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Worsening of ischemic damage in hearts from rats with selective growth hormone deficiency

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Abstract

The effects of growth hormone (GH) deficiency on cardiac function were studied in young male rats administered an anti-GH-releasing hormone (GHRH) serum from postnatal day 20 to 40. Dependence of heart abnormalities on GH deficiency was ascertained by giving a group of anti-GHRH serum-treated rats GH replacement therapy. Heart preparations from anti-GHRH serum-treated rats, undergoing low-flow ischemia, showed a progressive increase in left ventricular end-diastolic pressure with poor recovery of mechanical activity and increased coronary perfusion pressure upon reperfusion. Hearts from anti-GHRH serum + GH-treated rats, undergoing global reduction of the flow, showed only a minimal increase of left ventricular end-diastolic pressure and, upon reperfusion, cardiac mechanical activity recovered almost completely. Similar findings were also observed in heart preparations from control (normal rabbit serum-treated) rats. Infusion of acetylcholine (10^{-6} M) into heart preparations in the preischemic period increased coronary perfusion pressure values more markedly in hearts from normal rabbit serum- and anti-GHRH serum + GH-treated rats than in those from anti-GHRH serum-treated rats. These results indicate that selective GH deficiency in young male rats renders the heart more sensitive to ischemic damage and leads to an impairment of cardiac muscarinic receptor function.

Keywords: Growth hormone (GH) deficiency; Cardiovascular effects; Heart, rat; Ischemia

1. Introduction

Patients with hypopituitarism suffer a greater risk of cardiovascular mortality, in particular for myocardial infarction and heart failure, due to growth hormone (GH) deficiency (Rosen and Bengtsson, 1990). More recently, evaluation of cardiac structure and function by echocardiography in adult patients with GH deficiency demonstrated the existence of structural and functional abnormalities in these subjects (Merola et al., 1993). To gain more information on cardiac dysfunction in GH-deficient states, we have studied the mechanical activity of hearts obtained from animals deprived of GH secretion, using as an experimental model rats passively immunized against GH-releasing hormone (GHRH). Myocardial ischemia induced by hypoperfusion and endothelium-dependent relaxing function of the coronary vasculature were also evaluated in these GH-deficient animals. That these heart abnormalities result from GH deficiency was further ascertained by replacement therapy with GH in a group of rats treated with the anti-GHRH serum.

2. Materials and methods

2.1. Animals

Pregnant Sprague-Dawley rats (Charles River, Calco, Italy) were purchased and housed under controlled conditions (22 ± 2°C, 65% humidity, and artificial light from 06:00 to 20:00 h). After birth, all litters were culled to a standard size of 12 pups. At weaning (20 days), male rats were selected, randomly assigned to an experimental group (normal rabbit serum-treated, anti-GHRH serum-treated and anti-GHRH serum + GH-treated) and weighed.

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2.2. Antiserum to GHRH

The anti-GHRH serum was prepared by immunizing rabbits with a mixture of synthetic rat GHRH (Spiess et al., 1983) and methylated BSA emulsified in Freund's adjuvant, as previously described (Benoit et al., 1982). The biologic efficacy of the antiserum was assessed at various levels. The anti-GHRH serum has repeatedly been shown to significantly inhibit GH secretion and growth (Wehrenberg et al., 1984; Wehrenberg, 1986; Arsenijevic et al., 1989). In addition, the antiserum was tested for rat GHRH-binding capacity with ^{125}I-labeled rat GHRH. The antiserum dilution required to bind 30% of the tracer was approximately 1 : 30 000. Characterization of the antiserum showed that it was directed toward the GHRH carboxy terminal. It cross-reacted with synthetic human, bovine, and porcine GHRH by less than 4%. The antiserum did not cross-react with peptides that have considerable sequence homology with GHRH, including secretin, glucagon, vasoactive intestinal peptide, gastrin, motilin, bradykin, and angiotensin.

2.3. Treatments

Rats were treated every other day by s.c. administration of the anti-GHRH serum (250 μl/rat) or isovolumetric amounts of normal rabbit serum from postnatal day 20 to 40. A group of anti-GHRH serum-treated rats was given in addition biosynthetic human GH (0.4 μg/g body weight, s.c. bid, Genotropin, KabiVitrum, Stockholm, Sweden) for the same length of time. At 41 days of life, about 14 h after the last injection of GH, rats were killed by decapitation. Pituitaries were removed, immediately frozen on dry ice, and stored at −70°C until used. Blood was collected into EDTA-containing tubes and plasma was separated and stored at −20°C for insulin-like growth factor I (IGF-I) determination.

2.4. Pituitary GH mRNA

For the evaluation of GH mRNA levels, 10 pituitaries from each experimental group were collected in pools of two samples (five pools per experimental group). Total RNA was obtained by single-step acid guanidium-phenol-chloroform extraction (Chomczynski and Sacchi, 1987). Total RNA samples (20 μg/sample) were electrophoresed on 1.2% formaldehyde-agarose gel and transferred to a nitrocellulose membrane at room temperature for 24 h in 10 × saline sodium citrate (SSC) (1 × SSC = 0.1 M sodium chloride/0.01 M sodium citrate). Filters were hybridized with a rat GH cDNA sequence (Cella et al., 1994a,b) labeled by the Multiprime DNA labeling system (Amersham, Little Chalfont, UK) with α[^{32}P]dCTP to a specific activity of 10^9 dpm/μg DNA. Hybridization conditions were as previously reported (Cella et al., 1994a,b). Quantification of the hybridization signal was performed on a scanning densitometer (LKB XL Laser Densitometer, LKB, Uppsala, Sweden). Pituitary GH mRNA levels were expressed as percentage of control (normal rabbit serum-treated) values.

2.5. Plasma Insulin-like growth factor I (IGF-I) levels

Plasma IGF-I levels were evaluated by a homologous RIA in plasma extracted with 2 N HCl,12.5%, plus 87.5% ethanol, using reagents provided by the National Hormone and Pituitary Program (NHPP). The sensitivity of the assay was 100 pg/ml; intra- and interassay variation was less than 10%. The IGF-I plasma levels of 10 rats for each experimental group were determined.

2.6. Perfused rat heart preparations

As previously described (Berti et al., 1988), the hearts were rapidly removed and perfused retrogradely through the aorta with Krebs-Henseleit buffer (37°C) of the following composition (in mM): NaCl 118, KCl 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 5.5. The solution was gassed with 95% O_2/5% CO_2 and, after a 30-min equilibration period, the pH of the heart perfusate was 7.35. Left ventricular pressure was measured by a polyethylene catheter (with a small latex balloon on the top) inserted in the left ventricle cavity. The balloon was filled slowly with saline with a micrometer syringe until left ventricular end-diastolic pressure stabilized in the range of 5 mmHg. Coronary perfusion pressure and left ventricular pressure were monitored with Statham transducers (HP-280C) connected to a Hewlett-Packard dynograph (HP-7754A). The hearts were electrically paced at a frequency of 300 beats/min with rectangular impulses (1-ms duration; voltage 10% above threshold) by a Grass stimulator (S-88). The perfusion rate of each heart was adjusted to yield a coronary perfusion pressure (CPP) of 58–62 mmHg with a flow rate of 12 ml/min except during the ischemic period. Ischemia was induced by reducing the coronary flow to 2 ml/min with a perfusion pressure of 4–6 mmHg. Each heart was reperfused 40 min after the onset of ischemia at the preischemic flow rate (12 ml/min) for another period of 20 min. The increase of left ventricular end-diastolic pressure and coronary perfusion pressure during reperfusion was considered an index of the exacerbation of the ischemic picture.

In the preischemic period the perfused hearts were infused with acetylcholine (final concentration 10^{-6} M) for 1 min; heart function was evaluated as the increase of basal coronary perfusion pressure. All experiments were completed within 90 min.

2.7. Data analysis

Differences between groups in individual experiments were determined by Dunnett's *t*-test preceded by analysis

of variance. A value of $P < 0.05$ was taken to be statistically significant.

3. Results

3.1. Growth rate

Starting from day 28, i.e., 8 days after beginning the treatment, anti-GHRH serum-treated rats grew significantly less than normal rabbit serum-treated rats ($P < 0.05$); at the end of the experiment, rats of the control group weighed 195.2 ± 2.3 g, and the anti-GHRH serum-treated rats 169.6 ± 2.3 g (Table 1). In anti-GHRH serum + GH-treated rats, substitutive GH therapy completely counteracted the growth inhibitory effect of the antiserum, so that no significant difference between their body weight (194.1 ± 2.7 g) and that of normal rabbit serum-treated rats was observed at the end of the treatment (Table 1). In anti-GHRH serum-treated rats, heart weight was significantly reduced as compared to that of normal rabbit serum-treated rats (1281 ± 16.8 vs. 1519 ± 16.4 mg, $P < 0.01$) and anti-GHRH serum + GH-treated rats (1500 ± 11.0 mg) (Table 1). However, the ratio heart weight/body weight was not significantly different among the three groups, indicating that in anti-GHRH serum-treated rats the decrease of heart weight was proportional to the decrease in body weight (Table 1).

3.2. Pituitary GH mRNA levels

Pituitary GH mRNA levels were significantly reduced in anti-GHRH serum-treated rats (−49% vs. the normal rabbit serum-treated group, $P < 0.01$). Administration of GH did not modify pituitary GH mRNA levels in anti-GHRH serum-treated rats (data not shown).

3.3. Plasma IGF-I levels

Plasma IGF-I levels were significantly reduced in anti-GHRH serum-treated rats as compared to normal rabbit serum-treated controls (71.6 ± 5.0 vs. 163.1 ± 6.8 ng/ml, $P < 0.01$). Administration of GH restored plasma IGF-I levels to normal in anti-GHRH serum-treated rats (160 ± 5.7 ng/ml, P = NS vs. normal rabbit serum-treated group) (data not shown).

Table 1
Body weight, heart weight and ratio heart weight/body weight of young male rats (41 days of age) after 20-day treatment with normal rabbit serum (NRS), anti-GHRH serum (GHRH-Ab) or anti-GHRH serum + growth hormone (GHRH-Ab + GH)

	NRS	GHRH-Ab	GHRH-Ab + GH
Body weight (g)	195.2 ± 2.3	169.6 ± 2.3 [a]	194.1 ± 2.7
Heart weight (mg)	1519 ± 16.4	1281 ± 16.8 [a]	1500 ± 11.0
Heart wt/body wt (mg/g)	7.78	7.57	7.72

[a] $P < 0.01$ vs. NRS- and GHRH-Ab + GH values.

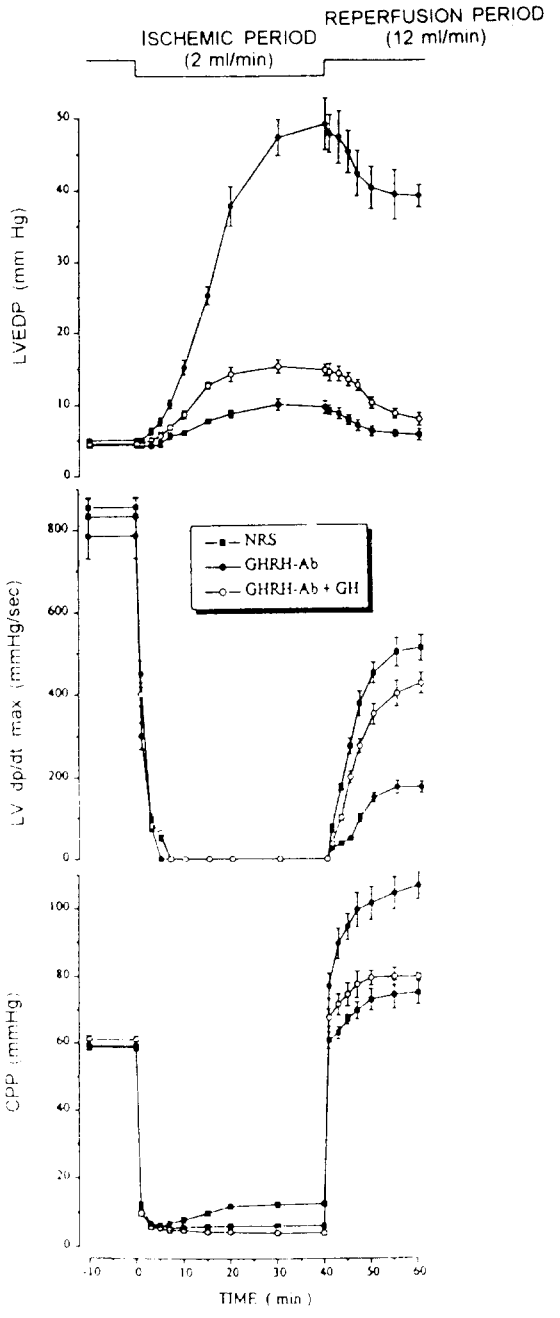

Fig. 1. Perfusion experiments with paced isovolumic heart preparations from normal rabbit serum (NRS)-, anti-GHRH serum (GHRH-Ab)-, and anti-GHRH serum + GH (GHRH-Ab + GH)-treated rats. Heart preparations were subjected to moderate ischemia and reperfusion (flow rate was reduced from 12 ml/min to 2 ml/min). LVEDP: left ventricular end-diastolic pressure; LV dP/dt max: maximal left ventricular dP/dt; CPP: coronary perfusion pressure. Each point represents the mean values and vertical bars the S.E.M. from 10 hearts.

3.4. Ischemia-reperfusion in isolated rat hearts

When the rate of perfusion of isovolumic heart preparations from normal rabbit serum-treated rats was reduced to

2 ml/min, peak systolic pressure developed pressure, maximum left ventricular dP/dt (LV dP/dt max) and minimum left ventricular dP/dt (LV dP/dt min) declined precipitously. Moreover, after complete ventricular standstill only a minimal elevation in left ventricular end-diastolic pressure (from 4.3 ± 0.4 to 9.4 ± 0.9 mmHg; $n = 10$) was observed at the end of the ischemic period (Fig. 1). Reperfusion produced a marked recovery (70%; $P < 0.01$) of cardiac mechanical activity (Fig. 1) with regular paced rhythm. In these preparations basal values of coronary perfusion pressure were in the range of 60 mmHg and rose during reperfusion to the range of 75 mmHg, indicating only a moderate increase in coronary artery resistance (Fig. 1). In contrast with the above results, when the rate of perfusion was reduced in hearts from anti-GHRH serum-treated rats, after ventricular standstill was reached, the left ventricular end-diastolic pressure increased progressively (from 5 ± 0.3 to 48.8 ± 3.5 mmHg; $n = 10$; $P < 0.0001$) (Fig. 1), indicating that a worsening of the ischemic damage was occurring. During reperfusion, due to a marked elevation of left ventricular end-diastolic pressure, a poor recovery of mechanical activity (Fig. 1) with a marked derangement of electrical pacing was observed. Moreover, the coronary perfusion pressure values during reperfusion increased by 81% over those recorded in the preischemic period (from 59.3 ± 1.5 to 107.3 ± 4.1 mmHg; $n = 10$; $P < 0.0001$) (Fig. 1). This increase in coronary artery resistance may be principally related to the pronounced left ventricular contracture due to the ischemic damage. When hearts from anti-GHRH serum + GH-treated rats were subjected to a global reduction of flow and reperfusion, the changes of left ventricular end-diastolic pressure during the ischemic and reperfusion period were not significantly different from those present in hearts excised from normal rabbit serum-treated rats (Fig. 1). Moreover, during reperfusion, the appearance of electrical pacing was prompt and no signs of dysrhythmia were recorded. This was associated with a recovery of cardiac contractility which was comparable to that observed in hearts from normal rabbit serum-treated rats (Fig. 1).

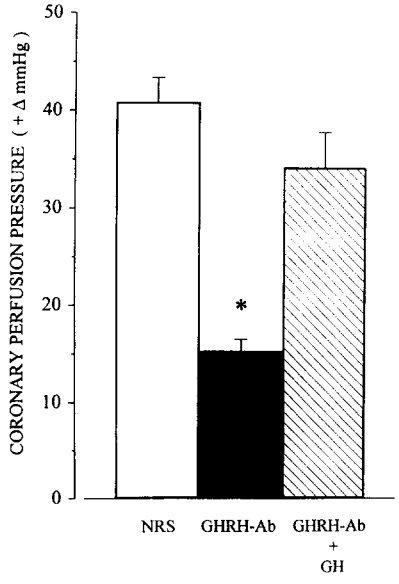

Fig. 2. Effects of acetylcholine (10^{-6} M) infusion for 1 min on coronary perfusion pressure in paced isovolumic heart preparations from normal rabbit serum (NRS)-, anti-GHRH serum (GHRH-Ab)- and anti-GHRH serum + GH (GHRH-Ab + GH)-treated rats. Each column is the mean ± S.E.M. of 10 determinations. $P < 0.01$ vs. NRS- and GHRH-Ab + GH-treated rats.

3.5. Acetylcholine activity

When acetylcholine was infused (10^{-6} M) in the preischemic period into hearts from normal rabbit serum-treated rats a marked increase of coronary perfusion pressure values was observed (Fig. 2), whereas the effect of acetylcholine infused into hearts from anti-GHRH serum-treated rats was significantly reduced (−63%) (Fig. 2). Acetylcholine completely recovered its vasopressor activity in hearts from anti-GHRH serum + GH-treated rats (Fig. 2).

4. Discussion

Passive immunization against rat GHRH provides an animal model of hypothalamic-driven GH deficiency (Arsenijevic et al., 1989; Cella et al., 1990; Wehrenberg et al., 1992) which mimics GH secretory events of GH-deficient humans. The efficacy of the anti-GHRH serum administration in decreasing GH secretion is demonstrated by the following findings. (1) anti-GHRH serum-treated rats had a growth rate significantly lower than normal rabbit serum-treated rats, starting 8 days after the beginning of treatment; GH prevented the reduction in growth rate at a dose shown capable to restore weight gain in hypophysectomized rats (Chomczynski et al., 1988). (2) Chronic administration of the anti-GHRH serum produced a significant reduction in pituitary GH mRNA levels, a result consistent with previous reports (Shakutsui et al., 1989; Cella et al., 1994a,b). (3) Anti-GHRH serum-treated rats had reduced plasma IGF-I levels and GH replacement therapy restored IGF-I concentrations, also confirming earlier reports (Arsenijevic et al., 1989; Cella et al., 1994b). Overall, anti-GHRH serum treatment from weaning and for 20 days (this study) markedly decreased GH synthesis and secretion, as demonstrated by the reduction in growth rate, pituitary GH mRNA and plasma IGF-I levels.

Anti-GHRH serum-treated rats had clear signs of cardiac dysfunction, consisting of an exacerbation of ischemic tissue damage during low-flow ischemia and reperfusion (with increased coronary artery resistance during reperfusion) and reduced functional activity of the muscarinic receptors in the coronary vascular bed. These heart abnormalities, which were reverted by 'ex-vivo' replacement therapy with GH, do not seem to be related to a decreased cardiac mass, since the ratio heart weight/body weight

was similar in the three experimental groups. Rosen and Bengtsson (1990) first reported that patients with hypopituitarism, when given the proper thyroid, adrenal and gonadal replacement therapy, without any specific GH replenishment, showed an increased mortality from cardiovascular disease, especially myocardial infarction and cardiac failure. As the deficit of GH secretion is an early event in pituitary failure (Lindholm et al., 1976), the authors pointed to GH deficiency as responsible for the increased cardiovascular mortality.

In a more recent study, Shahi et al. (1992) studied adult subjects with hypopituitarism and severe GH deficiency. In these subjects there was a significant correlation between serum levels of IGF-I, the principal biological marker of GH function, and left ventricular mass, suggesting that GH may still be necessary for the maintenance of cardiac size in adulthood. Furthermore, some patients showed left ventricular diastolic dysfunction and ischemic-like ST segment alterations during exercise testing (Shahi et al., 1992). The authors concluded that patients with GH deficiency have small coronary vessel disease or some undisclosed form of myocardial disease that may explain the increased incidence of cardiovascular mortality.

Our data showing a greater sensitivity of hearts from GH-deficient rats to low-flow ischemia are consistent with the findings of Shahi et al. (1992) and further strengthen the importance of GH in maintaining a normal cardiac function. The exacerbation of ischemic tissue damage during low-flow ischemia and the increased coronary resistance upon reperfusion appear clearly related to the reduced function of the GH/IGF-I axis. In fact, in hearts from anti-GHRH serum-treated rats given GH replacement both left ventricular end-diastolic pressure and coronary perfusion pressure values were not significantly different from those of hearts from normal rabbit serum-treated rats. The relationship between GH deficiency and exacerbation of the ischemic event (increased ventricular contracture) in the rat heart is difficult to explain. In this regard, impaired membrane function, resulting in changes in ion permeability, such as calcium loading, may have some relevance and deserves further investigation. In fact, an excess of intracellular free calcium could enhance ATP utilization and simultaneously diminish its production (Henry et al., 1977). The ensuing limitation of ATP availability is known to induce decreased myocardial compliance. Another interesting feature of our study was the impairment in the sensitivity to acetylcholine of vascular muscarinic receptors in perfused hearts of anti-GHRH serum-treated rats. These results are presently difficult to interpret and cannot be attributed to damage of the endothelial-dependent relaxing function. In fact, in view of the complex mechanism of action of acetylcholine in the coronary vasculature of the rat (Yang et al., 1993), an increased vasopressor effect of acetylcholine would have been anticipated. That the impaired muscarinic receptor function was clearly related to decreased somatotropic function was shown by the effectiveness of GH replacement in restoring to normal the vasopressor activity of acetylcholine. However, the molecular mechanism underlying GH-induced normalization of the activity of muscarinic receptors is far from being understood.

In all, our results point to an important role of GH (and/or IGF-I) in preserving the modulatory mechanisms of coronary vessels. Thus, GH deficiency is likely to induce coronary vessel dysfunction. The observation that patients with hypopituitarism, even though given thyroid, adrenal and gonadal replacement, may show ischemic-like ST segment changes during exercise (Shahi et al., 1992), strengthens this view.

Whether preservation of heart function is due to a direct action of GH on cardiac structures or is mediated by an increased level of circulating or/and tissue IGF-I still remains unclear and is worth further investigation. These possibilities, however, are not mutually exclusive, as implied by the reported presence of both GH (Mathews et al., 1989) and IGF-I (Sklar et al., 1989; Englemann et al., 1989) receptors in the rat heart.

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