Growth hormone increases the proliferation of existing cardiac myocytes and the total number of cardiac myocytes in the rat heart

Annemarie Brüel a,d,*, Tue E.H. Christoffersen b, Jens R. Nyengaard c

a Department of Connective Tissue Biology, Institute of Anatomy, Building 1233, University of Aarhus, 8000 Aarhus C, Denmark
b Laboratory for Molecular Cardiology, Rigshospitalet, Copenhagen, Denmark
c Stereology and Electron Microscopy Research Laboratory and MIND Center University of Aarhus, Denmark
d Department of Pathology, Aarhus University Hospital, Aarhus, Denmark

Received 22 December 2006; received in revised form 25 June 2007; accepted 28 June 2007
Available online 4 July 2007
Time for primary review 27 days

Abstract

Objective: Growth hormone (GH) is known to induce growth of the normal rat heart. Whether this growth is due solely to hypertrophy of the cardiac myocytes or whether a concomitant hyperplasia of the cardiac myocytes also takes place is currently not known. Therefore, the aim of the present study was to investigate whether GH induces hyperplasia in the left ventricle (LV) of sexually mature rats.

Methods: Three-month-old female Wistar rats were injected with GH (5 mg/kg/d) for 80 days, whereas a control group was injected with saline. Before perfusion–fixation, haemodynamic measurements were obtained. Isotropic, uniformly random sections were cut from the isolated LV, and the total number of myocyte nuclei and the average number of nuclei per myocyte were estimated using unbiased stereology. Immunohistochemistry was performed in order to detect proliferation (Ki-67), apoptosis (activated caspase-3), and stem cells (c-kit).

Results: GH increased the total number of cardiac myocytes by 33% [GH: 25.7×10^6 (0.09), controls: 19.3×10^6 (0.14), P<0.01, (mean (CV)], and this was accompanied by an increase in the percentage of Ki-67 positive myocytes by 216% [GH: 0.0179% (0.65), controls: 0.0057% (1.43), P<0.05]. No significant differences were found for caspase-3 positive or c-kit positive myocytes. GH increased insulin-like growth factor I (IGF-I) content of the myocardial tissue by 754% [GH: 247 ng/g wet weight (0.75), controls: 29 ng/g wet weight (0.58), P<0.01] and the LV wet weight by 50% [GH: 849 mg (0.15), controls: 567 mg (0.07), P<0.001], but did not influence the haemodynamic parameters.

Conclusion: These results strongly suggest that GH is able to stimulate cardiac myocytes to re-enter the cell cycle, divide, and thereby increase the number of cardiac myocytes in sexually mature rats.

© 2007 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Myocyte; Hypertrophy; Histo(patho)logy; Hormones; Growth factors

This article is referred to in the Editorial by I.M.C. Dixon (pages 377–378) in this issue.

1. Introduction

GH and IGF-I are important for normal growth of the heart and for maintaining cardiac mass and function (for a review see Isgaard [1]). GH has been shown to increase cardiac contractility and cardiac output in both humans and rats [2,3]. Furthermore, in rats with experimental heart failure GH has been shown to increase cardiac output [4,5] and long-term survival [6]. However, whether GH has potential in the treatment of patients with cardiovascular diseases remains unclear. A small unrandomised open study showed improvement of cardiac performance in GH-treated patients with heart failure [7], but subsequent data from other studies have been conflicting [8–11]. A recently published meta-analysis suggests that GH treatment may improve several relevant cardiovascular parameters in patients with heart failure [12]. However, the number of patients in the clinical studies is limited. Therefore, the authors propose that a large randomized placebo-controlled trial with long-term high-dose GH treatment of patients with heart failure is needed in order to clarify
this issue. Additionally, it has been proposed that different responses to GH therapy may be caused by GH resistance, which occurs in some patients [13].

Experimental studies demonstrate that excess GH is a potent stimulator of cardiac growth, which in normal rats may lead to concentric hypertrophy [3,14]. In contrast to concentric hypertrophy due to pressure overload like hypertension or aorta banding, GH-induced cardiac hypertrophy in rats is not dominated by fibrosis [15]. We have previously shown that GH induces a proportional growth of myocytes, capillaries, and connective tissue in the left ventricle (LV) of both young [16] and adult rats [17], indicating concomitant growth of these structures.

At present it is not known whether GH can increase the number of cardiac myocytes. However, it is known that GH can increase the total number of myocyte nuclei in rat LV [17–19]. Whether this is due to an increased number of nuclei of the individual myocytes, or an increased number of myocytes is not clear, as estimation of the number of individual myocytes has not previously been possible using traditional histological sections only. However, we have recently introduced a new method to estimate the total number of myocytes using histological sections [20]. Therefore, the aim of the present study was to investigate whether GH administered for 80 days to sexually mature female rats increases the total number of cardiac myocytes in LV.

2. Materials and methods

2.1. Experimental animals and design

Experiment 1: sixteen 3-month-old female Wistar rats (Mollegaard’s Breeding Centre, Ltd., Ejby, Denmark) were divided into 2 weight-matched groups with 8 animals in each group. One group was injected with recombinant human GH (Norditropin, Novo Nordisk, Gentofte, Denmark) for 80 days. The other group served as a control group, and was injected with saline. Experiment 2: twelve 3-month-old female Wistar rats were divided into 2 weight-matched groups with 6 animals in each group, and injected with either GH or saline for 80 days. GH was administered subcutaneously in the nape of the neck. The dosage was 5 mg/kg/day divided into 2 daily doses with at least 6 h interval. The rats had free access to standard rat chow (Altromin Diet 1314, Chr. Pedersen, Ringsted, Denmark) and tap water. Body weights were obtained once a week and at the end of the experiments. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996), and was approved by The Danish Animal Experiment Inspectorate. No rats died prematurely.

2.2. Haemodynamic studies

At the end of experiment 1, rats were anaesthetized by 3% isoflurane by inhalation. The right carotid artery was cannulated with a 2F Millar Mikro-tip pressure catheter (model SPR-407 Millar Instruments, Houston, TX) and the mean arterial pressure (MAP) was measured. The catheter was then advanced into the LV and the end diastolic pressure (LVEDP), the maximum contraction velocity (dP/dt max), and the maximum relaxation velocity (dP/dt min) were measured.

2.3. Left ventricle

After removing the catheter, the rats were perfusion-fixed through the heart with 0.1 M sodium phosphate buffered 4% formaldehyde (pH 7.0). The hearts were carefully removed, placed in fresh perfusion buffer for 24 h, and transferred to 70% ethanol. The left ventricle, including the interventricular septum (LV), was isolated by removing the atriae and the free wall of the right ventricle. In addition, the chordae tendineae and the trabecular muscles were removed. The wet weight of the LV was determined and converted into a volume, V(LV), by dividing the wet weight by 1.06 g/cm 3 [17].

2.4. Tissue shrinkage

We have previously found that shrinkage of plastic-embedded cardiac tissue is less than 10% [17,19]. However, in paraffin-embedded tissue, shrinkage is more pronounced. In the present study, the shrinkage of cardiac tissue embedded in paraffin was estimated to 35% (CV: 0.19) using Cavalieri’s principle as recently described in detail [17].

2.5. Sampling of the left ventricle

The sampling procedure was based on the smooth fractionator principle [21]. The left ventricle was serially sectioned into 2-mm-thick slices, and sectioned again perpendicular to the slices, providing 25–40 small tissue blocks per heart (depending on the heart size). The tissue blocks were then sampled by a smooth fractionator design and divided into 4 sets with 6–8 blocks per set.

In order to ensure isotropic orientation of the tissue blocks they were embedded in isectors, i.e. each tissue block was embedded in agar in rubber moulds with a spherical cavity [22]. The isectors were rolled on the laboratory table before they were embedded in either glycolmethacrylate (Technovit 7100, Heraeus Kulzer, Wehrheim, Germany) or paraffin (see Fig. 1A–H [20]). The use of isectors ensured that isotropic, uniformly random (IUR) sections were obtained from the tissue blocks [22].

2.6. Estimation of the total number of myocytes and non-myocytes of the LV

Estimation of the total number of myocytes in LV has been described in detail previously [20]. In brief, from the centre of each glycolmethacrylate block, one 40-μm-thick
IUR section was cut and stained with Mayer’s haematoxylin and 0.15% basic fuchsine. Using the optical disector principle, the numerical density of myocyte nuclei and non-myocyte nuclei was determined by focusing through a known sampling volume of cardiac tissue [23]. The total number of myocyte nuclei, \( N_{\text{myocyte nuclei, LV}} \), and non-myocyte nuclei, \( N_{\text{non-myocyte nuclei, LV}} \), were estimated by multiplying the numerical density with the \( V_{\text{LV}} \).

Using 2-μm-thick serial IUR paraffin sections, the lateral borders and intercalated discs were visualised using antibodies against type IV collagen and cadherin, respectively [20]. Using vertical windows (i.e. sections, where the myocytes have been cut along their longitudinal axis), myocytes were followed through the serial sections and the average number of nuclei per myocyte, \( \bar{N}_N(\text{nuclei/myocyte}) \), were determined. The total number of myocytes in LV, \( N_{\text{myocyte, LV}} \), was estimated by dividing the total number of myocyte nuclei by the average number of myocyte nuclei per myocyte.

2.7. Estimation of the volume density and total volume of myocytes, connective tissue, and capillaries

The method for estimation of the volume density and total volume of myocytes, connective tissue, and capillaries has recently been described in detail [19].

2.8. Estimation of length density and total length of myocytes

Two-μm-thick IUR paraffin sections were immunostained against type IV collagen and cadherin as described above. The method for estimation of the length density and total length of myocytes, has recently been described in detail [19]. As the parameter was obtained in paraffin sections, correction for shrinkage was applied.

2.9. Estimation of mean volume per myocyte and mean cross sectional area

In order to obtain the total volume of myocytes, connective tissue and capillaries, and total length of myocytes, the volume density and length density was multiplied by \( V_{\text{LV}} \).

The mean volume per myocyte was calculated as:

\[
\bar{v}_{\text{myocyte}} := \frac{V_{\text{myocyte, LV}}}{N_{\text{myocyte, LV}}}
\]

where \( V_{\text{myocyte, LV}} \) is the total volume of myocytes. The mean cross sectional area was calculated from the following equation:

\[
\bar{a}_{\text{myocyte}} := \frac{V_{\text{c,myocyte}}}{L_{\text{c,myocyte}}}
\]
Table 1
Estimates of total variance, biological variance, coefficient of error and mean of total counts per animal

<table>
<thead>
<tr>
<th></th>
<th>CVtot</th>
<th>CVbio</th>
<th>CEmet</th>
<th>Mean of total counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N(\text{myocyte nuclei, LV}))</td>
<td>0.11</td>
<td>0.08</td>
<td>0.07</td>
<td>162</td>
</tr>
<tr>
<td>(N(\text{non-myocyte nuclei}))</td>
<td>0.12</td>
<td>0.10</td>
<td>0.06</td>
<td>438</td>
</tr>
<tr>
<td>(\bar{N}_A(\text{nuclei/myocyte}))</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>91</td>
</tr>
<tr>
<td>(N(\text{myocyte}))</td>
<td>0.12</td>
<td>0.07</td>
<td>0.09</td>
<td>–</td>
</tr>
<tr>
<td>(l(\text{myocyte}))</td>
<td>0.12</td>
<td>0.12</td>
<td>0.02</td>
<td>831</td>
</tr>
<tr>
<td>(l(\text{cap}))</td>
<td>0.12</td>
<td>*</td>
<td>0.12</td>
<td>312</td>
</tr>
<tr>
<td>(l(\text{connective tissue}))</td>
<td>0.18</td>
<td>0.12</td>
<td>0.13</td>
<td>365</td>
</tr>
<tr>
<td>(L(\text{myocyte}))</td>
<td>0.19</td>
<td>0.13</td>
<td>0.14</td>
<td>494</td>
</tr>
</tbody>
</table>

\*The estimates of CE may vary quite a lot due to the limited amount of animals, therefore CEmet may sometimes be greater than CVtot, for which reason CVbio cannot be calculated.

where \(V_c(\text{myocyte})\) is the volume density of myocytes and \(L_v(\text{myocyte})\) is the length density of myocytes.

2.10. Sampling variance

Coefficients of variation and average count per animal are given in Table 1. The total coefficient of variation (CVtot) is calculated as the standard deviation (SD) divided by the mean. The coefficient of error (CEmet) is the error variance of the stereological methods and is based on the assumption that the estimates are ratio estimates [24]. The CEmet is based on the estimates of the numerical density of myocyte nuclei and the mean number of nuclei per myocyte. From the CVtot and CEmet, the biological variation, CVbio, can be determined from:

\[
CV_{bio} = \sqrt{CV_{tot}^2 - CE_{met}^2}
\]

2.11. Estimation of Ki-67, c-kit, and activated caspase-3 positive cells

Immunohistochemistry was performed on 2-μm-thick paraffin sections using an antibody against Ki-67 (1:50, monoclonal mouse anti-rat, M7248, DAKO, Denmark), c-kit (1:100, polyclonal rabbit anti-human, A4502, DAKO, Denmark) or activated caspase-3 (1:200, polyclonal rabbit anti-human, Asp175, Cell Signalling Technology, USA) as previously described in details [20]. The sections were counterstained with Mayer’s haematoxylin and alcoholic eosin. As negative controls, sections were incubated with mouse or rabbit serum instead of the primary antibody. As positive controls, sections from rat ovary, skin, or small intestine were used. The areas of the tissue sections (six per animal) were estimated using point counting at a final magnification of \(\times 156\).

The “number” of myocytes, “\(N_A\)” (myocyte/LV) per area was estimated as:

\[
\text{“}N_A\text{”(myocyte/LV)} := \frac{\sum Q(\text{myocyte})}{\sum P(\text{LV})} \cdot \left[\frac{p}{a(\text{frame})}\right]
\]

where \(Q(\text{myocyte})\) denotes the total number of myocyte profiles of the left ventricle, which were counted in an unbiased 2-dimensional counting frame. The area, \(a\), of the counting frame was 18645 \(\mu m^2\) at tissue level. \(P(\text{LV})\) is the total number of points hitting the left ventricle (the reference space), and \(p\) is the number of points used to count the points hitting the reference space (here 4). The “number” of non-myocytes per area, “\(N_A\)” (non-myocyte/LV), was also counted using Eq. (4), where \(Q(\text{myocyte})\) was replaced with \(Q(\text{non-myocyte})\). The number of myocyte profiles and non-myocyte profiles of the sections was estimated by multiplying the total section area with either “\(N_A\)” (myocyte/LV) or “\(N_A\)” (non-myocyte/LV). As very few cells were Ki-67 or caspase-3 positive, these cells were counted throughout the entire tissue sections using the \(x-y\)-stepper function at a final magnification of \(\times 1579\). The number of Ki-67-positive myocytes or non-myocytes was expressed as a fraction of the total number of myocytes or non-myocytes, respectively. The number of activated caspase-3 positive cells was expressed as number per tissue area.

The c-kit positive cells were counted using two serial sections: one immunostained for c-kit and the other stained with toluidine blue in order to visualise mast cells. Some of the cardiac mast cells expressed c-kit, but only the c-kit positive cells, that were not mast cells, were counted. The number of c-kit positive cells was expressed as number per tissue area.

2.12. IGF-I content in serum and myocardial tissue

At the end of experiment 2, the rats were anaesthetized by 3% isoflurane by inhalation. Blood was obtained from the LV, serum isolated and stored at \(-80\,^\circ\text{C}\). The heart was removed, and retrograde perfused through aorta with 50 ml 4 °C cold saline. The LV was isolated, and the wet weight determined. The LV was homogenised in 1 M acetic acid (5 ml/g tissue) on ice, centrifuged, re-extracted, and the resulting supernatants lyophilised and dissolved in a phosphate buffer [25]. IGF-I was determined in myocardial tissue and serum by an RIA-assay (Diagnostic Systems Laboratories, USA).

2.13. Statistics

In the text, data are presented as means (CVtot), whereas in the figures, data are presented as single animal values and means. CVtot is defined as the standard deviation (SD) divided by the mean. Differences between the groups were evaluated with the Mann–Whitney Rank Sum test. Differences were considered significant at \(P<0.05\).

3. Results

3.1. Body weight and wet weight of LV

GH increased the body weight by 64% [GH: 399 g (0.08), controls: 243 g (0.05), \(P<0.001\)], and LV wet weight by 50% [GH: 849 mg (0.15), controls: 567 mg (0.07), \(P<0.001\)] (Fig. 1A).
3.2. Haemodynamic studies

The haemodynamic parameters are shown in Table 2. No significant differences were found between the groups in systolic or diastolic blood pressure, mean blood pressure, LVEDP, dP/dt maximum, or dP/dt minimum.

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>131 (0.05)</td>
<td>132 (0.04)</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>100 (0.06)</td>
<td>101 (0.06)</td>
</tr>
<tr>
<td>Heart rate (per minute)</td>
<td>408 (0.07)</td>
<td>408 (0.07)</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>7.6 (0.54)</td>
<td>7.7 (0.51)</td>
</tr>
<tr>
<td>dP/dt maximum (mmHg/s)</td>
<td>9232 (0.14)</td>
<td>9196 (0.11)</td>
</tr>
<tr>
<td>dP/dt minimum (mmHg/s)</td>
<td>−9096 (0.11)</td>
<td>−8549 (0.11)</td>
</tr>
</tbody>
</table>

LVEDP: left ventricle end diastolic pressure, dP/dtmax: the maximum contraction velocity, dP/dtmin: the maximum relaxation velocity.

3.3. Estimation of numerical density and total number of myocyte nuclei

The numerical density of the myocyte nuclei was slightly decreased in the GH-treated group compared with the control group [GH: 57.1×10³ mm⁻³ (0.17), controls: 64.4×10³ mm⁻³ (0.09), P<0.05]. Fig. 1B shows the total number of myocyte nuclei. GH increased this parameter by 30% compared with the control group [GH: 44.9×10⁶ (0.08), controls: 34.5×10⁶ (0.12), P<0.001].

3.4. Estimation of the average number of nuclei per myocyte

Fig. 1C depicts the mean number of nuclei per myocyte. No significant difference was found between the two groups [GH: 1.75 (0.03), controls: 1.79 (0.04), NS].

3.5. Estimation of the total number of myocytes of the left ventricle

The total number of cardiac myocytes was increased by 33% in the GH-treated group compared with the control group [GH: 25.7×10⁶ (0.09), controls: 19.3×10⁶ (0.14), P<0.01] (Fig. 1D).

3.6. Estimation of volume density and total volume of myocytes, connective tissue, and capillaries

No significant difference was found between the two groups in the volume density of myocytes [GH: 0.931 (0.02), controls: 0.938 (0.01), NS] or connective tissue [GH: 0.037 (0.26), controls: 0.033 (0.10), NS]. However, GH increased

---

Fig. 2. A–D Rat myocardium: A: Ki-67 antibody stain showing a Ki-67 positive myocyte (arrow) and non-myocyte (arrowhead), B: activated caspase-3 antibody stain showing a positive non-myocyte (arrow), C and D: are serial sections, C: section 1, c-kit antibody stain showing three positive cells (arrows and arrowhead), D: section 2, toluidine blue stain showing toluidine blue granula in the cell (arrowhead), marked with arrowhead in C, indicating that this cell is a mast cell. Bar: 15 μm.
the capillary density by 14% [GH: 0.033 (0.16), controls: 0.029 (0.08), P < 0.05] compared with the controls. Furthermore, GH increased the total volume of myocytes by 49% [GH: 746 mm$^3$ (0.15), controls: 502 mm$^3$ (0.04), P < 0.001], the total volume of connective tissue by 61% [GH: 29 mm$^3$ (0.20), controls: 18 mm$^3$ (0.16), P < 0.001], and the total volume of capillaries by 73% [GH: 26 mm$^3$ (0.12), controls: 15 mm$^3$ (0.12), P < 0.001].

3.7. Length density, total length, mean volume, and mean cross sectional area of myocytes

GH did not significantly change myocyte length density [GH: $31 \times 10^2$ mm$^{-2}$ (0.21), controls: $34 \times 10^2$ mm$^{-2}$ (0.15), NS], whereas the total myocyte length was increased by 32% in the GH-treated group [GH: 2.42 km (0.19), controls: 1.83 km (0.18), P < 0.01]. No differences were found in either mean volume per myocyte [GH: 29109 μm$^3$ (0.14), controls: 26311 μm$^3$ (0.11), NS] or mean cross sectional area [GH 311 μm$^2$ (0.22), controls: 281 μm$^2$ (0.17), NS].

3.8. Estimation of numerical density and total number of non-myocyte nuclei

The numerical density of the non-myocyte nuclei was increased in the GH-treated rats compared with the controls [GH: $181 \times 10^3$ mm$^{-3}$ (0.05), controls: $156 \times 10^3$ mm$^{-3}$ (0.07), P < 0.01], as was the total number of non-myocyte nuclei [GH: $144 \times 10^6$ (0.12), controls: $84 \times 10^6$ (0.11), P < 0.001].

3.9. Estimation of Ki-67 positive cells

Fig. 2A, shows a Ki-67 positive myocyte as well as a non-myocyte. The number of Ki-67 positive cells was expressed as a fraction of the total number of myocytes in the sections. GH increased the fraction of Ki-67 positive myocytes by 214% [GH: 0.0179% (0.65), controls: 0.0057% (1.43), P < 0.05] (Fig. 3A) and the fraction of non-myocyte Ki-67 positive cells by 70% [GH: 0.63% (0.21), controls: 0.37% (0.27), P < 0.01] (Fig. 3B).

3.10. Estimation of activated caspase-3 positive cells

In Fig. 2B a non-myocyte positive for activated caspase-3 is shown. No myocytes were found to be positive for activated caspase-3 in either the GH-treated group or the controls. Furthermore, GH did not influence the number of caspase-3 positive non-myocyte cells [GH: $8.50 \times 10^{-8}$ μm$^{-2}$ (0.91), controls: $8.55 \times 10^{-8}$ μm$^{-2}$ (0.98), NS].

3.11. Estimation of c-kit positive cells

Fig. 2C and D show two serial sections. Three cells are c-kit positive (C), but one appears to be a mast cell (D). GH did not influence the number of c-kit positive cells [GH: $2.01 \times 10^{-7}$ μm$^{-2}$ (0.61), controls: $1.54 \times 10^{-7}$ μm$^{-2}$ (0.43), NS].

3.12. IGF-I content in serum and myocardial tissue

GH increased serum IGF-I by 95% [GH: 2447 μg/l (0.09), controls: 1255 μg/l (0.05), P < 0.01], and IGF-I content of the myocardial tissue by 752% [GH: 247 ng/g wet weight (0.75), controls: 29 ng/g wet weight (0.58), P < 0.01].

4. Discussion

In the present study, 3-month-old rats were injected with a pharmacological dose of GH for 80 days, and the effect on cardiac haemodynamics and proliferation of myocytes was studied. For the first time it is shown that, in sexually mature rats, GH induced a significant increase in the total number of cardiac myocytes (33%). This was accompanied by a significant two-fold increase in the number of Ki-67 positive myocytes. No changes in the haemodynamic parameters were found.

4.1. The effect of GH on the number of myocytes

GH increases IGF-I synthesis in the liver, and thereby the level of circulating IGF-I. Some of the anabolic and growth-promoting effects of GH are mediated by liver-derived IGF-I. However, Sjogren et al. have shown that normal cardiac growth took place in mice even when the IGF-I production from the
liver was reduced to 25% [26]. Peripheral tissues including the myocardium have been shown to express IGF-I [27]. Furthermore, cardiac myocytes express receptors for GH and IGF-I, and GH upregulates myocardial IGF-I mRNA indicating a possible autocrine/paracrine effect of IGF-I [27]. Therefore, GH may have direct effects on the cardiac myocytes as well as effects mediated by IGF-I. In the present study, GH induced a two-fold increase in serum IGF-I, which is consistent with previous studies [15,18]. Moreover, GH induced a seven-fold increase of the IGF-I content in myocardial tissue.

Female rats have a highly variable secretion pattern of endogenous GH. The peak levels reach 300–600 μg/l serum, whereas the concentration between peaks is 20–100 μg/l serum [28]. In rats, subcutaneous injections of 2 and 4 mg GH per kg result in a broad peak of GH with a maximum GH concentration of approximately 600 and 1200 μg/l serum, respectively, 2 h after injection [29]. Consequently, a dosage of 2.5 mg/kg twice a day is definitely within the pharmacological range, and results in very broad GH peaks twice a day.

In the present study, GH induced a substantial increase in the total number of cardiac myocytes in LV of normal rats. This increase was accompanied by a concurrent increase in the number of Ki-67 positive myocytes. Ki-67 is a non-histone nuclear protein, which seems to be required for cell proliferation [30]. In lymphocytes, it has been shown that the synthesis of Ki-67 protein is increased during the S phase and G2 phase, and it reaches a maximum during mitosis [31]. In the present study, the Ki-67 positive myocytes were similar in size to normal (Ki-67 negative) mature myocytes, indicating that GH was able to stimulate mature cardiac myocytes to re-enter the cell cycle.

GH increased the total volume of myocytes by 49%, which could be caused by either an increase in the size of the individual myocytes, an increase in the number of myocytes, or a combination of both. GH did not induce significant changes in either myocyte mean cross sectional area or myocyte mean volume, indicating that the mean dimensions of the myocytes did not differ between the groups. Consequently, the increase in the total volume of myocytes was mainly caused by the increase in myocyte number found in the GH-treated group.

Reiss et al. found an increased number of cardiac myocytes in transgenic mice overexpressing IGF-I compared with wild type mice [32]. The number of cardiac myocytes was determined by combining estimates from histological sections with volumes of isolated cardiac myocytes. These findings are in accordance with the results of the present study. However, it should be noted that in the transgenic mouse model IGF-I overexpression was initiated at the embryonic cell stage. In contrast, the present study showed that the number of cardiac myocytes could be increased even in sexually mature animals when GH was administered for a limited period of time.

Traditionally, the adult heart has been considered a post-mitotic organ where the cardiac myocytes were terminally differentiated without ability to divide. However, during the last two decades a series of experiment on cardiac tissue from both humans and animals has been conducted by Anversa’s group. These studies suggest that at least a subpopulation of myocytes re-enter the cell cycle and divide, and that a pool of cardiac stem cells may reside in the myocardium (for a review see [33]). The cardiac stem cells are found to be situated in niches mainly located in the atria and the apex [34]. The embryonic stem cell Isl1 has been shown to be able to differentiate into cardiac myocytes, smooth muscle cells, and endothelial cells [35]. The results of the present study strongly support the view that cardiac myocytes are capable of division. However, whether GH also stimulated existing cardiac stem cells to differentiate into mature cardiac myocytes is not clarified. In the present study, c-kit was used as a marker of stem cells. The number of c-kit positive cells in the GH-treated group was 31% higher than in the control group (mast cells excluded), but this difference was not statistically significant due to relatively large standard deviations.

A decrease in cardiac myocyte apoptosis would also lead to an increased number of cardiac myocytes. However, in the normal rat heart the rate of myocyte apoptosis is very low, whereas, for example in cardiac failure, the rate of apoptosis is increased. In a cardiac failure model, IGF-I has been shown to attenuate apoptosis in cardiac myocytes [36]. In the present study, activated caspase-3 was used as a marker of apoptosis, and it was shown that GH did not influence the number of cells positive for activated caspase-3. Consequently, it is not likely that the increased number of cardiac myocytes is caused by an inhibited cell apoptosis.

In contrast to the study by Cittadini et al. [3], the present study did not show an influence of GH on blood pressure, LVEDP, and LV contractility. However, data on the effect of GH on cardiac performance in rodents appear to be conflicting. In mice, Tanaka et al. found that GH (8 mg/kg/d for 2 weeks) did not influence contractility [37], whereas in rats, Cittadini et al. showed that GH (3.5 mg/kg/d for 4 weeks) increased contractility [3]. In the present study, we found that in rats GH (5 mg/kg/d for 80 days) did not influence contractility. In addition, Bollano et al. showed that in 8-month-old mice transgenic for GH, cardiac function was impaired [38]. An explanation of this discrepancy is that short-term GH excess could be beneficial for cardiac performance [3]. In contrast, long-term GH excess seems to be unfavourable [38], whereas medium-term GH excess does not seem to influence the cardiac performance (the present study). Therefore, the difference in duration of GH treatment may explain the discrepancy in contractility between the study of Cittadini and the present study. In addition, differences in dosage of GH and strain of rats may also be contributing factors.

4.2. Physiological or pathological hypertrophy

Cardiac hypertrophy exists in both a physiological and in a pathological form. The physiological or adaptive form, is found in response to e.g. exercise, and is characterised by a proportional growth of the myocyte and non-myocyte compartments, and by a normal or enhanced cardiac function. The
pathological or maladaptive form, is found in response to e.g. persistent hypertension or aorta stenosis, and is characterised by myocyte necrosis/apoptosis, fibrosis, and cardiac dysfunction (for review see [39,40]).

We found that GH increased the wet weight of the LV by 50%, which indicates a substantial cardiac growth. This growth was not accompanied by changes in the relative volume of myocytes or connective tissue, or by cardiac dysfunction, which is in accordance with the findings of previous studies [17,19]. Consequently, the present study indicates that GH induced a “physiological” hypertrophy of the LV characterised by a significant increase in the number of cardiac myocytes.

In cardiac myocytes, the two major pathways of IGF-I signalling are the phosphatidylinositol 3-kinase (PI3K)–Akt pathway and mitogen-activated protein kinase (MAPK) pathway. A recent study has shown that the PI3K–Akt pathway seems to be important for physiological cardiac hypertrophy [41]. In transgenic mice overexpressing the IGF-I receptor, the PI3K–Akt pathway was found to be activated [42]. Furthermore, overexpression of the IGF-I receptor induced a “physiological” cardiac hypertrophy with an enhanced systolic function without any signs of fibrosis [42]. The cardiac hypertrophy, induced by GH-injections, may, at least partly, be a result of an increased IGF-I activating the PI3K–Akt pathway.

4.3. Concluding remarks

One of the strengths of the present study is that GH was administered to genetically unmodified sexually mature rats. Another strength is the use of stereologically correct sampling and sectioning procedures, which allows estimation of the total number of cardiac myocytes in each individual LV. A weakness might be the size of the GH dose, which results in 2–3 times higher GH levels than the peak levels of endogenous GH in normal rats [28,29].

A matter of concern in relation to a potential therapeutic use of GH has been whether it is carcinogen, as experimental and epidemiological data suggest that GH/IGF-I may play a role in the development of cancer (for review see [43]). The proliferative and antiapoptotic effects of IGF-I, found in experimental studies, may accelerate carcinogenesis [44,45]. In addition, patients with acromegaly have an increased risk of colorectal cancer, but the risk of other types of cancer is not increased in patients with GH-induced acromegaly [46]. Thus, the relation between GH and cancer is still not clarified and is an ongoing matter of debate.

In conclusion, pharmacological doses of GH induced a considerable growth of the LV in sexually mature rats. This growth was accompanied by a significant and substantial increase in the total number of myocytes, and the number of Ki-67 positive myocytes. These results strongly suggest that GH is able to stimulate the cardiac myocytes to re-enter the cell cycle, divide and thereby increase the number of cardiac myocytes, which support the view that the heart is not a terminally differentiated organ.

Acknowledgements

This investigation was supported by Lægevidenskabens Fremme Foundation, The Aarhus University Research Foundation, Musikforlæggerne Agnes og Knut Mørks Foundation, Ingeniør A. F. Wedell Erichsens Legat, Eva and Henry Frænkedals Mindefond and The Foundation of 17.12.1981. MIND Center is supported by Lundbeck Foundation.

IGF-I levels were kindly determined by Jan Frystyk. The authors gratefully thank Anette Berg, Maj-Britt Lundorf, Eva K. Mikkelsen, and Kirsten N. Rasmussen for excellent technical assistance and Merete Fischer for linguistic revision. GH was kindly donated by Novo Nordisk.

References


