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The Role of GH/IGF-I Axis in Muscle Homeostasis during weightlessness

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Overview

Exposure to reduced gravity during space travel profoundly alters the loads placed on bone and muscle. Astronauts suffer significant losses of muscle and bone strength during weightlessness. Exercise as a countermeasure is only partially effective in remedying severe muscle atrophy and bone demineralization. Similar wasting of muscles and bones affects people on Earth during prolonged bed rest or immobilization due to injury (1-4). In the absence of weight bearing activity, atrophy occurs primarily in the muscles that act in low power, routine movements and in maintaining posture (2). Hormonal dysfunction could contribute in part to the loss of muscle and bone during spaceflight. Reduced levels of human growth hormone (hGH) were found in astronauts during space flight, as well as reduced GH secretory activity was observed from the anterior pituitary in 7-day space flight rats (5). Growth hormone has been shown to be required for maintenance of muscle mass and bone mineralization, in part by mediating the biosynthesis IGF-I, a small polypeptide growth factor (6). IGF biosynthesis and secretion plays an important role in potentiating muscle cell differentiation and has been shown to drive the expression of myogenin, a myogenic specific basic helix-loop-helix factor. IGF-I has also been shown to have an important role in potentiating muscle regeneration, repair and adult muscle hypertrophy (7-10).

Spin back to Earth Application

This project is not only applicable to mounting a countermeasure for muscle atrophy and bone demineralization induced during weightlessness in space flight, but also provides important new spin back applications to medical-related health problems on earth. Skeletal muscle mass is lost during aging from 25 years to senescence. Slow twitch muscle fiber is responsible for much of the age-related decline in muscle mass. The mechanisms responsible for the age-related decreasing muscle mass continues to be debated; although the likely contributory factors are decreased physical activity, hormonal influences, and neuromuscular alterations. For example, inactivity has been shown to cause muscle

atrophy in animals and humans of all ages. Orthopedic events, most commonly hip, vertebral or pelvic fracture, in the elderly are one of the causes of continuous bed rest in the elderly. It is known that non-ambulatory and underweight nursing home patients had increased rates of muscle atrophy after decreased mobility. It is also obvious that physical activity and the resultant stress imposed upon on the skeletal system improves bone mineralization. Bone mineral density is correlated with muscle strength in older men and women. Since a minimal amount of muscle mass is required for mobility and decreases in mobility reduce quality of life, skeletal muscle atrophy and the ability to rehabilitate are important components of aging that require a greater research emphasis. Previous studies have shown that in a significant number of normal elderly persons, growth hormone (GH) and insulin-like growth factors (IGFs) levels in serum are reduced. IGF-I is a potent anabolic factor that mimic most of the growth promoting actions of GH in vivo. Because the administration of either GH or IGF-I to GH-deficient animals under experimental conditions result in increased muscle and connective tissue mass, it has been proposed that these hormones may be important in maintaining muscle mass. We expect that growth hormone/IGF-I and exercise may serve as a means to reduce muscle atrophy in zero gravity. In the future, these hGH/IGF-I expression vectors developed by this proposal will have direct clinical and gene therapeutic applications for humans, since DNA vectors can now be directly injected into muscle and remain transcriptionally active for months expressing these important growth factors.

We hypothesize that the GH/IGF-I axis through autocrine/paracrine mechanisms will provide long-term muscle hypertrophy under conditions of prolonged weightlessness. This enhancement in muscle mass could occur at the level of systemic or local IGF-I production, production of modulating IGF binding proteins (IGFBPs), in serum form complexes which stabilize IGF-I from rapid turnover and binding with cell surface IGF-I receptors, a ligand-activated-tyrosine kinase and signal transduction to nuclear myogenic bHLH transcription factors.

Aim 1: To determine the role of overexpression of hGH, and IGF-I in transgenic mice on muscle . mass accretion under condition of weightlessness.

We constructed several DNA vectors, based upon the avian skeletal a-actin gene to drive human IGF-I expression in muscle cell culture and in transgenic mice (II). Transgene activity in single copy lines of mice was detected at approximately 50% of the endogenous a-skeletal actin gene in hind limb muscle of adult mice. Our most interesting observation to date is that overexpression of IGF-I in skeletal muscle causes muscle hypertrophy. We also developed Skeletalactin/hGH transgenic mice that demonstrated

serum levels between 300-1600 ng/ml of hGH. The Sk-actin-hGH hybrid gene elicited an approximate 25% gain in body weight. The expression of both GH/IGF-I will provide an ectopic source of hormone/factor that is independent of pituitary biosynthesis and secretion. We are now in position to test the proposition that the GH/IGF-I axis will provide long term hypertrophy/bone mineralization under conditions which accelerate muscle atrophy.

Experimental Design

It will now be possible to determine if hGH or IGF-I transgene expression from muscle will act as an effective countermeasure to muscle atrophy and bone demineralization. We plan to first use non-invasive evaluation of muscle anatomy and bone density via nuclear magnetic imaging (NMI). We are especially interested if the ectopic expression of hGH and IGF-I are effective in the maintenance of slow twitch muscle mass, since slow twitch muscle atrophy occurs more readily under weightlessness. An optimas morphometric package will be used to assess cross sectional diameter measurements of individual muscle fibers. IGF-I biosynthesis resulting from these human transgenic cDNA constructs will be measured in the presence of murine IGF-I by a sensitive sandwich RIA specific for a human IGF-I epitope (Diagnostic Systems Laboratories). Actin promoter activity will be evaluated by assessing the b-galactosidase activity using a standard ONPG assay and expressed as a specific activity per cellular protein as a means to evaluate transcriptional activity.

In collaboration with Dr. Eric Rabinovsky, we undertook analysis of the role of IFG-I as a neurotrophic agent to repair crushed motor neurons. The conduction rates (m/sec) were measured in sciatic nerves of SISII mice and their control litter mates following crush. After two weeks following crush the conduction rates in both IGF-I transgenics and control litter mates were about 1m/sec. Within three weeks of nerve crush, the IGF-I transgenic displayed a highly reproducible two to three fold increase in conduction velocity over the rates observed for nerves crushed in control mice. By four weeks following crush, the sciatic nerves of SIS II transgenic recovered over 65% of the non-crushed nerve conduction rates, and were at least twice as fast as the conduction rates measured for the sciatic nerve -crushed in control mice. Similarly, muscle weight gain or muscle growth and hypertrophy followed the return of nerve conduction activity, exemplified by the more rapid growth by the IGF-I transgenics in comparison to normal wild type mice. Demonstration of increased neural muscular restoration of IGF-I transgenics over control litter mates continued up to eight weeks post crush.

Closer inspection of the expression activity of specific myogenic genes provided some novel insights into the role of IGF-I in nerve-muscle repair. The levels of the g-acetylcholine subunit mRNA expressed in the muscle's of nerve-crushed and untreated control and IGF-I transgenic mice were measured as shown in Figure 1. It has been widely observed that Ach-g mRNA levels increase in response to nerve crush and denervation, consistent with the large increases in extrajunctional acetylcholine protein densities. Similarly, two weeks after sciatic nerve crush we observed robust enhanced muscular levels of Ach-a and Ach-g mRNAs. However, by three weeks following crush Ach-a and Ach-g mRNA levels were still at elevated levels in the muscle of wild-type mice, while both Ach mRNA levels returned to baseline levels in the muscle of IGF-I transgenics, that experienced nerve crush.

In a similar fashion, we also observed increased levels of myogenic basic helix-loop-helix factors, MyD and myogenein mRNAs, which becomes elevated during the repair process of crushed muscle. Expression of MyoD and myogenin was enhanced in both control and transgenic muscle, two weeks following nerve crush. However, by the third week following nerve crush, MyoD and myogenin mRNAs were already reduced to baseline control levels. High levels of myoD and myogenin messengers were still present in the muscles of three week post crushed muscle. These data indicate that the overexpression of IGF-I enhanced muscle and nerve repair function, consistent with the return of normal nerve conduction rates.

Summary

Growth hormone releasing hormone, a single polypeptide chain of 44 amino acid peptide aminated at the COOH terminus, was first identified by Guillemin et.al. (1982) Science 218, 585-587 from a pancreatic tumor of an acromegalic patient. Non-amidated forms of 40 and 37 amino acids have full biological activity and appear to be matured by protelytically processing from a 108 amino acid precursor. The pancreatic GHRH is identical to the GHRH of pituitary origin which was reported to be primarily involved in the biosynthesis and release of growth hormone by the pituitary gland. GHRH has biological activity in the fmole/ml range for increasing GH secretion and should be obtainable by simple formulation of the MVS system.

There are many potential uses for GHRH ranging from an injectable gene medicine for therapy for growth retardation in humans to treating astronauts in space. Currently, recombinant GHRH is

administered by daily injections. Ideally, long-term administration of GHRH would be our ultimate research objective.

We incorporated by recombinant DNA cloning the human growth hormone releasing hormone into a muscle specific vector. A synthetic human GHRH DNA was synthesized according to the following sequence :

NH2-Terminal Y A D A I F T N S Y R K V L G Q L S A R K L L Q D I M N R Q Q G E R N Q E Q G A K V R L

1. Successful secretion of GHRH requires processing of a larger precursor containing both a 31 amino acid presequence that is similar to a signal sequence and a 44 amino prosequence encoding the mature GHRH product. Processing of the precursor at paired Arg-Arg or Lys-Arg basic residues occurs as the result of an endoprotease, the product of KEX genes found in most cell types. The natural pro sequence is as follows:

 NH2 Terminal M P L W V F F F V I L T L S N S S H C S P P P P L T L R M R R / Y

This pro sequence was synthesized as a sticky ended DNA duplex and cloned upstream of the bovine GHRH cDNA, linkered, and then inserted into MVS.

2. **We validated biological activity of this vector for biosynthesis and secretion in mammalian myognenic cell types.** Levels of GHRH synthesis and secretion were assayed first in transient and stably-transfected myogenic cell lines. GHRH secreted into tissue culture media was detected by a sensitive radioimmunoassay. Secreted GHRH was used to increase growth hormone secretion from pig pituitary cells in culture and was equivalent to 10 ng of pure GHRH.

3 **We validated biological activity of this vector for biosynthesis and secretion in small mammals by direct injections into muscle.** We determined a near tripling of GH secreted into serum by RIA following a single injection of MVS-GHRH into leg muscle. Animal growth versus dosage of MVS-GHRH vector was determined by accurate weights and we determined an approximate 10% weight gain at the end of one month versus control litter mates.

Potentially, formulated MVS-GHRH DNA conjugates could be used in long-duration space flight to maintain normal GH levels in astronauts under conditions of microgravity.

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