A NOVEL NEUROPEPTIDE, Hym-65, EVOKES RELAXATION OF BODY COLUMN IN HYDRA

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We identified a novel neuropeptide (Hym65) that evoked relaxation of Hydra body column.Hym 65 was originally isolated as a candidate for bioactive peptide during the course of a systematic screening of bioactive peptide in Hydra (the Hydra Peptide Project). However, since its activity was not observed by Differential Display PCR (DD-PCR), it was set aside as non-signaling molecule. In search for peptide genes in EST database, we developed datamining algorithm and found a clone that encodes the Hym65 peptide sequence. This gene encodes signal peptide, Hym65 sequence flanked by an amidation motif (GKK) at the C-teminal side, and another peptide similar to Hym 65. The mass analysis of the native peptide confirmed that the peptide did have an amidated C-terminus as predicted by the precursor sequence. Using in situ hybridization, Hym65 gene was shown to be expressed in a subpopulation of neurons distributed throughout the body. The peptide was shown to evoke relaxation of the body column of epithelial (nerve free) Hydra. This strongly suggests that the peptide is released from neurons and exerts its effect directly on muscle cells.

STUDY ON ENDOCRINE-RELATED MOLECULES IN PLANARIAN

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In primitive organisms as planarian, the endocrine system is still obscure. The existence and function of their steroid/thyroid hormone could not be detected. In many organisms, their steroid/tyroid hormone receptor family contains highly conserved DNA binding domain. Recently, we reported that one gene, which conteins the DNA binding domain of steroid/tyroid hormone receptor family was cloned and it expressed in ovary of sexualized worm. In this study, we have searched some endocrine related genes of *Dugesia ryukyuensis* with EST-Database constructed from sexualized worms. The FTZ-F1(Ad4BP/SF-1) homolog that is concerned with sex determination in vertebrate was found in the detabase and the gene was expressed in ovary of sexualized worm. These data suggest that planarian worms would have an endocrine-like system, which concerned in formation of reproductive organs as vertebrate.

DEGRADATION OF THYROID HORMONE RECEPTOR β BY UBIQUITIN-PROTEASOME SYSTEM IN THE TAILS OF METAMORPHOSING *XENOPUS LAEVIS*

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The degradation of steroid hormone/thyroid hormone (TH) nuclear receptor superfamily proteins by the ubiquitin (Ub)-proteasome system (UPS) has been demonstrated. UPS is the major mechanism for regulation of proteins with short half-lives, such as transcription factors. TH receptor (TR) β is one such protein, which regulates target gene transcription in a ligand-dependent manner. Here, we focused on the degradation of TR β by UPS during amphibian metamorphosis. Aliquots of recombinant GST-fused *Xenopus* TR β protein were incubated in the presence of the tail lysate from stage 57 *Xenopus* tadpoles with or without E1 Ub activating enzyme mixture, which was necessary for initiation of ubiquitination of the substrate proteins. The reaction products were separated by SDS-PAGE and western blotting analysis was performed to detect bands derived from GST-TR β using anti-TR β antibodies. No anti-TR β-positive band was seen in specimens treated with tail lysate, while control specimens showed a band. These results suggested that the tail lysate was capable of degrading ubiquitinated TR β *via* the proteasome.

INDUCTION BY THYROID HORMONE OF A NOVEL ANTIMICROBIAL PEPTIDE GENE IN THE SKIN OF *RANA TAGOI* AND ITS SUPPRESSION BY BISPHENOL A

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Temporins are antimicrobial peptides (AMP) discovered in the skin of frogs of the Ranid genus. We amplified and cloned *Rana tagoi* temporin cDNA by RT-PCR and detected increases in temporin transcript levels in parallel with metamorphosis. To examine the direct effects of thyroid hormone (TH) on temporin gene expression, several adult *R. tagoi* frogs were immersed in 10^{-8} M tri-iodo-thyronine (T_3) for 72 h. At the end of the incubation period, total RNA was extracted from the skin of these animals and subjected to semi-quantitative analysis of temporin gene transcripts by RT-PCR. While T_3 did not show obvious effects on temporin gene expression, another cDNA was amplified by the same RT-PCR. The deduced amino acid sequence of this cDNA encoded a novel hydrophobic short peptide, and the peptide synthesized with this amino acid sequence showed antimicrobial activities against Gram-positive and -negative bacteria. In addition, this TH-inducible novel AMP gene expression was strongly suppressed by administration of the anti-thyroid hormonal endocrine disruptor, Bisphenol A, in a dose-dependent manner.

EFFECTS OF BISPHENOL A ON THE THYROID HORMONE RECEPTOR GENE EXPRESSION IN HUMAN CELL LINES

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Bisphenol A (BPA), a solvent for plastics, is a weak estrogenic endocrine disruptor. In addition, our and other recent studies clearly demonstrated that this compound possesses anti-thyroid hormonal activities through binding to thyroid hormone receptors (TRs). As BPA exerts effects on thyroid hormone (TH)-dependent TR gene transcription, the rates of both spontaneous and TH-inducible amphibian metamorphosis were reduced by addition of BPA. In the present study, we established semi-quantitative RT-PCR systems for measurement of the levels of expression of human TR gene isoforms, TRα1, TRα2, TRβ1, and TRβ2, in human cells in culture. Using these systems, we examined the effects of BPA on TR gene expression in human cell lines HT1080 and HeLa. Our results indicated that while each TR isoform in both cell lines showed different patterns of gene expression after treatment with 10^{-8} M tri-iodo-thyronine (T_3) for 48 h, treatment with 10^{-5} M BPA slightly reduced the levels of each TR isoforms mRNA as compared with controls in both cell lines under the same incubation conditions.

IDENTIFICATION OF TWO FORMS OF BULLFROG CORTICOTROPIN RELEASING FACTOR RECEPTORS

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In non-mammalian vertebrates, CRF has been considered to be the major TSH-releasing factor. In the bullfrog, homologous CRF revealed to have a potent TSH-releasing activity. As a step to study the action of CRF at the receptor level, molecular cloning of bullfrog cDNA encoding CRF receptors was attempted. The specific cDNAs (630 bp) was amplified by reverse-transcribed PCR of a total RNA from the bullfrog hypothalamus. Using the partial cDNAs as probes, two types of CRF receptor full-length cDNAs (type 1: 2275 bp, type 2: 1600 bp) were obtained from a bullfrog hypothalamus cDNA library. Bullfrog CRF receptor type 1 and type 2 amino acid sequences showed 77-94% and 81-92% identity with other vertebrates CRF receptor type 1 and type 2, respectively. To investigate the expression of each CRF receptor mRNA in the bullfrog brain and pituitary, in situ hybridization was performed.

STUDIES OF GHRELIN-INDUCED GROWTH HORMONE AND PROLACTIN RELEASE FROM THE BULLFROG PITUITARY

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We have previously demonstrated that bullfrog ghrelin enhances the in vitro release of both growth hormone (GH) and prolactin (PRL) from the bullfrog pituitary cells in a concentration-dependent manner. Firstly, in this study, we tested frog ghrelin for its GH- and PRL-releasing activities in vivo. Fifteen minutes after a single intraperitoneal injection of frog ghrelin to juvenile frogs, a marked elevation of plasma GH and PRL levels were observed. The levels returned to the initial ones 60 min after injection. This indicates that the peptide released from the stomach into the general circulation may cause the release of GH and PRL by acting on the pituitary. Secondly, we ascertained whether somatostatin (SS) exerts an inhibitory effect upon the release of GH induced by frog ghrelin from the pituitary in vitro. Mammalian SS, which is homologous to frog SS1, did not suppress the frog ghrelin-inducible GH release, whereas SS suppressed the GH release caused by bullfrog GH-releasing peptide (frog GRP). The result suggests that the mode of action of frog ghrelin is different from that of frog GRP.