DETECTION OF ANDROGEN RECEPTOR-LIKE PROTEIN IN PLANARIANS

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In vertebrate, sex hormones are synthesised and secreted in overies or testes, and are important in the sexualization. While, it is not enough to research the hormones in invertebrate, especially in platyhelminthes. Sexual individuals of planarians have the sexual organs; overies, testes, yolk glands, copulatory apparatus, and so on. To examine whether sex hormones are present or not in planarian, we tested cross-reaction with anti human androgen receptor antibody. In western blotting analysis using the total protein of a mature *Bdellocephala brunnea*, a single band was detected at about 110 kDa like that of mouse testis. In section immunohistochemical staining on *B. brunnea*, cyanophilic glands and the gland ducts of adhesive organ were especially positive with the receptor anti body, though the sexual organs were not detectable. *Phagocata teshirogii* and *Polycelis sapporo* not having the adhesive organ showed the positive reaction in the anterior and posterior gland cells and the ducts.

ENDOCRINE SYSTEM IN PLANARIAN

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Planarians have a remarkable capacity of regeneration, and switch between asexual and sexual reproduction in natural conditions. They have very simple triblastic systems, but the endocrine system has not been detected in planarian. However, we have reported that 17β-estradiol or bisphenol A have effects on the formation and/or the maturation of female sexual organs. In addition, a gene contains DNA binding domain of steroid/thyroid receptor family has been cloned, and the gene expression is in ovary of sexualized worms. We expect that planarian have an endocrine-like system that is in vertebrate, and some molecules which play some roles in reproduction system in planarian.In this study, we have searched some endocrine related genes of *Dugesia ryukyuensis* with EST data base (10,745 clones) constructed from sexualized worms. We found three clones, Fushi tarazu facter-1 (FTZ-F1) homolog that is belong to steroid/thyroid receptor family, and two cytochrome P-450 homolog which might be related to the steroid synthesis. These results suggest a possibility that planarian worms have an endocrine-like system.

PRESENCE OF MEMBRANE ECDYSONE RECEPTOR IN THE ANTERIOR SILK GLAND OF THE SILKWORM, *BOMBYX MORI*

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Nongenomic action of an insect steroid hormone, 20-hydroxyecdysone (20E), has been implicated in several 20E-dependent developmental events including the programmed cell death (PCD) of *Bombyx mori* anterior silk glands (ASGs), but no information is available for the mode of action. We provide several lines of evidence for a putative membrane receptor (mEcR) located in the plasma membrane of ASGs as an integral membrane protein. The membrane fractions did not contain conventional EcR as revealed by western blot analysis using anti EcR-A antibody. The mEcR exhibited saturable binding for [^{3}H]Ponasterone A (k_d = 17.3 nM, B_{max} = 0.82 pmol/mg). Association and dissociation kinetics revealed that [^{3}H]ponA associated with and dissociated from mEcR within minutes. The present results clearly show that insect tissue membranes contain ecdysone receptors, and that the steroid membrane receptors are commonly present in insects and mammals.

IDENTIFICATION OF THE *CHICKEN GnRH-II* GENE IN THE CHICKEN AND CHARACTERIZATION OF ITS DISTINCTIVE GENOMIC ORGANIZATION AND EXPRESSION PATTERN

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Gonadotropin-releasing hormone (GnRH) plays a pivotal role in the regulation of reproductive functions in vertebrates. Of all the structural variants of GnRH, chicken GnRH-II (cGnRH-II, or GnRH-II) has been found to be universally present in and uniquely conserved among jawed vertebrates without any sequence substitutions. GnRH-II peptide was first identified from the chicken; however, no precursor sequences have been cloned from Aves. Here we report the first identification of the full-length avian GnRH-II precursor cDNA/gene. Its distinctive structure, expression pattern and molecular phylogeny were also determined.

ANALYSIS OF GROWTH HORMONE IN MOUSE PERIPHERAL TISSUES

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Growth hormone (GH) mRNA is expressed not only in pituitary but in various peripheral tissues in mouse. Two isoforms for GH mRNA are detected in these tissues, which transcript differently. Now, immunostaing with the anti-ratGH antiserum was performed on murine stomach, GH like immunoreactive materials exists in the stomach cells.

REGULATION OF EXPRESSION OF GH RECEPTOR mRNA IN THE MOUSE ANTERIOR PITUITARY GLAND

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Growth hormone (GH) is the most important protein anabolic agent in the body and is essential for protein synthesis thoughout the lifespan. The numerous biological actions of GH are mediated and regulated by a membrane-bound GH receptor (GHR) and circulating soluble GH binding protein (GHBP).The mouse GHR and the GHBP are products of a single gene which are generated by alternative splicing. The present study is aimed to investigate the regulation of GHR, GHBP mRNA gene expression in mouse using RT-PCR method. In the anterior pituitary, secretary cell types of GHR and GHBP expressing cells ware detected in somatotrophs and mammotrophs and gonadotrophs by in situ hybridization using GHR cDNA as a probe. This result showed that somatotrophs express GHR, suggesting that GH acts in an autocrine and/or paracrine manner to regulate pituitary functions. Further in this study, we used primary cultured cells obtained from mouse pituitaries and semi-quantitatively analyzed the mRNA levels of GHR, GHBP and GH in the cultured cells treated with rat GH by RT-PCR. GH treatment increased GHR and GHBP mRNA levels. These results suggest that GHR and GHBP mRNA expression is regulated by GH.

LOW-TEMPERATURE-ARREST OF ANURAN METAMORPHOSIS

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The anuran metamorphosis is the phenomenon in which aquatic tadpoles transform to terrestrial frogs, and is controlled by thyroid hormones (THs). The induction of amphibian metamorphosis by exogenous THs exhibits a pronounced low-temperature inhibition. Tadpoles maintained for several months at 5C after treated with THs, showed no response to the THs. To elucidate molecular mechanisms of the temperature sensitive responses in amphibian TH-signaling pathway, we have examined the effects of temperature on 3,3',5-L-triiodothyronine (T_3)inding to the major plasma TH-binding protein transthyretin (TTR)), uptake into erythrocyte, intracellular transport to nucleus, binding to nuclear receptors (TRs), and transcription of TH-responsive genes, in bull frog. Our results indicated that the major molecular event causing the low-temperature arrest of amphibian metamorphosis occurs after T_3 entry into the nucleus but before or during the transcriptional activation of the *tr* genes. TTR binding T_3 in plasma and the cellular TH uptake system on the plasma membrane may only contribute to slow accumulation of T_3 into nucleus by decreasing the amount of T_3 taken up from extracellular fluid at 4C.

SEQUENCE ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF TWO TYPES OF mRNAs ENCODING PAIRED DOMAIN PROTEIN, PAX8, FROM RAINBOW TROUT THYROID GLAND

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Pax8 is a paired domain-containing transcription factor essential for thyroid-specific gene expression and the organogenesis of the thyroid. We have identified two distinct cDNAs encoding Pax8 isoforms (Pax8a and -b) from rainbow trout thyroid gland. Sequence analysis suggested that Pax8a and -b mRNAs can be translated from the same gene, but spliced alternatively. The Pax8a mRNA is predicted to encode the orthologue of the tetrapod Pax8, which conserves three characteristic domains: i.e. the paired domain, octapeptide, and partial homeodomain. On the other hand, the Pax8b mRNA has an insertion in the coding region, which causes a smaller Pax8 variant lacking the carboxy-terminal portion. RT-PCR analysis showed that both Pax8a and -b mRNAs were expressed in the thyroid and kidney, but Northern blot analysis detected only Pax8a mRNA in these organs. In situ hybridization confirmed that the trout Pax8 mRNA is expressed in the thyroid follicular epithelial cells. For functional analysis, we are now examining whether the promoter of rat thyroidal peroxydase (TPO) gene can be transactivated with the Pax8a or -b by luciferase assay.