cDNA fragments for ERα and β-2 (termed β), from testis by RT-PCR. The nucleotide sequences of isolated cDNAs showed high homology with those for ERα and β of other teleosts, respectively. Genetically sex-controlled male carp were fed diets containing estradiol-17β (0.01-1mg/g-diet) for 4 months. Whole body mRNA levels for the three subtypes of ER were measured by quantitative RT-PCR. The mRNA levels of all subtypes were observed to increase dose-dependently. Especially, ERα mRNA showed most prominent increase, while ERγ was most abundant in control. The findings suggested that ERα was mainly expressed and may play a major role in response to exogenous estrogen.

GLUCOCORTICOID RECEPTOR GENE EXPRESSION IN THE BRAIN OF FROG *RANA NIGROMACULATA*

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Previous studies using amphibians have evidenced that corticosterone, which is a major glucocorticoid biosynthesized in the frog adrenals by the cytochrome P45011β, aldo enzyme catalysis, is involved in the courtship behavior. We have recently demonstrated using RT-PCR and *in situ* hybridization methods that a brain of the frog *Rana nigromaculata* expresses *cytochrome P45011β*, aldo mRNA, suggesting the possibility that the frog brain produces corticosterone. In the present study, we analyzed *glucocorticoid receptor* (*GC-R*) gene expression in the frog brain. Firstly, we isolated the *Rana GC-R* cDNA fragment, which showed 74.6% homology at the nucleic acid level with that of *Xenopus laevis GC-R* cDNA. RT-PCR analysis demonstrated that the *Rana* brain expressed *GC-R* mRNA, of which the expression level was comparable to that in the adrenals, without a clear-cut sex difference. In addition, the *GC-R* gene expression was detected throughout the frog brain, such as the telencephalon, diencephalon, midbrain, and cerebellum. These results suggest that corticosterone biosynthesized in the frog brain may participate in several brain functions in addition to the courtship behavior.

EXPRESSION OF GONADOTROPIN RECEPTORS IN THE SERIAL SEX CHANGING GOBY, *TRIMMA OKINAWAE*

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Molecular cloning of the goby, *Trimma okinawae*, FSH and LH receptors is reported together with their expression throughout their serial sex change. The cDNAs encoding the receptors were isolated from ovaries using RT-PCR and RACE procedures. The LH and FSH receptor cDNAs encoded 691 and 699 amino acids, respectively. Identity between the two deduced amino acid sequences was only 39.4%; however, high similarity was found in the transmembrane region. Expression of the goby gonadotropin receptors was essentially restricted to the testis and ovary. Minimal expression of the LH receptor was seen in the spleen. Transcript abundance of the two receptor genes in ovary and testis of the same individual was measured by quantitative real-time RT-PCR. Expression appears to be related to sexual phase with quick location switching of the two genes after social manipulation to stimulate sex change. This differential expression of the two gonadotropin receptor genes may play a critical role in the sex change.

DYNAMICS OF MESSENGER RNA ENCODING GROWTH FACTORS IN THE ANTERIOR PITUITARY DURING THE RAT ESTROUS CYCLE

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Growth of pituitary secretory cells is regulated by growth factors produced in the pituitary gland. During the estrous cycle in rats, the number of pituitary cell is changed. Mammotroph proliferation and PRL secretion is stimulated by estrogen. Estrogen action on cell proliferation is mediated by growth factors in an autocrine or paracrine manner. Transforming growth factor-α (TGF-α) synthesized in somatotrophs acts as one of estrogen-induced growth factors. TGF-α, a member of epidermal growth factor (EGF), binds to EGF receptors. The aim of the present study was to clarify expression of several growth factors and EGF-R during the estrous cycle. In the present study, we used 3-month-old female Sprague-Dawley rats and the pituitary glands were collected at various stages during the estrous cycle. The mRNA levels of TGF-α, EGF, EGF-R and PRL were semi-quantitatively analyzed by RT-PCR. TGF-α mRNA levels were elevated on diestrus 1. EGF-R mRNA levels were elevated on proestrus. EGF and PRL mRNA levels were not changed.

INTERLEUKIN-18 RECEPTOR α, β GENE EXPRESSION IN THE MURINE ENDOMETRIUM

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Interleukin-18 (IL-18) is a pro-inflammatory cytokine. IL-18 was considered to act mainly in an immune system. The aim of our study was to clarify the function of IL-18 in mammalian reproductive systems. To investigate the IL-18 function, we studied the expression of IL-18 receptor (IL-18R), consisting of a binding chain termed IL-18Rα and a signaling chain termed IL-18Rβ. We detected IL-18Rα chain andβ chain mRNAs in uteri and ovaries of adult mice using RT-PCR analysis. Our previous studies show that IL-18Rα mRNA levels varied during the estrous cycle, and therefore the expression of IL-18Rα gene is thought to be controlled by steroid hormones. To clarify the effect of estradiol-17β and progesterone treatment on IL-18Rα expression, IL-18Rα mRNA levels was semi-quantitatively analysed by RNase protection assay. Estradiol-17β and progesterone treatment both decreased IL-18Rα mRNA levels in ovariectomized mice. These results suggest that mouse uteri are target organs of IL-18 and that female steroid hormones have a suppressive effect on the expression of IL-18Rα mRNA.

EFFECT OF TRANSFORMING GROWTH FACTOR-BETA ON THE PROLIFERATION OF MOUSE UTERINE ENDOTHELIAL CELLS

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The proliferation and differentiation of mouse uterine endometrial epithelial and stromal cells are regulated by sex steroids and growth factors produced within the endometrium. We have shown that the expression of transforming growth factor-beta (TGF-beta) in mouse uterine endometrial cells. We have also shown that estradiol-17beta (E2) treatment increased TGF-beta2 mRNA levels, but did not change TGF-beta1 and beta3 mRNA levels. Progesterone (P4) treatment inhibited E2-induced TGF-beta2 mRNA expression. This study was aimed at clarifying the effect of TGF-betas on the proliferation of uterine endometrial cells. The endometrial epithelial and stromal cells from ICR immature female mice were cultured, and effects of TGF-betas on DNA-replication were studied by measuring the uptake of bromodeoxyuridine (BrdU). TGF-beta1 (1 ng/ml, 24 h) treatment decreased the uptake of BrdU in epithelial cells, did not in stromal cells. These results suggest that TGF-beta1 is one of the mitogenic growth factors for mouse uterine endometrial cells.

CHARACTERIZATION OF VAGINAL STROMAL CELL LINES AND SEARCH FOR INDUCTIVE FACTORS OF EPITHELIAL STRATIFICATION

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Because of complex hormonal and tissue-to-tissue interactions *in vivo*, it is difficult to understand the mechanisms of epithelial stratification and involvement of estrogen in the vagina. We have developed a 3D co-culture system of epithelial and stromal cell lines established from mouse vaginas. In the present study, 3 stromal cell lines (SV-4b4b2, SV-6c4a1b and MV-1e6g1a) were compared in induction of epithelial stratification and responsiveness to estrogen. SV-4b4b2 induced stratification, but had no responsiveness to estrogen in spite of expression of estrogen receptor alpha. SV-6c4a1b induced stratification and responded to estrogen. MV-1e6g1a had responsiveness to the hormone and no induction of stratification. The results suggested heterogeneous cell populations in the vaginal stroma. In co-culture, epithelial cells were not stratified when they were separated from stromal cells by filter. However, separation by collagen gel induced stratification, suggesting that epithelial cells require extracellular matrices for stratification.

MODULATION OF GENE EXPRESSION BY INTERACTIONS BETWEEN EPITHELIAL AND STROMAL CELLS OF OVIDUCTAL CELL LINES NEWLY ESTABLISHED FROM A NEONATAL MOUSE

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We previously reported that epithelial cell lines established from an oviduct of a adult $p53^{-/-}$ mouse were classified into cilial, secretory and undifferentiated cell type. And stromal cell lines were classified into 2 types; One is inducible of *foxj1* expression in cilial epithelial cells, and the other is inducible of *mogp-1* expression in secretory epithelial cells. However, neither *mogp-1* nor *foxj1* expression was detected in undifferentiated epithelial cell lines, OA-2c cells, and co-culture with any stromal cell lines had no induction of these genes in OA-2c cells.In this study, the stromal cell lines established from neonatal oviduct were co-cultured with OA-2c cells. The results of co-culture showed that OA-2c cells were acquired *foxj1* expression after exposure to neonate-derived stromal cells. The acquisition was confirmed by the result that the *foxj1* expression of OA-2c cells was increased by co-culture with adult-derived stromal cells enhancing *foxj1* expression. These results suggest that neonate-derived stromal cells have ability to determine epithelial function and adult-derived stromal cells have ability to enhance the gene expression on determined epithelial cells.