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Growth Hormone-Independent Cardioprotective Effects of Hexarelin in the Rat*

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ABSTRACT

We previously reported that induction of selective GH deficiency in the rat exacerbates cardiac dysfunction induced by experimental ischemia and reperfusion performed on the explanted heart. In the same model, short-term treatment with hexarelin, a GH-releasing peptide, reverted this effect, as did GH. To ascertain whether hexarelin had non-GH-mediated protective effects on the heart, we compared hexarelin and GH treatment in hypophysectomized rats. Hexarelin (80 μg/kg sc), given for 7 days, prevented exacerbation of the ischemia-reperfusion damage induced by hypophysectomy. We also demonstrate that hexarelin prevents increases in left ventricular end diastolic pressure, coronary perfusion pressure, reactivity of the coronary vasculature to angiotensin II, and release of creatine kinase in the heart perfusate. Moreover, hexarelin prevents the fall in prostacyclin release and enhances recovery of contractility. Treatment with GH (400 μg/kg sc) produced similar results, whereas administration of EP 51389 (80 μg/kg sc), another GH-releasing peptide that does not bind to the heart, was ineffective. In conclusion, we demonstrate that hexarelin prevents cardiac damage after ischemia-reperfusion, and that its action is not mediated by GH but likely occurs through activation of specific cardiac receptors. (*Endocrinology* **140:** 4024–4031, 1999)

A GROWING BODY of evidence suggests that GH plays an important role in maintaining cardiovascular health, and alterations of the somatotropic function are frequently associated with abnormalities of cardiac structure and function (1). Hypopituitary patients show left ventricular (LV) diastolic dysfunctions and ischemic-like ST segment changes during exercise testing (2). As a result, these patients are at increased risk of cardiac mortality caused by myocardial infarction and heart failure (3). The decline of exercise capacity may explain the increased cardiovascular mortality of hypopituitary patients. The reversibility of cardiovascular abnormalities during GH treatment in hypopituitary patients (4) supports the view that long-term GH replacement therapy may be beneficial in adults with overt GH deficiency (GHD).

We have shown that heart preparations from rats rendered GHD by passive immunization against GHRH are more sensitive to postischemic ventricular dysfunction than those from control rats (5). In these animals, *in vivo* GH replacement was effective in improving ischemic damage, and its effects were similar to those of hexarelin under identical experimental conditions (6).

Hexarelin is a highly effective GH secretagogue (GHS) (7), and its cardiac effects are likely mediated by GH (6). However, previous studies indicated that the GH-secreting activity of hexarelin was largely impaired in the GHD rat model (8). Like other GHSs, hexarelin requires the presence of endogenous GHRH for maximal stimulation of GH secretion (8, 9), because passive immunization against GHRH blunts hexarelin-induced GH secretion (10). Alternatively, hexarelin activity in the heart may be only partially dependent on GH or even independent of GH. In fact, the recent demonstrations of specific binding sites for GHS-like compounds in the heart (11, 12) suggest that hexarelin may have direct cardiac effects.

To address this issue, we compared the effects of hexarelin with those of GH on the mechanical and metabolic alterations induced by low-flow ischemia and reperfusion in isolated hearts obtained from hypophysectomized rats treated for 1 week with hexarelin, GH, or saline. To study the specificity of hexarelin on cardiac function, we compared its effects with those of EP 51389, a synthetic tripeptide with strong GH-releasing activity (11, 13). The structure of EP 51389 is distinct from that of hexarelin; and therefore, it is unable to displace hexarelin from its cardiac binding sites (11). In the hypophysectomized rats, hexarelin or EP 51389 cannot stimulate the release of pituitary GH; therefore, the cardiac effects of these peptides must be GH-independent.

Materials and Methods

Animals and treatments

Adult male intact and hypophysectomized Sprague Dawley rats (155–160 g body weight) were purchased from Charles River Italia (Calco, Como, Italy) and were housed under controlled conditions (22 ± 2 C, 65% humidity, and artificial light from 0600 h to 2000 h). Control and hypophysectomized rats were weighed every day during all experiments. Beginning 2 weeks after their arrival, control rats were treated sc, once a day for 7 days, with 1 ml/kg physiological saline and

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then killed by cervical dislocation. Their hearts were rapidly dissected and mounted for the *in vitro* procedures (see below). Hypophysectomized rats, 14 days after arrival, were randomly assigned to four experimental groups (8 animals each) and treated sc, once a day for 7 days, with: 1) saline (1 ml/kg); 2) GH (400 μg/kg); 3) hexarelin (80 μg/kg); or 4) EP 51389 (80 μg/kg). All hypophysectomized rats were killed by cervical dislocation, 16 h after the last injection. Completeness of hypophysectomy, which was performed by the transauricolar route according to Falconi and Rossi (14), was assessed by visual inspection of the sella turcica and by plasma GH determination. Trunk blood was collected for RIA of GH and insulin-like growth factor I (IGF-I) concentrations, and the hearts were rapidly dissected for ischemia and reperfusion experiments. IGF-I concentrations in cardiac muscles were also determined.

All experimental protocols were approved by the Review Committee of the Department of Pharmacology and met the Italian guidelines for use of laboratory animals, which conform with the European Communities Directive of November 1986 (86/609/EEC).

GH assay

Plasma GH concentrations were measured using a double-antibody RIA (15). Results were expressed as ng/ml, relative to the National Institutes of Health standard rat GH RP-2, the potency of which was 2 U/mg. The minimum detectable value of rat GH was 1 μg/liter; intraassay variability was 6%. To avoid possible interassay variations, all samples were assayed in a single RIA. Reagents for GH RIA were a kind gift of the National Hormone and Pituitary Program, NIDDK, NICHHD, USDA.

IGF-I assay in plasma and heart

Plasma samples were cryoprecipitated in 87.5% ethanol and 12.5% HCl 2N, as previously described by Brewer *et al.* (16). Hearts were weighed and frozen in liquid nitrogen. Single hearts were subsequently pulverized, and IGF-I was extracted using 1 mol/liter ice-cold acetic acid (5 ml/g tissue), as previously described by D'Ercole *et al.* (17). After centrifugation at 600 $\times$ *g* for 10 min, the supernatants were frozen at -20 C, lyophylized, and reconstituted with assay buffer (2 ml/g fresh weight). Total IGF-I plasma levels and heart IGF-I concentrations were determined using a commercially available RIA kit (Amersham Pharmacia Biotech Italia, Milan, Italy). The sensitivity of the assay was 50 pg/ml; intraassay variability was less than 10%. To avoid possible interassay variations, all samples were assayed in a single RIA.

Perfused heart preparations

The isolated hearts were perfused, retrograde fashion, through the aorta with gassed Krebs Henseleit solution (37 C), as previously described by Berti *et al.* (18). The perfusion rate was adjusted to yield a coronary perfusion pressure (CPP) of 55–60 mm Hg with a flow rate of 12 ml/min. LV pressure (LVP) was measured by inserting a small latex balloon into the ventricular cavity and filling it with saline until LV end-diastolic pressure (LVEDP) stabilized in the range of 5 mm Hg. The preparations were electrically paced at a frequency of 300 beats/min with rectangular pulses (1 msec duration; voltage, 10% above threshold) by a Grass stimulator (model S-88, Grass Instruments, Quincy, MA).

The hearts of the experimental groups of hypophysectomized and intact rats were allowed to stabilize for 20 min and subsequently exposed to the low-flow ischemia and reperfusion protocol (see below).

Ischemia was induced by reducing the coronary flow to 2 ml/min (CPP, 4–6 mm Hg) for a period of 40 min. At the end of this period, reperfusion was resumed at the preischemic flow rate of 12 ml/min for another period of 20 min. In this study, CPP and LVP were monitored with Statham transducers (HP-1280C) connected to a dynograph (HP-7754A; Hewlett-Packard Co., Waltham, MA). LVEDP (which is an index of stiffness and difficulty in relaxation of cardiac cells) and postischemic LV-developed pressure (LVDP, which measures the strength of contractility of cardiac myocytes, calculated as the peak LVP minus LVEDP) were also evaluated. Furthermore, the reactivity of the coronary vasculature to angiotensin II was evaluated to assess the integrity of endothelium-dependent relaxant mechanisms. Angiotensin II (1 μg; Sigma Chemical Co., St. Louis, MO) was injected as a bolus into the perfusion system at the beginning of each experiment.

Creatine kinase (CK) in heart perfusate

CK activity, a biochemical marker of myocardial cell lesions, was determined in heart perfusates, which were collected in an iced-cooled beaker before flow reduction and during the 20 min of reperfusion. CK activity was evaluated according to the method of Bergmeyer *et al.* (19) using a commercial kit (Roche Molecular Biochemicals, Milan, Italy). Total CK was determined spectrophotometrically (Lambda 16, Perkin-Elmer Italia, Monza, Italy) and expressed as U/20 min·g wet tissue.

6-Keto-PGF$_{1\alpha}$ in heart perfusate

Because prostacyclin (PGI_2) generation plays an important role in maintaining flow within vessels and protecting the heart against ischemia, PGI_2 release in the heart perfusates was measured by assaying the levels of its stable metabolite, 6-Keto-$PGF_{1\alpha}$. Heart perfusates were collected during the 5 min immediately preceding flow reduction and during the first 10 min of reperfusion. The concentrations of 6-Keto-$PGF_{1\alpha}$ were evaluated according to the enzyme immunoassay method described by Pradelles *et al.* (20) using a commercially available kit (detection limit 3 pg/ml; Amersham Pharmacia Biotech) and are expressed in ng/min.

Statistical analysis

Data were analyzed for statistical significance by one-way ANOVA followed by the Tukey-Kramer test for multiple comparisons. A value of $P < 0.05$ was considered significant. The area under the curve (AUC) was assessed following the trapezoid method.

Drugs

Hexarelin [His-D-2-Me-Trp-Ala-Trp-D-Phe-Lys-NH_2] and biosynthetic human GH (Genotropin) were kind gifts from Pharmacia & Upjohn, Inc. (Stockholm, Sweden). EP 51389 [Aib-D-2-Me-Trp-D-2-Me-Trp-NH_2] was synthetized by Europeptides.

Results

Growth rate

On the day of their arrival (experimental day -14) there were no significant differences in mean body weight between intact and hypophysectomized rats (157.1 $\pm$ 1.3 and 160 $\pm$ 1.5 g, respectively), whereas on the first day (experimental day 1) of treatment, the mean body weight of the four groups of hypophysectomized rats were significantly lower than that of intact rats (152.5 $\pm$ 2.3 and 172.0 $\pm$ 3.5 g, respectively; $P < 0.05$). As expected, during this time interval, the body weight of the latter group had increased progressively, whereas that of hypophysectomized rats had declined significantly. Irrespective of treatments, the mean body weights of hypophysectomized rats remained significantly lower than intact animals for the duration of the study. Administration of GH to hypophysectomized rats induced a significant increase of body weight on day 7 of treatment (from 143 $\pm$ 3 to 158 $\pm$ 5 g; $P < 0.05$), whereas hexarelin and EP 51389 failed to do so (final weight 145 $\pm$ 3 g and 139 $\pm$ 3 g, respectively; Table 1). No treatment altered the heart/body weight ratio in hypophysectomized rats, indicating that proportional changes in body and heart weight had occurred (Table 1). Plasma GH concentrations were below the detection limit of the assay in all hypophysectomized rats (data not shown).

Ischemia-reperfusion in hearts from hypophysectomized rats

When ischemia-reperfusion was induced in hearts from saline-injected hypophysectomized rats, a marked aggravation of the ischemic damage occurred. In this instance, during the ischemic phase, the values of LVEDP gradually increased (peak, 82.5 ± 3.2 mm Hg; $P < 0.01$) and, at the end of reperfusion, remained elevated at 66.6 ± 3.1 mm Hg ($P < 0.01$) (Figs. 1 and 3). As a consequence, electrical pacing was not reestablished, and cardiac rhythm disturbances were associated with a severe impairment of heart contractility. Moreover, upon reperfusion, CPP values were significantly increased (58.7 ± 5.2 mm Hg over the preischemic values; $P < 0.01$), denoting severe coronary vasoconstriction caused, in part, by heart stiffness (Fig. 2). Treatment of hypophysectomized rats with hexarelin notably protected the isolated hearts from ischemia-reperfusion damage, such that CPP values were in the range of those determined in preparations from intact rats (Figs. 1–3). At the end of the ischemic and reperfusion periods, LVEDP values were, respectively, 31.3 ± 2.5 mm Hg ($P < 0.01$) and 13.1 ± 1.7 mm Hg ($P < 0.05$); and CPP values of hypophysectomized rats were not statistically different from those of intact rats (Fig. 2). Similar results were obtained with heart preparations from hypophysectomized rats given GH. In this case, LVEDP, CPP (Fig. 2), and LVDP (Fig. 3) values were not statistically different from those obtained from hearts of hypophysectomized rats given hexarelin. In contrast, heart preparations from hypophysectomized rats given EP 51389 generated LVEDP values, recorded during ischemia (peak 62.5 ± 2.9 mm Hg) and at the end of reperfusion (50.7 ± 3.4 mm Hg), that were consistently greater than the corresponding preischemic values (Fig. 2). Dysrhythmia was present during reperfusion. At

TABLE 1. Heart and body weights in intact and hypophysectomized rats on the final day of the experiment

	Heart weight (mg)	Body weight (g)	Heart weight/ body weight (mg/g)
Intact	928.0 ± 19.5	212.6 ± 8.4	4.365 ± 0.226
Hypox + saline	614.5 ± 15.6	143.3 ± 3.6	4.288 ± 0.166
Hypox + GH	679.8 ± 21.3	158.4 ± 5.4[a]	4.292 ± 0.114
Hypox + hexarelin	607.4 ± 14.7	145.1 ± 3.4	4.186 ± 0.133
Hypox + EP 51389	605.2 ± 22.2	139.5 ± 3.0	4.338 ± 0.171

Data are the mean ± SEM of eight rats. Treatments are as described in the legend of Fig. 2.

[a] $P < 0.05$ *vs.* hypox + saline.

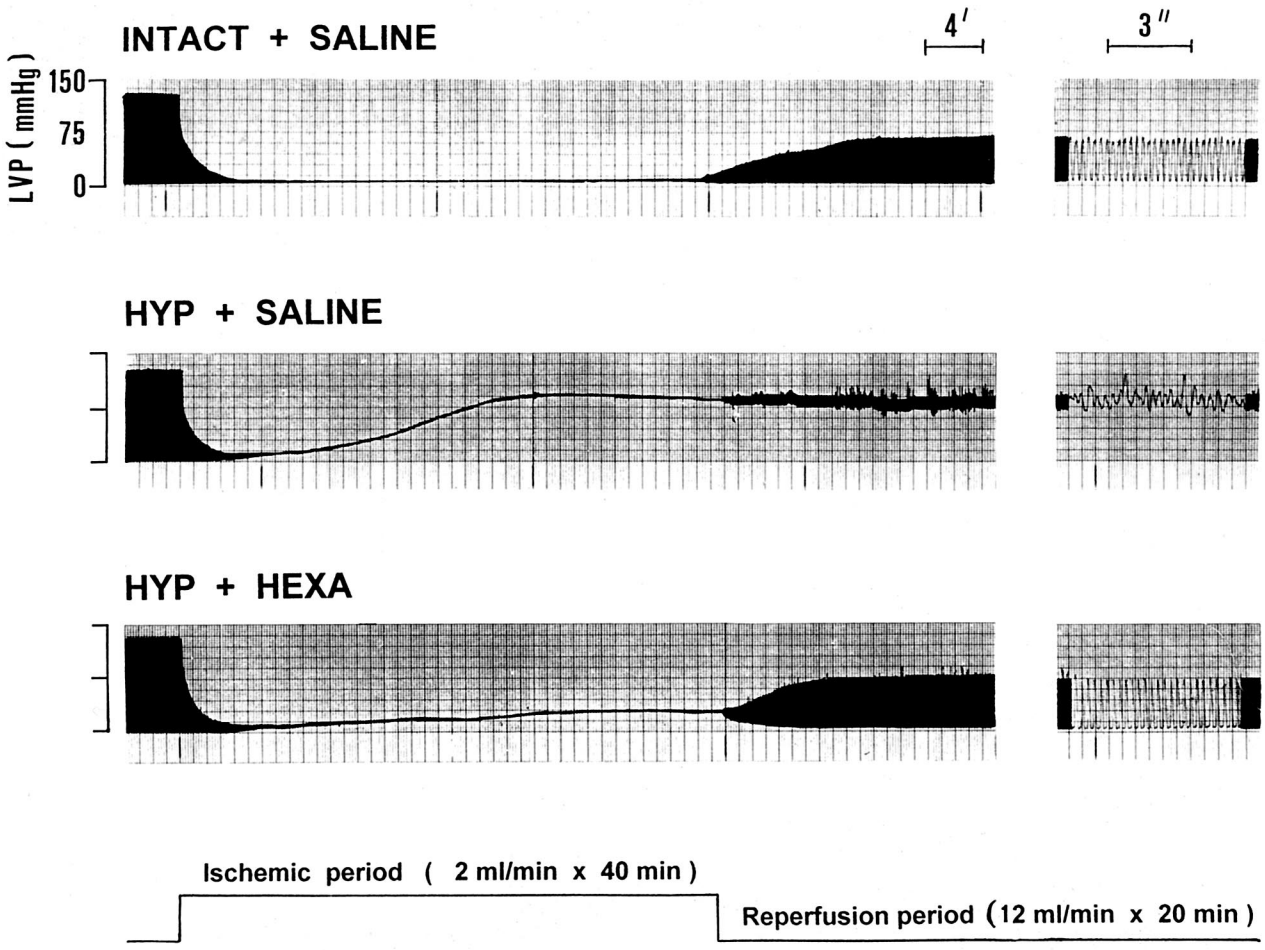

FIG. 1. Representative ischemia-reperfusion tracings obtained from hearts of intact and hypophysectomized rats. Rats were treated *in vivo* for 7 days as follows: INTACT + SALINE (intact rats treated with 1 ml/kg sc of saline); HYP + SALINE (hypophysectomized rats treated with 1 ml/kg sc of saline); HYP + HEXA (hypophysectomized rats treated with 80 μg/kg sc hexarelin).

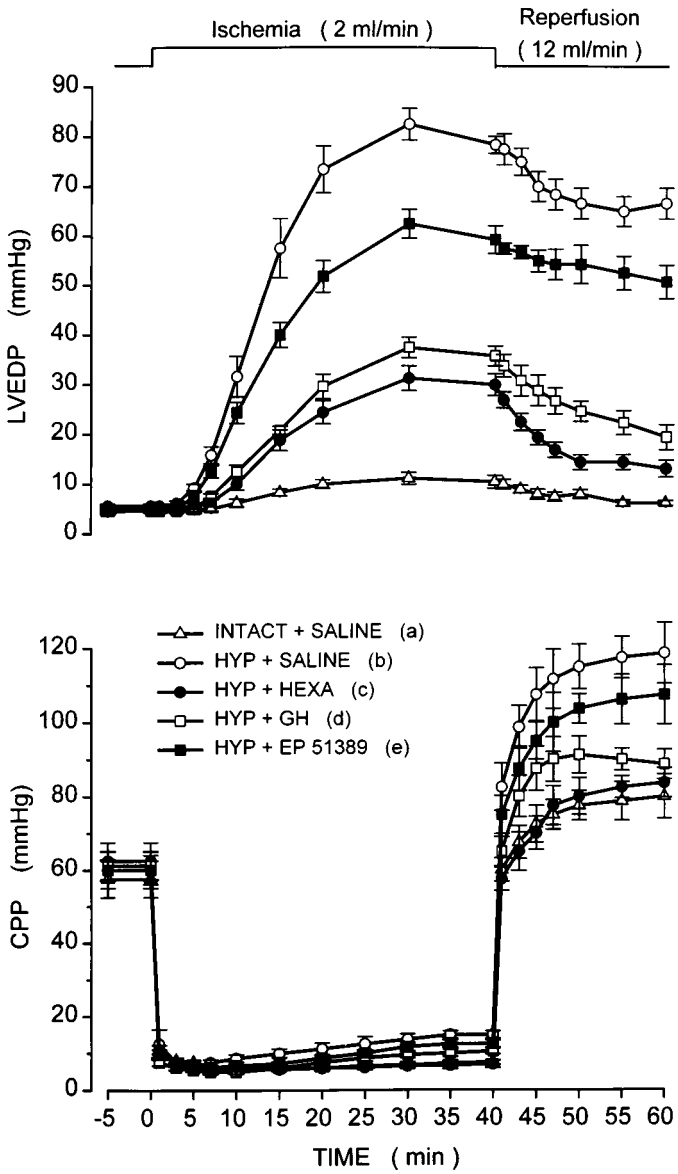

FIG. 2. Perfusion experiments with paced heart preparations. Drugs or saline were administered *in vivo* from experimental day 1–7. a, INTACT + SALINE; b, HYP + SALINE; c, HYP + HEXA; d, HYP + GH (hypophysectomized rats treated with 400 μg/kg sc of GH); e, HYP + EP 51389 (hypophysectomized rats treated with 80 μg/kg sc of EP 51389). Each point represents the mean ± SEM of the determinations obtained from eight hearts in each group. *Upper panel*, LVEDP (mm Hg). The corresponding AUCs, calculated according to the trapezoid method (from 0–60 min), are: a, 439 ± 38; b, 3637 ± 261; c, 1172 ± 137; d, 1490 ± 184; e, 2755 ± 204. Statistical differences are: b *vs.* a, $P < 0.01$; b *vs.* c and d, $P < 0.01$; b *vs.* e, $P < 0.05$; a *vs.* c and a *vs.* d, $P < 0.05$. *Lower panel*, CPP (mm Hg). Statistical analysis of AUCs, relative to the reperfusion period (from 40–60 min), shows that b *vs.* a, $P < 0.01$.

the end of the reperfusion period, these hearts recovered only 35% of their preischemic contractility values. In line with these results, the CPP values were elevated to values higher than basal values, *i.e.* 47.5 ± 3.2 mm Hg ($P < 0.01$) at the end of reperfusion.

CK activity

The level of CK activity released in the perfusates is a biochemical marker of necrotic lesions. The CK activities found in heart perfusates, collected during the reperfusion period, exhibited a strong correlation with the degree of myocardial ischemic injury present in the five experimental groups. The total amount of CK released, during 20-min reperfusion, from hearts of hypophysectomized animals was almost 3-fold higher ($P < 0.01$) than that found in perfusates of intact rats (Fig. 4). Treatment with GH or hexarelin reduced, by almost 50% ($P < 0.05$), the amount of CK released by the hearts of hypophysectomized rats during reperfusion. In contrast, heart preparations from hypophysectomized rats given EP 51389 generated CK activity in amounts similar to those released by hearts from saline-injected hypophysectomized rats (Fig. 4).

6-Keto-PGF$_{1\alpha}$ generation and angiotensin II activity

Hypophysectomy greatly impaired the basal formation of cardiac PGI_2, thus hindering the expected increase in its formation during early reperfusion (Fig. 5). The rate of 6-Keto-$PGF_{1\alpha}$ production in hearts from hypophysectomized rats was reduced by 55% and 54% in the preischemic and reperfusion periods, respectively. Treatment with GH or hexarelin prevented this fall in the rate of 6-Keto-PGF_{1a} production during the preischemic period. At reperfusion, the rate of formation of the eicosanoid in hearts from hexarelin- or GH-treated hypophysectomized rats was diminished only by 16% and 22%, respectively, and was not significantly different from that of intact rats. In contrast, in hearts from hypophysectomized rats given EP 51389, the rate of formation of 6-Keto-$PGF_{1\alpha}$ was still reduced by 47% and 49% in the preischemic and reperfusion periods, respectively (Fig. 5).

The functional integrity of the vascular endothelium was evaluated by measuring the reactivity of the coronary vessels to angiotensin II. The vasoconstriction induced by angiotensin II was significantly higher in hearts of hypophysectomized rats than in those from intact rats. In fact, injection of angiotensin II into the perfusion system of hearts from hypophysectomized saline-treated rats caused a CPP rise of 59.6 ± 1.5 mm Hg (Fig. 6), which was 3.7-fold higher ($P < 0.01$) than that recorded in hearts from intact rats (16.2 ± 2.5 mm Hg). Treatment with GH or hexarelin significantly reduced, by almost 50%, the effect of angiotensin II in hypophysectomized rats. In contrast, EP 51389 failed to protect the vascular endothelium from the ischemic damage. In fact, in heart preparations from rats treated with EP 51389, the rise in CPP values (46.5 ± 2.4 mm Hg) was 2.9-fold higher ($P < 0.01$) than that measured in preparations from intact rats (Fig. 6).

IGF-I concentrations in plasma and heart

GH administration induced a significant increase of IGF-I plasma concentrations in hypophysectomized rats (93.5 ± 9.2% increment over those of saline-injected rats, $P < 0.01$), whereas hexarelin and EP 51389 had no effect on plasma IGF-I levels. Hexarelin administration induced a trend toward an increase of heart IGF-I concentrations, though this

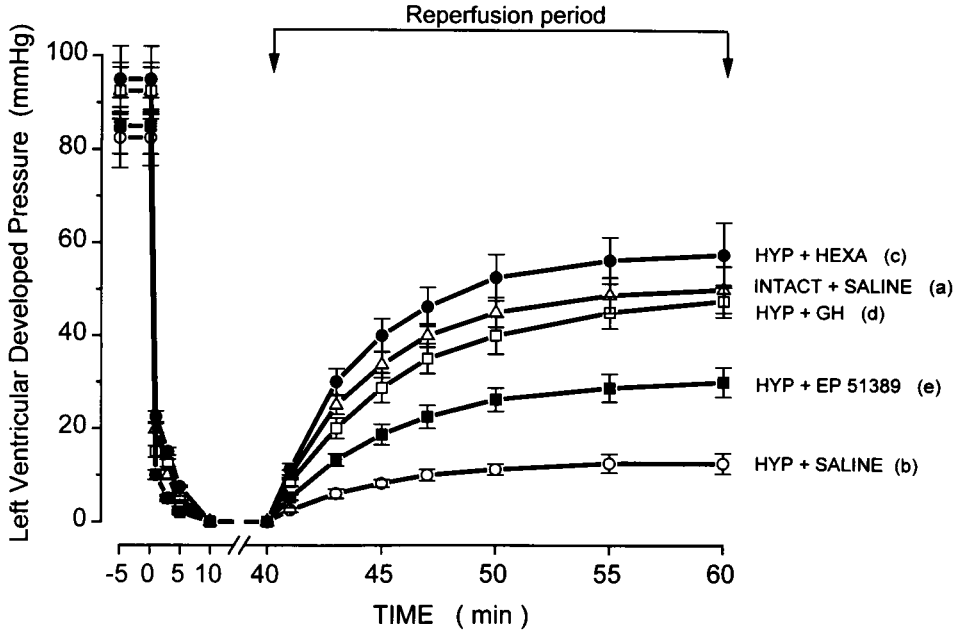

FIG. 3. LVDP in paced heart preparations subjected to global low-flow ischemia and reperfusion. Treatments are as described in the legend of Fig. 2. Each point represents the mean ± SEM of eight determinations. The calculated AUC values of LVDP (in mm Hg; time from 40–60 min) are: a, 781 ± 72; b, 196 ± 28; c, 907 ± 109; d, 701 ± 94; e, 451 ± 57. Statistical differences: b *vs.* a, $P < 0.01$; e *vs.* b, $P < 0.05$.

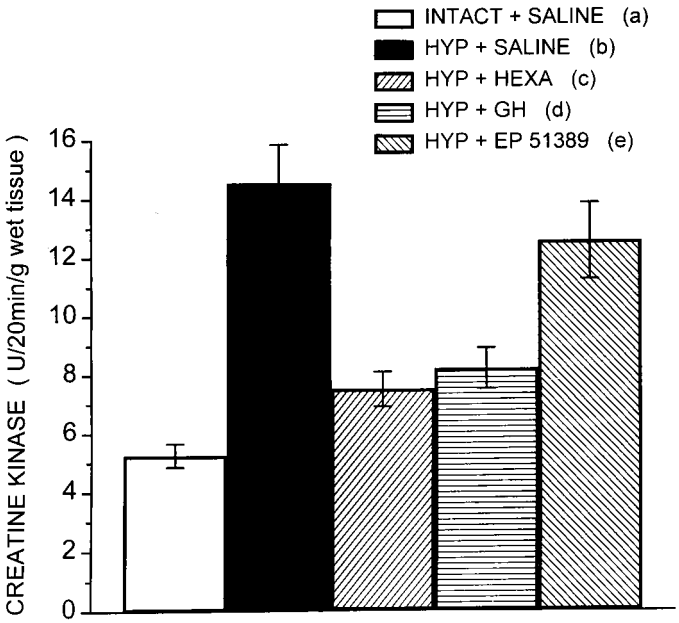

FIG. 4. CK activity, determined in the heart perfusates collected during the 20 min of reperfusion. Treatments are as described in the legend of Fig. 2. Values are the mean ± SEM of eight determinations. Statistical differences: b *vs.* a and e *vs.* a, $P < 0.01$; c *vs.* b and d *vs.* b, $P < 0.01$; a *vs.* c and a *vs.* d, $P < 0.05$.

increase did not reach statistical significance; GH and EP 51389 did not affect heart IGF-I concentrations (Table 2).

Discussion

Experimental and clinical studies have demonstrated that GH influences cardiac function and structure in several ways, but its mechanism of action is largely unknown. We have previously shown that, in young-adult male rats, the induction of selective GHD heightens myocardial ischemic damage when the hearts are exposed *in vitro* to global flow limitation followed by reperfusion (5). Both GH and hexarelin, given *in vivo* for 1 week, were similarly competent in reverting the effects of GHD. Because hexarelin is a very powerful GHS (7), its cardiac effects could have been mediated by endogenous GH. To ascertain whether hexarelin has non-GH-mediated protective effects on the heart, we compared GH and hexarelin treatment in hypophysectomized rats. Consistent with those data indicating that GH is needed to maintain optimal heart contractility, our results show that heart global flow limitation and reperfusion induced significantly greater myocardial damage in hearts from hypophysectomized rats than in those of intact animals. Compared with intact rats, hearts from hypophysectomized rats presented severe signs of ischemic and postischemic ventricular dysfunction, arrhythmia, increased CK activity in the perfusates, and constriction of the coronary vascular bed. Substitution with GH reduced ischemic cardiac injury. Hearts from GH-treated hypophysectomized rats exhibited a quicker recovery of contractility than the preparations from the saline group and more promptly followed the imposed electrical pacing. This treatment also normalized CK activity in the perfusates and the vasoconstriction of the coronary vasculature induced by angiotensin II. GH may have acted directly on the myocardium, stimulating specific receptors, and/or indirectly, through an increase in circulating IGF-I levels. GH receptors are expressed in the heart (21), and their number changes under different experimental settings, including volume overload (22). Chronic *in vivo* administration of GH can increase contractility of cardiac papillary muscles (23), and physiological doses of GH can improve systolic function in an experimental model of heart failure (24).

In this study, hexarelin had a strong protective activity against ischemia and reperfusion-induced myocardial damage, very similar to that observed for GH. Hexarelin pretreatment effectively reduced the ventricular contracture of the perfused heart during ischemia, and it reduced CK ac-

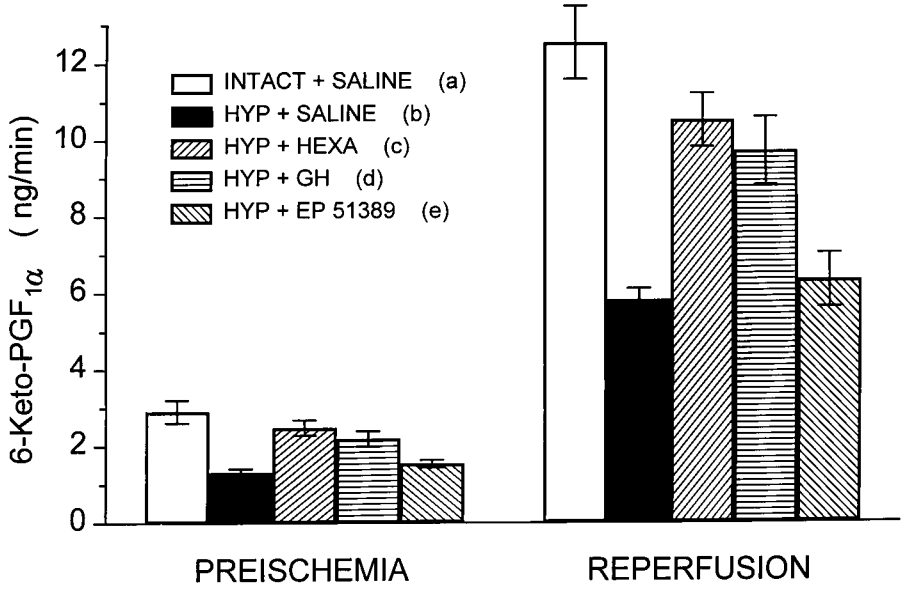

FIG. 5. Rate of 6-Keto-PGF_{1a} release in the heart perfusates during preischemia and reperfusion periods. Treatments are as described in the legend of Fig. 2. Each column represents the mean ± the SEM of eight determinations. Statistical differences during both preischemia and reperfusion are: b *vs.* a, $P < 0.01$; b *vs.* c and b *vs.* d, $P < 0.01$.

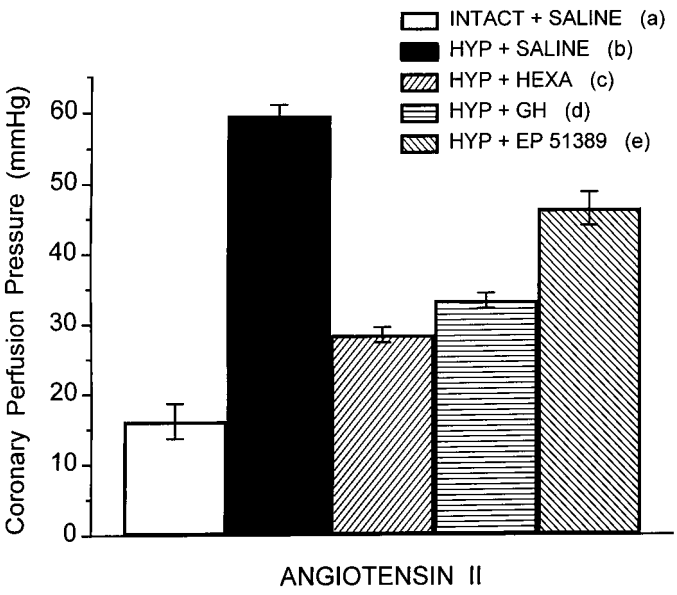

FIG. 6. Vasopressor activity of angiotensin II (1 μg/bolus) injected in paced heart preparations during preischemia. Treatments are as described in the legend of Fig. 2. Each column represents the mean ± the SEM of eight determinations. Statistical differences: b *vs.* a, $P < 0.01$; b *vs.* c and b *vs.* d, $P < 0.01$; b *vs.* e, $P < 0.05$.

tivity in the heart outflow at reperfusion. These events were paralleled by a more efficient recovery of LVDP, a prompt compliance of the heart to follow the external electrical pacing, and a reduction of the CPP. The protective effect of hexarelin was also demonstrated by maintenance of 6-Keto-$PGF_{1\alpha}$ production, as well as restoration of the coronary vessel reactivity to angiotensin II.

In the heart, PGI_2 production is a critical cytoprotective mechanism for resisting the damage caused by ischemia. In fact, PGI_2-mimetics (25, 26) or PGI_2 releasers (27) are known to improve heart mechanics in ischemic hearts by reducing ventricular contracture (heart rigidity) and calcium ion overload within cardiac myocytes. Moreover, stabilization of cardiac lysosomes, provided by a normal generation of PGI_2 in cardiac tissues, may represent another possible link with the beneficial effects disclosed by hexarelin in the present experiments. Cardiac lysosomes contain several acid hydrolases, including proteases and phospholipases. If these enzymes are released into the cell cytoplasm, they may contribute to the degradation of structural proteins and membrane phospholipids. During ischemia, leakage of lysosomal enzymes is reported to occur before the irreversible damage of myocardium (28). PGI_2 has been reported to be a potent stabilizer of lysosomes in the isolated cat liver (29) and in ischemic myocardium of intact animals (30).

The mechanism by which hexarelin exerts its beneficial effects on cardiac function in hypophysectomized rats is obviously independent of GH. Data obtained with the tripeptide EP 51389 are consistent with this view. This molecule is as effective as hexarelin in stimulating GH secretion in the rat (11, 13), but it is far less effective in protecting the heart from ischemia (this study). The messenger RNA (mRNA) encoding a receptor specific for peptidyl and nonpeptidyl GHS has recently been cloned (31), and it has been reported that it is expressed in several peripheral organs of the male rat, including the heart (32, 33). Interestingly, EP 51389 effectively displaces hexarelin from its hypothalamic binding sites, and poorly from cardiac membranes (11), which suggested the presence of multiple receptor subtypes for GHS. More recently, evidence for GHS receptor subtypes in rat pituitary and heart, distinct from that previously cloned, was obtained using a photoactivable analog of hexarelin (12, 34).

A possible interpretation of our findings is that hexarelin, via stimulation of specific cardiac receptors, triggers cytoprotective mechanisms conferring resistance to ischemic insults.

The local generation and release of IGF-I may have contributed to the overall protective effect of hexarelin and GH. IGF-I has a positive inotropic effect in healthy male volunteers (35), increases force development in isolated rat pap-

TABLE 2. Levels of IGF-I in plasma and cardiac muscles of hypophysectomized rats

	IGF-I levels			
	Saline	GH	Hexarelin	EP 51389
Plasma (ng/ml)	70.3 ± 7.9	131.2 ± 17.2[a]	73.3 ± 5.1	65.4 ± 7.7
Heart (ng/g)	12.3 ± 3.1	18.7 ± 3.6	22.5 ± 5.0	12.9 ± 1.5

Data are the mean ± SEM of eight rats. Treatments are as described in the legend of Fig. 2.
[a] $P < 0.05$ *vs.* saline-treated rats.

illary muscles (36), and increases free cytosolic Ca^{2+} concentrations in cultured cardiomyocytes (36). However, in our experiments, neither GH, hexarelin, nor EP 51389 had significant effects on IGF-1 titers in the heart. The cardioprotective effects of GH may have been mediated by an elevation of plasma IGF-I levels. In fact, it has been shown that IGF-I stimulates nitric oxide (NO) release from cultured endothelial cells, and NO is an important regulator of vascular function (37). In contrast, hexarelin did not stimulate plasma levels of IGF-I. The existence of a direct functional relationship between hexarelin and NO formation in cardiac endothelial cells is yet to be explored.

Our data showed that ablation of the pituitary gland also resulted in the hyperreactivity of coronary smooth muscle cells to angiotensin II, a phenomenon previously observed in rats with selective GHD (5). This finding, together with the clear-cut reduction of PGI_2 generation, further emphasizes the involvement of the somatotropic axis in the mechanism(s) regulating the vascular tone.

It is well known that NO generation by endothelial cells plays a prominent role in the regulation of vascular tone and in the modulation of vasoconstrictor activity, whereas the contribution of PGI_2 to this mechanism is rather poor (38). PGI_2, released by the endothelium, is mainly directed toward the vascular lumen, so that its major activity would be the antiplatelet effect and not vasodilatation. This would imply that a dysfunction of NO production in the coronary vascular bed of the hypophysectomized rat should be considered for understanding the hyperreactivity to angiotensin II. Alterations of the vasopressor acetylcholine activity in perfused hearts obtained from rats with selective GHD already has been reported (5).

The mechanism(s) through which hexarelin and GH preserve the functional integrity of cardiac endothelial cell function and normalize PGI_2 production in hypophysectomized rats is unknown. Whatever the mechanism(s) involved, it is intriguing that both hexarelin and GH were able to counteract the increased sensitivity of the coronary vasculature to vasoconstrictors in the hypopituitary state. This effect was not observed with EP 51389, which emphasizes the specificity of hexarelin action on the heart.

In conclusion, our findings demonstrate that short-term pretreatment with hexarelin counteracts ischemic damage in perfused hearts of hypophysectomized rats. This protective activity is likely exerted through specific cardiac receptors and is independent of its GH-releasing properties. These data suggest that the GHS may be of therapeutic value in the prevention of primary and, possibly, secondary myocardial ischemic events in humans.

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