Protein remodeling of the heart ventricles in hereditary hypertriglyceridemic rat: effect of ACE-inhibition

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Summary

The aim of this study was to determine whether protein remodeling of the heart ventricles and remodeling of the aorta were present in hereditary hypertriglyceridemic (hHTG) rats and whether treatment with the angiotensin-converting enzyme inhibitor, captopril, could prevent these alterations. Three groups of rats were investigated in a four week experiment: control Wistar/C rats, hHTG rats, and hHTG rats given captopril (100 mg/kg/day) (hHTG + Cap). In the hHTG group, the increased systolic blood pressure (SBP) was associated with hypertrophy of the LV and RV. Protein profile analysis revealed an enhancement of metabolic protein concentration in both ventricles. The concentration of total collagenous proteins was not changed in either ventricles. However, alterations in composition of cardiac collagen were detected, characterized by higher concentration of hydroxyproline in pepsin-insoluble fraction and lower concentration of hydroxyproline in pepsin soluble fraction in the LV. Hypertrophy of aorta, associated with the reduction of nitric oxide dependent relaxation, was also present in hHTG rats. Captopril normalized SBP, reduced left ventricular hypertrophy (LVH), diminished metabolic protein concentration in both ventricles, and improved NO-dependent relaxation of the aorta. Furthermore, captopril partially reversed alterations in hydroxyproline concentration in soluble and insoluble collagenous fractions of the LV. We conclude that hypertrophy of both ventricles and the aorta is present in hHTG rats, along with protein remodeling of both ventricles. Captopril partially prevented left ventricular hypertrophy development and protein remodeling of the myocardium.

Introduction

Hypertrophy of the left ventricle, although an adaptive compensatory mechanism, involves the risk of increased cardiovascular morbidity and mortality. It is generally believed that prevention of hypertrophic growth or regression of established hypertrophy diminishes the increased cardiovascular risk. However, the success of pharmacological treatment of LVH may vary, depending on the nature of the hypertrophy, the period of hypertrophic growth, and the drug used [1–3]. Thus, the particular pharmacological intervention should be tested separately for each model and period of myocardial hypertrophy.
Nonobese, hereditary hypertriglyceridemic (hHTG) rats provide an interesting model of hypertension associated with hypertriglyceridemia and glucose intolerance [4, 5]. This model of LVH is also characterized by adrenergic overactivity and insulin resistance [6], and mimics the well known metabolic syndrome in man. Despite numerous studies performed on this model investigating humoral [6, 7], metabolic [8–10], and genotype alterations [5], there is little data focused on cardiovascular system disorders [11–13]. Pressure haemodynamic overload can be expected to induce the adaptive growth of the heart, and alteration of vascular and myocardial structure, which could be modified by the specific neurohormonal and metabolic disorders.

We investigated whether protein remodeling of the left and right ventricles and remodeling of aorta is present in hHTG rats and whether these potential alterations could be modified by angiotensin converting enzyme (ACE) inhibitor captopril.

Material and methods

Animals and treatment

The experiments were performed on male Wistar and hHTG rats, 12 weeks old, which were fed a standard pellet mixture. Three groups of rats were investigated: control Wistar /C/ rats (n = 10), hHTG rats (n = 7) and hHTG rats given captopril (100 mg/kg/day) (hHTG + Cap) (n = 7).

Systolic blood pressure (SBP) was measured by the non-invasive method of tail cuff plethysmography at the beginning of the experiment and at the end of each week, for 4 weeks (28 days). After 4 weeks the animals were sacrificed, the body weight (BW), the heart weight (HW), the left and right ventricle weights (LVW, RVW) were determined, and the relative weights of both ventricles (LVW/BW and RVW/BW) were calculated.

Protein profile analysis of heart ventricles

The samples from both LV and RV were rapidly weighed and transferred into precooled homogenization test tubes. They were frozen subsequently to –50 °C. Tissue samples were later thawed, a 40-fold volume was achieved by adding 50 mmol·l⁻¹ sodium–potassium–phosphate buffer, pH 7.4, containing 10 mmol·l⁻¹ EDTA and 1% Triton X, homogenized and centrifuged at 15,000 × g; supernatant was used for the determination of metabolic proteins. The pellet was resuspended and fractions of contractile and collagenous proteins were obtained in a stepwise manner by extracting contractile proteins into a supernatant with phosphate buffer (100 mmol·l⁻¹, pH 7.4, containing 1.1 mmol·l⁻¹ KCl). The pellet was briefly washed with 0.5 mmol·l⁻¹ acetic acid, then extracted with 0.5 mol·l⁻¹ CH₃COOH-pepsin, concentration was kept in the range 1:100–1:50. After 24 h at 4 °C, the extracts were centrifuged. The supernatant contained the fraction of soluble collagenous proteins. The pellet was further suspended in 1.1 mol·l⁻¹ NaOH and left for 45 min at 105 °C. This fraction contained insoluble collagenous proteins. The protein profile procedure yielded three basic fractions: (a) metabolic proteins (containing predominantly mitochondrial and cytosolic enzyme systems for aerobic and anaerobic substrate utilization), (b) contractile proteins (complex of contractile, regulatory and modulatory proteins of myofibrils – see Pelouch et al. [14]), (c) structural collagenous proteins (the fraction included collagens, elastins, proteoglycans and glycoproteins), which can be divided into two fractions: (1) soluble collagenous proteins constituted mainly by collagen I and III, and (2) insoluble collagenous proteins including collagen aggregates, elastins and other proteins of extracellular matrix. This methodological approach has been described in detail elsewhere [15,16]. Protein concentration in individual fractions was determined according to Lowry et al. [17] and expressed per g of tissue wet weight. Hydroxyproline concentration was estimated in soluble, insoluble and total collagenous proteins [15].

Zymography

Metalloproteinase (MMP) activity in cardiac tissue extracts was determined by gelatin zymography performed by standard procedures by using a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) matrix containing gelatin (1 g·l⁻¹). Cardiac tissue (20 mg) was minced into small fragments, agitated for 24 h at 4 °C in 10 volumes of buffer containing 10 mmol·l⁻¹ cacodylic acid, 150 mmol·l⁻¹ NaCl, 1 μmol·l⁻¹ ZnCl₂,