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The control of sexual dimorphism by growth hormone and the gonadal steroids.

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Introduction

The principal regulator of post natal growth is the growth hormone (GH)/insulin-like growth factor 1 (IGF-1)/myostatin axis through the intracellular signallers STAT5a and STAT5b. In the majority of species, males have a significantly larger body size and muscle mass than females (sexual dimorphism). Sexual dimorphism of skeletal muscle appears to be secondary to interactions between the GH/IGF-1/myostatin axis and the gonadal steroids (testosterone and estrogens), although the exact interactions are not fully known. The current literature reports that, based on body weight, these interactions occur through STAT5b, as male STAT5b knockout (KO) mice have the same body weight as female STAT5b KO and wild-type mice. STAT5a was previously thought to be redundant as there is no significant difference in body weight between both male and female STAT5a KO and wild-type mice. Our recent work reveals that sexual dimorphism of muscle mass normalised to bone length persists between male and female STAT5b KO mice. This data is not known for STAT5a KO mice. Therefore, we hypothesised that STAT5a has a role in skeletal muscle in the interactions between the GH/IGF-1/myostatin axis and the gonadal steroids.

Methods

C2C12 myoblasts were treated with viral STAT5a and/or STAT5b RNA interference (siRNA) with a scrambled vector as a control. Under differentiation conditions, the C2C12 myoblasts were treated with GH (100 ng/mL), testosterone (30 nmol/L) or 17β-estradiol (10 nmol/L) for 24 hours. RNA was harvested and quantitative PCR was performed.

Results

GH increased IGF-1Ea mRNA in the control ($P < 0.001$) and STAT5b ($P < 0.05$) siRNA treated myoblasts, but this was prevented by STAT5a or STAT5a/STAT5b siRNA treatment. Testosterone and 17β-estradiol had no effect on IGF-1Ea mRNA, except for 17β-estradiol increasing IGF-1Ea mRNA in STAT5b siRNA treated myoblasts alone ($P <0.05$). GH and testosterone individually increased androgen receptor (AR) mRNA in the control ($P < 0.05$) and STAT5b ($P <0.001$) siRNA treated myoblasts with no significant change in STAT5a or STAT5a/STAT5b siRNA treated myoblasts. Similarly, GH and 17β-estradiol individually increased estrogen receptor alpha (ERα) mRNA in the control ($P < 0.001$) and STAT5b ($P <0.001$) siRNA treated myoblasts with no significant change in STAT5a or STAT5a/STAT5b siRNA treated myoblasts.

Conclusions

STAT5a is the key intracellular signaller of GH in the upregulation of IGF-1 mRNA. GH augments the response of the gonadal steroids by upregulating both AR and ERα mRNA and this is also STAT5a

dependent. Intriguingly, the upregulation of AR mRNA by testosterone and ERα mRNA by 17β-estradiol was prevented by STAT5a knockdown and the mechanism for this is not known. However, the augmentation of the gonadal response by GH through STAT5a provides a further mechanism to the sexual dimorphism of skeletal muscle. Furthermore, to our knowledge we are the first group to report the regulation of the ERα by GH in skeletal muscle.

Future directions

We had planned to commence our *in vivo* study last year to investigate the interactions of the GH/IGF-1/myostatin axis and the gonadal steroids on gonadectomised male and female mice with testosterone or 17β-estradiol implants. However, there was a major delay due to the new regulations preventing the export of testosterone pellets from the United States. As an alternative, we are now using silastic tubing filled with testosterone or 17β-estradiol and sealed with silastic adhesive and the study has commenced using FVB mice. The study will be completed by the end of this year as well and we also anticipate completing the analysis of quantitative PCR and Western blotting from our *in vitro* treatments above. Additionally, we have completed the muscle and serum sample collection of 28 patients with growth hormone deficiency before and 6 months after GH replacement, 12 healthy control participants and 5 control participants with growth hormone deficiency without GH replacement. These samples will be processed and analysed over the upcoming months. Together, these studies should elucidate the regulation of the GH/IGF-1/myostatin axis in muscle, the interactions with gonadal steroids and further our understanding of the regulation of muscle and sexually dimorphic growth. We expect several publications from our work and we will acknowledge and thank the Waikato Medical Research Foundation for their support of our research.

Expenditure

Cell culture reagents	$ 1200
Reverse transcription reagents	$ 1800
RNA extraction reagents	$ 1100
PCR consumables (Roche)	$ 4400
Silastic tubing (Dow Corning)	$ 800
Silastic adhesive (Dow Corning)	$ 600
Estradiol/testosterone ELISA assays	$ 3600
Total	**$ 13500**