A NOVEL MUSCLE CELL GROWTH FACTOR, S-MYOTROPHIN, PROMOTES THE EXPRESSION OF FAST MYOSIN IN MYOTUBES DEVELOPED FROM C2C12 CELLS

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Background

Skeletal muscle fibers are innervated by motor neurons. Skeletal muscle is atrophied by the denervation of a motor neuron. It had been speculated by muscle biologists that muscle growth is regulated by unknown growth factor(s) under the control of motor nerve. We obtained an interesting result in 1998[1] that a water-soluble substance of 12kDa mass in the atrophied muscle is significantly less than that in non-treated counterpart. Based on this finding, we purified a novel muscle cell growth factor (hypertrophic factor) from porcine skeletal muscle and named as S-myotrophin[1]. S-myotrophin is a peptide containing saccharides in its molecule. S-myotrophin significantly increased creatine kinase activity of cultured chick skeletal muscle cells (multinucleated myotubes) and intracellular protein content. Immunoblot analysis confirmed that the amounts of myosin and actin in the myocytes were greatly increased by S-myotrophin stimulation as in the case of IGF-I stimulation[2] Morphological observations using anti-desmin antibody staining procedure demonstrated that the size of both S-myotrophin and IGF-I treated myotubes was appreciably larger than that of control myotubes[2] These results suggest that S-myotrophin is a potent mediator of skeletal muscle cell hypertrophy through the accumulations of muscle structural proteins.

Objectives

The objective of the presnt study was to investigate the effect of S-myotrophin on the expression of actin, myosin and creatine kinase as well as the expression of myoD and myogenin in myotubes prepared from C2C12 cells. To examine the difference in the expression of adult, fast and slow types of myosin in the cells was also the major concern of the present study.

Materials and Methods

S-Myotrophin was prepared from porcine skeletal muscle according to a modified procedure of Hayashi *et al.*.(2001). C2C12 myoblasts were incubated on COL-I coated cover glass (Iwaki, 12mm) previouosly set in each of 24 wells plate in a mixture of DMEM and FBS at the concentration of $1x10^5$ cells/well. During early stage of the incubaton of myoblasts, a mixture of DMEM and 10%FBS was used. After myoblasts grew to confluent state, the medium was changed to differentiation medium (a mixture of DMEM and 1%FBS) and incubated until the differentiation of the cells from myoblasts to myotubes was recognized. S-Myotrophin (final conc.=100ng/ml.) was added to myotubes and the resulting mixture was incubated at 37°C and 5% CO_2 for 24hr.

After the incubation, myotubes were fixed with 4% paraformaldehyde or with 100%methanol, followed by the inactivation of intrinsic peroxydase of the myotubes with 0.3% H_2O_2 dissolved in 100% methanol. The inactivated myotubes were histochemically stained with antibodies using R.T.C.VECTASTAINUNIVERSALElite ABC KIT (Vector Laboratories,Inc.). As primary antibodies of the histochemical staining, myoD, myogenin (Santa Cruz Biochemistry), adult, slow and fast myosins(Sigma), and MM-CPK(Biogenesis) were used. Peroxidase-labelled secondary antibody was coupled to the primary antibodies. The immunologically stained samples were observed with a microscope(Microphot-S)(Nikon,Ltd.).

After S-myotrophin treated and control myotubes were solubilized, the solubilied samples were electrophoresed according to the procedure of Lammni.[3] Proteins on the gels were electrically transferred to PVDF membranes. Then, the PVDF membranes were immunologically stained with primary antibodies and with the secondary antibody. Color development produced by peroxidase was made by DAB. Bands on the PVDF membranes were incorporated into a scanner [EpsonGT-7000U] and were image-processed with Photoshop LE (Adobe System Inc.) and were then printed out. The intensity of each blotted band was analyzed with a densitometer.

Results and Discussion

MyoD and myogenin are the factors which determine the fate of mesodarmal stem cells to myoblasts and also work for the maintainance of the function of the determined cells. In the present experiment, the expression of myoD and myogenin in the myotubes were significantly increased by the addition of S-myotrophin. S-myotrophin also induced hypertrophy of myotubes as well as the increase in the numbers of myotubes. The level of the expression of actin and MM-CPK in S-myotrophin treated myocytes was higher than that of control myocytes. Adult myosin in myocytes increased by the addition of S-myotrophin. The expression of slow myosin decreased after the addition of S-myotrophin, while the expression of fast myosin increased (Fig. 1). Attitude of calcineurin in S-myotrophin stimulated myocyte hypertrophy is now under investigation.

Conclusion

In conclusion, myotrophin promoted the differentiaton of C2C12 cells from myoblasts to myotubes and also promoted the hypertrophy of the myotubes by activating the expression of myoD and myogenin and then by activating the expression of muscle specific proteins. During the hypertrophy of myotubes, the expression of fast type of myosin was prominent.

Pertinent Literatures

1) Toshiya Hayashi *et al.*(1998) *Intern.J.Biochem.Cell Biol.*, **30**, 897-908.
2) Toshiya Hayashi *et al.*(2001) *ibid.*, **33**, 831-838.
3) U.K.Laemmli(1970) *Nature*, **227**, 68-685.

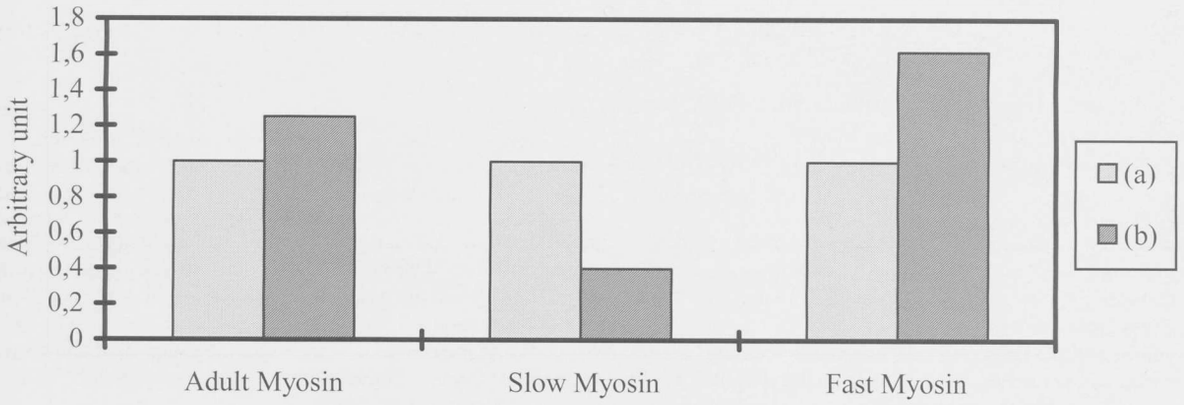

Fig.1. Densitometric analysis of three types of myosins of S-myotrophin treated and control myotubes

Ordinate indicates the intensity of color development of three types of myosin if the intensity of the unit of control sample is 1. (a) Control myotubes; (b) S-myotrophin treated myotubes