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The Growth Hormone Secretagogue Hexarelin Improves Cardiac Function in Rats after Experimental Myocardial Infarction*

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ABSTRACT

Several studies have shown that GH can enhance cardiac performance in rats after experimental myocardial infarction and in humans with congestive heart failure. In the present study, the hemodynamic effects of hexarelin (Hex), an analog of GH-releasing peptide-6 and a potent GH secretagogue, were compared with the effects of GH. Four weeks after ligation of the left coronary artery male rats were treated sc twice daily with hexarelin [10 μg/kg·day (Hex10) or 100 μg/kg·day (Hex100)], recombinant human GH (2.5 mg/kg·day), or 0.9% NaCl for 2 weeks. Transthoracic echocardiography was performed before and after the treatment period. GH, but not Hex, increased body weight gain. GH and Hex100 decreased total peripheral resistance ($P < 0.05$) and increased stroke volume ($P < 0.05$ and $P < 0.01$, respectively) and stroke volume index ($P = 0.06$ and $P < 0.01$, respectively) *vs.* NaCl. Cardiac output was increased by GH and Hex100 ($P < 0.05$), and cardiac index was increased by Hex100 with a borderline significance for GH ($P = 0.06$). In conclusion, Hex improves cardiac function and decreases peripheral resistance to a similar extent as exogenous GH in rats postmyocardial infarction. The mechanisms of these effects are unclear; they could be mediated by GH or a direct effect of Hex on the cardiovascular system. (*Endocrinology* **141:** 60–66, 2000)

GH SECRETAGOGUES (GHS) are a heterogeneous group of synthetically produced peptides [GH-releasing peptides (GHRP)] and nonpeptides that stimulate GH secretion in both animals and human subjects. A pair of specific, G protein-coupled GHS receptors (GHS-R) were recently cloned in human and rat (1, 2), but new data suggest that there are other GHS-R types, possibly related to the cloned receptors (3). With cloning of GHS-R the idea of an as yet unidentified endogenous ligand as an additional regulator of GH secretion is supported. The functional type Ia GHS-R is expressed in the hypothalamus and the pituitary, and there seems to be a negative feedback of GH on expression of the GHS-R (4). GHRP-6, which is the most extensively studied GHS, exerts its effect at both hypothalamic and pituitary sites. The main action is exerted in the hypothalamus, where the release of GH-releasing hormone (GHRH) is increased by GHRP-6, and somatostatin is functionally antagonized (5–7). In addition, GHRP-6 promotes the release of GH directly from rat and human pituitary cells (8, 9). However, the mechanisms behind the GH-releasing properties of GHS are not completely understood.

The hexapeptide hexarelin (Hex) is chemically more stable and a more potent GH secretagogue than its analog GHRP-6 (10). Its actions have been investigated in both experimental and clinical studies (11–16). The results of many studies have shown that Hex increases GH secretion, but there are few studies of the clinical long term effects. However, Hex has been reported to accelerate growth in short children, influence parameters of bone formation and muscle morphology in aged dogs, and restore sodium channel properties in skeletal muscle in aged rats (17–19).

The myocardium expresss functional receptors for both GH and insulin-like growth factor I (IGF-I), and it is now well known that the GH/IGF-I axis has an important role during cardiac development and for maintaining the structure and function of the heart (20). In GH deficiency one of the clinical signs is an impaired cardiovascular performance, which may be reversed after GH substitution therapy, particularly in terms of increased stroke volume and reduced peripheral resistance (21). GH has also been shown to improve systolic function in normal subjects (22). In recent studies of patients with congestive heart failure due to dilated cardiomyopathy, both acute administration of GH and more chronic treatment have been shown to exert major hemodynamic effects (23, 24). The most important findings in these studies were improved parameters of systolic heart performance, decreased peripheral resistance, and enhanced ventricular work despite a reduction in oxygen consumption and energy production. These promising results could not be confirmed in a small placebo-controlled study despite increased levels of serum IGF-I (25). However, there is also abundant experi-

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mental evidence for enhanced cardiac performance by GH, among them several studies using a rat model of experimental myocardial infarction (MI) (26–29). Besides a pronounced vasodilatation and lowering of total peripheral resistance by GH/IGF-I, enhanced myocardial contractility is likely to contribute (30–32). Moreover, in animal models of GH excess there is a shift toward a myosin isoform with a lower adenosine triphosphatase activity, which may decrease the energy demand of the contractile process (30).

Recently, two groups have shown cardiac effects of Hex, including protection from ischemia in rats (33, 34) and acute hemodynamic effects in humans (35). In the present study we have characterized, *in vivo* and noninvasively, the hemodynamic effects of Hex. We have used echocardiography in a rat model 4 weeks after experimental myocardial infarction and compared the effects of Hex with those of GH.

Materials and Methods

Animals

The experiments were conducted on male Sprague Dawley rats (ALAB, Sollentuna, Sweden), weighing 115–120 g at the time of surgery. All animals were fed with standard rat pellets and tap water *ad libitum* and housed in cages in groups of five animals at 26 C with 60% humidity and a 0500–1900 h light regimen. The study protocol was approved by the ethics committee for animal experiments at the University of Göteborg (Göteborg, Sweden), and the investigation conforms to the Guide for the Care and Use of Laboratory Animals (NIH publication 85–23, revised 1985).

Study protocol: animal model of myocardial infarction

During short lasting methohexital sodium (75 mg/kg, ip; Brietal, Eli Lilly & Co., Indianapolis, IN) anesthesia, the rats were intubated and artificially ventilated with a respirator with room air (4 ml/beat·kg; 78 beats/min and 40/60% inspiration/expiration ratio) using a Carlsson ventilator (no. 8908, Mölndal, Sweden). A left thoracotomy was performed, exposing the left ventricular (LV) wall of the rats. The left coronary artery was ligated by positioning a suture between the pulmonary artery out-flow tract and the left atrium. The lungs were thereafter hyperinflated, positive end-expiratory pressure was applied, and the thorax was immediately closed (36). All rats (n = 56) were operated upon within 3 days. Ten rats died during surgery or the postoperative phase.

After a recovery period of 4 weeks, the remaining operated rats (n = 46) were examined by transthoracic echocardiography. Again, all rats were examined within 3 days in the corresponding order as they were operated upon 4 weeks previously. The rats were anesthetized using ketamine hydrochloride (Ketalar, Parke-Davis, Barcelona, Spain; 50 mg/kg) and xylazine hydrochloride (Rompun vet, Bayer Corp., Leverkusen, Germany; 10 mg/kg, ip). Nine rats died during anesthesia. The chests were shaved, the rats were placed prone on the left decubitus, and echocardiography was performed (see below), blinded for treatment. According to infarction size, all rats with MI were distributed into five groups based on a score system described in echocardiographic methods section. Animals with no echocardiographic signs of MI were excluded from the study before randomization (n = 3). According to infarction size, the rats were then randomized to the different treatment groups, and treatment was started with recombinant human GH (2.5 mg/kg·day; n = 8), hexarelin [10 μg/kg·day (Hex10; n = 9) and 100 μg/kg·day (Hex100; n = 8)], or 0.9% NaCl (n = 9). GH and hexarelin were injected sc twice daily, and NaCl was injected once daily. Recombinant human GH (Genotropin) and hexarelin (His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH_2) were gifts from Pharmacia & Upjohn, Inc. (Stockholm, Sweden).

After the treatment period the rats were anesthetized and subjected to echocardiography a second time, also blinded for treatment. The examination was finished within 3 days so that the treatment period for all rats was 14 days. No rats died during the second echocardiographic examination. Untreated intact rats (n = 6) were used as normal controls and were examined with echocardiography only at the end of the experiment. After completion of the second echocardiographic examination, blood was sampled from the inferior caval vein, and the rats were killed by rapid excision of the heart. The atria were trimmed free from the ventricles, the right ventricle (RV) was separated from the LV plus septum, and the parts were weighed. Left kidney and liver tissue were collected, and the kidney was weighed. Blood samples were centrifuged, and serum was kept at −20 C until assayed for IGF-I concentration.

Echocardiography

All Doppler echocardiographic recordings were performed by an investigator blinded to treatment allocation, and interpretations were performed by two independent observers, also blinded to treatment. Echocardiographic examination of the rats was performed before treatment and at the end of the treatment period, as previously described (28, 37). All rats were evaluated by a commercially available echocardiographic system equipped with a 10-MHz linear array transducer (Acuson, Sequoia-512, Mountain View, CA). Electrocardiographic electrodes were placed for timing of intracardiac events. Optimal image sequences were recorded on the S-VHS videorecorder and saved as digital cine loops (three consecutive heart cycles, high resolution zoom with a high frame rate) on the optical disc for future analysis. Images were obtained through the left parasternal rib spaces by placing the transducer against the chest wall from below. A long axis view of the LV was obtained, and subsequently perpendicular to this, a two-dimensional short axis view of the LV was acquired at the level between the papillary muscles and mitral valve. By superior angulation in the short axis view, the pulmonary artery was visualized and recorded along the transecting beam with and without color Doppler display. Pulsed wave Doppler signals and velocity time integrals were obtained with the sampling site placed in the pulmonary artery well below the valve level, avoiding turbulent flow or higher velocities from the valve area. Measurements of main pulmonary artery (MPA) diameter were performed at the level of the pulsed wave sampling site from digitized cine loops in three consecutive heartbeats.

The size of the myocardial damage/infarction was estimated visually at the baseline examination from the two-dimensional short axis images as follows: no akinetic or severely hypokinetic segments (no damage), grade 0; akinesis/hypokinesis comprising up to 60° circumference (small damage), grade 1; 61–120° (moderate damage), grade 2; 121–180° (large damage), grade 3; and more than 180° (very large damage), grade 4. Average infarction size grades in the groups were 2.4 ± 0.4 (NaCl), 2.4 ± 0.4 (GH), 2.4 ± 0.3 (Hex10), and 2.5 ± 0.3 (Hex100; mean ± SEM).

Short axis views of the LV were recalled and displayed. Images were selected at end-diastole, end-systole, and short axis LV, and myocardial areas were measured. The mean of three tracings from one to three beats was used depending upon the quality of recorded beats. Area fractional shortening was calculated as (diastolic − systolic)/diastolic LV short axis area.

Stroke volume (SV) was calculated as product of MPA mean velocity time integral (mean of three consecutive pulmonary artery velocity profiles) and corresponding MPA mean area. To minimize measurement errors in longitudinal comparison of SV, we used the baseline estimation of pulmonary artery dimension also at the end of treatment, however adjusted by a coefficient determined by the relative gain in body weight (BW). Multiplying stroke volume by heart rate yielded cardiac output (CO). Total peripheral resistance (TPR) was calculated as mean arterial pressure (MAP)/CO.

Meridional wall stress was determined at 2 weeks according to Mirsky by the formula (38): $1.33 \times MAP \times (ESA/ESMA) \times 10^3$ kdyn/cm^2. For the calculation of end-systolic wall stress, we used MAP as an equivalent of end-systolic pressure (39). ESA indicates end-systolic short axis area of the LV cavity, and ESMA indicates LV end-systolic short axis myocardial area.

Blood pressure measurement

An arterial catheter was placed during anesthesia in the aortic arch via the left carotid artery. MAP was recorded on a Grass model 7D polygraph (Quincey, MA) via a Statham P 23 DC transducer (Gulton Statham Transducers, Inc., Costa Mesa, CA). Blood pressure values were calculated as means of 30 samples taken during 60 sec.

Serum IGF-I

The serum concentration of IGF-I was determined by a hydrochloric acid-ethanol extraction RIA using authentic IGF-I for labeling (Nichols Institute Diagnostics, San Juan Capistrano, CA). The assay was performed according to the manufacturer's protocol after centrifugation of precipitated serum proteins at 4 C followed by neutralization with Tris base and another centrifugation at 4 C.

Isolation of RNA

RNA was isolated using Trireagent (Sigma, St. Louis, MO) according to the manufacturer's protocol. Briefly, tissue was homogenized in Trireagent using a Polytron (Brinkmann Instruments, Inc., Westbury, NY). After centrifugation, isopropanol was added to the supernatant, followed by centrifugation. The RNA pellet was washed with 75% ethanol and then dissolved in diethylpyrocarbonate water.

IGF-I probe

A sequence corresponding to the base coordinates 267–286 and 366–385 (145 bp) of the IGF-I complementary DNA (40) was subcloned into the *Sap*I-*Xho*I sites of pCRII (Invitrogen, Carlsbad, CA) and subsequently used to generate sense messenger RNA (mRNA) as synthetic standard and antisense probe. RNA antisense probes were generated from linearized plasmids (*Sap*I) using T7 RNA polymerase. Synthetic standard was generated using *Xho*I-linearized plasmid and Sp6 RNA polymerase.

Solution hybridization and ribonuclease (RNase) protection assays

A solution hybridization assay (41) was used to quantify IGF-I mRNA in cardiac, liver, and kidney tissue, respectively. The hybridization was performed at 70 C for 24 h in 0.06 M NaCl, 20 mM Tris-HCl (pH 7.5), 4 mM EDTA, 0.1% SDS, 0.75 mM dithiothreitol, 25% formamide, and ^{35}S-labeled IGF-I probe. After the addition of 100 μg herring sperm DNA, the samples were treated with 40 μg/ml RNase A and 2 μg/ml RNase T_1 (Sigma). Trichloroacetic acid-precipitated protected hybrids were then collected on glass-fiber filters (GF/C, Whatman, Maidstone, UK) and counted in a scintillation counter. The signal was compared with a standard curve based on known amounts of synthetic IGF-I mRNA, and results were related to total RNA content.

To visualize the hybridization of labeled probe to mRNA, a RNase protection assay was performed using RPA II kit 1410 (Ambion, Inc., Austin, TX). Briefly, samples of 40 μg total cellular RNA were hybridized at 45 C overnight with 5×10^5 cpm of the ^{35}S-labeled rat IGF-I mRNA antisense probe, then digested with RNase. The RNA:RNA hybrids were precipitated, resuspended, and separated on a 6% polyacrylamide/8 M urea gel. The signals from protected fragments were visualized on a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA), showing a major protected fragment of 145 bp (data not shown).

Statistical analysis

Values are given as the mean ± SEM. Heart and kidney weights (only when normalized to BW); serum IGF-I; IGF-I mRNA in liver, kidney, and cardiac tissue; and hemodynamic variables available only at the end of the experiment were compared using one-way ANOVA/*post-hoc* Fisher's protected least significant difference test between the MI groups and unpaired *t* test between intact controls and MI NaCl rats. When data were available before and after treatment (*i.e.* BW and echocardiographic variables), a one-way ANOVA/*post-hoc* Fisher's protected least significant difference test was performed on the changes in the four MI groups, but no statistical comparisons were performed with intact rats.

Results

Body weight gain, heart weights, serum IGF-I, and tissue IGF-I mRNA (Fig. 1 and Table 1)

After 2 weeks of treatment, BW gain was increased in GH compared with NaCl rats. In both Hex groups, the BW gain was significantly lower than that in GH rats and did not differ from that in NaCl rats (Fig. 1A). Total heart weight/BW was higher in NaCl *vs.* intact rats ($P < 0.05$). There were no significant differences in kidney weight/BW or LV and RV weight/BW (Table 1). There were no significant differences in serum IGF-I between NaCl-treated and Hex- or GH-treated rats, although there was a tendency toward increased values in the GH group. IGF-I mRNA expression in the liver did not differ between any of the groups. Cardiac IGF-I mRNA was increased in NaCl *vs.* intact rats, but was not further increased by treatment with GH or Hex (Table 1). Kidney IGF-I mRNA was increased in the GH group *vs.* NaCl, whereas IGF-I mRNA expression in both Hex groups was comparable to expression in the NaCl group and significantly lower than that in the GH group (Fig. 1B).

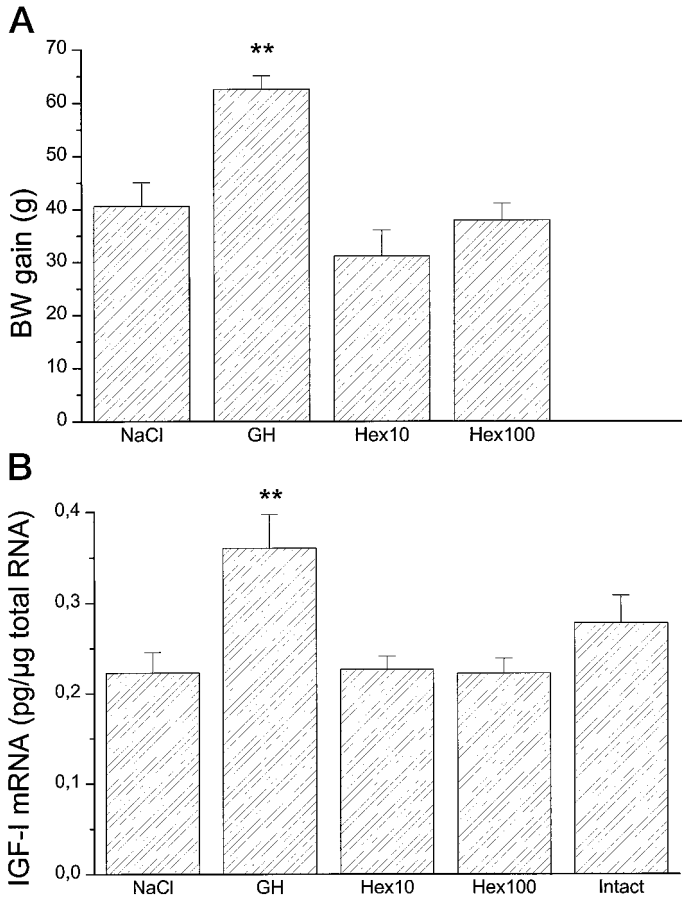

FIG. 1. BW gain (A) after 2 weeks treatment of MI rats with NaCl, recombinant human GH (2.5 mg/kg·day), Hex10, or Hex100 and kidney IGF-I mRNA in MI and intact rats (B). Data are presented as the mean ± SEM. **, $P < 0.01$ *vs.* NaCl, Hex10, and Hex100 rats.

Doppler echocardiographic heart dimensions, volumes, and performance (Figs. 2–4 and Table 2)

There was a significant effect on SV by GH and Hex100 *vs.* NaCl, whereas Hex10 had no effect on SV (Fig. 2). When normalized to BW, the effect was still significant for Hex100 with a borderline *P* value ($P = 0.060$) for GH. GH and Hex100 also increased CO compared with NaCl and Hex10 (Fig. 3). CO/BW was increased by Hex100, with a borderline signif-

TABLE 1. Body weight gain, heart weights, serum IGF-I, and liver and cardiac IGF-I mRNA

	MI NaCl	MI GH	MI Hex 10	MI Hex100	Intact
BW_{BL} (g)	399 ± 15	415 ± 8	413 ± 7	411 ± 10	
BW_{2W} (g)	439 ± 16	478 ± 10	444 ± 8	449 ± 10	442 ± 11
Kidney wt (g)	1.53 ± 0.07	1.61 ± 0.04	1.52 ± 0.04	1.59 ± 0.05	1.60 ± 0.06
Kidney wt/BW (g/100 g)	0.35 ± 0.01	0.34 ± 0.01	0.34 ± 0.10	0.35 ± 0.01	0.36 ± 0.01
Heart wt (g)	1.63 ± 0.04	1.82 ± 0.09	1.56 ± 0.05	1.68 ± 0.09	1.42 ± 0.04
Heart wt/BW (g/100 g)	0.38 ± 0.02[a]	0.38 ± 0.02	0.35 ± 0.01	0.37 ± 0.02	0.32 ± 0.004
LV wt (g)	1.00 ± 0.03	1.10 ± 0.02	1.05 ± 0.04	1.06 ± 0.05	0.95 ± 0.02
LV wt/BW (g/100 g)	0.23 ± 0.005	0.23 ± 0.004	0.24 ± 0.006	0.24 ± 0.009	0.22 ± 0.003
RV wt (g)	0.34 ± 0.02	0.38 ± 0.04	0.30 ± 0.007	0.36 ± 0.05	0.27 ± 0.008
RV wt/BW (g/100 g)	0.079 ± 0.008	0.080 ± 0.01	0.067 ± 0.001	0.080 ± 0.01	0.061 ± 0.0009
S-IGF-I (μg/L)	1290 ± 80	1400 ± 110	1230 ± 45	1220 ± 55	1450 ± 30
Liver IGF-I mRNA (pg/μg RNA)	4.55 ± 0.18	4.52 ± 0.29	4.71 ± 0.17	4.87 ± 0.09	5.02 ± 0.06
Cardiac IGF-I mRNA (pg/μg RNA)	0.88 ± 0.04[b]	0.83 ± 0.07	0.79 ± 0.04	0.87 ± 0.08	0.26 ± 0.01

LV, Left ventricular; RV, right ventricular; BL, at baseline; 2 wk, after 2 weeks ± of treatment. Values are the mean ± SEM.
[a] $P < 0.05$ *vs.* intact rats.
[b] $P < 0.01$ *vs.* intact rats.

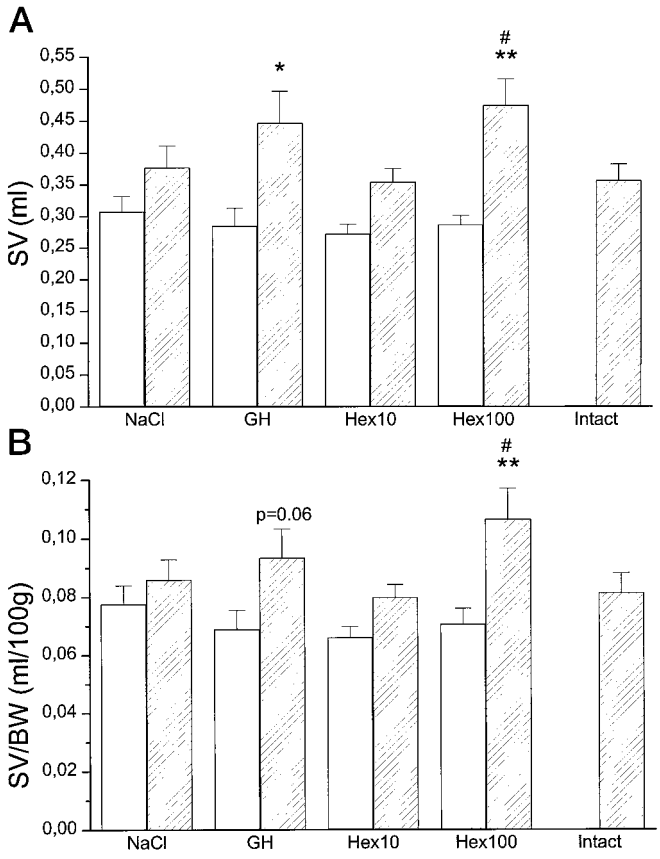

FIG. 2. SV measured with Doppler echocardiography (A) and SV normalized to BW (SV/BW; B) at baseline (*open bar*) and after 2 weeks of treatment (*hatched bar*) of MI rats with NaCl, recombinant human GH (2.5 mg/kg·day), Hex10, or Hex100. Intact, untreated rats were examined only at the end of the experiment. Data are presented as the mean ± SEM. The *P* value for GH *vs.* NaCl rats is indicated in the figure. *, $P < 0.05$; **, $P < 0.01$ *vs.* NaCl rats. #, $P < 0.05$ *vs.* Hex10 rats.

icance for GH ($P = 0.056$). At the end of the treatment period, TPR was lower in the GH and Hex100 groups compared with that in NaCl and Hex10 rats (Fig. 4). No improvement of other systolic parameters by GH/Hex treatment could be detected (Table 2). No significant effects were seen on diastolic parameters, *i.e.* deceleration rate and early *vs.* late LV filling velocities ratio (data not shown). Heart rate did not differ between the groups. MAP was lower in the GH group *vs.* that in the NaCl group, but was not significantly changed by Hex treatment, although there was a tendency toward lower MAP in the Hex100 group. End-systolic wall stress was increased in all NaCl rats *vs.* intact rats, but there were no significant effects of treatment. There were no effects on LV geometry variables such as ESA and EDA.

Discussion

In the present study we have used echocardiography to assess the hemodynamic effects of the GH secretagogue Hex in a rat model of impaired cardiac function after MI. The main findings were that both GH and Hex treatments increased SV and CO and lowered TPR. Previously, our group and others have reported increased SV, CO, and other systolic variables by GH treatment after MI (26–28, 32). However, this is the first time Hex or any other GHS has been shown to have hemodynamic effects in the MI model.

Only one systolic parameter was significantly affected by Hex and GH treatment, *i.e.* stroke volume. However, SV as well as its derivatives, CO and TPR, are considered to be important variables in heart failure (42). Moreover, the effects on stroke volume and cardiac output were still present when normalized to BW. The small changes in cardiac dimensions, *e.g.* ESA and EDA, were not significantly different between treatments and were not sufficient as an explanation of the effect on SV.

The mechanisms behind the hemodynamic effects of GH may be complex. The decrease in MAP/TPR may be a nitric oxide-mediated effect (43), and a reduction of afterload may, in turn, result in an increase in SV. However, there are also studies providing evidence of an effect on cardiac contractility by GH (30, 31); recent findings by Tajima *et al.* suggest an up-regulation of the sarcoplasmatic Ca^{2+}-adenosine triphosphatase and enhancement of the contractile reserve (32).

Previous studies have shown that Hex has a stimulatory effect on GH secretion (15), and the similarities in hemodynamic profile would indicate that its actions may be mediated through GH. However, no other signs of increased GH

TABLE 2. Echocardiographic measurements and mean arterial blood pressure

	MI NaCl	MI GH	MI Hex 10	MI Hex100	Intact
ESA_{BL} (cm^2)	0.49 ± 0.08	0.45 ± 0.05	0.52 ± 0.05	0.54 ± 0.06	
ESA_{2W} (cm^2)	0.58 ± 0.04	0.61 ± 0.07	0.58 ± 0.05	0.67 ± 0.03	0.25 ± 0.01
EDA_{BL} (cm^2)	0.82 ± 0.07	0.75 ± 0.07	0.77 ± 0.04	0.83 ± 0.04	
EDA_{2W} (cm^2)	0.85 ± 0.05	0.95 ± 0.07	0.86 ± 0.08	0.91 ± 0.03	0.53 ± 0.03
EDA/BW_{BL} (cm^2/100 g)	0.21 ± 0.02	0.18 ± 0.02	0.19 ± 0.01	0.20 ± 0.01	
EDA/BW_{2W} (cm^2/100 g)	0.20 ± 0.01	0.20 ± 0.02	0.19 ± 0.02	0.20 ± 0.007	0.12 ± 0.006
AFS_{BL}	0.43 ± 0.05	0.41 ± 0.03	0.34 ± 0.04	0.36 ± 0.05	
AFS_{2W}	0.31 ± 0.03	0.36 ± 0.06	0.32 ± 0.05	0.26 ± 0.03	0.53 ± 0.009
HR_{BL} (beats/min)	284 ± 8	296 ± 9	292 ± 10	297 ± 10	
HR_{2W} (beats/min)	281 ± 12	301 ± 12	286 ± 8	288 ± 8	290 ± 10
MAP (mm Hg)	123 ± 8	103 ± 7[a]	120 ± 5	110 ± 5	109 ± 4
ESWS (10^3 kdyn/cm^3)	163 ± 21[b]	129 ± 13	133 ± 15	156 ± 13	65 ± 5

ESA, End-systolic area of the left ventricular cavity; EDA, end-diastolic area of the left ventricular cavity; AFS, area fractional shortening; HR, heart rate; MAP, mean arterial pressure; TPR, total peripheral resistance; ESWS, end-systolic wall stress. BL, at baseline; 2 wk, after 2 weeks treatment. Values are the mean ± SEM.

[a] $P < 0.05$ *vs.* NaCl-treated rats.

[b] $P < 0.01$ *vs.* intact rats.

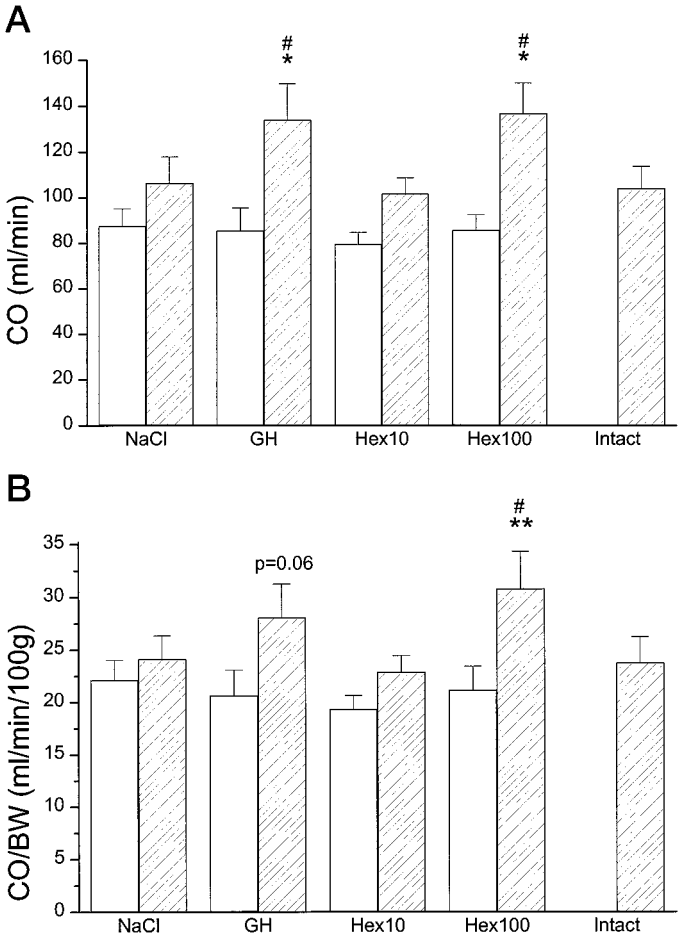

FIG. 3. CO measured with Doppler echocardiography (A) and CO normalized to BW (CO/BW; B) at baseline (*open bar*) and after 2 weeks of treatment (*hatched bar*) of MI rats with NaCl, recombinant human GH (2.5 mg/kg·day), Hex10, or Hex100. Intact, untreated rats were examined only at the end of the experiment. Data are presented as the mean ± SEM. The *P* value for GH *vs.* NaCl rats is indicated in the figure. *, $P < 0.05$ *vs.* NaCl rats; **, $P < 0.01$ *vs.* NaCl rats; #, $P < 0.05$ *vs.* Hex10 rats.

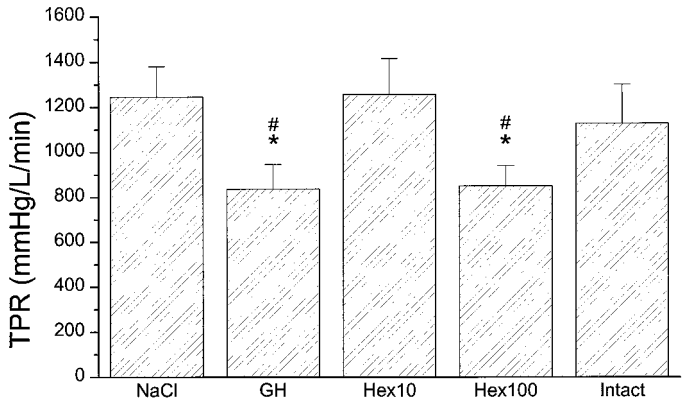

FIG. 4. Total peripheral resistance after 2 weeks of treatment of MI rats with NaCl, recombinant human GH (2.5 mg/kg·day), Hex10, or Hex100 and in intact rats. Data are presented as the mean ± SEM. *, $P < 0.05$ *vs.* NaCl rats; #, $P < 0.05$ *vs.* Hex10 rats.

activity by Hex was detected in our study, including BW gain and kidney IGF-I mRNA levels, where GH had clear-cut effects. Notably, the GH-releasing effect of Hex has not been studied in this rat MI model, and serum levels of GH in Hex animals are unknown. Thus, Hex may exert its hemodynamic effects through small increases in GH, not sufficient for increasing BW gain and kidney IGF-I mRNA. Moreover, different secretion patterns may evoke differential effects at the organ level.

Recent studies have given support for a direct, GH-independent action of Hex on the myocardium. Locatelli *et al.* studied the effects of ischemia and reperfusion on cardiac function in hypophysectomized rats and found that Hex administrated before ischemia preserves cardiac performance (34). Bisi *et al.* reported an increased LV ejection fraction, peaking 30 min after a single iv injection of Hex in normal male subjects, not seen after GH administration despite similar peak values of GH after the two substances (35). Expression of mRNA for the cloned GHS receptor in rat heart has been reported (44), although this has not been found by

other groups (45, 46). Ong *et al.* reported Hex binding to rat cardiac membranes and suggest the existence of a different GHS-R subtype in the myocardium (3, 47). These data on direct cardiac effects are in alignment with our results of systolic effects of Hex without other signs of increased GH activity. Moreover, the existence of GHS/Hex receptors in the vascular endothelium has been suggested, as Hex treatment seems to alter the reactiveness of the aortic ring in hypophysectomized male rats (48). These new findings could also be in accordance with the decrease in TPR by Hex100 treatment in our study.

Our finding of increased BW gain in GH-treated rats is in line with previous observations (26, 27), and increased IGF-I mRNA expression in kidney gives further support for peripheral effects by GH. As the liver is the major source of circulating IGF-I (49), the effects on liver IGF-I mRNA are closely linked to serum IGF-I. We found no significant effect of GH (or Hex) on serum or liver IGF-I in MI rats. A possible explanation may be increased cytokine activity, *e.g.* tumor necrosis factor-α, observed in heart failure (50), which may cause a reduction in the liver production of IGF-I (51). Local expression of IGF-I mRNA in the heart was increased after MI as previously demonstrated (28), but GH (and Hex) failed to increase it further when studied at this single time point 6 weeks after MI, which is consistent with previous results from our laboratory (28). Thus, although the activation of specific transcripts or regional differences in IGF-I expression not have been excluded here, activation of cardiac IGF-I expression appears to be of minor importance for both GH and Hex effects on hemodynamics after MI.

In contrast to GH, Hex-treated animals did not gain more weight than controls, suggesting the absence of anabolic effects mediated by GH. Other groups have also reported no effect of Hex on BW gain in male rats (33). The lack of Hex effect on serum IGF-I is in accordance with several previous studies of administration of Hex or GHRP-6 to animals and humans, with different routes of administration (18, 33, 52–54). However, data are conflicting, and increased levels of serum IGF-I after Hex/GHRP-6 treatment have also been reported in some studies (15, 17, 55–57).

There are now several studies of the cardiac and peripheral hemodynamic effects of GH and/or IGF-I, and among them, promising clinical data pointing to a future role of GH/IGF-I in cardiovascular therapy (24). GHS have the ability to amplify the physiological pulsatile GH secretion, and several of the GHS are orally bioavailable, in contrast to GH (13). In the current study we report that Hex improves variables of *in vivo* cardiac function, comparable to GH. Further investigation of the cardiovascular effects of Hex and other GHS is mandatory and should include studies of the direct action of GHS on the myocardium.

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