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Upregulation of the angiotensin-converting enzyme 2/angiotensin-(1–7)/Mas receptor axis in the heart and the kidney of growth hormone receptor knock-out mice

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ABSTRACT

Objective: Growth hormone (GH) resistance leads to enhanced insulin sensitivity, decreased systolic blood pressure and increased lifespan. The aim of this study was to determine if there is a shift in the balance of the renin-angiotensin system (RAS) towards the ACE2/Ang-(1–7)/Mas receptor axis in the heart and the kidney of a model of GH resistance and retarded aging, the GH receptor knockout (GHR−/−) mouse.
Design: RAS components were evaluated in the heart and the kidney of GHR−/− and control mice by immunohistochemistry and Western blotting (n = 12 for both groups).
Results: The immunostaining of Ang-(1–7) was increased in both the heart and the kidney of GHR−/− mice. These changes were concomitant with an increased immunostaining of the Mas receptor and ACE2 in both tissues. The immunostaining of AT1 receptor was reduced in heart and kidney of GHR−/− mice while that of AT2 receptor was increased in the heart and unaltered in the kidney. Ang II, ACE and angiotensinogen levels remained unaltered in the heart and the kidney of GH resistant mice. These results were confirmed by Western blotting and correlated with a significant increase in the abundance of the endothelial nitric oxide synthase in both tissues.
Conclusions: The shift within the RAS towards an exacerbation of the ACE2/Ang-(1–7)/Mas receptor axis observed in GHR−/− mice could be related to a protective role in cardiac and renal function; and thus, possibly contribute to the decreased incidence of cardiovascular diseases displayed by this animal model of longevity.

1. Introduction

The growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis has important physiological functions in maintaining normal growth, body composition, proliferation and differentiation of various cell types, regulation of lipid, carbohydrate and fat metabolism, development and maintenance of the immune system as well as control of heart, kidney and brain functions [1,2]. Deletion of growth hormone receptor (GHR) gene leads to GH resistance, very low levels of circulating IGF-1, reduced body size, signs of delayed aging, and a remarkable increase in longevity [3,4]. Growth hormone receptor deficient mice (GHR−/−) also display enhanced whole-animal insulin sensitivity [5–7], hypoinsulinemia and significantly lower fasting and nonfasting glucose levels in adult male and female (10 months old) GHR−/− mice compared with wild-type (WT) controls [5,7,8]. Moreover, GHR−/− mice show elevated percentage of adiposity [9], together with lower cholesterol, triglycerides and apolipoprotein B circulating levels than wild type mice [10,11].

The GH/IGF-1 system is also important for the maintenance of both renal and cardiovascular structure and function [12–14]. Derangements in the GH/IGF-1 axis are associated with chronic renal failure [13]. Accordingly, overexpression of GH in transgenic mice, is associated with development of severe glomerulosclerosis by about 6 months of age [15]. In contrast, GHR−/− mice are protected from diabetic nephropathy [16].

Previous studies described a close relationship between the GH/IGF-1 and the renin-angiotensin system (RAS). Growth hormone has been shown to stimulate the RAS as shown by an increase in circulating levels of angiotensinogen, aldosterone and plasma renin activity in human subjects [17,18] and dwarf rats [19]. In addition, it was demonstrated

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that GH can increase the AT1 receptor density in primary cultures of rat astrocytes [20] and in the remnant kidney of uninephrectomized male rats [21].

Although GHR −/− mice have reduced cardiac weight and volume, the preservation of health and the increased lifespan of these animals, suggest presence of compensatory mechanisms that tend to overcome the negative physiological effects on the cardiovascular system of a disrupted GH/IGF-1 axis [12,14]. This is demonstrated by echocardiography measurements that show that GHR −/− mice have a decreased systolic cardiac function that correlates with decreased systolic blood pressure observed in 18-week-old GHR −/− females [12] and in 9-month-old GHR −/− males [14], reduced levels of plasma renin and increased levels of circulating K^+ and aortic endothelial nitric oxide synthase (eNOS) expression, with no changes in total aldosterone levels [12].

The RAS is classically conceived as a coordinated hormonal cascade involved in the control of cardiovascular, renal, and adrenal functions, mainly through the actions of angiotensin (Ang) II [22], that is generated in the circulation and locally in numerous organs by renin and angiotensin-converting enzyme (ACE) [22,23]. The description of local RAS highlighted several non-hemodynamic effects of Ang II and led to the identification of new roles in physiological and pathophysiological processes, including inflammation, cell proliferation and fibrosis [22,24,25]. Angiotensin (Ang) II acts through two pharmacologically distinct G protein-coupled receptors, angiotensin type 1 (AT1) and the type 2 (AT2) receptors which have counter-regulatory actions in the cardiovascular and renal system [26,27]. Activation of the AT1 receptor, promotes vasoconstriction, reactive oxygen species (ROS) production; extracellular matrix remodeling and inflammation response, tissue injury and insulin resistance [26,28]. In line with these reports, it was also demonstrated that blockade of AT1 receptor could represent a crucial determinant of health and extended lifespan [29]. On the other hand, the AT2 receptor inhibits cell growth, inflammation and fibrosis; and exerts a cardio-protective role against ischemia–reperfusion injury and acute myocardial infarction [25,27]. Advances in the field led to the recognition of other active components of the RAS metabolism, such as Ang III, Ang IV, and Ang-(1–7) [30,31], the angiotensin-converting enzyme (ACE) 2, that forms Ang-(1–7) directly from Ang II and indirectly from Ang I [32], and the Ang-(1–7) specific G protein-coupled receptor Mas [33]. The ACE2/Ang-(1–7)/Mas receptor axis in general opposes the vascular and proliferative effects of Ang II [31]. Angiotensin-(1–7) arises as a potential regulator of endothelial function [31]; and several observations point towards an active role of Ang-(1–7) in metabolic actions [34–36].

Cardiovascular diseases are commonly associated with alterations of the GH/IGF-1 axis and involve an imbalance within the RAS. However, to date, there is scant information available regarding the effects of disturbances in the GH/IGF-1 axis on the in vivo expression of the main components of the RAS. Therefore, our hypothesis is that GHR −/− mice may display modifications on the expression of cardiac and renal main components of the RAS towards a protective status of this system that could explain at least in part their increased lifespan. This study was designed to evaluate local levels of Ang II and Ang-(1–7); AT1, AT2 and Mas receptor, as well as ACE, ACE2 angiotensinogen (AGT) and endothelial nitric oxide synthase (eNOS) in the heart and the kidney of GHR −/− mice by both immunohistochemistry and Western blotting analysis.

2. Materials and methods

2.1. Animals

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the appropriateness of the experimental procedure, the required number of animals used, and the method of acquisition were approved by the Southern Illinois University Laboratory of Animal Care and Use Committee. Two to three-month old GHR −/− and normal male mice (n = 12) were produced in our breeding colony derived from GHR −/− animals provided by Dr. J. J. Kopchick (Ohio University, Athens, OH, USA). Wild-type littermates of GHR −/− mice served as controls for this study. Mice were housed three to five per cage in a room with controlled light (12 h light per day) and temperature (22 ± 2 °C). The animals had free access to food (Lab Diet Formula 5008, containing a minimum of 23% protein and 5% fat and a maximum of 5% fiber; Purina Mills Inc., St. Louis, MO, USA) and tap water.

2.2. Tissue collection

The heart and kidneys from GHR −/− and control mice were perfused with physiological saline solution through the abdominal aorta until they were free of blood. Afterwards, tissues were removed and weighed. For immunohistochemical studies, whole heart as well as whole decapsulated kidneys were cut longitudinally, fixed in phosphate-buffered 10% formaldehyde (pH 7.2), and embedded in paraffin. A piece of each tissue was preserved at −80 °C for immunoblotting determinations.

2.3. Blood pressure determination

Systolic and diastolic blood pressures were measured using a computerized noninvasive tail-cuff system based on Volume Pressure Recording (Kent Scientific Corporation, Northwest Connecticut, USA). Conscious animals were allowed to enter a restraining holder freely and were kept in the cylinder for 10 min before the determination. The blood pressure session consisted of 50 cycles; the first 20 cycles were considered acclimatization cycles and were not recorded.

2.4. Immunohistochemistry

Paraffin-embedded tissues were cut at 3 μm and subjected to immunohistochemistry. Briefly, the sections were deparaffinized with xylene, rehydrated through graded series of ethanol to water, and then incubated in blocking solution (PBS plus 1% bovine serum) at room temperature for 1 h. Then, the sections were incubated overnight at 4 °C with one of the following primary antibodies: rabbit polyclonal antibody anti-Ang II (1:100 dilution; H002-12) and anti-Ang-(1–7) (1:50 dilution; H002-24; Phoenix Pharmaceutical, Inc., Burlingame, CA, USA); polyclonal anti-Ang-(1–7) Mas receptor (1:100 dilution; AAR-013; Alomone Labs, Ltd., Jerusalem, Israel); polyclonal anti-AT1 receptor (1:100 dilution; sc-579), anti-AT2 receptor (1:100 dilution; sc-9040), anti-ACE (1:100 dilution; sc-12187) and anti-ACE2 (1:100 dilution; sc-17720; Santa Cruz Biotechnology, Santa Cruz, CA). All antibodies were diluted with blocking solution. Immunostaining was carried out with an avidin-biotin-peroxidase complex kit and counterstained with hematoxilin [37]. Specificity of the Ang II and Ang-(1–7) staining was tested by preincubating the corresponding primary antibodies for 30 min at room temperature with a 1 μM solution of Ang II or Ang-(1–7) peptides (Bachem Americas, Torrance, CA, USA), [37]. Histological sections were studied in each animal using a light microscope Nikon E400 (Nikon Instrument Group, Melville, NY, USA). All tissue samples were evaluated independently by two investigators without prior knowledge of the group to which the mouse belonged. Histological evaluation of tissues was assessed on 20 consecutive microscopic fields at 400× magnification, where each field represents 1.13 mm2, resulting in a total explored area of 22.6 mm^2. Data were averaged and results were expressed as a percentage per mm^2. In the case of the kidney, independent analysis in glomeruli and tubules was performed in order to evaluate potential differences in the expression of Ang II, Ang 1–7, ACE, ACE2, AT1, AT2 and Mas receptor. All measurements were carried out using an image analyzer Image-Pro Plus ver. 4.5 for windows (Media Cybernetics, LP. Silver Spring, MD, USA).