Mechano growth factor

Research on mechano growth factor: its potential for optimising physical training as well as misuse in doping

G Goldspink

Mechano growth factor can produce rapid increases in muscle and strength, giving it considerable therapeutic and doping potential

The sequencing of the human genome showed that there are only about 40 000 genes. However, there are many more proteins. This is because some genes are spliced to produce different protein/peptides which usually have different biological functions. Combining physiological and molecular biology methods made it possible for our team to identify and characterise a local muscle growth/repair factor (MGF). This we found is derived from the insulin-like growth factor I (IGF-I) gene by alternative splicing, but, owing to a reading frame shift, MGF has a unique C-terminal peptide. After resistance exercise, the IGF-I gene is spliced towards MGF which ''kick starts'' hypertrophy and repair of local muscle damage by activating the muscle stem cells as well as anabolic processes. Interestingly, loss of muscle mass in old age and in certain diseases is associated with an impaired ability to express MGF. In these conditions it seems that the muscle stem (satellite) cell pool is not adequately replenished.

CLONING OF MGF AND OTHER HUMAN MUSCLE IGF-I SPLICE VARIANTS

For some time it has been apparent that muscle mass and strength must be under the control of local growth factors because if one exercises a particular muscle, it is that muscle and not all the muscles of the body that undergo hypertrophy. A little over 10 years ago, our group set out to clone the factor(s) that are involved in autocrine regulation of muscle mass. For this purpose we needed to have an animal model in which we could make muscle grow rapidly. Previous work had shown that the tibialis anterior muscle in the mature rabbit, when held in the stretched position by plaster cast immobilisation combined with low voltage electrical stimulation, increased in mass by 35% in just over a week.[1] It was known that muscles adapt to a new functional length by adding sarcomeres in series at the ends of the existing myofibrils. However, if muscles are also subjected to electrical stimulation, they increase in girth as well as length. Total RNA in these muscles was found to increase by about four times within a couple of days. We also studied specific messenger RNAs using a technique known as differential display and detected an mRNA that was expressed in exercised but not in resting muscles.[2] This was converted into cDNA and sequenced, and the genome database showed that it was derived from the IGF-I gene. This local type of IGF-I we called mechano growth factor (MGF) as it was expressed in response to mechanical stimuli and because it has a different downstream (C-terminal) sequence from the liver or systemic types of IGF-I. From physiological experiments it became apparent that the muscle forms of IGF-I have different functions and that in the case MGF its unique C-terminal peptide has a special function of activating and replenishing the muscle stem (satellite) cell pool. As with the central nervous system, skeletal muscle is a post-mitotic tissue. Therefore there has to be an effective local cellular repair mechanism otherwise cell death will ensue. The extra nuclei required for growth and repair come from the muscle stem (satellite) cells fusing with the muscle fibres. This is also one of the early events in the hypertrophy process. MGF is responsible for replenishing the pool of muscle stem cells,[3 4] and this provides the means by which strength adaptation occurs after exercise and/or local muscle damage.

SPLICING OF THE IGF-I GENE IN RESPONSE TO EXERCISE AND HORMONES

Previous research had shown that resistance exercise which results in muscle hypertrophy is associated with an increase in IGF-I expression.[2 5 6] However, these studies failed to distinguish between the different types of IGF-I. As mentioned above, the way MGF was discovered was by studying the RNA transcript of exercised and non-exercised muscle.[2] Shortly after this, the group of Ken Baldwin and Greg Adams in the United States[7] showed that MGF is expressed earlier than IGF-IEa in response to exercise. Using specific primers (gene probes), we measured the mRNA concentrations of MGF and IGF-IEa using quantitative polymerase chain reaction mechanically in overloaded rodent muscle[8] as well as in human volunteers in which muscle biopsy specimens were taken 2.5 hours after a single bout of high intensity exercise of knee extensor muscles.[9] In young muscle, MGF mRNA concentrations were significantly increased as a result of resistance exercise, but no significant change was observed in older muscle when subjected to the same degree of mechanical overload. However, elderly male volunteers when given growth hormone combined with exercise training produced increased concentrations of MGF,[10] which could be correlated with increased muscle cross sectional area as determined from computed tomography scans. Figure 1 shows the way the IGF-I gene is spliced after exercise and in response to hormones.

It was noted that in exon 5 of MGF in the human there is a 49 base insert (52 in the rat) which results in a reading frame shift. Amino acids are coded for by triplets of bases. As the exon 5 insert is not a multiple of 3, the downstream peptide sequence of MGF is different from that of the other kinds of IGF-I. This region has important functional consequences as the carboxy peptide of some IGF-I isoforms is involved in the recognition of the specific binding proteins that stabilise these growth factors. At least two forms of systemic IGF-I are expressed by muscle even at rest. However, it is apparent that in response to exercise and/or damage, MGF is expressed locally and that it has a dual action. This includes activating the muscle stem cell pool through its C-terminal domain (encoded in exons 5 and 6) and increasing anabolic effects as the result of its IGF-I receptor binding domain (encoded in exons 3 and 4), which all the IGF-I genes possess.

GENE TRANSFER AND ENHANCEMENT OF MUSCLE MASS AND STRENGTH

One of the methods we used to establish the biological action of MGF was to engineer a gene into which its cDNA was inserted into a vector. To our

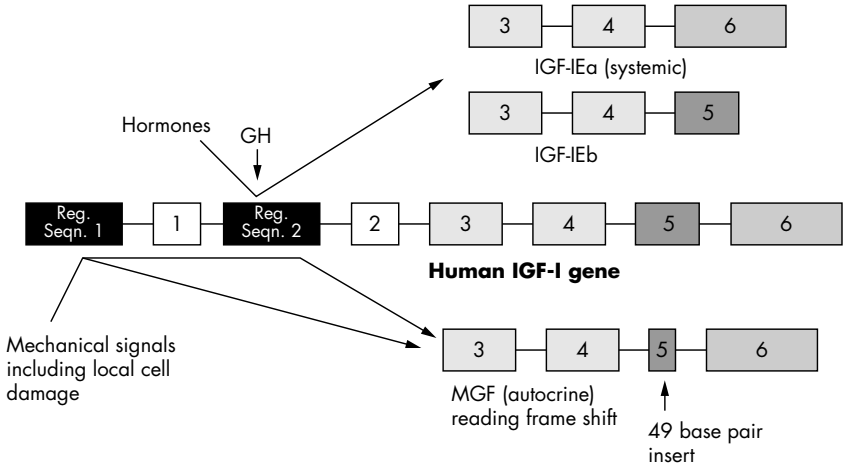

Figure 1 Splicing of the insulin-like growth factor (IGF) gene to produce different forms of IGF-I in human muscle. Mechano growth factor (MGF) is produced locally in response to exercise and it differs from the two systemic types of IGF-I as the 49 base insert in the exon creates a reading frame shift so that the downstream or C-terminal peptide sequence is different. This unique peptide has been found to be involved in activating the muscle stem cells and to ''kick start'' the tissue repair and/or hypertrophy processes. In the elderly, who are growth hormone (GH) deficient, there is an improvement in MGF expression when administration of recombinant human GH is combined with exercise. Reg Seqn, Regulatory sequence.

surprise a single intramuscular injection into a mouse muscle resulted in a 25% increase in mean muscle fibre cross section area within three weeks.[11] Similar experiments have been carried out using the systemic or liver type of IGF-I in an adenoviral vector under the control of a muscle regulatory sequence. However, this took four months to produce a 15% increase and is probably due to the anabolic effect of IGF-I, which is common to all the splice variants.[12] The use of the DNA of IGF-I and particularly MGF is therefore a prime candidate for gene doping for enhancement of athletic performance. This presents the anti-doping agencies with a challenge, as the range of vectors available for use in engineering these genes enable them to be designed so that the gene product can be delivered locally or systemically and also so that they can be switched on and switched off after they have been introduced into the body. Our research unit is using the extremely sensitive and specific reverse transcriptase polymerase chain reaction to amplify a vector and/or the enhancer cDNA as a means of detecting gene doping. We also know that MGF as well as IGF-I exist as class I and class 2 isoforms and that the ratios of these in serum change if they are introduced as the peptide or by gene transfer. Therefore there is the possibility of detecting the misuse of these strength generating substances even if delivered in the form of ''gene doping''.

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COMMENTARY

The control of muscle growth is an important area of research in clinical and sports medicine. The team lead by Geoff Goldspink in London have made a significant contribution to the characterisation and function of growth factors in skeletal muscle. This leader is a summary of their work to date. It details the regulatory control and biological functions of spliced variants of the IGF-I gene and the potency and mechanism of action of the protein products. Genuine therapeutic potential is much in evidence from this work, but with athletes ever more informed of the potential of gene doping the article also serves to remind us of the challenges that lie ahead if we are to tackle ''gene doping'' for performance gain.

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