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- Brief Communication -

Effect of hCG on Connexin 43 mRNA Expression in Goldfish Ovary

Cheol-Young Choi†

Department of Biology, The University of Alabama at Birmingham, Birmingham, AL 35284, USA

This study examined whether the connexin (Cx) is an essential protein during oocyte maturation in the ovary of the goldfish (*Carassius auratus*). In mature female goldfish ovaries, at late vitellogenic stage, human insulin-like growth factor-I (IGF-I; 20 M) and human chorionic gonadotropin(hCG; 20 IU/㎖) were injected. Twelve hr after the injection, mature female goldfish ovaries were removed and stored at -80C until analysis by RT-PCR. From the goldfish Cx43 cDNA sequence (GenBank accession number AB078505), two degenerate primers were designated. *In vivo*, 12 hr after the treatment with hCG, goldfish Cx43 mRNA expression level was increased, while the levels of IGF-I was not changed. Goldfish Cx43 mRNA expressed after, but not before the hCG treatment. These results suggest that Cx43 mRNA was judged to be a gene, which was transcribed during oocyte maturation induced by hCG.

Key words: Human chorionic gonadotropin (hCG), Connexin 43, Goldfish, Maturational competence

In this study, human chorionic gonadotropin (hCG) induced oocyte maturation in ovaries of goldfish. It is known that priming with an injection of hCG influences the induction of oocyte maturation *in vitro* (Degani and Boker, 1992; York *et al.*, 1993; Yoshizaki *et al.*, 1994). Kagawa *et al.* (1998) reported that GTH-II is involved in the final maturation of red seabream oocytes. In the oocytes of Atlantic croaker (Patio and Thomas, 1990) and red seabream (Kagawa *et al.*, 1994), actinomycin D, an inhibitor of protein synthesis, blocked gonadotropin -induced oocyte maturational competence. These reports suggest that the production of new proteins through a mechanism of GTH-stimulated protein synthesis is essential for the development of maturational competence (Patio and Thomas, 1990; Kagawa *et al.*, 1994).

The objective of the study wasto clarify whether human insulin-like growth factor-I (IGF-I) and hCG can induce of maturational competence. The increased production of connexin (Cx) mRNA appeared to be selective because the levels of certain Cx mRNA were increased with gonadotropin induction of maturational competence. The present study was designed to measure changes of Cx43 mRNA levels, in the ovaries of goldfish induced by treatment with hCG, during the induction of maturational competence.

Goldfish (*Carassius auratus*) ranged from 9 to 14 ㎝ in length were purchased, and kept at 17-18℃ in a semi-recirculating tank. In mature female goldfish ovaries, at late vitellogenesis stage, IGF-I (20 ㎛M, Sigma) and human chorionic gonadotropin (hCG; 20 IU/ml, Sigma) were injected. Twelve hrs after the injection, mature female goldfish ovaries were removed and stored at -80℃ until analysis by RT-PCR. For control, physiological saline was injected into mature females, at the time of IGF-I and hCG injection. From the goldfish Cx43 cDNA sequence, two primers were designated as follows: Cx43

†Corresponding author : Cheol-Young Choi, Tel : +1-205-975-4632, Fax : + 1-205-975-6097, E-mail : choi_cy@hotmail.com

primers: [5′-TAG TTC TGG GAA CAG CAG TG-3′] and [5′-AGA TGG TCT TCT CCG TAG GT-3′] as described in a previous study (Choi and Kim, 2003). Approximately 100 mg of ovaries were extracted for total RNA, using a total extraction kit (Amersham). The extracted RNA samples were treated with RNase-free DNase (Promega) according to the supplier's procedures. Three micrograms of total RNA extracted from ovary of goldfish was reverse transcribed with an oligo(dT) primer and M-MLV reverse transcriptase (Gibco/BRL) according to the manufacture's instructions. PCR reactions contained 3.0 $\mu\ell$ of the RT reaction, 200 μM each of dNTPs, 10 μM each of primers, 5U of *Taq* DNA polymerase in 50 $\mu\ell$ of buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl2). Primers for goldfish 18S rRNA was designed as an internal standard based on the GenBank accession number AF047349, which was described in a previous study (Choi *et al.*, 2003). Expression levels of Cx43 mRNA, and on the 18S rRNA for positive control, were carried out using total RNA extracted from ovaries 12 hrs after hCG and IGF-I injection.

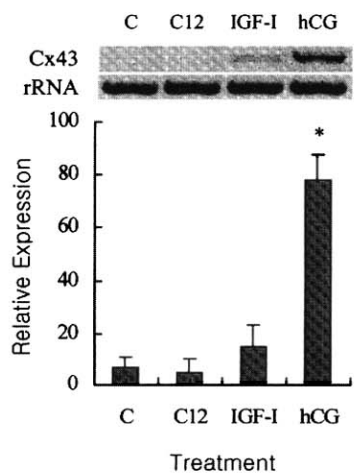

Fig. 1. Effect of human insulin-like growth factor (IGF-I; 20 M) and human chorionic gonadotropin (hCG; 20 IU/ml) on the Cx43 mRNA expression in goldfish (*Carassius auratus*). Three micrograms of total RNA extracted from each sample of hormone-treated ovaries was used as a template for the analysis of Cx43 gene expression by RT-PCR. The expression level of Cx43 mRNA, each samples were standardized to the goldfish 18S ribosomal RNA (rRNA) signal and expressed as a percentage of the control level.An asterisk indicates a significant difference compared with the respective control value (P 0.01). Values are means ± S.E. (N=5). C0, control; C12, ovaries 12 hr after physiological saline injection.

In the present study, *in vivo*, RT-PCR analyses revealed that expression level of Cx43 mRNA was increased 12 hrs after the treatment with hCG. These results suggest that Cx43 mRNA was highly expressed after, but not before, the oocyte maturational competence after treatment with hCG (Fig. 1). Choi and Kim (2003) reported that lower transcription of goldfish Cx43 levels was obtained in immature ovaries, which was found strongly expressed in mature ovaries. However, IGF-I does not affect the level of Cx43 expression (Fig. 1). This agrees with previous reports (Kagawa *et al.*, 1994; Choi and Takashima, 2000) that IGF-I transcription is not involved in the induction of oocyte maturational competence. The findings suggest that goldfish Cx43 mRNA was transcribed during oocyte maturation induced after treatment with hCG.

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