Fish Physiology and Biochemistry **28:** 355–356, 2003.
© 2004 *Kluwer Academic Publishers. Printed in the Netherlands.*

Regulation of steriodogenic enzyme mRNAs in rainbow trout (*Oncorhynchus mykiss*) ovarian follicles *in vitro*

I. Nakamura[1], M. Kusakabe[1] and G. Young[1,2]
[1]*Dept of Zoology, University of Otago, PO Box 56, Dunedin, New Zealand;* [2]*Dept Biological Sciences & Center for Reproductive Biology, University of Idaho, Moscow, ID 83844-3051, USA*

Abstract

The effect of gonadotropins, forskolin and IGF-I on steroidogenic enzyme gene expression and E2 production by the rainbow trout follicle were measured. The results indicate that 3β-HSD gene expression is regulated by gonadotropins partially through the cAMP/PKA mechanism. The effect of IGF-I on P450arom mRNA levels suggests that IGF-I is a major regulator of P450arom gene expression.

Introduction

Pituitary gonadotropins (GTHs) are major regulators of oogenesis through their stimulation of sex steroid synthesis by ovarian follicles. In teleosts, GTHs stimulate production of estradiol-17β (E2) which induces oocyte growth through its control of vitellogenin synthesis in the liver during vitellogenesis. In salmonids, it is known that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) both stimulate steroid production by ovarian follicles. The cAMP/ protein kinase A (PKA) pathway partially mediates the actions of FSH and LH on ovarian steroidogenesis in brook trout follicles (Planas et al. 1997). Insulin-like growth factor I (IGF-I) has also been implicated in regulating ovarian steroid production in mammals and other vertebrates including fish (Maestro et al. 1997; Behl and Pandey 1999; Kagawa et al. 2003). However, the precise mechanisms by which these hormones regulate steroidogenesis, especially regulation of steroidogenic enzyme genes remains unclear. The aim of this study was to determine the effects of FSH, LH and IGF-I on expression of steroidogenic enzyme genes and steroid production in ovarian follicles of rainbow trout. We measured mRNAs encoding P450 aromatase (P450arom), 3β-hydroxysteroid dehydrogenase (3β-HSD) and P450 side-chain cleavage enzyme (P450scc), and E2 production by ovarian follicles *in vitro*.

Materials and methods

Two-year-old female rainbow trout (Wanaka Trout Hatchery, New Zealand) were obtained from breeding stock held in flow-through fresh water under ambient conditions. Pre-vitellogenic follicles or early or late vitellogenic follicles were used for incubations. At each stage, follicles from three females were isolated from ovarian connective tissue and then incubated for 18 h in the rainbow trout ringer in the presence or absence of forskolin (10 μM), salmon FSH, LH or human IGF-I (all at 100 ng/ml). E2 levels in incubation media were measured by RIA. Following total RNA extraction, samples were subjected to Northern blot. Hybridization signals were normalized to those of 18S.

Results

E2 production by follicles at all stages was stimulated by forskolin. FSH and LH stimulated E2 production by vitellogenic follicles. FSH stimulated a significant increase in P450arom mRNA, but only in early vitellogenic follicles. By contrast, IGF-I consistently stimulated 3–7 fold increases in P450arom mRNA in follicles at all stages. 3β-HSD mRNA levels were increased significantly in early vitellogenic follicles incubated with forskolin or with FSH or LH. P450scc

mRNA levels were not consistently affected by any treatment.

Discussion

GTHs and forskolin stimulated E2 production in rainbow trout follicles, as reported previously. The stimulatory effects of GTHs and forskolin on 3β-HSD mRNA levels indicate that gonadotropins regulate 3β-HSD gene expression, partly through a cAMP/PKA mechanism. FSH also appears to regulate transcription of the P450arom gene. The consistent stimulatory effects of IGF-I on P450arom mRNA levels suggest that IGF-I has a role in regulating E2 synthesis.

Acknowledgements

This study was funded by Marsden Fund contract UOO815 and by an Otago Research Committee grant to GY. We thank Dr Penny Swanson for the gift of salmon GTHs.

References

Behl, R. and Pandey, R.S. 1999. Effect of insulin-like growth factor-1 on steroidogenesis in cultured carp ovarian follicles: interactions with estradiol. Indian J. Exp. Biol. 37: 138–142.

Kagawa, H., Gen, K., Okuzawa, K. and Tanaka, H. 2003. Effects of luteinizing hormone and follicle-stimulating hormone and insulin-like growth factor-I on aromatase activity and P450aromatase gene expression in the ovarian follicles of red seabream, Pagrus major. Biol. Reprod. 68: 1562–1568.

Maestro, M.A., Planas, J.V., Moriyama, S., Gutierrez, J., Planas, J. and Swanson, P. 1997. Ovarian receptors for insulin and insulin-like growth factor I (IGF-I) and effects of IGF-I on steroid production by isolated follicular layers of the preovulatory coho salmon ovarian follicle. Gen. Comp. Endocrinol. 106: 189–201.

Planas, J.V., Goetz, F.W. and Swanson, P. 1997. Stimulation of brook trout ovarian steroidogenesis by gonadotropins I and II is mediated by the cyclic adenosine 3′,5′-monophosphate/protein kinase A pathway. Biol. Reprod. 57: 647–654.