IDENTIFICATION OF TWO SUBTYPES OF THYROID HORMONE RECEPTOR cDNAs IN THE LEOPARD GECKO, *EUBLEPHARIS MACULARIUS*

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Thyroid hormone plays various important physiological roles in such as development, metabolism and metamorphosis in vertebrates. To gain better understanding of the physiological significance of thyroid hormone as a pleiotropic hormone for various biological functions, reptiles could be very appropriate animal species. Reptilia is the only animal class belonging to both amniotes and ectotherms. As they inhabit widely around the world, they acquire various biological functions to adapt to their diverse environments. In this reason, exploring in such species is necessary. Thyroid hormone receptor (TR), which mediates the effects of thyroid hormone, belongs to nuclear receptor superfamily, and their molecular data are now available in most of vertebrate species except reptiles. In the present study, we cloned and characterized full cDNA sequences of two TR subtypes from the leopard gecko, *Eublepharis macularius*, and analyzed tissue distribution of their mRNA expression. This is the first identification of both TR cDNAs in reptilian species.

PHYSIOLOGICAL ROLE OF INTERLEUKIN-18 IN THE MOUSE PITUITARY

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Interleukin-18 (IL-18) is an inflammatory cytokine, and belongs to a member of an IL-1 family. We had shown that IL-18 and IL-18 receptor α (R α) mRNA levels in the mouse pituitary were expressed from 3 weeks of age. The present study was aimed at clarifying physiological roles of IL-18 in the mouse pituitary. We studied expression of IL-18, IL-18Rα, IL-18β, IL-1β converting enzyme (ICE), IL-12 and interferon (IFN)- γ mRNAs in the adult mouse pituitary by semi-quantitative RT-PCR method. Injection of lipopolysaccharide (LPS), bacterial endotoxin, significantly increased IL-18Rα, IL-18Rβ, IL-12 and IFN-γ mRNAs levels at 6 hr after the injection. These mRNAs levels were returned to basal levels 24 hr after the injection. IL-18 mRNA were not changed by LPS. ICE mRNA levels were significantly increased at 6 and 24 hr after the injection, suggesting the increased production of ICE. ICE is known to produce mature of IL-18. Hence, it is probable that the increase of ICE mRNA levels leads to the production of mature molecular of IL-18. There results suggest that the mouse pituitary have an IL-18 system and pituitary IL-18 may be involved in inflammatory response elicited by LPS.

TPIT BINDING SITE AND NurRE SITE ARE INVOLVED IN RAT POMC GENE EXPRESSION

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Proopiomelanocortin (POMC) is a precursor peptide of adrenocorticotropin (ACTH) and other melanocortin peptides. Differentiation of POMC-expressing corticotrophs depends on the expression of Tpit, a member of T-box transcription factor. CRH-induced POMC expression is mediated by accumulation of intracellular cAMP followed by activation of PKA and expression of nuclear orphan receptor Nur77. The present study was aimed at clarifying the involvement of Tpit binding site and NurRE (Nur response element) site in rat POMC gene expression. Promoter activity of rat POMC gene was studied using firefly luciferase reporter system. 5' flanking region of rat POMC gene was fused to firefly luciferase gene and transfected into AtT-20 corticotrophic cells. Deletion and point mutation of Tpit binding site reduced the promoter activity of POMC gene. Deletion and point mutation of NurRE site abolished CRH, or cAMP-induced POMC promoter activation. These results suggest that Tpit is responsible for basal transcription activity of POMC gene and Nur transcription factors are required for CRH-cAMP signaling pathway for the activation of POMC gene promoter.

EXPRESSION OF ESTROGEN RECEPTOR ISOFORMS IN THE PITUITARY GLANDS OF DIABETIC MICE

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Estrogen stimulates proliferation of various cells including anterior pituitary cells in mice, but failed to increase the mitotic activity of pituitary cells in diabetic mice. The biological actions of estrogen are mostly conveyed through interaction with nuclear estrogen receptor (ER). The two different isoforms of ERs, major isoform ERα and minor isoform ERβ, express with some tissues, such as the pituitary. However, the molecular mechanisms for the control of pituitary responsiveness to estrogen remain unknown. The present study is aimed to investigate the regulation of ERα, ERB mRNA gene expression in mice using semi-quantitative RT-PCR method. Streptozotocin-treatment decreased ER α mRNA, but did not change ER β mRNA in diabetic mice. These results suggest that diabetes-induced ER α mRNA expression decrement reduce pituitary responsiveness.

IDENTIFICATION OF *CIONA INTESTINALIS* CALCITONIN AND ITS RECEPTOR

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Calcitonin is a vertebrate hormone peptide that has a critical role in calcium homeostasis and ion excretion, but no orthlogous peptide has not ever been identified in any invertebrate species. Database-searching on the genome/cDNA database of the ascidian *Ciona intestinalis* led to detection of a cDNA encoding a calcitonin-like sequence (CLSTR ID; 32230). The putative peptide sequence conserved several amino acids essential for functions of vertebrate calcitonins. We cloned this cDNA from the neural complex and designated the peptide as 'Ci-CT'. We also detected and cloned a Ci-CT receptor (Ci-CT-R) candidate (CLSTR ID: 32782) homologous to vertebrate calcitonin receptors and an accessory protein (Ci-RCP; CLSTR ID: 05350) homologous to vertebrate calcitonin receptor component protein, RT-PCR demonstrated that the Ci-CT gene is expressed in the heart and endodtyle as well as neural complex, whereas the expression of Ci-CT-R gene was observed in the Neural complex. Moreover, the HEK293 cells stably transfected with Ci-CT-R and Ci-RCP or Ci-CT-R alone were found to elevate cAMP production upon administration of Ci-CT.

THE COMPREHENSIVE PREDICTION OF PEPTIDE RECEPTOR GENES IN THE *CIONA INTESTINALIS* GENOME

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Neuropeptides and hormone peptides play integral roles in physiological function of vertebrates. Analysis of relationship between peptides and their receptors in ascidians is expected to contribute to our understanding of evolutionary history of vertebrate neuron and endocrine system. The peptide receptors are belonged to G-protein coupled receptor (GPCR) that displays the uniform topology with the seven transmembrane domains. Taking advantage of this characteristic of the receptor, we extracted candidates of Ciona GPCRs using the tmap program that predict the transmembrane segments. We have identified 161 candidates of GPCR. Using sequence similarity among transmenbrane domains of GPCRs, we next predict additional GPCRs. We will extract peptide receptors from GPCRs, and analyze phylogeny of these receptors.

PROLACTIN INHIBITS OSTEOCLASTIC ACTIVITIES IN THE SCALES OF GOLDFISH

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In teleosts, prolactin is a known hypercalcemic hormone that regulates calcium flux in the gill. However, there is little information about its role in bone metabolism. Recently, we developed an in vitro assay system with goldfish scales to measure both the osteoclastic and osteoblastic activities. Using this assay system, we, for the first time, demonstrated that salmon prolactin significantly inhibited the osteoclastic activities. Even 10 pg/ml of prolactin significantly suppressed the scale osteoclastic activities during a 6-hr incubation. A high dose (100 ng/ml) of salmon growth hormone and salmon somatolactin increased the osteoclastic activities during an 18-hr incubation, although a low dose of these hormones (100 pg/ml to 10 ng/ml) did not change them. Therefore, while the action of prolactin in osteoclasts is specific, that of the growth hormone and somatolactin is not. In the case of osteoblastic activities, prolactin, growth hormone, and somatolactin promoted their activities at 10 to 100 ng/ml. Our present data indicates that prolactin regulates osteoclastic and osteoblastic activities and is involved in the teleost bone metabolism.

CHANGES OF MELATONIN CONCENTRATIONS IN THE SCALE OF FEMALE GOLDFISH DURING REPRODUCTIVE PERIOD

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We have previously reported that goldfish scales synthesize melatonin, which has a suppressive function in osteoclasts, and show seasonal variations in the concentration of melatonin with higher levels in the spring. Vitellogenin, an egg yolk precursor molecule, contains appreciable amounts of calcium, and its mobilization takes place predom-