ghrelin and other GHS may have pharmacologic potential (10) but also have pleiotropic actions with a high possibility of unexpected side effects and potentially serious disadvantages. Thus, the administration of GHRH offers a potentially highly physiologic approach based on direct action without known side effects or the necessity to activate the GH/IGF-1 axis (11, 12). Furthermore, synthetic GHRH agonists, such as JI-38 (GHRH-A) are more potent and longer-acting agents than native GHRH (13). Here we tested the hypothesis that GHRH-A has a favorable cardiac effect, attenuating infarct size and the progressive decrease of cardiac structure and function following MI. In addition, we investigated the conjecture that GHRH directly activates signaling within the heart (10) and exerts effects on cellular reparative pathways.

Results

As depicted in Fig. S1*A*, baseline body weight (BW) was similar in all groups. In the placebo group, MI significantly reduced BW from 225 ± 4 to 208 ± 3 g ($P < 0.05$), an effect that was fully prevented by administration of GHRH-A (from 231 ± 5 to 225 ± 3 g). Conversely, rat recombinant GH (rrGH) increased BW from 217 ± 4 to 256 ± 3 g ($P < 0.01$). Heart weight (HW) was increased in concert by rrGH (850 ± 38 mg) in comparison with placebo (674 ± 14 mg) or GHRH-A (695 ± 26 mg) ($P < 0.0001$ for both; Fig. S1*B*). Accordingly, the HW/BW ratios (Fig. S1*C*) were similar in all groups.

GH and IGF-1 Levels. To test the impact of rrGH and GHRH-A on the GH-IGF-1 axis, we measured circulating levels of these hormones (Fig. S2 *A* and *B*). Whereas treatment with GHRH-A did not increase serum levels of either GH or IGF-1 relative to placebo, treatment with rrGH led to marked increases in GH (679 ± 196 vs. 64 ± 23 ng/mL; $P < 0.01$) and IGF-1 (1,052 ± 91 vs. 553 ± 46 ng/mL; $P < 0.01$) compared with placebo.

Echocardiographic Measurements. Next, we measured the impact of GHRH-A and rrGH on cardiac structure and function after MI. Baseline echocardiography documented similar parameters of LV dimension and function in all groups (Fig. 1 *A–D* and Table S1). As expected, MI led to a time-dependent increase in LV chamber dimensions and a reduction in ejection fraction (EF) and fraction shortening (FS). Treatment with GHRH-A, but not with rrGH, attenuated the MI-induced increase in LV end-systolic diameter. In addition, the reduction in EF due to MI was ameliorated by GHRH-A (47 ± 4% vs. 38 ± 3%; $P < 0.05$)

Author contributions: R.M.K.-T., N.L.B., J.L.V., A.V.S., and J.M.H. designed research; R.M.K.-T., K.T., L.M.T., A.V.T., G.L., R.D., F.R., and A.K. performed research; J.L.V. and A.V.S. contributed new reagents/analytic tools; R.M.K.-T., K.T., L.M.T., A.V.T., G.L., M.H., Y.S., F.R., A.K., Q.H., and J.M.H. analyzed data; and R.M.K.-T., N.L.B., A.V.S., and J.M.H. wrote the paper.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0914138107/DCSupplemental.

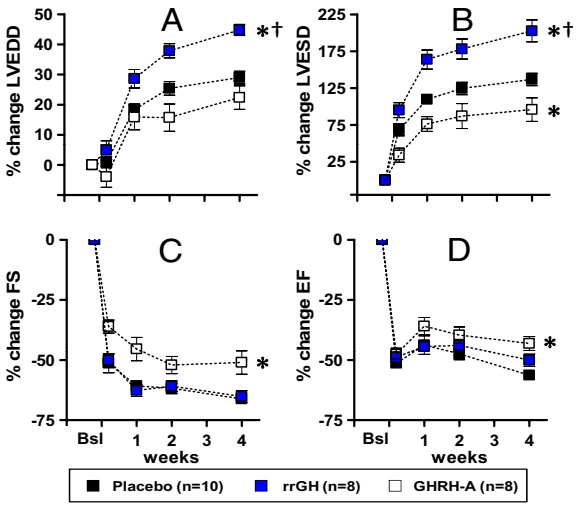

Fig. 1. Impact of rrGH and GHRH-A on LV chamber size and function. Changes over time in (*A*) LV end-diastolic diameter (LVEDD) (*$P < 0.05$ vs. placebo; †$P < 0.01$ vs.GHRH-A), (*B*) LV end-systolic diameter (LVESD) (*$P < 0.01$ vs. placebo; †$P < 0.001$ vs.GHRH-A), (*C*) EF (*$P < 0.05$ vs. placebo), and (*D*) FS (*$P < 0.05$ vs. placebo vs.GHRH-A).

but not by rrGH [44 ± 2%; P = nonsignificant (NS)], both compared with placebo. Similarly, a reduction in FS from 55 ± 1% to 18.5 ± 0.9% ($P < 0.05$) due to MI was improved in the GHRH-A (28.7 ± 3.3%; $P < 0.05$) but not in the rrGH group (20.3 ± 1.3%; P = NS), both compared with placebo.

Hemodynamic Measurements. To directly assess the impact of these interventions on cardiac contractile performance and to separate the effects of GHRH-A on cardiac contractility and cardiovascular loading conditions, we performed in vivo hemodynamic analysis (Fig. 2 and Table 1). Treatment with GHRH-A but not rrGH caused an increase in both stroke volume and cardiac output relative to placebo. This increase in cardiac performance was attributed, at least partially, to a reduction in ventricular afterload, measured as arterial elastance. Interestingly, arterial elastance was actually increased with rrGH. LV end-systolic and end-diastolic pressures were similar in all groups. Consistent with the echocardiographic data, EF was higher in the GHRH-A than in the placebo or rrGH groups. Similarly, stroke work was increased the GHRH-A group vs. the placebo or rrGH groups. With regard to myocardial contractility, the peak rate of pressure rise (dP/dt_{max}) was increased in the GHRH-A group in comparison with the placebo and rrGH groups, whereas there were no significant differences in the peak rate of pressure decline (dP/dt_{min}) and the relaxation time constant; however, treatment with GHRH-A trended to increase preload-recruitable stroke work and the relationship between dP/dt_{max} and end-diastolic volume. Conversely, the ratio between arterial elastance and end-systolic elastance trended to be lower in the GHRH-A group.

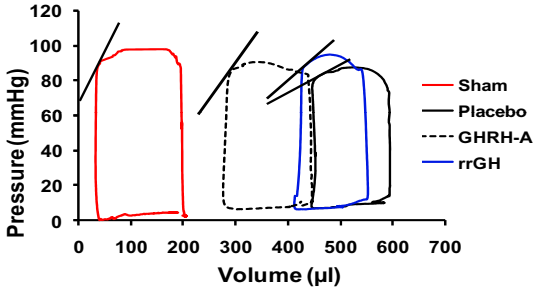

Fig. 2. Representative pressure-volume loops and corresponding end-systolic pressure volume relationships (ESPVR) from Sham (non-infarcted), MI Placebo, MI rrGH, and MI GHRH-A groups. As depicted, MI causes a rightward shift in the loop to increased volumes and depression in the slope of the ESPVR. GHRH-A but not rrGH reduces the volume and restores the slope of ESPVR toward normal.

Histopathology. MI size (Fig. 3*A*) in rrGH and placebo groups was similar (45 ± 2% vs. 41 ± 1%, respectively), whereas GHRH-A rats had reduced MI size (36 ± 3%; $P < 0.05$ vs. placebo and rrGH). The reduced infarct burden was also reflected in the percentage of ventricular fibrosis (Fig. 3*B*), which was strikingly reduced with GHRH-A (20 ± 1%) in comparison with placebo (29 ± 1%) and rrGH (27 ± 1%; $P < 0.01$ for both), whereas capillary density (Fig. S3*A*) was higher in rrGH (0.02 ± 0.002/mm^2) than in the placebo or GHRH-A groups (0.01 ± 0.001/mm^2 and 0.006 ± 0.001/mm^2, respectively; $P < 0.001$ for both). The width of myocytes was not different among groups (Fig. S3*B*).

GHRH Receptor. The presence or absence of GHRHR was detected in frozen sections of pituitary, heart, and skeletal muscle under fluorescent and confocal microscopy (Fig. 4*A* and Table S2), and the intensity of the fluorescence of the GHRHR was measured in paraffin tissues of treated and nontreated rats (Fig. S4). The expression of GHRHR was confirmed by Western blotting (Fig. 4 *B* and *C*), and the GHRHR was also detected within cardiomyocytes (Fig. 4*D*). In addition, using real-time quantitative PCR, we demonstrated the presence of mRNA for GHRHR in rat heart (Fig. S5 *A* and *B* and Tables S3 and S4), and the radioligand binding studies revealed that the ischemic rat heart samples showed specific high-affinity binding sites for GHRH antagonist, JV-1-42 ligand, characterized by a K_d of 0.86 nM and a B_{max} of 51.28 fmol/mg protein.

Impact on Cellular Division and Proliferation. Immunostaining for Ki67$^+$ myocytes and nonmyocytes revealed no differences between the border and infarct zones; however, in the remote zone, the expression of Ki67$^+$ cells was higher in the rrGH group relative to the placebo and GHRH-A groups ($P < 0.01$ for both) (Fig. 5 *A* and *B*). Next we measured the proliferation of endogenous c-kit$^+$ cardiac precursor cells. Importantly, the expression of c-kit$^+$ cells (mast cells excluded) per cubic millimeter was higher ($P = 0.02$) in both treated groups than in placebo (Fig. 6).

TUNEL staining (Fig. 5*C*) did not show differences between groups. On the other hand, real-time quantitative PCR revealed that the expression of an antiapoptotic gene (Bcl2) was up-regulated in GHRH-A ($P = 0.07$), whereas the proapoptotic gene (Bax) trended to be down-regulated in the same group ($P = 0.207$). Accordingly, the ratio between Bax and Bcl2 expression was significantly reduced in the GHRH-A group in comparison with placebo- or rrGH-treated rats ($P = 0.03$).

Discussion

The main finding of the present study is that GHRH-A has a cardioprotective role in vivo after acute MI. Animals receiving GHRH-A had improved cardiac structure and function and reduced infarct size. In addition, cardiac fibrosis, which is one of the main biologic determinants of poor prognosis in heart failure and strongly associated with severe arrhythmias, diastolic dysfunction, and sudden death (14), was markedly reduced in the GHRH-A group but not in the rrGH group. The cardiac effects of GHRH agonist seem to be direct, not involving the GH/IGF-1 axis, because the circulating levels of these hormones were not increased by GHRH-A treatment.

Table 1. Hemodynamic parameters and indices of systolic and diastolic function derived from left-ventricular pressure–volume relationships

Parameter	Placebo (n = 8)	rrGH (n = 6)	GHRH-A (n = 8)
Heart rate (bpm)	256 ± 6.6	247 ± 6.8	270 ± 17
Integrated performance			
EF (%)	29.8 ± 1.4	26.2 ± 1.7	36.9 ± 2.6*†
SW (mmHg × μL)	9,424 ± 1158	6,920 ± 790	12,000 ± 866*†
SV (μL)	131 ± 20	98 ± 13	161 ± 12‡
CO (mL/min)	30.5 ± 5.0	22.5 ± 3.4	40.1 ± 3.1*†
Ea/Ees	4.1 ± 0.7	4.4 ± 0.6	3.2 ± 0.3
Afterload			
LVESP (mmHg)	85 ± 1.8	91 ± 2.3	83 ± 1.1
Ea (mmHg/μL)	0.8 ± 0.1	1.1 ± 0.2	0.5 ± 0.004*†
Preload			
LVEDP (mmHg)	9.8 ± 0.6	10 ± 1.8	8 ± 0.5
LVEDV (μL)	413 ± 56	351 ± 38	421 ± 26
Contractility			
dP/dt_{max} (mmHg/s)	6,198 ± 194	6,243 ± 313	6,986 ± 163‡
dP/dt_{max}-EDV (mmHg/s per μL)	22.3 ± 8.1	17.5 ± 4.3	42.5 ± 12.9
Ees (mmHg/μL)	0.26 ± 0.09	0.33 ± 0.12	0.19 ± 0.02
PRSW (mmHg)	45 ± 3.6	48 ± 5.0	53 ± 2.1
Lusitropy			
dP/dt_{min} (mmHg/s)	3,986 ± 177	4,028 ± 334	3,989 ± 106
Tau-G (ms)	16.8 ± 0.9	19.9 ± 0.4	16.9 ± 0.5

All values represent mean ± SEM. SW, stroke work; SV, stroke volume; CO, cardiac output; Ea/Ees, ratio between arterial elastance (Ea) and end-systolic elastance (Ees); LVESP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; dP/dt_{max}, peak rate of the pressure rise; dP/dt_{max}-EDV, relationship between dP/dt_{max} and end-diastolic volume; PRSW, preload recruitable stroke work; dP/dt_{min}, peak rate of pressure decline; Tau-G, relaxation time constant calculated by Glantz method.

*$P < 0.05$ vs. placebo.

†$P < 0.01$ vs. rrGH.

‡$P < 0.05$ vs. placebo and rrGH.

The present findings can be viewed in the context of previous evaluations of the GH/IGF-1 axis that have yielded variable results. The inconsistent and contradictory effects of GH or IGF-1 administration on experimental post-MI models have been shown to be dependent on the timing of the treatment, the stage of the disease at treatment initiation, and different dosing regimens (8) and might be related to the heterogeneous origin of treatment (15). In most studies, early treatment with recombinant human GH improves cardiac function and reduces LV remodeling (5, 16, 17), whereas other studies did not show beneficial effects (15, 18). Similarly, treatment with rrGH did not show beneficial effect in rats with large MI (19). Conversely, in rats all studies starting late after MI showed improvement on cardiac function (20–22). Importantly, all treatments with recombinant human GH in rats had a clear limitation, due perhaps to the production of anti-GH antibodies after 2 weeks of treatment (23). Therefore, the long-term effects (either beneficial or deleterious) remain unknown in these models (8).

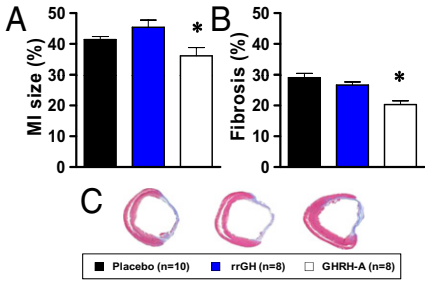

Fig. 3. Impact of rrGH and GHRH-A on myocardial infarct burden. Bar graphs showing (*A*) circumferential infarct size and (*B*) percentage of fibrosis. (*C*) Representative Masson's trichrome-stained histologic sections for infarct size measurement. The circumferential infarct size (MI%) was significantly attenuated in the GHRH-A group (*P = 0.011 vs. placebo and rrGH). Similarly, the percentage of fibrosis was reduced in the GHRH-A group (*P = 0.0002 vs. placebo and rrGH).

Our findings demonstrate that rrGH markedly increases BW, HW, and circulating levels of GH and IGF-1 but does not improve cardiac function or prevent remodeling; on the contrary, rats treated with rrGH exhibited larger chambers and worse EF. These results are in agreement with a study that showed that GH caused adverse effects on the process of LV remodeling (18).

An alternative approach for increasing systemic levels of GH is the administration of GHS such as ghrelin (24) or a synthetic GHS peptides such as hexarelin (20, 25). Nagaya et al. (24) showed that ghrelin improved LV function and attenuated cardiac remodeling in a chronic heart failure model; however, these results were attributed to both GH/IGF-1–dependent and GH-independent vasodilatory effects of ghrelin. Similarly, Tivesten et al. (6) showed that hexarelin increased stroke volume and reduced total peripheral resistance. In contrast, Shen et al. (19) reported increased survival rate but no hemodynamic beneficial effect of GH-releasing peptide in dogs subjected to transient coronary occlusion, suggesting that these effects were mediated by GHS receptors rather than through the GH/IGF-1 axis (i.e., by a GH independent pathway). To date, only one study in vitro has shown cardioprotective and a direct effect of GHRH (10). In that study, GHRH cardioprotection was demonstrated in isolated rat hearts subjected to ischemia–reperfusion injury, whereas in our work, cardiac function was assessed by echocardiography and in vivo closed-chest LV catheterization in rats subjected to a permanent occlusion.

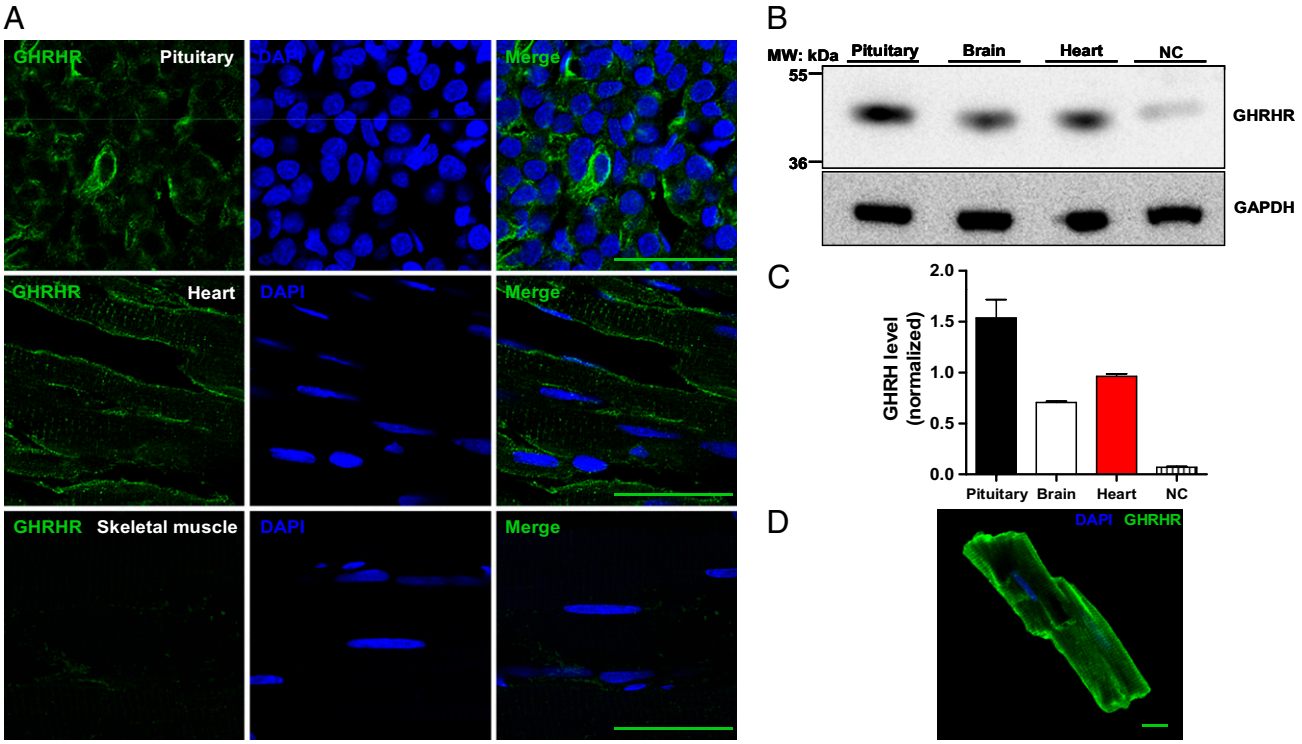

Fig. 4. Presence of GHRHR in the heart and cardiomyocytes. (*A*) Cryosections of pituitary (*Top*), heart (*Middle*), and skeletal muscle (*Bottom*) incubated with GHRHR (green). (Scale bars, 50 μm.) GHRHR specificity is demonstrated by intense immunohistochemical reactivity in pituitary (positive control) and heart; negative results are observed in skeletal muscle (negative control). (*B*) Western blotting detected a 47-kDa protein corresponding to GHRHR. Molecular weight markers are indicated on the left side of the panel and NC corresponds to negative control. (*C*) GHRHR protein abundance measured by Western blotting analysis and expressed in arbitrary units. (*D*) Representative confocal micrograph image showing the presence of GHRHR (green) on cardiomyocyte sarcolemmal membrane. (Scale bar, 10 μm.)

The mechanism underlying the differences between GHRH and GH effects is unclear. Postreceptor signaling cascades can be one reason for differences in activity between GHRH and GH. GHRH actions involve the stimulation of GHRHR, a G protein–coupled receptor that activates at least two transduction pathways, the adenyl cyclase/cAMP/protein kinase A pathway via the $G_s\alpha$ subunit (26) and the Ras/MAPK pathway through the βγ subunits (27).

The activation of the ERK1/2 signaling pathway has been connected with several cellular activities, such as proliferation, differentiation, and survival, and ghrelin has previously been shown to activate both ERK1/2 and the serine threonine kinase Akt (28). GHRH induces activation of cAMP and a significant activation of the Akt and ERK1/2 survival pathways, as has been demonstrated by Western blotting after GHRH administration. The PI3k/Akt pathway is a well-known signaling pathway for cell protection, and recently Granata et al. (10) reported that ERK1/2 and PI3k/Akt are involved in survival effects induced by GHRH and found that GHRH increased ERK1/2 and Akt phosphorylation, cAMP, and phosphorylation on serine 133 of cAMP response element–binding protein. Recently, Lorenz et al. (29) proposed that specific phosphorylation events on ERK 1/2 integrate differing upstream signals to induce hypertrophy. Hexarelin has also previously been shown to promote neuroprotection through activation of the PI3/Akt pathway (30). Moreover, the PI3k/Akt pathway controls cell size, including cardiomyocyte size (31), and is associated with cardiomyocyte hypertrophy and apoptosis (30, 32).

Traditionally, the adult heart has been considered a postmitotic organ where the cardiac myocytes were terminally differentiated without ability to divide. However, several investigators (33–35) have suggested that at least a subpopulation of myocytes re-enters the cell cycle and divides, and that a pool of cardiac stem cells may reside in the myocardium. In the present study, the expression of $Ki67^+$ cells was significantly higher at the remote zone but only in the rrGH group, and this was accompanied by an increase in capillary density in the same group. Previous study has docu-

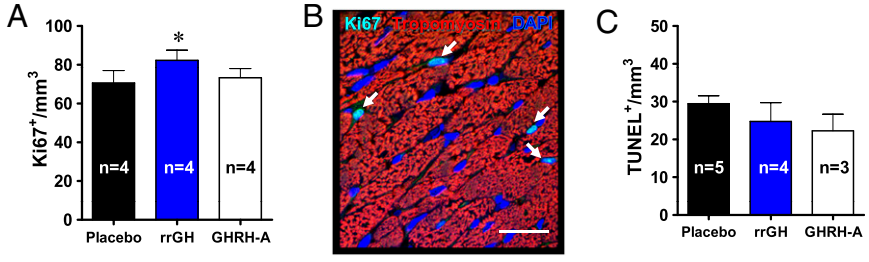

Fig. 5. Impact of rrGH and GHRH-A on cell division and apoptosis. (*A*) Bar graph showing the expression of cells positively stained for Ki67 in the remote zone. *$P < 0.05$ vs. placebo and GHRH-A. (*B*) Representative confocal micrograph image of $Ki67^+$ cells (cyan, white arrows), tropomyosin (red), and DAPI (blue). (Scale bar, 20 μm.) (*C*) Bar graph showing expression of $TUNEL^+$ cells per unit area.

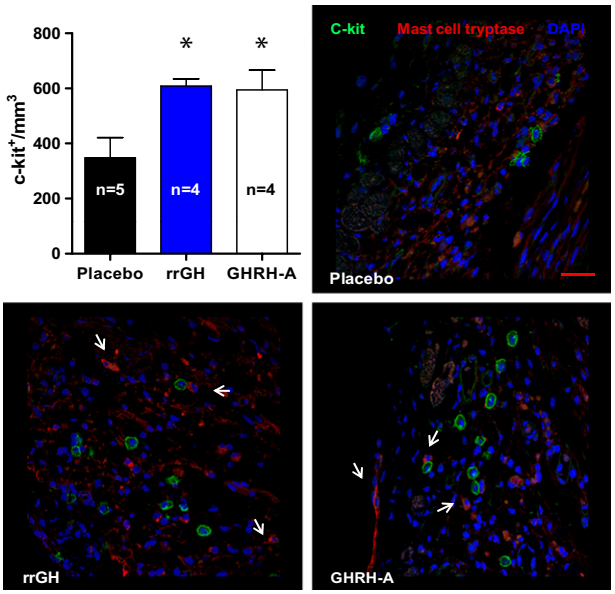

Fig. 6. Representative images of c-kit$^+$ cells in the infarct zone observed under confocal microscopy. Panels depict representative triple staining for mast cell tryptase (red), c-kit (green), and nuclei (blue) obtained from placebo (*Top Right*), rrGH (*Bottom Left*), and GHRH-A (*Bottom Right*). Arrows correspond to examples of mast cells. (Scale bar, 20 μm.) *Top Left:* Bar graph showing the increased expression of c-kit$^+$ cells per unit area in rrGH- and GHRH-A–treated rats. *$P < 0.05$ vs. placebo.

mented that GH is able to stimulate mature cardiac myocytes to reenter the cell cycle and divide and thereby increase their number in rat myocardium (36). A reduction in apoptosis would also lead to an increased number of cardiac myocytes, but in our study, surprisingly, the reduction in apoptosis in both treated groups was lower and not statistically significant when assessed by TUNEL assay; however, at a molecular level, changes in the expression of Bax and Bcl2 supported an antiapoptotic effect of GHRH-A.

We also examined the abundance of cardiac precursor cells, which showed increased c-kit$^+$ cells expression (clusters) in the infarct zone in both treated groups; recruitment of c-kit$^+$ stem cells is associated with improvement in cardiac performance (37). Brüel et al. (36) also reported that the number of c-kit$^+$ cells in a GH-treated group was 31% higher than that of the control group, but it was not statistically significant. Given the observation of similar increases in c-kit cells with GH and GHRH, yet greater reverse remodeling with GHRH, it is attractive to speculate that GHRH may stimulate cardiopoiesis to a greater extent. An alternate explanation is that the c-kit cells may traffic and/or proliferate to a greater or earlier extent. Finally, the findings of an antiapoptotic milieu might suggest improved survival of differentiation cardiac precursor cells (CPCs). Future work is required to evaluate the direct effects of GHRH on CPCs. Besides, CPCs possess the IGF-1/IGF-1 receptor system (38), which potentiates their survival and growth (39). Further studies are needed to ascertain whether GHRH-A or rrGH stimulated existing cardiac stem cells to differentiate into mature cardiac myocytes.

In summary, the present findings document that GHRH activation in the heart leads to MI size reduction, favorable hemodynamics, and recovery of functional performance to a greater degree than that due to GH following myocardial injury, and that this occurs without stimulation of BW or HW. These findings support ongoing basic and translational research into GHRH signal transduction mechanisms within the heart.

Materials and Methods

Animal Model. MI induced by coronary artery ligation was performed in female 6-month-old Fisher-344 rats, as described previously (40). Animals were randomly assigned to receive placebo, GHRH-A (JI-38, 50 μg/kg), or rrGH (0.5 mg/kg), starting 2 h after surgery. All treatment was given s.c. twice daily for 4 weeks. The Institutional Animal Care and Use Committee of University of Miami approved all protocols and experimental procedures.

Drugs. The rrGH was supplied by Dr. A. F. Parlow from the National Hormone and Pituitary Program (University of California, Los Angeles-Harbor), and GHRH-A (JI-38) was made in the laboratory of one of us (A.V.S.) (12, 13).

For additional information, see *SI Material and Methods*.

ACKNOWLEDGMENTS. We thank J. B. Fernandez for experimental assistance; Dr. I. H. Schulman for help with cryosections; Dr. G. McNamara for assistance with confocal imaging; and Irene Margitich for help with manuscript review. This work was supported by National Institutes of Health Grants R01-AG025017, RO1-HL084275, RO1-HL65455, and RO1-HL094848 and by National Heart, Lung, and Blood Institute Grant U54-HL081028 (all to J.M.H.). The studies in the laboratory of A.V.S. were supported by the Medical Research Service of the Veterans Affairs Department, the South Florida Veterans Affairs Foundation for Research and Education, and the University of Miami, Miller School of Medicine, Department of Pathology and Medicine, Division of Hematology/Oncology (all to A.V.S.).

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