Chicken Insulin-Like Growth Factor-I Stimulates Protein Synthesis of Chicken Embryo Myoblasts Cultured in Serum-Free Medium

K. Kita* and J. Okumura
Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

ABSTRACT : The effect of chicken IGF-I on protein synthesis of chicken embryo myoblasts cultured in serum-free medium was examined. When myoblasts were expanded to approximate 20-30% of well, the medium was changed to the serum-free medium including 0, 2, 20, 200 or 2000 ng/ml of recombinant chicken IGF-I. The culture medium including 10% fetal calf serum (FCS) was used as positive control. After 1 day of incubation, protein synthesis was measured by the incorporation of [^{3}H]-L-leucine. Thereafter cells were continued to incubate for further 18 hours, and the radioactivity in the protein was measured as an index of protein synthesis. The values for protein synthesis cultured in the serum-free medium without chicken IGF-I or with 2000 ng/ml of chicken IGF-I were the lowest. Protein synthesis was elevated with increasing chicken IGF-I concentration from 0 to 20 ng/ml. The values for protein synthesis in the 20 ng/ml and 200 ng/ml IGF-I groups were about half of that of the FCS group. The present study revealed that the potency of chicken IGF-I at the levels of 20 to 200 ng/ml to stimulate myoblast protein synthesis was about half of that of 10% FCS. ***(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 1 : 17-20)***

Key Words : IGF-I, Protein Synthesis, Serum-Free Medium, Myoblasts, Chickens

INTRODUCTION

Avian serum as well as mammalian serum is potent to stimulate cell proliferation and for inducing protein synthesis in chicken embryonic fibroblasts cultured *in vitro* (Kita et al., 1996a). In that study, the serum derived from fasted chickens had low potency to stimulate protein synthesis, and the lowered potency was associated with a decrease in blood concentration of insulin-like growth factor-I (IGF-I). The IGF-I found in the chicken has been shown to consist of 70 amino acids (Ballard et al., 1990). After hatching, plasma IGF-I concentration and hepatic gene expression of IGF-I increase rapidly with advancing age, reach a peak before sexual maturity, and then decline gradually (Huybrechts et al., 1985; Johnson et al., 1990; McGuinness and Cogburn, 1990; Kikuchi et al, 1991). Recently it was reported that muscle protein synthesis in the chicken during early stages of post-hatching growth might be partly regulated by changes in plasma IGF-I concentration (Kita et al., 1998b). Myoblast primary culture has been widely used for investigating cell proliferation and differentiation. However, the direct influence of chicken IGF-I on protein synthesis of avian myoblasts has not been clarified so far. Therefore, in the present study, we examined the effect of recombinant chicken IGF-I on protein synthesis by chicken embryo myoblasts cultured in serum-free medium.

* Address reprint request to K. Kita. Laboratory of Grassland Science, University Farm, Graduate School of Bioagricultural Sciences, Nagoya University, Togo, Aichi 470-0151, Japan. Tel: +81-5613-7-0203, Fax: +81-5613-7-0203, E-mail: kitak@agr.nagoya-u.ac.jp.
Received August 2, 2000; Accepted October 27, 2000

MATERIALS AND METHODS

Primary culture of chicken embryo myoblasts

Ten fertilized eggs from single comb White Leghorn hens maintained in our laboratory were incubated for 14 days. At this time 5 embryos were used. The materials and methods for preparation of chicken embryo myoblasts were described previously (Kita et al., 1998a). Myoblasts were seeded on a Type-1 collagen-coated 24-well plate at the low level of cell density (500 cells/cm^2) and incubated in the culture medium. The culture medium was prepared by mixing Dulbecco's modified Eagle's medium (DMEM, Sigma Chemical Company, MO, USA) and Medium 199 (M199, GIBCO LABORATORIES Life Technologies Inc., NY, USA) (DMEM:M199 1:1) including 0.25 mg/ml Fungizone (GIBCO LABORATORIES Life Technologies Inc., NY, USA), and 50 μg/ml gentamycin (Wako Pure Chemical Industries, Ltd., Osaka, Japan). To investigate the influence of chicken IGF-I on myoblast protein synthesis, varying levels of recombinant chicken IGF-I (GroPep Pty. Ltd., Adelaide, SA, Australia) were added to the culture medium. When myoblasts were expanded to approximate 20-30% of well, the medium was changed to DMEM:M199 (1:1) including 0, 2, 20, 200 or 2000 ng/ml of chicken IGF-I. The culture medium including 10% fetal calf serum (FCS) was used as positive control. After 1 day of incubation, protein synthesis was measured by using the method of Ballard (1982) modified by Kita et al. (1996a). The medium was taken out from the well and then 1 ml of fresh medium including L-[^{3}H]leucine (2.11 TBq/mmol, 37 MBq/ml, Amersham LIFE SCIENCE, Ltd., Tokyo, Japan) was added into the well, in which the

radioactivity was 1 μCi/ml. Thereafter cells were continued to incubate for further 18 hours, and then the medium was removed and cells were rinsed with ice-cold Dulbecco's phosphate buffered saline twice. The intracellular free amino acids were removed by washing with ice-cold trichloroacetic acid twice. After rinsing with ice-cold water, 1 ml of 0.5 M NaOH/0.1% Triton X-100 was added and incubated at room temperature for 30 min. After dissolving protein by pipetting, the radioactivity in NaOH/Triton X-100 solution was measured as an index of protein synthesis.

Statistical analysis of data was performed by one-way ANOVA using the General Linear Model Procedures of SAS (SAS/STAT Version 6, SAS Institute, Cary, NC, USA).

RESULTS

Figure 1 illustrates chicken embryo myoblasts

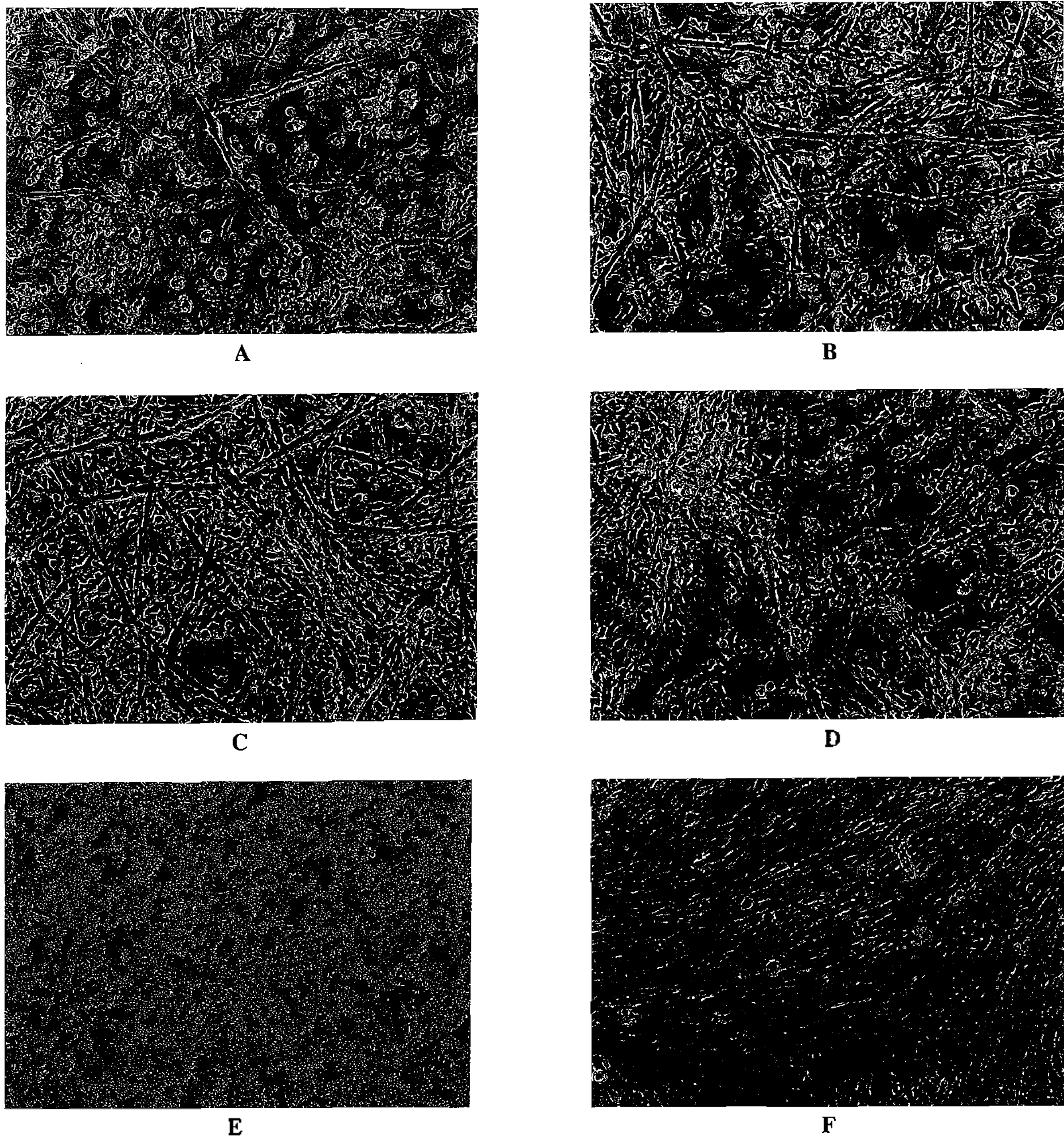

Figure 1. Chicken emryo myoblasts cultured for 2 days in the medium (DMEM:Medium 199 1:1) containing either 10% fetal calf serum (FCS) or varying levels of chicken IGF-I (0, 2, 20, 200 and 2000 ng/ml). (A) Chicken IGF-I 0 ng/ml, (B) Chicken IGF-I 2 ng/ml, (C) Chicken IGF-I 20 ng/ml, (D) Chicken IGF-I 200 ng /ml, (E) Chicken IGF-I 2000 ng/ml, (F) 10% FCS. Magnification was $\times$200.

cultured in the serum-free medium with various concentrations of chicken IGF-I on day 2 of incubation. When myoblasts were cultured in the serum-free medium without chicken IGF-I, growth of myoblasts was not observed. In the serum-free medium including 2 ng/ml of chicken IGF-I, cells were expanded to 60-70% of well and were fused to form multinucleated myotubes. When IGF-I level was increased to 20 ng/ml, cells became confluent and myotube formation was observed. Myoblasts cultured in the serum-free medium including 200 ng/ml of IGF-I were expanded to be confluent similarly to those cultured in 10% FCS. When chicken IGF-I concentration was 2000 ng/ml, all myoblasts died.

Figure 2 shows the effect of various concentrations of chicken IGF-I on protein synthesis by myoblasts cultured in the serum-free medium. The values for protein synthesis cultured in the serum-free medium without chicken IGF-I or with 2000 ng/ml of chicken IGF-I were the lowest. Protein synthesis was elevated as chicken IGF-I concentration increased from 0 to 20 ng/ml. There was no significant difference in protein synthesis between 20 to 200 ng/ml of chicken IGF-I. The values for protein synthesis in the 20 ng/ml and 200 ng/ml IGF-I groups were about half of that of the FCS group.

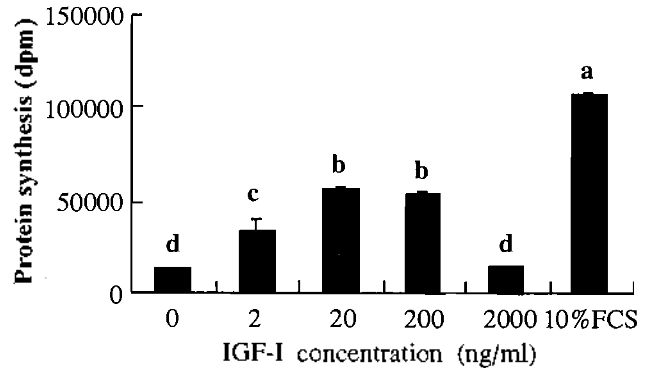

Figure 2. Protein synthesis by chicken embryo myoblasts cultured in the serum-free medium (DMEM:Medium199 1:1) containing varying levels of chicken IGF-I (0, 2, 20, 200, and 2000 ng/ml). The 10% of fetal calf serum (FCS) was added in serum-free medium as positive control. Incorporation of ^{3}H-leucine into protein was measured after 1 day of incubation as an index of protein synthesis. Vertical bars represent means ±SEM. The number of experiments was four. Means not sharing the same letter are significantly different at $p<0.05$ (a, b, c, d).

DISCUSSION

In the present study, the effect of recombinant chicken IGF-I on protein synthesis by chicken embryo myoblasts cultured in the serum-free medium was investigated. The physiological concentration of plasma IGF-I in the chicken is about 20 ng/ml (Kita et al., 1996b). The levels of medium IGF-I concentrations studied in the present study varied from 0 to 2000 ng/ml, and ranged very widely from deficient to excess levels. It has been well recognized that mammalian and avian cells cultured *in vitro* require whole serum obtained from animals. As shown in figure 1, when FCS was withdrawn from the medium, chicken embryo myoblasts ceased to proliferate. But the addition of chicken IGF-I (2-200 ng/ml) into the serum-free medium brought about proliferation and formation of multinucleated myotubes of chicken embryo myoblasts. These results are in good agreement with the results reported by Vandenburgh et al. (1991). In this report, 250 ng/ml of human IGF-I induced rapid cell hyperplasia and myofiber hypertrophy of chicken embryo myoblasts cultured in the serum-free medium. Although chicken IGF-I substitutes eight amino acids compared to human IGF-I, the potency of chicken IGF-I to stimulate myoblast cell growth and proliferation seems to be equal to that of human IGF-I.

Previously we reported that when chicken embryo fibroblasts were cultured *in vitro* with serum derived from fasted chickens, protein synthesis of fibroblasts was decreased compared with the well-fed chicken serum group (Kita et al., 1996a). In this study, the lowered potency for stimulating protein synthesis resulted from the decrease in serum IGF-I concentration. As shown in figure 2, the stimulation of proliferation and myotube formation by chicken IGF-I involved an increase in the rate of protein synthesis, which is consistent with the previous finding reported by McElligott et al. (1988). The present study revealed that the potency of chicken IGF-I at the levels from 20 to 200 ng/ml to stimulate myoblast protein synthesis was about half of that of 10% FCS. However, when chicken IGF-I concentration was 2000 ng/ml, all myoblasts died and the rate of protein synthesis was the lowest and similar to the 0 ng/ml IGF-I group. As the physiological level of plasma IGF-I concentration in the chicken is 20 ng/ml (Kita et al., 1998b), 2000 ng/ml of chicken IGF-I was an excess for the chicken. However, there has been no reports on the negative influence of IGF-I at excess levels and, this issue should be studied further.

REFERENCES

Ballard, F. J. 1982. Regulation of protein accumulation in cultured cells. Biochem. J. 208:275-287.

Ballard, F. J., R. J. Johnson, P. C. Owens, G. L. Francis, F. Z. Upton, J. P. McMurtry and J. C. Wallace. 1990. Chicken insulin-like growth factor-I: amino acid sequence, radioimmunoassay, and plasma levels between

strains and during growth. Gen. Comp. Endocrinol. 79:459-468.

Huybrechts, L. M., D. B. King, T. J. Lauterio, J. Marsh and C. G. Scanes. 1985. Plasma concentration of somatomedin-C in hypophysectomized, dwarf and intact growing domestic fowl as determined by heterologous radioimmunoassay. J. Endocrinol. 104:233-239.

Johnson, R. J., J. P. McMurtry and F. J. Ballard. 1990. Ontogeny and secretory pattern of plasma insulin-like growth factor-I concentrations in meat-type chickens. J. Endocrinol. 124:81-87.

Kikuchi, K., F. C. Buonomo, Y. Kajimoto and P. Rotwein. 1991. Expression of insulin-like growth factor-I during chicken development. Endocrinology 128:1323-1328.

Kita, K., K. Hiramatsu and J. Okumura. 1998a. Influence of chicken embryo extract on protein synthesis of chicken embryo myoblasts depends on cell density. Asian-Aus. J. Anim. Sci. 11:713-717.

Kita, K., T. Shibata, M. Aman Yaman, M. A. Conlon, T. Sasaki, N. Saito and J. Okumura. 1998b. Relationship of insulin-like growth factor-I to muscle protein synthesis infasted chicks. Jpn. Poult. Sci. 35:263-270.

Kita, K., M. Miyazaki and J. Okumura. 1996a. Influence of food-deprived chicken serum on protein synthesis of chicken embryo fibroblasts. Jpn. Poult. Sci. 33:339-346.

Kita, K., F. M. Tomas, P. C. Owens, S. E. Knowles, B. E. Forbes, Z. Upton, R. Hughes and F. J. Ballard. 1996b. Influence of nutrition on hepatic IGF-I mRNA levels and plasma concentrations of IGF-I and IGF-II in meat-type chickens. J. Endocrinol. 149:181-190.

McElligott, M. A., L. -Y. Chaung, V. Baracos and E. A. Gulve. 1988. Prostaglandin production in myotube cultures. Influence on protein turnover. Biochem. J. 253:745-749.

McGuinness, M. C. and L. A. Cogburn. 1990. Measurement of developmental changes in plasma insulin-like growth factor-I levels of broiler chickens by radioreceptor assay and radioimmunoassay. Gen. Comp. Endocrinol. 79:446-458.

Vandenburgh H. H., P. Karlisch, J. Shansky and R. Feldstein. 1991. Insulin and IGF-I induce pronounced hypertrophy of skeletal myofibers in tissue culture. Am. J. Physiol. 260:C475-484.