sequences, followed by three amino acids GRR or GKR which seem to be a protease recognition site, as other vertebrate GnRH precursor. RT-PCR analysis showed that this RNA product existed preferentially in adult neural complex. Additionally, two cDNAs, which might play a role in GnRH peptide maturation, were isolated from neural complex cDNA library. One coded a glutaminyl-peptide cyclotransferase and another coded a peptidyl-glycine alpha-amidating monooxygenase. Although expression of these genes in adult neural complex is only indirect evidence, matured and functional GnRH peptides may be produced from Ci-GnRHLP protein by these enzymes. To confirm this possibility, we prepared antiserum against synthetic Ci-GnRH peptide. Now we attempt to analyze expression patterns of Ci-GnRH peptide by immunoblott analysis and histoimmunostaining method.

INHIBITION OF GONAD-STIMULATING SUBSTANCE SECRETION FROM RADIAL NERVES OF STARFISH *ASTERINA PECTINIFERA* BY SALMFAMIDE-1

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In starfish, the peptide hormone gonad-stimulating substance (GSS) secreted from nervous tissue stimulates oocyte maturation to induce 1-methyladenine production by ovarian follicle cells. SALMFamide-1 (S1) is also known to be a neural peptide in starfish. This study examined effect of S1 peptide on GSS secretion from radial nerves of starfish *Asterina pectinifera*. When isolated radial nerves were treated with high potassium seawater (200 mM K^+), GSS secreted was observed in the media. S1 peptide inhibited the GSS secretion from radial nerves. 50% inhibition was obtained by about 0.1 mM S1. On the contrary, another neural peptide, SALMFamide-2 did not inhibit GSS secretion. This suggests that S1 peptide play a role for regulation of GSS secretion during breeding season.

INDUCTION OF OOGENESIS BY INJECTIONS OF SALMON PITUITARY HOMOGENATES IN HYPOPHYSECTOMIZED IMMATURE JAPANESE EEL

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In In teleosts, oogenesis takes place under the control of gonadotropic hormones (GTH). The Japanese eel, *Anguilla japonica*, dose not undergo the gonadal development under aquarium conditions. Therefore, artificial induction of gonadal maturation has been attempted using exogenous hormonal treatments, such as injections of salmon pituitary homogenates (SPH) that contain GTHs. However, it is not known whether in the eel SPH-induced oogenesis proceeds solely because of the stimulation by exogenous GTHs, or if endogenous GTHs are also necessary. In this study, we examined the involvement of endogenous GTHs in artificially matured eel. Intact and hypophysectomized immature eels received weekly SPH injections (40 mg/kg body weight) to induce ovarian development. After 12-20 injections of SPH, both groups of eels reached the migratory nucleus (MN) stage. Histological studies of the eel ovaries at the MN stage revealed no differences between the groups. Moreover, the levels of serum vitellogenin, estradiol-17β and 11-ketotestosterone were similar in the two groups of eels. These results suggested that eel oogenesis during artificial maturation is regulated only by exogenous GTHs.

THE ROLE OF ANDROGEN IN EARLY OOGENESIS IN THE JAPANESE EEL (*ANGUILLA JAPONICA*).

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The most potent androgen identified in teleost is 11-ketotestosterone (11KT). Although 11KT has recently also been found in the blood of several female fish, its role in oocyte development has not been evaluated. To investigate this matter, we examined the role of 11KT in previtellogenic oocyte growth in the Japanese eel. Immature female eels, at the perinucleolus stage, were fed diets containing 11KT or estradiol-17β (E2) for four months. After two months, almost all of the eels entered the oil droplet (OD) stage, with neither E2 nor 11KT showing any effects. However, after four months, both the E2- and the 11KT-treated eels seemed to have larger oocytes than the controls. Moreover, the oocytes of 11KT-treated eels tended to have more ODs than those of the control and the E2-treated fishes. Female eels at the OD stage were then implanted for one month with silastic capsules containing either 11KT or E2. Implantation with 11KT significantly increased the size of the oocytes and the number of ODs, whereas the control and the E2-implanted eels showed no changes. These results suggested that 11KT plays an important role in controlling previtellogenic oocyte growth.

CHANGES IN IMMUNOLOCALIZATION OF STEROIDOGENIC ENZYMES (P450SCC, P450C17, P450AROM) IN GONAD OF THE JAPANESE EEL

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Gonadal development is controlled by sex steroids which are synthesized from cholesterol by P450scc, P450c17 and P450arom. In this study, we produced antibodies for the recombinant P450s, and observed changes in their immunolocalization in the ovary during artificial maturation of the Japanese eel, *Anguilla japonoca*. To characterize the antisera of the above P450s, ovarian homogenate was subjected to immunoblot analysis . Each P450 antiserum specifically recognized a protein approximately 59 kDa in size. In testes and interrenal gland (adrenal cortex), P450scc and P450c17 were immunolocalized in steroidogenic cells, which were found in clusters. In immature ovaries, a few cells which were immunoreactive to P450scc and P450c17 were localized as clusters of steroidogenic cells. With advancing ovarian development, the number of P450scc and P450c17 immunoreactive cells increased. These cells were localized in the outer layer of the ovarian follicle until the late vitellogenic stage. In contrast, P450arom seemed to be consistently localized in the innermost follicle layer. Furthermore, it was suggested that each of the P450 antisera will be efficacious in studies with other teleosts.

SEARCH OF THE COELOMIC FLUID SPECIFIC PROTEINS IN FEMALE FISH

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In gymnovarian type fishes, such as Salmonids and eels, it is known that viscous fluid is secreted from the coelomic wall into the coelom at the time of ovulation. This coelomic fluid (CF) possesses some physiological functions such as maintenance of fertility and activation of sperm mobility. Therefore, this fluid may contain various physiologically active substances, but little is known about them. In the present study, we searched CF specific protein (CFSP) in salmonids. Electrophoretic analyses using SDS-PAGE revealed specific bands in the coelomic fluid from Masu salmon (about 25 KDa), Chum salmon (about 18 and 19 KDa) and Japanese huchen (about 16 and 25 KDa) under reduced conditions. In Western blot analyses, the Masu and Chum salmon bands had an immunoreaction to the rabbit-anti-Masu salmon CFSP-antibody; however the Japanese huchen bands did not. In addition, histological observation of coelomic epithelial cells, regarded as a synthetic site of CFSP, was also performed. Thickness of the epithelial cells increased together with the formation of cilia during sexual maturation.

EFFECTS OF CORTISOL ADMINISTRATION ON SEX DETERMINATION, BODY COLOR AND GROWTH IN FISH.

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It is known that environmental factors, such as temperature and pH, influence sex determination during the larval and juvenile stages in many fishes. For example, masculinization of genetic females results from treatments with high temperatures or low pHs. Thus, extreme environments may act as stressors on sex determination. In addition, it is well known that serum cortisol levels increase under stress conditions. Therefore, in the present study, the effects of cortisol administration on sex determination were examined in goldfish and Japanese flounder. Each fish was fed diets containing various doses of cortisol before the sex determination period. After the treatment, sexing was carried out by histological observation of the gonads. In addition, the ratio of abnormal pigmentation was recorded in Japanese flounder. The proportion of males increased in both species in a dose-dependent fashion. In the Japanese flounder, treatment with a higher dose of cortisol resulted in lower growth, abnormal pigmentation, and incomplete metamorphosis. These results suggested that cortisol influences not only sex determination, but also body color and growth.

BACULOVIRUS-MEDIATED PRODUCTION OF THE RED SEABREAM FSH AND LH IN SILKWORM LARVAE

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Two distinct gonadotropins (GTHs; FSH and LH) have been demonstrated in many teleost species. However, their physiological roles remain unclear because of the absence of purified GTHs. Recent advances in recombinant gene technology have made it possible to produce large quantities of pure protein. In particular, the baculovirus expression system is capable of carrying out post-translational modifications and multi-subunit protein complexes can be synthesized. In this study, we attempted to produce the red seabream GTHs in silkworm larvae using baculovirus. The cDNAs for the coding region of GTH subunits (α and β) were used to prepare recombinant viruses expressing either the individual subunits separately. Western blot analyses revealed that on co-infection with recombinant viruses, the silkworm larvae produced GTHs as a heterodimeric form in hemolymph. The biological activities of recombinant FSH and LH were comparable to those of the natural GTHs in the *in vitro* 11-KT production in sliced testis. These data suggest that the baculovirus expression system results in a facile method for the production of biologically active