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Growth hormone modulates the degradative capacity of muscle nucleases but not of cathepsin D in post-weaning mice

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Abstract

We determined whether recombinant human growth hormone (rhGH) administration might modulate the enzyme degradative capacity of the muscle lysosomal system and influence muscle growth. Muscle cathepsin D, acid RNase and DNase II activities are determined in the gastrocnemius muscle of rhGH-treated post-weaning female BALB/c mice. Linear regressions were used to analyze the relationships of each enzyme with their respective substrate. GH induced a depletion-recovery response of muscle growth through a mechanism which is similar to catch-up growth. In these conditions, cathepsin D activity decreased with age in all animals (GH: 40%; saline: 79%), showing a substantial developmental decline that could reflect changes in the rate of protein breakdown. However, the degradative capacity of cathepsin D was paradoxically unmodified in rhGH-mice compared with saline mice (according to the enzyme vs. substrate linear regression slope), in spite of the increase in enzyme activity elicited by GH. This suggests that the muscle protein breakdown is not increased by GH-treatment in post-weaning mice. The enhancement of muscle protein deposition as indicated by the augmented muscle cell size (protein:DNA ratio) of rhGH-mice (increased 178% from 25 to 50 days) vs. saline, can be attributed to a higher muscle K_{RNA}. In contrast, acid RNase and DNase II activities directly participate in muscle RNA and DNA degradation. Both nucleases were inhibited by GH treatment (a decrease of 48% and 63%, respectively, vs. saline at 50 days). The decrease in RNase activity suggests an inverse relation between the rate of protein synthesis (high) and acid RNase activity (low), leading to spare muscle RNA for synthesizing protein during catch-up growth. Also, low DNase II activity could contribute to inhibiting of muscle DNA degradation, facilitating muscle growth. Thus, GH seems to act as a direct modulator of the degradative capacity of skeletal muscle nucleases but not of cathepsin D, influencing DNA and RNA degradation during the depletion-recovery response to GH of gastrocnemius muscle in female post-weaning mice.

Keywords: rhGH; Lysosomal enzymes; Skeletal muscle

1. Introduction

Lysosomes are implicated in the turnover of cytoplasmic soluble constituents and of cellular organelles, including even nuclei (Roberts et al., 2003). Degradation of proteins, DNA and RNA in mammalian cells is mediated by a wide variety of lysosomal enzymes. Endopeptidase, aspartic protease (cathepsin D) (Yamamoto, 1995), and the endonucleases: acid deoxyribonuclease (DNase II) (Tsukada et al., 2001) and acid ribonuclease (RNase) (Sorrentino and Libonati, 1997), are known to be involved in intracellular protein, DNA and RNA degradation respectively, within the lysosomal compartment in various organic tissues including muscle. Cathepsin D may degrade mainly endocytosed and autophagocytosed cell constitutive proteins and act on hydrophobic residues of the polypeptide chain (Yamamoto, 1995), while DNase II cleaves deoxyribonucleotide linkages in native and denatured DNA, yielding products with 3′-phosphates. Similarly, acid RNase preferentially cleaves to phosphodiester linkages adjacent to a pyrimidine and purine nucleotide of RNA, vía a 2, 3′-cyclic phosphate intermediate, to form oligo or mononucleotides with a terminal 3′phosphate (Barnard, 1969). It has recently been reported that cathepsin D (Kagedal et al., 2001) and DNase II (Samejima and Earnshow, 2005) might participate in the apoptotic cell death. The lysosomal pathway is regarded to be a minor contribution to the proteolytic pathway of bulk skeletal muscle compared to the ubiquitin proteasome (Ub) dependent

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system (Taillander et al., 1996). Thus, the stimulation of muscle protein degradation in a wide variety of catabolic states results mainly from a general activation of the Ub pathway including increased content of Ub–protein conjugates (Wing et al., 1995) and of mRNA encoding Ub (Medina et al., 1995), certain ubiquitination enzymes (Lecker et al., 1999) and multiple proteasome subunits (Lecker et al., 2004). One of the main reasons for considering that the autophagic lysosomal system plays a minor role in skeletal muscle has been that typical lysosome structures have rarely been seen in normal muscle tissues. Nevertheless, the characterization of cathepsin D (Roberg and Öllinger, 1998) as well as the existence of a lysosomal apparatus with a full complement of acid hydrolases (Tassa et al., 2003), and the identification of LC3 (Kanazawa et al., 2002), a mammalian homolog of yeast Apg8p, which is defined as one of the autophagy-specific proteins associated with the autophagosomal membranes (Kabeya et al., 2000) have been reported recently in skeletal muscle.

Nutrient supply and/or endocrine factors exercise a primary function during muscle development and various catabolic states to modify muscle rates of protein breakdown (Tawa and Goldberg, 1994) and the expression of hydrolytic enzymes such as proteinases (Bechet et al., 2005). Muscle protein degradation in fasting or diabetes requires glucocorticoids and a fall in levels of insulin (Price et al., 1996). Administration of thriyodotironine (T3) raised the content of proteasomes (as well as lysosomal proteases) in muscle and increased the proteolytic process (Tawa et al., 1997). In contrast, IGF-I stimulates muscle protein synthesis and hypertrophy via the phosphatidylinositol 3-kinase (PI·K)–Akt pathway (Rommel et al., 2001), and prevented the dexamethasone (Dex)-induced increase in overall and myofibrillar protein breakdown. An important component of growth stimulation by IGF-I through the PI3K–Akt pathway is its ability to rapidly suppress transcription of the atrophy-related E3 atrogin-1 and other atrogenes, thereby blocking the atrophy program (Sacheck et al., 2004). However, data on the cellular mechanism of action of GH on muscle protein, DNA and RNA catabolism and the role played by the lysosomal enzymes are controversial. Thus, no significant effect of GH treatment on enzyme proteolytic activity in muscle of GH-treated pigs (Blanchard et al., 1993) or on the Ub pathway mRNAs (Chrysis and Underwood, 1999) has been reported. Also GH appears to act on lysosomal enzymes of proliferative chondrocytes in the growth plate (Gevers et al., 1996). In addition the administration of ghrelin, an endogenous GH secretagogue, changes lysosomal enzymes activities in the blood serum of female rabbits (Witek et al., 2005).

The administration of GH to growing animals enhances growth rate, decreasing rates of lipid deposition and improving rates of protein accretion (Boyd and Bauman, 1989). Vann et al. (2000) found in young, rapidly growing animals in the fully fed state, that GH treatment improves protein balance by reducing protein degradation rather than by stimulating protein synthesis. However, the effect of GH on tissue growth seems to vary between different phases of growth. Thus, GH administration at a dose of 120 μg kg^{-1} BW on pigs during the 10 to 25 kg growth phase (Harrell et al., 1997) does not improve the rate or efficiency of gain during the first half of the study, but increases growth rate and improve feed utilization during the second half. Part of the growth regulation by the GH/IGF-I system affects amino acid oxidation and the efficiency of amino acid use (Boyd et al., 1991). GH challenge decreases plasma urea nitrogen levels at all ages but the magnitude of the response is age-dependent, with greater reductions occurring in older pigs (Harrell et al., 1999), indicating that protein accretion response to GH treatment is enhanced in later growth phases. These effects on amino acid metabolism parallel age-dependent changes in the GH/IGF-I axis, resulting in only 20 to 30% increases in circulating IGF-1 in young pigs, compared to a 300% increase in older pigs (Harrell et al., 1999). Thus, somatotropin appears to have minimal effects during the prenatal and early post-weaning growth phase, but has substantial effects in later stages of development.

Previously we found that exogenous administration of recombinant human growth hormone (rhGH) to weaned BALB/c male and female mice induces a biphasic response on feed intake and body weight, resulting in a delay in the growth process from 25 to 30 days of age, and causes decreases in skeletal muscle weight and myonuclei number (López-Oliva et al., 2000). During the second stage (from 35 to 50 days of age), hyperphagic behaviour allows rhGH-mice to recover their body and muscle weight to control values through compensatory muscle hypertrophy (López-Oliva et al., 2001; Agis-Torres et al., 2002).

Since the cellular mechanism of action of GH on the protein, DNA and RNA catabolism of skeletal muscle is relatively unknown, in this work we studied whether GH administration might modulate the degradative capacity of muscle lysosomal system and influence muscle growth. Muscle cathepsin D, acid RNase and DNase II activities are determined in the gastrocnemius muscle of rhGH-treated post-weaning female BALB/c mice. Linear regressions were used to analyze the relationships of each enzyme with their respective substrate.

2. Materials and methods

2.1. Experimental design

The animal protocol was approved by the Laboratory Animal Care Advisory Committee of the Faculty of Pharmacy, Universidad Complutense de Madrid, Spain. 60 weaned (21 days of age) female BALB/c mice (*Mus musculus*) with an average body mass of 12 ± 1 g were used and supplied by the breeding unit *Nutrición y Fisiología* (Spanish Government licence: ES280790000085) at the Faculty of Pharmacy. All animals were caged individually in metabolic cages maintained at 23 ± 1 °C with 12 h light:dark cycles. Mice were assigned randomly to two groups of 30 mice each and injected subcutaneously with either saline (S: NaCl 9 g L^{-1} water) or rhGH (GH: 74 ng g body $mass^{-1}$, in 20 μL saline) (kindly donated by Novo Nordisk Pharma S.A. Madrid) every 2 days. Feed intake and water were available *ad libitum*.

Body mass (BM) and feed intake (FI) were recorded daily. Six partial experimental periods were established within the total experimental period of 29 days. The initial day of every partial period started at 21 days of age, whereas the final days were at age 25, 30, 35, 40, 45 or 50 days.