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Cardiac ischemia and impairment of vascular endothelium function in hearts from growth hormone-deficient rats: Protection by hexarelin

Vito De Gennaro Colonna *, Giuseppe Rossoni, Micaela Bernareggi, Eugenio E. Müller, Ferruccio Berti

Department of Pharmacology, Chemotherapy and Medical Toxicology, University of Milan, Via Vanvitelli 32, 20129 Milan, Italy

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Abstract

The ability of hexarelin, an effective growth hormone (GH)-releasing hexapeptide, to reverse the worsening of cardiac dysfunction in GH-deficient animals was studied in young male rats passively immunized by administration of an anti-GH-releasing hormone (GHRH) serum. Heart preparations from anti-GHRH serum-treated rats, undergoing low-flow ischemia and reperfusion, showed: (1) a progressive increase of left ventricular end-diastolic pressure during the ischemic period and a poor recovery of contractility at reperfusion with a consistent decrease of the left ventricular-developed pressure; (2) a decreased rate of formation of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), a stable metabolite of prostacyclin, in perfusates from preischemic and reperfusion periods; (3) an increased vasopressor activity of angiotensin II. Hexarelin (80 μg/kg, bid, s.c.), administered for 15 days to anti-GHRH serum-treated rats, restored to normal the impaired somatotropic function and counteracted the ischemic damage, improving postischemic left ventricular developed pressure to values higher than those of controls. Furthermore, both the generation of 6-keto-$PGF_{1\alpha}$ and the vasopressor activity of angiotensin II reverted to those of control preparations. Administration of hexarelin to control rats induced a considerable improvement of postischemic ventricular function of the perfused hearts which was similar to that present in preparations from anti-GHRH serum-treated rats given hexarelin. This protective activity was divorced from any further stimulation of somatotropic function. Collectively, these data indicate that, in GH-deficient rats, hexarelin is capable of restoring somatotropic function and has a beneficial effect in myocardial ischemia and reperfusion damage. In addition, the increased responsiveness of the coronary vasculature to angiotensin II and the decreased generation of prostacyclin in hearts from GH-deficient rats would indicate that for prevention of injury and dysfunction of the vascular endothelium a normal somatotropic function is mandatory. © 1997 Elsevier Science B.V.

Keywords: Hexarelin; GH (growth hormone-)deficiency; Ischemia-reperfusion; Heart, rat; 6-keto-prostaglandin $F_{1\alpha}$; Angiotensin II

1. Introduction

Over the past few years, significant advances have been made in our understanding of the cellular and molecular mechanisms involved in growth hormone (GH) action, including its effects on cardiac tissue (Saccà et al., 1994). Furthermore, experimental evidence points to a role of GH in cardiac pathophysiology. It has been suggested, in fact, that the increased cardiovascular mortality, in particular a high incidence of myocardial infarction and heart failure, seen in patients with treated hypopituitarism, but without any GH replenishment, may be due to GH deficiency (Rosen and Bengtsson, 1990). Consistent with this view, in young adults with congenital GH deficiency and impairment of systolic function, GH administration for 6 months restores ventricular mass and function (Amato et al., 1993).

Recently, we reported (De Gennaro Colonna et al., 1996) that heart preparations from a rat model of GH deficiency, when subjected to global low-flow reduction and reperfusion, are significantly more sensitive to ischemic damage than heart preparations from control rats. In these rats, GH replacement therapy prevented ischemic and postischemic myocardial abnormalities.

These findings prompted us to investigate in the present study whether hexarelin, a recently synthesized hexapeptide with a strong GH-releasing activity (Deghenghi et al., 1994), like GH is capable of reversing cardiac ventricular dysfunction in the same rat model of GH deficiency. In

* Corresponding author. Tel.: +39-2-70146237; fax: +39-2-70106838.

this experimental context, the endothelium-dependent relaxing function of coronary arteries was also investigated.

2. Materials and methods

2.1. Animals and treatments

Male Sprague–Dawley rats (Charles River, Calco) at weaning (20 days) were selected and randomly assigned to four experimental groups of 10 animal each and treated with: 1, normal rabbit serum (controls); 2, anti-GHRH-serum (GH-deficients); 3, normal rabbit serum + hexarelin; 4, anti-GHRH-serum + hexarelin.

The anti-GHRH-serum was prepared as previously described (De Gennaro Colonna et al., 1996) and rats were treated every other day by subcutaneous administration of the antiserum (250 μl/rat) or isovolumic amounts of normal rabbit serum from postnatal day 20 to 40.

Hexarelin (His-D-2-Me–Trp–Ala–Trp-D-Phe–Lys–NH_2, Pharmacia, Stockholm) (80 μg/kg, bid, sc) was given to rats from postnatal day 25 to 40 in addition to normal rabbit serum or anti-GHRH-serum treatment. Rats were killed by cervical dislocation 14 h after the last injection of hexarelin. Anterior pituitaries were removed, immediately frozen on dry ice and stored at -20°C until used for determination of the level of GH mRNA, which was performed as previously described (De Gennaro Colonna et al., 1996) using a rat GH cDNA sequence labelled by the multiprime DNA labelling system (Amersham, Little Chalfont). In addition, blood was collected into EDTA-containing tubes and the separated plasma was used for the radioimmunological evaluation of plasma levels of insulin-like-growth factor-1 (IGF-1), as reported by Daughaday et al. (1980).

2.2. Perfused rat heart preparations

Hearts from animals in the four experimental groups were perfused retrogradely through the aorta with gassed Krebs–Henseleit solution (37°C), as described by Berti et al. (1988). The perfusion rate for each heart, electrically paced at a frequency of 300 beats/min, was adjusted to yield a coronary perfusion pressure of 55–60 mm Hg with a flow rate of 12 ml/min. Left ventricular pressure was measured by inserting a small latex balloon into the ventricular cavity and filling it with saline until left ventricular end-diastolic pressure stabilized in the range of 5 mm Hg.

Ischemia was induced by reducing the coronary flow to 2 ml/min (perfusion pressure 4–6 mm Hg). Reperfusion started 40 min after the onset of the flow reduction and lasted for another 20 min. Postischemic left ventricular-developed pressure (= peak left ventricular systolic pressure minus left ventricular end-diastolic pressure) was recorded.

The vasopressor activity of angiotensin II (1 μg injected as a bolus into the perfusion system) on coronary vasculature was recorded at the beginning of each experiment.

In this study, Hewlett-Packard (Waltham, MA) and Grass (Quincy, MA) instruments were used.

2.3. 6-Keto-$PGF_{1\alpha}$ in heart perfusates

Prostacyclin (PGI_2) generation was measured in the heart perfusates as 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), using the enzyme immunoassay previously described by Pradelles et al. (1985). Particularly, the concentration of this eicosanoid was determined in the heart perfusates collected for 5 min immediately before flow reduction and during the first 10 min of reperfusion. The rate of formation of 6-Keto-$PGF_{1\alpha}$ was evaluated by using a specific kit (Cayman, Chemical, Ann Arbor, MI, Catalog No. 416011) and was expressed in ng/min.

2.4. Statistical analysis

Differences between data for groups in individual experiments were analyzed for statistical significance by one-way analysis of variance and Student's *t*-test (two-tailed) for unpaired samples. A value of $P < 0.05$ was considered significant. Results are expressed as means ± S.E.M. or/and areas under the curve (AUC) evaluated according to the trapezoid method.

Table 1
Body and heart weights and markers of somatotropic function in control and GH-deficient rats treated or not with hexarelin

Treatment	Body weight (g)	Heart weight (mg)	Heart weight/body weight (mg/g)	Pituitary GH mRNA (%)	Plasma IGF-1 (ng/ml)
NRS	193.1 ± 2.2	1475 ± 10.1	7.63	100	169 ± 5.0
GHRH-Ab	168.2 ± 2.1 [a]	1295 ± 9.0 [a]	7.69	−51.2 ± 1.7 [a]	93 ± 2.4 [a]
HEXA	190.0 ± 1.5	1451 ± 10.2	7.60	−2.7 ± 4.4	158 ± 5.2
GHRH-Ab + HEXA	192.8 ± 1.8	1480 ± 12.8	7.67	−7.0 ± 6.1	157 ± 2.4

Treatment legend as in Fig. 1. Figures related to body and heart weights and plasma levels of IGF-1 are mean values ± S.E.M. of 10 determinations. Figures related to pituitary GH mRNA are mean values ± S.E.M. of 5 determinations.
[a] $P < 0.01$ vs. NRS.

3. Results

3.1. Growth rate and somatotropic function

As shown in Table 1, during treatment with anti-GHRH-serum, rats grew significantly less than those of the remaining experimental groups and the same was true for the weight of the heart recorded at the end of the experiment. However, in all experimental groups, the heart weight/body weight ratio was not statistically different, indicating that in the GH-deficient rats a proportional decrease of body and heart weight had occurred. Pituitary GH-mRNA and plasma IGF-1 levels were reduced by 51.2% ($P < 0.01$) and 43% ($P < 0.01$), respectively, in GH-deficient rats, but long-term administration of hexarelin restored both indices of somatotropic function to those of control rats. In contrast, hexarelin in normal rabbit serum-treated rats did not modify basal somatotropic function, pituitary GH mRNA and plasma IGF-1 levels being not statistically different from those of controls.

3.2. Ischemia-reperfusion in isolated rat hearts

The global reduction of the perfusion flow of paced isovolumic left heart preparations from normal rabbit serum-treated rats resulted in minor ischemic damage at reperfusion (Figs. 1 and 2). In fact, based on a prompt restoration of electrical pacing and on a minimal increase in resting tension, heart contractility, expressed as LV-developed pressure, recovered within 20 min to 58.1% of the basal values ($P < 0.01$). In contrast, when hearts from GH-deficient rats were subjected to ischemia and reperfusion, severe aggravation of the ischemic trend occurred (Figs. 1 and 2). In this instance, the values of LVEDP at the end of the ischemic and reperfusion periods were 5.4-($P < 0.001$) and 7.8-($P < 0.001$) fold as high as the corresponding control values.

The poor recovery of heart contractility at reperfusion was associated with a persistent rhythm disturbance and a LVDP 2.6-fold lower ($P < 0.01$) than that of control hearts (Fig. 2). Moreover, at the end of this period coronary perfusion pressure was only 52% ($P < 0.01$) of that of controls, and this was in part due to a certain degree of heart stiffness (Fig. 1).

Hexarelin treatment strikingly inhibited the ischemic damage in the isolated hearts from GH-deficient rats, so that the results obtained were now rather similar to those of control preparations (Figs. 1 and 2). In fact, at the end of the ischemic and reperfusion periods, the values of LVEDP were only 1.4- ($P < 0.01$) and 2.3- ($P < 0.01$) fold higher than the corresponding control values, respectively (Fig. 2); CPP was hardly modified, being at the end of reperfusion not statistically different from that of controls (Fig. 1). It is noteworthy that the postischemic LV-developed pressure of these hearts was even greater than

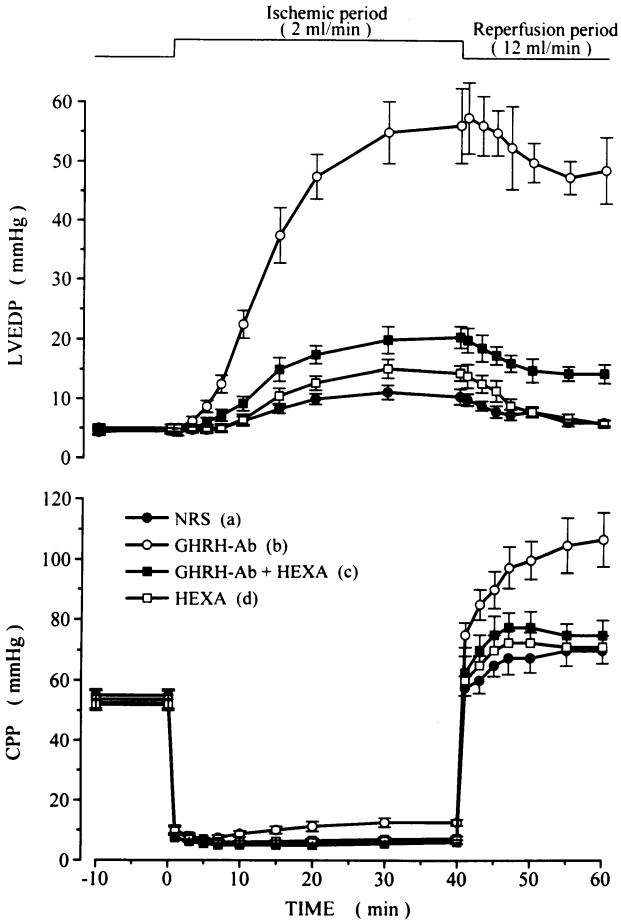

Fig. 1. Perfusion experiments with paced isovolumic left heart preparations from normal rabbit serum (NRS, group a), anti-GHRH serum (GHRH-Ab, group b), anti-GHRH serum + hexarelin (GHRH-Ab + HEXA, group c) and hexarelin (HEXA, group d) treated rats. Each point represents the mean values and vertical bars the S.E.M. from 10 hearts. LVEDP = left ventricular-end diastolic pressure; CPP = coronary perfusion pressure. The LVEDP-AUC values are: group a, 499 ± 55; group b, 2563 ± 197; group c, 916 ± 84; group d, 622 ± 68. Statistical significance: b vs. a, c and d, $P < 0.001$; c vs. d and a, $P < 0.05$; d vs. a, P = NS. AUC: in ordinate, LVEDP in mm Hg; in abscissa, time from 0 to 60 min.

that of controls, since the recovery of heart contractility at the end of reperfusion was 77.6% (Fig. 2).

Administration of hexarelin to normal rabbit serum-treated rats provided a degree of protection against ischemia-reperfusion damage which was not different from that present in hearts from anti-GHRH-serum + hexarelin-treated rats (Figs. 1 and 2). In fact, the values of the corresponding areas under the curves related to LVDP were not statistically different (Fig. 2).

3.3. 6-Keto-$PGF_{1\alpha}$ generation in perfused rat hearts

It is well known that a reperfused organ releases various mediators such as catecholamines and prostacyclin (Berti et al., 1986). In the present study, the rate of formation of 6-keto-$PGF_{1\alpha}$ during preischemia and reperfusion in hearts from anti-GHRH-serum treated rats was markedly reduced

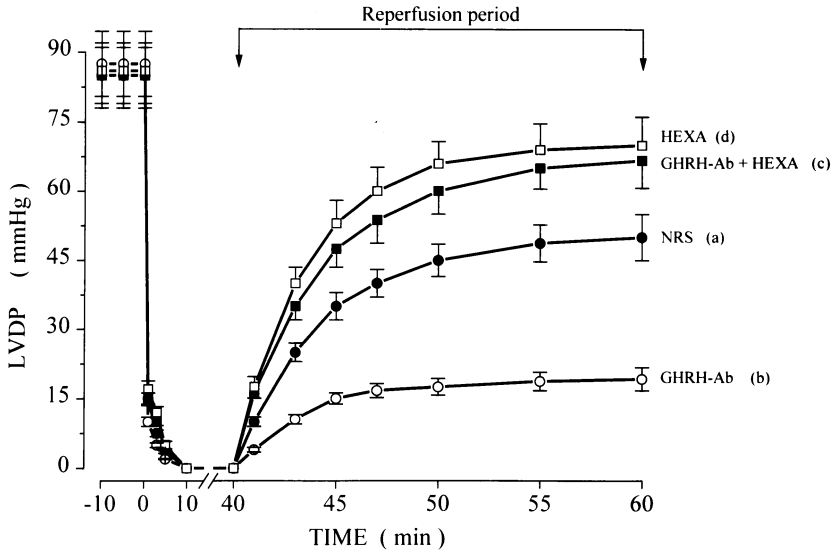

Fig. 2. Left ventricular developed pressure (LVDP) in isovolumic left heart preparations subjected to global low-flow ischemia and reperfusion. Each point represents the mean values and vertical bars the S.E.M. from 10 hearts. The LVEDP-AUC values are: group a, 877 ± 54; group b, 386 ± 29; group c, 1154 ± 82; group d, 1256 ± 108. Statistical significance: b vs. d, c and a, $P < 0.01$; d and c vs. a, $P < 0.05$; d vs. c, P = NS. For abbreviations see legend of Fig. 1.

(50%; $P < 0.01$) as compared to that of hearts from controls rats (Fig. 3). In contrast, the rate of formation of 6-keto-$PGF_{1\alpha}$ in hearts from anti-GHRH-serum + hexarelin-treated rats was not statistically different from that of hearts from controls in both the experimental periods (Fig. 3). In fact, the rate of formation of 6-keto-$PGF_{1\alpha}$ in hearts from controls and GH-deficient rats treated with hexarelin during the preischemia was 2.7 ± 0.2 and 2.3 ± 0.2 ng/min, respectively, and at reperfusion was 9.4 ± 0.8 and 7.9 ± 0.8 ng/min, respectively. In contrast, treatment of control rats with hexarelin did not significantly modify the rate of formation of 6-keto-$PGF_{1\alpha}$ (Fig. 3).

3.4. Vasopressor activity of angiotensin II

During preischemia the bolus injection of angiotensin II (1 μg) into the isolated heart from GH-deficient rats induced a marked rise in CPP which was 3.8-fold higher ($P < 0.001$) than that recorded in hearts from control rats. The hyperreactivity of the coronary vasculature to angiotensin II was not present in hearts from anti-GHRH

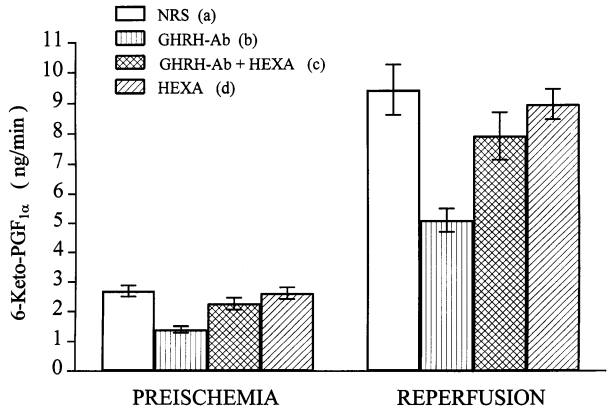

Fig. 3. Rate of release of 6-Keto-$PGF_{1\alpha}$ in perfusates of isovolumic left heart preparations from rats of the 4 experimental groups. Each column represents the mean values and vertical bars the S.E.M. from 10 hearts. Perfusates were collected for 5 min before flow reduction (preischemia) and during the first 10 min of reperfusion. Statistical significance: a vs. b, $P < 0.001$; a vs. c and d, P = NS. For abbreviations see legend of Fig. 1.

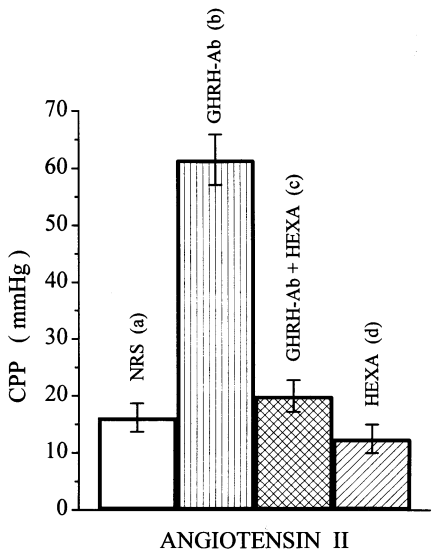

Fig. 4. Vasopressor activity of angiotensin II (1 μg) injected in isovolumic left heart preparations during the preischemic phase. CPP, coronary perfusion pressure. Each column represents the mean values and vertical bars the S.E.M. from 10 hearts. Statistical significance: b vs. a, c and d, $P < 0.001$; a vs. c and d, P = NS. For abbreviations see legend of Fig. 1.

serum + hexarelin-treated rats. In fact, in this instance, CPP (20 ± 2.8 mm Hg) was in the range of values recorded in hearts from controls (16.2 ± 2.5 mm Hg). When isolated hearts from control rats treated with hexarelin were challenged with angiotensin II, the response of the coronary vasculature (12.5 ± 2.5 mm Hg) was in the range of that recorded in untreated control preparations (Fig. 4).

4. Discussion

In a recent study from our group (De Gennaro Colonna et al., 1996), rats passively immunized against GHRH, a suitable model of selective GH deficiency (Wehrenberg et al., 1984; Wehrenberg, 1986; Arsenijevic et al., 1989; Cella et al., 1994a,b), exhibited clear signs of cardiac dysfunction, consisting of an exacerbation of ischemic tissue damage during low-flow ischemia and reperfusion, with increased coronary artery resistance upon reperfusion. 'Ex vivo' replacement therapy with GH restored the heart abnormalities to normal. In the present study, the possibility of restoring cardiac function in anti-GHRH serum-treated rats was tested with the use of hexarelin, an effective GH secretagogue (Deghenghi et al., 1994).

Rats given the anti-GHRH serum were truly GH deficient, as shown by decreased growth rate, pituitary GH mRNA and plasma IGF-1 levels (Arsenijevic et al., 1989; Shakutsui et al., 1989; Cella et al., 1994a,b). In these rats, administration of hexarelin restored somatotropic function, as indicated by normalization of these biological markers. Restoration of GH mRNA levels in anti-GHRH serum-young-adult male rats given hexarelin at the same dose as that used in these experiments was already reported by Torsello et al. (1997).

The mechanism(s) underlying the action of hexarelin is not fully understood. This peptide may modulate GH secretion by acting directly on the pituitary (Pong et al., 1991; Smith et al., 1993) or/and at hypothalamic level by modulating the release of somatostatin (Clark et al., 1989; Bowers et al., 1991) and/or GHRH (Bercu et al., 1982; Clark et al., 1989; Dickson et al., 1993) and/or an unknown factor (Bowers et al., 1991). Whatever the mechanism of action might be, hexarelin-induced restoration of somatotropic function appears to be instrumental to the striking improvement of the postischemic ventricular function recorded in the isolated hearts. However, the observation that hexarelin induced a clear-cut protection against myocardial damage also in control rats without modifying somatotropic function raises the important issue of its true mechanism of action and suggests that the peptide may also act directly on the heart. In support of this view, mRNA coding for a receptor related to growth hormone-releasing secretagogues, such as hexarelin, has been recently detected in rat cardiac tissue (Grilli et al., 1997). Furthermore, the possibility cannot be ruled out and is currently being investigated that hexarelin, besides increasing plasma IGF-1 levels, may stimulate local IGF-1 biosynthesis or induce accumulation of the peptide in the heart by interfering with its degradation (Zapf, 1995).

In this connection, an increased responsiveness of the cardiac myofilaments to IGF-1 made locally available by hexarelin should be considered and this might explain the more pronounced improvement of the isolated heart contractility upon reperfusion. Reportedly, IGF-1 has positive inotropic effects in healthy male volunteers (Donath et al., 1996) and on rat papillary muscle, with increasing force development and rise in free peak Ca^{2+} in isolated cardiac myocytes (Freestone et al., 1996). Furthermore, the ability of IGF-1 to limit reperfusion injury in rat hearts subjected to ischemia (Buerke et al., 1995) and in functionally impaired hearts of rats undergoing myocardial infarction (Duerr et al., 1995) has been clearly demonstrated.

Another interesting feature of GH deficiency that emerges from these experiments was the reduced formation of 6-keto-$PGF_{1\alpha}$ in isolated hearts, not only during the preischemic phase but in particular during reperfusion. This may bear some relevance to the aggravation of the ischemic damage detected in the hearts of these animals. In fact, in hearts from anti-GHRH serum plus hexarelin-treated rats, the attenuation of the ischemic damage was associated with a recovery of prostacyclin release within the range of values of control preparations. It is well known that insufficient production of primary prostaglandins may be associated with further aggravation of tissue damage, in particular in early reperfusion (Berti et al., 1988). Along this line, it has been already reported that prostacyclin mimetics (Araki and Lefer, 1980; Farber et al., 1988) or prostacyclin releasers (Berti et al., 1987; Hohlfeld et al., 1991) prevent ventricular contracture of ischemic hearts and improve heart mechanics at reperfusion.

Another important finding of our study was the hyperreactivity of coronary smooth muscles to angiotensin II in heart preparations from GH-deficient rats. This, especially when viewed in conjunction with a clear-cut reduction of prostacyclin generation by the cardiac tissues, not only denotes damage of the vascular endothelial-dependent relaxant mechanism but also emphasizes the crucial role of somatotropic function in maintaining the integrity of the vascular endothelial cell lining. In fact, in heart preparations from anti-GHRH serum plus hexarelin-treated rats, the recovery of the somatotropic function was associated with normalization of the vasopressor activity of angiotensin II and with preserved generation of prostacyclin. The competence of the latter to modulate the vasopressor activity of endothelin 1 in the isolated perfused rabbit heart has already been demonstrated (Berti et al., 1993).

Changes in coronary perfusion pressure in response to acetylcholine have been already reported in isolated hearts from GH-deficient rats, in which replacement therapy with GH restored the physiologic response of the coronary vasculature to the neuromediator (De Gennaro Colonna et

al., 1996). In spite of the complexity of the mechanism underlying the vasopressor response to acetylcholine (Yang et al., 1993), these previous data and our present results support the idea that for a modulatory response of the vascular tissue to vasoconstriction, a preserved somatotropic function is needed.

Collectively, the present results indicate that hexarelin given to rats with selective GH deficiency is capable of restoring somatotropic function to an extent similar to that induced by GH replacement therapy. However, the beneficial effect of hexarelin on the postischemic ventricular dysfunction of control rats would involve, at least in part, a direct action on the myocardiocytes and is unrelated to restoration of pituitary GH mRNA and plasma IGF-1 levels. Finally, the hyperreactivity of the coronary vasculature to angiotensin II in hearts from GH-deficient rats, which was fully antagonized by hexarelin, would indicate that a normal function of the GH/IGF-1 axis is crucial for preventing vascular endothelial injury and dysfunction. However, presently we cannot ignore the possibility that the effects induced by hexarelin may be totally or in part due to intrinsic GH-independent properties of the peptide.

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