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Growth Factor Involvement in Tension-Induced Skeletal Muscle Growth

Herman H. Vandenburgh, Ph.D.

Department of Pathology

Brown University Program in Medicine

Providence, RI 02912

and

Department of Pathology and Laboratory Medicine

The Miriam Hospital

Providence, RI 02906

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Correspondence Address:

Dr. H.H. Vandenburgh

Dept. Laboratory Medicine

164 Summit Ave.

Providence, RI 02906

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Description of Research

Long-term manned space travel will require a better understanding of skeletal muscle atrophy which results from the microgravity found in space. Astronaut strength and dexterity must be maintained for normal mission operations, for emergency situations, and for the rigors of reentry into the earth's atmosphere. A biochemical understanding of how gravity, by increasing the tension on muscle cells, helps to maintain muscle size and strength, should ultimately allow pharmacological intervention to prevent muscle atrophy in microgravity. Because of the limited availability of performing experiments in space, a number of earth-based model systems have been designed to study the relationship of muscle tension to muscle growth (reviewed in Vandenburgh, 1987a). These models include the casting of an animal's limb such that the muscles are held under increased (stretched) or decreased (shortened) tension, placing weights on limb muscles to increase their load; and suspending an animal's hinquarters above the ground in order to reduce skeletal muscle use.

Our laboratory has developed several tissue culture model systems for studying the relationship of tension to skeletal muscle growth (Vandenburgh and Kaufman, 1979; Vandenburgh, 1987b; 1987c). Tissue culture cells have been extensively used for studying basic biochemical growth processes in other cell types and allow easier manipulation of experimental conditions than whole animals. Cultured cells have been especially helpful in studying the role of growth factors in normal and pathological cell growth (reviewed in Rozengurt, 1986). We are utilizing

one of our tissue culture model systems to determine the role played by exogenous and endogenous growth factors in tension-induced skeletal muscle growth. Briefly, this model involves isolating muscle cells from 12 day avian embryos and growing them on a highly elastic substratum material in a humidified CO_2 incubator at 37°C (Vandenburgh, 1987b; 1987c). The muscle cells, growing in a complex medium, initially proliferate, and then fuse to form multinucleated, spontaneously contractile myotubes in a manner very similar to growth and differentiation in vivo (Coleman and Coleman, 1969). These cells actively synthesize actin and myosin and incorporate them into cross-striated myofibrillar material. These in vitro differentiated myofibers can be subjected to different patterns of mechanical activity with our "Mechanical Cell Stimulator" which simulates in vivo muscle activity. The activity patterns used to stimulate muscle growth are generated by an Apple IIe computer. In effect, we can subject muscle fibers in a defined in vitro environment to different types of "exercise" programs, and study their growth response on a molecular level. The results obtained using avian muscle should be applicable to mammalian muscle, since most basic molecular mechanisms utilized by Nature are identical or very similar in different classes of vertebrates.

Accomplishments

We have shown previously that increased tension on the cultured muscle cells in a complex medium stimulates their growth (Vandenburgh and Kaufman, 1979). These factors include the medium growth factors found in serum and embryo extract supplements as well as endogenous growth factors produced by the muscle tissue itself (Vandenburgh, 1983; Summers et.al., 1985; Kardami et.al., 1985). The hypothesis currently under investigation is whether extracellular serum factors present in the growth medium are essential for mechanical stimulation of skeletal muscle growth *in vitro* (Hypothesis I). The protocol being used is to allow the avian muscle cells to differentiate into spontaneously contractile myofibers in the Cell Growth Chamber of our Mechanical Cell Stimulator in complete culture medium (with 10% horse serum, 5% chicken embryo extract). Once developed, the cells are switched to medium, with or without added growth factors. Repetitive mechanical stimulation of the cells is initiated and at varying times, groups of cells are removed for determination of their growth rate by measuring protein:DNA ratios and by morphological measurements of the myotube's cross-sectional area. In an earlier study, it was found that a passive stretch of the muscle fibers (12% for 6 to 12 hr) in the absence of exogenous growth factors (serum) decreased the rate of atrophy of the cells but did not stimulate them to grow; growth occurred only in the presence of serum growth factors (Vandenburgh, 1983). It was of interest to determine whether a more dynamic stretch/relaxation stimulus in our new Mechanical Cell Stimulator could stimulate myotube growth in the absence of exogenous growth factors. Based on recent metabolic studies, the cells undergoing continuous stretch/relaxation in this system are being actively "worked"

(Hatfaludy et.al., 1987b). Based on myotube diameter measurements after 2 to 4 days of continuous activity, myotube growth occurs in the presence of serum growth factors but not in their absence (Hatfaludy et.al., 1987a). As with the static model system, mechanical activity in the dynamic system prevents muscle cell wasting in the absence of growth factors, but does not lead to cell growth. Thus, our conclusion to Hypothesis I is that both static and active passive stretching of muscle cells differentiated in tissue culture can prevent muscle fiber wasting in basal medium, but cell growth requires the presence of exogenous growth factors which are as yet undefined. We will now proceed to analyze the role played by endogenous prostaglandins in these processes.

Significance of Accomplishments:

New muscle tissue culture techniques have been developed to grow skeletal myofibers which differentiate into more adult-like myofibers (Vandenburgh et.al., 1987a; 1987b). Mechanical stimulation studies of these muscle cells in a newly developed Mechanical Cell Stimulator can now be utilized to study growth processes in skeletal muscle. We have defined conditions in the Mechanical Cell Stimulator where mechanical activity can either prevent muscle wasting or stimulate muscle growth. The role of endogenous and exogenous growth factors in tension-induced muscle growth is being investigated under the defined conditions of tissue culture.

Grant-Supported Publications:

(see Literature Cited for full references)

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