EFFECT OF TESTOSTERONE ON P70S6K PHOSPHORYLATION IN SKELETAL MUSCLE CELL COLTURES

[1]Maria G. Dattolo, [1]Rocco Saviano, [1]Giovanna La Rana, [1]Antonio Calignano, [2]Maria Miniaci and [1]Pietro Scotto

[1]Dept.of Experimental Pharmacology, University of Napoli "Federico II";[2]Dept.of Experimental and Clinical Medicine, University of Catanzaro, Italy

Testosterone, has a well-known anabolic effect related to both muscle hypertrophy (1) and upregulation of a number of proteins (2). Most of its effects are exerted by a direct binding to the androgen receptors (AR) located in the cytoplasm. In hormone absence, the AR is associated with proteins (hsp) that prevent the interaction with the cellular transcription apparatus. Upon binding testosterone, the receptor undergoes an activating conformational change facilitating its translocation into the nucleus and association with specific DNA sequences leading to the induction of transcription (3). Testosterone is used to increase muscle mass but its mechanism of action is poorly understood. Since activation of the mTOR-p70S6 kinase pathway is associated to protein synthesis (4), we aimed to investigate, in vitro, the possibility that testosterone might act trough a nongenomic mechanism involving this pathway. Rat H9c2 myoblasts, were cultured in DMEM with 1% FBS to promote cell differentiation. Myocytes (2 x 10^6 cells/well) were incubated with a supra-physiological dose of testosterone enanthate (10^{-5} M) for 2h at 37°C in air and 5% CO_2. Cells were lysed and total protein extracts were collected for Western blot analysis. We detected an increased expression (70% above control) of intracellular phospho-p70S6k (the activated form of p70S6k). This increase was completely inhibited by pre-incubation of the cells with flutamide and cyproterone acetate (10^{-3} M), two drugs acting as androgen antagonists, indicating that this effect is mediated by AR binding. Furthermore, cells incubated with testosterone in the presence of rapamycin (mTOR inhibitor; 20 nM) did not show increase of p70S6k phosphorylation suggesting that mTOR-p70S6k plays a key role in muscle hypertrophy. Our data agree with the hypothesis that increased p70S6k phosphorylation due to testosterone receptor binding may be an important mechanism leading to protein synthesis in cultured muscle cells.

1. Bhasin S, Storer T, Berman N, Callegari C, Clevenger B, Philips J, Bunnel T, Tricker R, Shirazi C and Casaburi R. (1996) N Engl J Med. 335: 1-7.
2. Rooyackers OE and Nair S. (1997) Annu Rev Nutr. 17: 457-485.
3. Beato M. (1989) Cell. 56: 335-344.
4. Kubica N, Bolster D R, Farrell P A, Kimball S C, Jefferson L S. (2005) J Biol Chem. 280: 7570-7580.