The effect of estrogen on Akt signalling and protein synthesis in C2C12 mouse skeletal myotubes

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The maintenance of skeletal muscle mass is a critical component of health in both chronic wasting diseases and aging. A considerable amount of progress has been made in the understanding of the signalling pathways that mediate skeletal muscle hypertrophy and atrophy. Akt is seen as a key molecular protein involved in the maintenance of skeletal muscle mass as it has the dual ability to positively influence protein syntheses and negatively regulate protein degradation in its active state (Glass, 2003). Potential mechanisms which may assist with maintaining skeletal muscle mass are the estrogen hormones. Estrogens increase the proliferation of mouse and rat myoblasts and can also attenuate immobilization-induced skeletal muscle atrophy in rats *in vivo* (Kahlert *et al.*, 1997). No studies have investigated the effect of estrogens on the activation of skeletal muscle hypertrophy and atrophy signalling pathways. Estrogens may contribute to maintaining skeletal muscle mass *via* their activation of the Akt signalling pathways. Therefore, the aims of the present study were to determine if treatment of C2C12 myotubes with either 17β-estrodiol or estrone increases the activity of Akt and its downstream anabolic signalling proteins, GSK, p70s6k and 4E-BP1 and decreases its catabolic stimulating targets, FOXO, atrogin-1 and MuRF-1. A secondary aim was to determine if this was associated with an increased rate of protein synthesis.

C2C12 myotubes were incubated at 37°C in serum free DMEM without phenol red containing 10 000 units/ml penicillin, 10 000 μg/ml streptomycin, and 250μg/ml amphotericin B for 24h. Myotubes were then stimulated with 17-β estradiol (10nM) for 24h. Phosphorylated and total proteins for Akt, p70S6k, GSK3β, 4E-BP1, FOXO and atrogin-1 were measured using western blotting techniques. Atrogin-1 and MuRF1 mRNA levels were measured using real time-PCR. Protein synthesis rates were measured by incorporation of [^{3}H]-tyrosine into the myotubes during the last hour of treatment.

Compared to control myotubes, treatment with 17β-estradiol increased the ratio of phosphorylated to total protein contents for Akt, GSK-3β and P70^{s6k} by, 1.62, 1.53 and 2.2 fold, respectively (n=6 per group; $p < 0.05$). There was, however, no difference in the ratios of phosphorylated to total 4E-BP1 or Foxo3a or Atrogin-1 and MuRF1 mRNA. Protein synthesis rates remained unchanged.

This study demonstrates that in C2C12 mouse myotubes, 17β-estradiol treatment increases the phosphorylation of the hypertrophy signalling protein, Akt, and its downstream hypertrophy signalling targets, GSK-3β and P70^{s6k}; no associated changes in protein synthesis were observed. Future studies should investigate the ability of 17β-estradiol to activate these proteins in a model of myotube catabolism and to determine if protein degradation is attenuated.

Glass DJ. (2003) *Nature Cell Biology*, **5**, 87-90.

Kahlert S, Grohe C, Karas RH, Lobbert K, Neyses L. Vetter H. (1997) *Biochemical and Biophysical Research Communications*, **232**, 373-378.