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Growth hormone- and pressure overload-induced cardiac hypertrophy evoke different responses to ischemia-reperfusion and mechanical stretch

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Abstract

Objective. To compare the molecular, histological, and functional characteristics of growth hormone (GH)- and pressure overload-induced cardiac hypertrophy, and their responses to ischemia-reperfusion and mechanical stretch.
Design. Four groups of male Wistar rats were studied: aortic banding ($n = 24$, AB) or sham ($n = 24$, controls) for 10 weeks, and GH treatment ($n = 24$; 3.5 mg/kg/day, GH) or placebo ($n = 24$, controls) for 4 weeks. At 13 weeks, the rats were randomly subjected to: (i) assessment of basal left ventricular mRNA expression of sarcoplasmic reticulum calcium-ATPase (SERCA-2), phospholamban (PLB), and Na^+–Ca^{2+} exchanger (NCX) and collagen volume fraction (CVF) (*Protocol A*, 8 rats in each group); (ii) left ventricular no-flow ischemia with simultaneous evaluation of intracellular Ca^{2+} handling and ATP, phosphocreatine (PCr) and inorganic phosphate (Pi) content (*Protocol B*, 12 rats in each group); or (iii) left ventricular mechanical stretch for 40 min with assessment of tumor necrosis-α (TNF-α) mRNA (*Protocol C*, 4 rats in each group). *Protocol B* and *C* were carried out in a Langendorff apparatus.
Results. In *Protocol A*, no difference was found as to myocardial mRNA content of Ca^{2+} regulating proteins and CVF in GH animals vs controls. In contrast, in the AB group, myocardial mRNA expression of SERCA-2 and PLB was downregulated while that of NCX and CVF were increased vs. controls ($p < 0.05$). In *Protocol B*, recovery of left ventricular function was significantly decreased in AB vs GH groups and controls and this was associated with 1.6-fold increase in intracellular Ca^{2+} overload during reperfusion ($p < 0.05$). Baseline ATP content was similar in the four study groups, whereas PCr and Pi was lower in AB vs GH rats and controls. However, the time courses of high-energy phosphate metabolic changes did not differ during ischemia and reperfusion in the four study groups. In *Protocol C*, no detectable TNF-α mRNA level was found in the left ventricular myocardium of GH treated rats and controls at baseline, while a modest expression was noted in AB animals. Mechanical stretch resulted in de novo myocardial TNF-α mRNA expression in GH group and controls, which was dramatically increased in AB animals (≈5-fold above baseline, $p < 0.001$).
Conclusions. The data show that cardiac hypertrophy activated by short-term GH treatment confers cardioprotection compared with pressure overload with regard to molecular and histological characteristics, and responses to ischemia-reperfusion and mechanical stretch.

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1. Introduction

Augmenting muscles mass is one of the means by which the heart faces a hemodynamic burden, in addition to the recruitment of neurohormonal mechanisms and the use of the Frank–Starling mechanism [1]. However, load-induced hypertrophy (*pathologic hypertrophy*) is characterized by interstitial fibrosis, molecular remodeling with reduced sarcoplasmic reticulum and increase of sarcolemmal content of Ca^{2+} regulating proteins, and progressive cell death [2]. Several studies have documented that post-ischemic recovery of mechanical function is impaired in load-induced hypertrophy [3–6]. However, little data is available with regard to the pathophysiological mechanisms of such impaired recovery, particularly its dependency on perturbations of intracellular Ca^{2+} handling and/or energy metabolism, nor myocardial TNF-α expression has been tested in response to acute mechanical stretch in hypertrophied hearts.

On the other hand, myocardial growth stimulated by the activation of the growth hormone/insulin-like growth factor I (GH/IGF-I) axis appears more "*physiologic*" than load-induced hypertrophy, at least in the short-term, insofar as it is associated with unchanged capillary density, no interstitial remodeling, normal or even augmented systolic and diastolic function, and reduced apoptotic rate [7,8]. Such observations have prompted several experimental investigations focused on GH/IGF-I activation in heart failure by genetic manipulation or pharmacological means [8–11].

However, no study has compared vis-à-vis the molecular and histological characteristics of GH- and load-induced hypertrophy. In addition, there is no information as to whether these two kinds of hypertrophy entail different responses to ischemic and mechanical injury. The current study was performed to clarify these issues using classical models of ischemia-reperfusion and mechanical stretch in vitro.

2. Methods

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 experimental procedures were approved by the local Animal Care Committee. Wistar rats of male sex were used and the study design is shown in Fig. 1. Transverse banding of ascending aorta (AB) was performed placing a tantalum hemoclip in rats aged 3 weeks, as previously described [12]. Sham rats underwent the same surgical procedures, although the clip was not placed. Growth hormone treatment (3.5 mg/kg body weight rhGH per day via two subcutaneous injections) or placebo (normal saline) were randomly started in rats aged 9 weeks and continued for 4 weeks. Ex vivo studies were performed at 13 weeks of age. No death was observed during surgery and before the final experiments. Timing of AB and GH treatment, and even more GH dosage, were established on the basis of pilot experiments (unpublished data) showing that at 13 weeks of age, AB and GH-treated rats achieved similar Δ left ventricular growth responses (see below).

2.1. Assessment of cardiac growth in AB and GH-treated rats

Although the optimal method for comparing heart weights in the rat is unknown, normalizing left ventricular weight to tibial length relates cardiac size to the amount of lean body tissue and to cell size more than does normalization to body weight [13]. Accordingly, at the end of the final experiments, right hind legs of the rats were removed by disarticulating the femurs from the acetabulum at the hip. The tibias were dissected free of soft tissue and frozen at −20 °C. Four radiographic films (X-Omat XTL2, Eastman Kodak Co) of the tibias were then obtained, and the tibial length of each animal was assessed with a caliper from the radiograph.

2.2. Protocol A

A total of 32 hearts ($n = 8$ in each group) underwent basal molecular analysis and histochemistry. Immediately after rats were sacrificed, left ventricles were carefully separated form right ventricles and stored for subsequent analysis.

2.2.1. Basal myocardial mRNA expression of Ca^{2+} regulating proteins ($n = 4$ in each group)

Total RNA was prepared from left ventricular myocardium according to the method of Chomczynski et al., as previously described [14]. For sarcoplasmic reticulum calcium-ATPase (SERCA-2), Na–Ca^{2+} exchanger (NCX) and phospholamban (PLB), the polymerase chain reaction products which were 523, 429 and 535 bp respectively, were cloned into a Bluescript vector. They were subsequently transformed into com-