Effects of Growth Factors on Bovine Myogenesis in Vitro

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[ABSTRACT]

The present study was conducted to examine the effects of growth factors (aFGF, Insulin, IGF-1, IL-1, DXMS) on bovine myoblasts during terminal differentiation in vitro. Bovine myoblasts were isolated from *M. longissimus thoracis* of Japanese black steers and cultured in 1:4 mixtures of Dulbecco's medium and M-199 medium. The myoblasts were bipolar and spindle in shape, stained by both MHC and desmin antibody, and underwent myotube formation. When growth factors were added to the cultures, aFGF(10^{-9}M), insulin(10^{-6}-10^{-7}M), IGF-1(10^{-9}M) and IL-1(10^{-9}M) augmented the cell growth of bovine myoblasts. Especially, short-term treatment of aFGF and IL-1 provided a growth-promoting stimulus to bovine myoblasts. Insulin (10^{-6}M) and IGF-1(10^{-7}-10^{-8}M) caused a significant increase of myotubes by the myoblast fusion. The results indicate that aFGF and IL-1 act as a myotrophic factors, especially, a competence factor, and insulin and IGF-1 effect as a progression factor and/or myotube forming factor on bovine myoblasts during terminal differentiation in vitro.

[INTRODUCTION]

A large number of in vitro studies using primary myogenic cells isolated from skeletal muscle of mouse, rat, chicken and swine (1-3) have showed that the cell growth and terminal differentiation of myoblasts are controlled by several growth factors. However, the myogenesis of bovine myoblasts, especially, transformation from cell proliferation to terminal differentiation is not always understood. We describe the possible role of growth factors in regulating the myogenesis of bovine myoblasts in vitro in the present study.

[MATERIALS AND METHODS]

Primary cultures were carried out with myoblasts prepared by enzyme dissociation from *M. longissimus thoracis* of Japanese black steers (1-6 months old). The tissues were minced and suspended in 20 ml of enzyme solution containing 1mg/ml collagenase, 1mg/ml hyaluronidase, 1000 u/ml of dispase and 0.1mg/ml of DNAase in PBS+ and agitated for 20 min at 37°C. The supernate was filtered through a mesh screen of 25μm. Bovine myoblasts were suspended in growth medium mixed with 8:2:1 of medium 199, Dulbecco's modified Eagle's medium and FCS, and 1.5% swine fetal extract (SFE). The cells were enriched by the combination procedure of a differential adhesion method and a selection method of myoblasts by cytochalasin B treatment(1-3).

Bovine myoblasts were cultured in collagen-coated 24 well plates at an initial density of 2-5x10^4 cells/well and maintained at 37°C in a 5% CO_2 atmosphere. SFE was added to the cultures at a concentration of 1.5%. Acidic fibroblast growth factor (aFGF, Bio-Science Lab.: 10^{-8}-10^{-10}M), insulin (Gibco: 10^{-6}-10^{-8}M), dexamethasone (DXMS, Sigma: 10^{-5}-10^{-7}M), insulin-like growth factor 1 (IGF-1, Toyobo: 10^{-7}-10^{-9}), interleukin 1 (IL-1: 10^{-7}-10^{-10}M) were added to the cultures when myoblast cultures were subconfluent.

For immunostaining (2,4), bovine myoblasts which were cultured on collagen-coated Lab Tek Chamber slides or collagen-coated cover slips were fixed with 4% paraformaldehyde in PBS+ for 30 min. The cells were reacted with the monoclonal antibodies against MHC, desmin and IL-1, and visualized with FITC-conjugated F(ab')$_2$ of sheep anti-mouse IgG. The nuclei were stained with PI solution. FITC/PI two-color observation was carried out with a confocal laser microscope (MRC-1024, BIO-RAD).

[RESULTS AND DISCUSSION]

The fusion and differentiation of bovine myoblasts into multinucleated cells were observed in the primary cultures. The myoblasts were bipolar and spindle in shape, stained by MHC or desmin antibody and fused to form myotubes.

An addition to the cultures of aFGF (10^{-9}M), IL-1 (10^{-9}M), insulin (10^{-6}-10^{-7}M) and IGF-1 (10^{-9}M) stimulated the cell growth of bovine myoblasts (Fig.1). Especially, 4hr-treatment of aFGF and IL-1 except for insulin and IGF-1 at the beginning of experiment provided a growth-promoting stimulus to bovine myoblasts (Fig.2). Furthermore, a lot of myoblasts and all myotubes were stained with IL-1 antibody. The results suggest that IL-1 stimulates autocrine growth of bovine myoblasts. On the other hand, an addition of insulin (10^{-6}M) and IGF-1 (10^{-7}-10^{-8}M) caused the increase of myotube formation although the fusion index was not different between insulin and IGF-1 treatment. aFGF and IL-1 were less effective on the myotube formation. aFGF (10^{-9}M) reduced the fusion of bovine myoblasts. DXMS did not influence on the cell growth and myotube formation of bovine myoblasts. Our study indicates that aFGF and IL-1 act the growth of bovine myoblasts as a competence factor and insulin and aFGF effect not only as a progression factor on the cell growth but as a myotube forming factor on bovine myoblasts during terminal differentiation in vitro.

Fig.1. Effect of aFGF and IGF-I during Myogenesis of Bovine Myoblasts

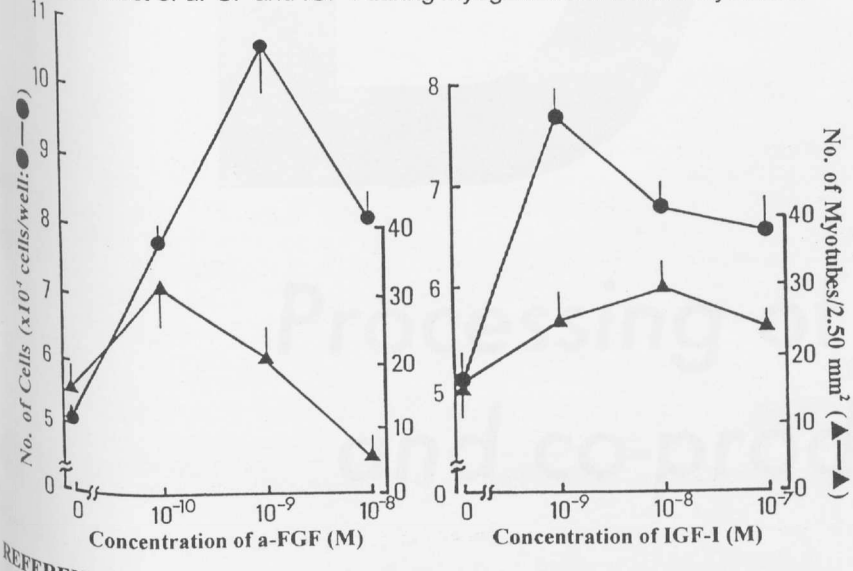
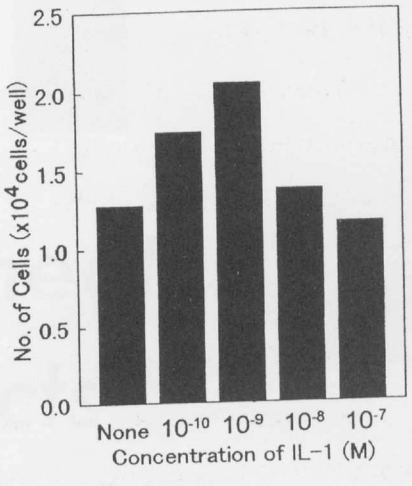


Fig.2. Effect of IL-1 on the Growth of Bovine Myoblasts



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