Abstract

There is a large body of evidence that biological aging is related to a series of long-term catabolic processes resulting in decreased function and structural integrity of several physiological systems, among which is the cardiovascular system. These changes in the aging phenotype are correlated with a decline in the amplitude of pulsatile growth hormone secretion and the resulting decrease in plasma levels of its anabolic mediator, insulin-like growth factor-1 (IGF-1). The relationship between growth hormone and biological aging is supported by studies demonstrating that growth hormone administration to old animals and humans raises plasma IGF-1 and results in increases in skeletal muscle and lean body mass, a decrease in adiposity, increased immune function, improvements in learning and memory, and increases in cardiovascular function. Since growth hormone and IGF-1 exert potent effects on the heart and vasculature, the relationship between age-related changes in cardiovascular function and the decline in growth hormone levels with age have become of interest. Among the age-related changes in the cardiovascular system are decreases in myocyte number, accumulation of fibrosis and collagen, decreases in stress-induced cardiac function through deterioration of the myocardial conduction system and β-adrenergic receptor function, decreases in exercise capacity, vessel rarefaction, decreased arterial compliance and endothelial dysfunction leading to alterations in blood flow. Growth hormone has been found to exert potent effects on cardiovascular function in young animals and reverses many of the deficits in cardiovascular function in aged animals and humans. Nevertheless, it has been difficult to separate the effects of growth hormone deficiency from age-related diseases and associated pathologies. The development of novel animal models and additional research are required in order to elucidate the specific effects of growth hormone deficiency and assess its contribution to cardiovascular impairments and biological aging. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Biological aging can be physiologically characterized as the interaction of various catabolic processes affecting bone, muscle, brain, and cardiovascular structure and function. One potential explanation for the increase in catabolism with age is a decline in plasma levels of anabolic hormones including estrogen, androgen and numerous peptidergic trophic factors or hormones. Growth hormone is one such hormone which has been characterized as an important regulator of postnatal somatic growth. Growth hormone pulse amplitude declines with age due to alterations in the normal dynamic interactions of two hypothalamic hormones, somatostatin and growth hormone-releasing hormone. Generally, both adult-onset growth hormone deficiency and aging are manifest as a decrease in lean body mass, increase in adiposity, decline in bone density, decreased skin thickness, decreased
immune function, decline in learning and memory, and impaired myocardial function. Growth hormone replacement reverses many of these changes; however, the effects of growth hormone and its anabolic mediator, insulin-like growth factor-1 (IGF-1), on the cardiovascular system are complex and influenced by both age and disease. In this review, we detail the current knowledge of the regulation of growth hormone and its anabolic mediator, IGF-1. The alterations in this system with age are described and the implication for the regulation of cardiovascular function in aged animals discussed.

2. Growth hormone and aging

2.1. General characteristics of growth hormone

Pure bovine growth hormone is a single chain polypeptide of 191 amino acids first isolated from the pituitary gland by Li et al. [1]. It was subsequently shown to stimulate fatty acid mobilization, amino acid uptake, DNA, RNA and protein synthesis, thus regulating cell division and tissue hypertrophy. In humans, growth hormone is released in pulsatile bursts from the pituitary gland with the majority of secretion occurring nocturnally in association with slow-wave sleep [2–5]. Similar pulses are observed in rodents, except that high-amplitude secretory pulses occur every 3.5 h in males [6] and hourly in females. Although the precise function of this ultradian pattern remains unknown, the pulsatile nature of growth hormone release has been confirmed in every species examined to date and appears to be essential to optimize biological potency of the hormone. The regulation of these pulses involve at least two hormones released by the hypothalamus; growth hormone-releasing hormone (GHRH) which increases growth hormone release [7,8] and somatostatin which inhibits its release [9]. The dynamic interactions between these hormones are responsible for high amplitude, pulsatile growth hormone secretion. It is generally believed that somatostatin tone is dominant during trough periods, whereas when somatostatin is suppressed, growth hormone is released in response to secretion of GHRH [10].

Once growth hormone is secreted from the anterior pituitary, it binds with high affinity to the growth hormone receptor which is found in tissues throughout the body. Plasma growth hormone is carried by a binding protein which is homologous to the cleaved extracellular domain of the growth hormone receptor [11]. The growth hormone molecule exhibits two binding sites for the growth hormone receptor resulting in dimerization of the receptor, a step that is required for biological activity of the hormone [12]. Activation of the growth hormone receptor stimulates the synthesis and secretion of insulin-like growth factor-1 (IGF-1), a small peptide (about 7.5 kD) structurally related to proinsulin. IGF-1 circulates in the blood at high concentrations and acts as a mitogen, stimulating DNA, RNA and protein synthesis [13]. Although most of the circulating IGF-1 is derived from liver, growth hormone may also have a role in regulating the synthesis and secretion of IGF-1 from many tissues, thereby directly influencing the paracrine or local activities of the hormone [14–17].

IGF-1 circulates in blood either free (t½ is about 15–20 min) or bound to specific binding proteins that prolong the half-life of the peptide. Presently, six IGF-1 binding proteins (IGFBPs) have been identified and constitute an elaborate system for regulating IGF-1 activity. IGFBPs function to regulate the availability of IGF-1 to its receptor in target tissues [18], and specific proteases are required to cleave the IGF-1 protein from its binding protein complex. Once IGF-1 binds to the type 1 IGF receptor, it initiates autophosphorylation of the receptor, IRS-1 (insulin receptor substrate) phosphorylation, and tyrosine phosphorylation. Subsequent activation of Ras/Raf, PI 3-kinase and MAP kinase results from IGF receptor activation [17]. Although it was initially proposed that all of the actions of growth hormone were mediated through IGF-1, several studies have provided evidence that growth hormone has direct effects on specific tissues and/or synergizes with IGF-1 [19,20].

2.2. Age-related changes in growth hormone and IGF-1

Initial studies in elderly humans designed to investigate the relationship between growth hormone and physiological changes associated with age reported a decline in the ability of individuals to secrete growth hormone in response to several stimuli, including insulin-induced hypoglycemia and arginine administration [21]. Subsequent studies revealed a loss of nocturnal surges of growth hormone [22] and a decrease in plasma IGF-1 that paralleled the decline in growth hormone pulses [23,24]. These early studies in humans have been confirmed by numerous investigators (see review by Corpas et al. [3]) and it is now evident that the decline in high-amplitude growth hormone secretion and plasma IGF-1 concentrations is one of the most robust and well characterized endocrine events that occur with age.

Shortly after the documentation of decreases in growth hormone secretion in aged humans, studies confirmed that the amplitude of growth hormone pulses also decreased with age in rodents [25] (see Fig. 1) and that these decreases were associated with a decline in plasma IGF-1 [26]. These studies immediately progressed to an investigation of the mechanisms responsible for the decline in growth hormone secretion. Several studies in both humans and animals documented a decreased in vivo pituitary response to GHRH with age [27–29]. However, over the next several years, numerous studies attempting to detail the deficits within the pituitary gland produced controversial results which ultimately were attributed to either
differential responses of older animals to the pharmacological agents used to suppress endogenous growth hormone pulses during in vivo testing [30] or to technical limitations involved with culturing anterior pituitary cells from older animals [31].

2.3. Hypothalamic hormone regulation

Research efforts were eventually directed to an analysis of hypothalamic releasing and inhibiting hormones after studies revealed that: (1) acute, in vivo, administration of somatostatin antiserum increased growth hormone release identically in both young and old animals [32], (2) passive immunization with somatostatin antiserum restored the in vivo deficiency in pituitary response to GHRH [30], and (3) stimulation of hypothalamic slices of old animals in a superfusion system released greater amounts of somatostatin than in those of young animals [33]. Our laboratory has found that older animals release increased amounts of somatostatin peptide from pituitary extracts, suggesting an age-related increase in the release of this peptide from hypothalamic neurons [34]. These results provided compelling evidence that increased somatostatin tone may be an important factor in the decline in growth hormone pulses with age. These conclusions were supported by research demonstrating that administration of cholinergic agonists or arginine, considered to preferentially inhibit somatostatin release, was capable of restoring growth hormone secretion in the elderly [34,35].

Although these studies did not address the synthesis and release of GHRH, a decline in this hormone is another contributing factor in the decrease in growth hormone secretion with age. GHRH mRNA decreases with age [36] and the feedback relationship between growth hormone and hypothalamic neurons is impaired [37]. In the latter study, growth hormone increased somatostatin mRNA and decreased GHRH mRNA in young animals, but in older animals GHRH neurons were non-responsive. As a result, it was concluded that deficiencies within the hypothalamus involving regulation of both GHRH and somatostatin are responsible for the decrease in growth hormone secretion with age.

2.4. Tissue responsiveness to growth hormone

Although a decline in the amplitude of growth hormone pulses is an important determinant of the decrease in plasma IGF-1, more recent studies demonstrate that growth hormone induced IGF-1 secretion is diminished in elderly individuals and suggest that resistance to the action of growth hormone may be a secondary contributing factor in the low plasma IGF-1 concentrations [38]. In rodents, a two-fold increase in growth hormone receptors has been observed with age but this increase fails to compensate for the reduction in growth hormone secretion [39,40]. A more detailed investigation revealed that the $K_D$ and apparent size of the growth hormone receptor were not altered with age [W.E. Sonntag, unpublished observations] whereas the capacity of growth hormone to induce IGF-1 gene expres-
sion and secretion was 40–50% less in old than in young animals. The activated growth hormone receptor associates with JAK2 with subsequent phosphorylation of both proteins [41] (see review by Roupas and Herington [42]) (Fig. 2). Several other intracellular proteins are subsequently phosphorylated, including mitogen-activated protein kinase (MAP kinase), S6 kinase and the STAT proteins (signal transducer and activator of transcription). The result is an increase in c-fos, c-jun, serine phosphatase inhibitor-1 and IGF-1 gene expression [43]. Although diminished signal transduction has been described for both the insulin and IGF-1 receptors in aged animals [44,45], studies have not addressed alterations in growth hormone receptor signaling. Xu et al. [40] subsequently reported that phosphorylation of JAK2 and the growth hormone receptor complex were suppressed in aged rodents in response to growth hormone and that these decreases were accompanied by a decline in MAP kinase activity. More recent studies demonstrate that growth hormone induced STAT3 activation and nuclear translocation are also decreased with age [46]. Although these studies did not address the etiology of the decrease in JAK2 activity, they established that diminished growth hormone receptor signal transduction is a contributing factor in the decrease in IGF-1 expression with age.

3. Effects of growth hormone on the cardiovascular system

3.1. Preclinical studies

The concept that growth hormone has effects on the
cardiovascular system and may reverse age-related changes in cardiovascular function is based on both in vivo and in vitro studies. Cardiac myocytes contain receptors for growth hormone [47–49] and cultured myocytes are responsive to growth hormone administration [49]. Growth hormone has been shown to regulate cardiac growth in vivo (including myocyte growth) [50]. Growth hormone has also been demonstrated to increase LV pump function, and has favorable effects on LV remodeling and contractile processes in pigs with congestive heart failure (CHF) [51]. Two studies have suggested that stimulation of the IGF-1 receptor results in phosphorylation of the β-adrenergic receptor [52,53] and may partially reverse the age-related decline in β-adrenergic activity. Therefore, it is likely that growth hormone, either directly or through IGF-1, has potent effects on the heart and has the capacity to improve myocardial function in aged animals or in response to specific diseases that affect the cardiovascular system.

Although these studies provide evidence for a role of growth hormone and IGF-1 on the cardiovascular system, the conclusions are often based on the use of high doses of human growth hormone in rodents that introduce several potentially confounding variables. Human growth hormone is both antigenic and prolactinogenic which can potentially limit anabolic effects and introduce non-specific actions of the hormone. For rodent models, these two problems can be avoided by utilizing either bovine or porcine growth hormone which exhibit higher sequence homology to rat growth hormone. Finally, the high pharmacological doses of growth hormone used in these studies may cause acromegalic-like side effects, insulin resistance, and an increased risk of pathogenesis with long term use.

Despite these limitations, administration of growth hormone to rats in physiological doses has been demonstrated to ameliorate many age-related changes in the cardiovascular system. First, the age-related rarefaction of both brain [54] and myocardial vessels [55] was partially reversed by administration of bovine growth hormone. It is of interest that in this latter study the effects of growth hormone on microvascular density were limited to specific regions of the heart and it was suggested that the actions of growth hormone may be limited by fibrosis which was prevalent in the aged animals. Growth hormone administration to aged rats also increased coronary blood flow (see Fig. 3) [55] and cardiac myofilament contractility (see Fig. 4) [56]. These results suggest that lower, physiological doses of growth hormone are able to reverse some of the changes in cardiovascular structure and function with age and raise the concept that age-related decreases in growth hormone have the potential to contribute to impairments in cardiovascular function observed in aged animals.

Several investigators have examined the differential and

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Fig. 3. Pseudocolor enhanced photographs depicting changes in blood flow using 14C-iodoantipyrine in the base of the rat myocardium. Sections (20 μm) are representative of the changes observed in (A) young Brown Norway × Fischer 344 rats treated with saline (6 months), (B) old rats treated with saline (30 months), and (C) old rats treated with growth hormone (30 months); from Khan et al. [55] with permission.
synergistic cardiac effects of growth hormone and IGF-1. Cittadini et al. [57] administered recombinant human growth hormone, recombinant human IGF-1, and a combination of both hormones to 3-month-old Sprague–Dawley rats. Echocardiography revealed that combination therapy with growth hormone and IGF-1 did not have synergistic effects on myocardial growth or function. However, cardiac output and stroke volume were increased in response to IGF-1 and/or in response to the combination of growth hormone and IGF-1 compared to controls and growth hormone treated animals. Another study examined the in vitro effects of growth hormone and IGF-1 on intracellular calcium [58]. No acute effects of growth hormone were found on cardiac function, although IGF-1 caused an increase in isovolumic developed pressure without a change in intracellular $[\text{Ca}^{2+}]$. Further studies of isolated ferret papillary muscles demonstrated that the effects of IGF-1 on contractility could be blocked by wortmannin, an inhibitor of PI-3 (phosphatidylinositol-3 kinase), an intracellular kinase stimulated by IGF-1 receptor activation [59]. These results suggest that IGF-1 is able to increase contractility in vitro by altering myofilament calcium sensitivity, not through increasing intracellular calcium. Studies utilizing combination therapy with growth hormone and IGF-1 suggest that some of the effects of growth hormone on the heart are mediated through IGF-1 and that both hormones exert independent and interacting effects on cardiac structure and function. However, a limitation of these studies is that the differential effects of growth hormone and IGF-1 could be due to physiological differences in the doses of hormones. While it is established that growth hormone and IGF-1 affect cardiac structure and function, further studies are required to determine the specific dose of growth hormone necessary to improve cardiovascular function while minimizing pathological effects.

3.2. Growth hormone therapy in animal models of congestive heart failure

Growth hormone administration has been considered as a possible treatment for heart failure [60] and has been shown to improve left ventricular contractility in rats with experimental heart failure and cardiomyopathy. In an experimental model of heart failure, involving left coronary artery ligation [61], growth hormone administration increased stroke volume and decreased mean arterial pressure through a decrease in systemic vascular resistance. LV contractility, as measured by LV $dP/dt$, was also improved by growth hormone. Except for an increase in
body weight and decrease in mean arterial pressure, growth hormone administration did not have a significant effect in control animals. This study suggests that improvements in LV contractility, mean arterial pressure, and stroke volume occur with administration of growth hormone in animals with experimental heart failure.

A recent study by Ryoke et al. [62] examined the effects of growth hormone administration on LV dysfunction in cardiomyopathic hamsters. This strain becomes cardiomyopathic due to an autosomal recessive mutation of the gene for D-sarcoglycan, part of the dystrophin complex [63,64]. LV contractility improved with growth hormone treatment but to a greater degree in the younger versus older animals. LV wall stress at end systole was reduced by growth hormone administration in 4-month-old animals whereas wall stress increased at end diastole in response to growth hormone in 10-month-old animals. It was therefore concluded that growth hormone administration has beneficial effects on LV contractility and wall stress but the response is blunted with age.

Tajima et al. [65] recently proposed a possible mechanism whereby growth hormone can increase myocardial contractility and contractile reserve. This group showed that 14 days of growth hormone treatment to rats with postinfarction heart failure improved cardiac function. Contractile reserve was depressed in rats after experimental myocardial infarction, as would be expected. However, growth hormone treatment restored myocyte contractility and this effect was correlated with increases in SERCA-2 (sarcoplasmic reticulum Ca\(^{2+}\) ATPase-2) mRNA and protein in the left ventricle. This suggests that growth hormone can restore contractility by modulating synthesis of proteins involved in excitation–contraction coupling.

3.3. Clinical studies of growth hormone in congestive heart failure

Growth hormone/IGF-1 deficiency has been implicated as a potential cause of decreases in cardiac mass and ventricular wall thickness in humans. Amato et al. [66] demonstrated that administration of recombinant human growth hormone to growth hormone deficient adults increased left ventricular wall thickness and mass. Another double-blind study utilized long term administration of growth hormone to adult-onset growth hormone deficient humans and demonstrated improved exercise performance and cardiac output [67]. Doppler-echo techniques used in this study revealed that patients administered growth hormone had an increased LV mass index and interventricular septum size as well as improved exercise performance. This study suggested that long-term growth hormone replacement therapy is generally well-tolerated and increases LV mass index and stroke volume.

Studies in humans have also suggested that growth hormone deficiency can result in cardiomyopathy. A positive correlation between mean nocturnal growth hormone plasma levels and ejection fraction was demonstrated in twelve patients with cardiomyopathy [68]. In a single case report of a patient with hypopituitarism, dilated cardiomyopathy was partially reversible with growth hormone administration [60] and growth hormone withdrawal resulted in recurrent thinning of the myocardial wall and a decrease in LV ejection fraction. Although this is a single report of a patient experiencing Sheehan’s syndrome (postpartum hypopituitarism) and both cortisol and thyroxine were also involved in the 3-month hormone replacement therapy, the findings suggest that the cardiomyopathy associated with Sheehan’s syndrome may be due, in part, to growth hormone deficiency.

Genth-Zotz et al. [69] demonstrated that growth hormone replacement improved cardiac function in patients with ischemic cardiomyopathy. Interestingly, growth hormone was administered at a relatively low dose—half the dose shown to be effective in growth hormone deficient cardiomyocytes [60]. In this study, exercise capacity, cardiac output, myocardial wall thickness increased while end systolic and diastolic volume indices decreased. However, there was no increase in LV ejection fraction. The effects were maintained for 3 months and began to diminish after withdrawal of growth hormone therapy. It is unknown whether these effects were mediated by increased IGF-1 levels or changes in myocardial structure. In conclusion, growth hormone administration is beneficial to cardiomyopathic humans and individuals with adult-onset growth hormone deficiency.

4. Animal models of growth hormone deficiency

The complexities of studying the interactions of growth hormone with disease processes in humans have raised questions concerning the precise role(s) of growth hormone deficiency and replacement in normal cardiovascular function. However, it has been difficult to separate the direct effects (or absence of effects) of growth hormone in aged animals and humans from numerous pathologies that occur with age. Despite the studies implicating alterations in growth hormone and IGF-1 in the process of biological aging, a model to mimic age-related changes in these hormones has been elusive and further progress in the field requires the development of a proper animal model to distinguish the effects of growth hormone deficiency from the effects of aging.

Over the past decade, several animal models of growth hormone deficiency (or excess) have been developed [70] but the interpretation of these studies are limited since the alterations in growth hormone do not resemble changes found in the aging human or the animals exhibit other endocrine deficiencies that confound the interpretation of the data. For example, transgenic animals with high (supraphysiologic) levels of growth hormone exhibit abnormal growth in a number of tissues (similar to acromega-
ly in humans) and develop early pathological changes in tissues which limit lifespan. On the contrary, many of the studies conducted in dwarf rodents (Ames and Snell dwarf animals) as well as the recently developed growth hormone receptor knockout mouse are of limited use as a model of growth hormone deficiency for aging studies since (1) there are alterations in numerous endocrine systems besides growth hormone and IGF-1, (2) developmental differences exist due to the absence of these hormones during critical phases of growth and introduce non-specific differences between the dwarf and wild-type animals, and (3) there are significant decreases in body size. Ames dwarf mice, for example, lack pituitary cells that produce growth hormone, prolactin and thyroid stimulating hormone due to a mutation of the Prophet of pit-1 (prop-1) gene [70]. These primary multiple endocrine deficiencies, secondary endocrine anomalies (e.g. excess cortisol and impaired insulin secretion) and issues related to development limit the interpretation of data from this model since it would not be evident whether the resulting effects were directly related to growth hormone deficiency.

More recent models have used the dw/dw rat that exhibits an unknown mutation in the growth hormone secretory axis resulting in dwarfism. The hormonal deficiency is isolated to growth hormone with levels about 10% of normal while plasma IGF-1 declines to 50% of levels found in heterozygote littermates. Analysis also indicates that these animals have no changes in basal glucose, insulin, glucocorticoids or T/T. Cittadini et al. [71] have recently published studies examining various parameters of myocardial structure and function in this model. One study investigated postinfarction healing in dwarf animals [71]. Two subsequent studies examined the effects of growth hormone deficiency and human growth hormone administration on cardiac morphology and function. Using echocardiography, Longobardi et al. [72] were able to demonstrate an improvement in cardiac output and increase in myocyte area with growth hormone administration. Another study reported a decrease in maximal calcium activated force and $\beta$-adrenergic responsiveness as measured in an LV papillary muscle preparation, and a subsequent increase in myocyte area, developed tension per cross-sectional area and maximal calcium activated force [73]. While limited due to the antigenic and prolactogenic effects of human growth hormone, these were the first reports of deficits in cardiovascular structure and function in growth hormone deficient animals. Furthermore, these studies suggest that growth hormone replacement can reverse many of the deficits seen in cardiovascular function in growth hormone deficient rats.

Recent studies in our laboratory have provided additional information related to the utility of this animal model. Our results indicate that the dwarf animal has pancreatic insufficiencies which may require a modification in protocol in order to separate growth hormone deficiency from other endocrine anomalies. Specifically, the pancreas in dwarf rats appears underdeveloped and does not secrete adequate insulin compared to control rats when challenged during a glucose tolerance test. However, dwarf animals administered growth hormone for 10 weeks appear to have restored pancreatic function. Growth hormone replacement also increases body weight and plasma IGF-1 to levels found in heterozygous controls. The dw/dw model is currently being used to assess the effects of adult onset growth hormone deficiency by comparing dwarfs treated and withdrawn from growth hormone therapy to dwarfs that are continuously treated with growth hormone. This animal model appears to resolve many of the issues related to the developmental effects of growth hormone deficiency and will undoubtedly be useful in advancing our understanding of the consequences of growth hormone deficiency on the aged cardiovascular system.

5. Conclusions

The hypothesis that a decline in growth hormone and IGF-1 has an important role in mechanisms of aging has gained substantial support in recent years since it has been demonstrated that administration of these hormones to aged animals restores cellular protein synthesis [74] and increases lean body mass [75], immune function [76,77], skin thickness [78] and vertebral bone density [75,79,80]. Furthermore, the age-related decrease in these hormones also appears to be involved in the decline in cardiovascular function with age since the results of several studies suggest that growth hormone replacement can improve or reverse many of these deficits. Recent studies from our laboratory demonstrate that low doses of growth hormone have beneficial effects on myocardial structure and function in aged animals, e.g. increasing coronary flow, capillary density, and myofilament contractility. However, the effects of growth hormone and IGF-1 deficiency may not be fully recognized since aged animals are compromised by pathological changes and current, alternative animal models of growth hormone deficiency have been compromised by multiple endocrine deficiencies. The development of new models of growth hormone deficiency that are specific to growth hormone will advance our understanding of the role of growth hormone in the maintenance of tissue function and in aging.

Despite the evidence that growth hormone administration may be beneficial to the cardiovascular system, these results must be balanced with possible side effects of growth hormone replacement. Growth hormone is potentially diabetogenic and recent studies have established IGF-1 as a risk factor for lung, breast, prostate and colorectal cancer [81]. These issues indicate that further studies are essential to overcome the concerns of treating normal aging humans with growth hormone. More re-
search on the dual physiological and pathological actions of this hormone is required before more extensive clinical studies are undertaken.

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