Cloning and characterization of rainbow trout inhibin cDNA

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INTRODUCTION

Three activin subforms have been purified from ovarian follicular fluid of porcine resulting from combinations of two β A chains (activin A),1) two β B chains (activin B),2) two β B chains (activin B), or one β A and β B chain (activin AB).3) Two forms of inhibin have been found in follicular fluid. Inhibins are composed of an inhibin α subunit linked to either an activin β A subunit (inhibin A) or an activin β B subunit (inhibin B).4) Activin was originally discovered by its ability to stimulate the secretion of follicle-stimulating hormone (FSH) from cultured anterior pituitary cells.1-3) In contrast, inhibin suppresses pituitary follicle-stimulating hormone (FSH) secretion.5)

Inhibin and activin are also biochemically related proteins thought to play an important role in paracrine regulation of testicular function.5) mRNAs of all three subunits, inhibin α and activin β A and β B, have been demonstrated in the testes of embryonic, prepubertal, and adult rats either through measurements of mRNA levels in testicular homogenates or through in situ hybridization.7-13) The localization and distribution of inhibin and activin in the ovary and testis of mammals have been studied. Previously, we cloned and characterized the cDNA for the β A and β B subunits of rainbow trout activin.15) However, there is no information of the inhibin gene of fish.

In the present study we cloned and sequenced the rainbow trout inhibin cDNA.

RESULTS AND DISCUSSION

The inhibin cDNA consisted of 1,305 bp, which encoded 352 amino acid residues. The DDBJ/EMBL/GenBank accession number of rainbow trout inhibin cDNA is AB044566. The deduced amino acid sequence is aligned with the sequences of other inhibins in Fig. 1. The amino acid sequence identities of the whole region of inhibin among rainbow trout, chicken,6) and mammals17) ranged from 35 to 43 %. The identities of the mature regions of these proteins were higher than the identities of their whole sequences and were 50-60 %.

Recently, several types of activin/inhibin families of mammals and Xenopus laevis have been cloned and characterized18-21) The coding regions of these genes have a significant homology in amino acid and nucleotide sequences. However, there is no significant homology in the 3' non-coding region of activin/inhibin family genes. The 3' non-coding region of rainbow trout activin β A and β B and inhibin also have no significant identity to each other. Based on this result, we used the 3' non-coding region as probes for in situ hybridization for characterization of the cell localization of the α-unit, β A-subunit, and β B-subunit. Distribution of inhibin α and activin β A and β B in different ovarian and testis compartments were studied in rainbow trout by in situ hybridization with complementary RNA probes. In testis tissue, inhibin α and activin β A and β B were expressed only in
the testicular interstitia between the seminal lobules, where Sertoli cells and Leydig cells are distributed. The localizations and intensities of the reactions were constant throughout the maturation of the testis. Within ovarian tissue, the theca cell layers of follicles showed strong reactions of Dig-labeled anti-sense mRNA probes hybridizing against inhibin a and activin βA and βB in all samples over the same sampling period. In regressing oocytes, a positive reaction was observed in the granular cell layer of the follicles.

Fig. 1 Alignment of inhibin a amino acid sequences. The DDBJ/GenBank/EMBL Accession number are: chicken (151215), human (A24248), mouse (48243), and cow (A25732).

REFERENCES