RADIOIMMUNOASSAY AND IMMUNOHISTOCHEMISTRY USING AN ANTISERUM AGAINST BULLFROG GHRELIN

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Ghrelin is an acylated peptide secreted by the stomach. The bullfrog ghrelin we have previously identified is unique in that its amino acid position 3 is threonine and acylated. In the present study, we established radioimmunoassay for bullfrog ghrelin using an antiserum against the bullfrog ghrelin [13-28], and their plasma and stomach ghrelin concentrations were measured. We also examined localization of ghrelin-immunopositive (ghrelin-ip) cells employing the antiserum and of ghrelin mRNA-expressing (ghrelin-ex) cells using a specific probe for the bullfrog ghrelin gene. Immunoreactive ghrelin levels in the plasma and stomach were approximately 150 fmol/ml, and 130 pmol/g, respectively. Using the RIA combined with reverse-phase HPLC, we found that the major form in the plasma was des-acyl ghrelin, whereas the major form in the stomach extract was acylated ghrelin. Intracellular Ca mobilization assay using rat GHSR-expressing cells revealed that the acylated forms are biologically active. In histochemistry, both ghrelin-ip and ghrelin mRNA-expressing cells were observed in the mucosal layer of the stomach.

NOVEL NEWT HYPOTHALAMIC NEUROPEPTIDES: CLONING OF A cDNA ENCODING THE PRECURSOR POLYPEPTIDE AND IDENTIFICATION AND LOCALIZATION OF ITS TRANSCRIPTS

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We previously identified bullfrog neuropeptide having a C-terminal LPLRFamide sequence that stimulated GH release and therefore was designated frog GH-releasing peptide (fGRP). The fGRP precursor polypeptide encoded fGRP and its related peptides that contained a C-terminal LPXRFamide (X = L or Q) sequence. In view of our interest in amphibian neuroendocrinology, we investigated novel newt LPXRFamide peptides which may possess hypophysiotropic activities. In this study we first cloned a cDNA encoding LPXRFamide peptides from the newt brain. The deduced precursor polypeptide encoded four LPXRFamide peptides. We then isolated these four LPXRFamide peptides by HPLC purification. Mass spectrometric analysis revealed that these peptides were derived from the precursor in the newt brain as endogenous ligands. The localization of precursor mRNA was revealed in the suprachiasmatic nucleus (SCN). Mature peptides were restricted to the SCN and median eminence. Taken together, the newt hypothalamus expresses novel neuropeptides containing the C-terminal LPXRFamide sequences which may be involved in the release of pituitary hormones.

IDENTIFICATION OF NOVEL RFAMIDE PEPTIDES IN THE BRAIN OF BROWN HAGFISH, *PARAMYXINE ATAMI*

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We previously identified novel hypothalamic RFamide peptides having their C-terminal LPXRFamide (X = L or Q) sequences (LPXRFamide peptides) in a variety of vertebrates. These peptides act as novel hypothalamic factors to regulate the release of pituitary hormones. On the other hand, neuropeptide FF and AF with a C-terminal PQRFamide sequence (PQRFamide peptides) have also been identified as pain modulatory peptides. To understand the evolutional origin of these peptides in vertebrates, we sought to identify novel RFamide peptides in the brown hagfish, one of the most ancient vertebrates. Three novel RFamide peptides were identified from the hagfish brain. All of the identified peptides had the C-terminal PQRFamide motif (hagfish PQRFamide peptides). Hagfish PQRFamide peptides showed high homology to PQRFamide peptides and low homology to LPXRFamide peptides. A cDNA encoding hagfish PQRFamide peptides was further cloned. The deduced precursor polypeptide encoded the three identified PQRFamide peptides. The phylogenetic analysis of hagfish precursor polypeptide revealed that the hagfish PQRFamide peptides may be ancestral peptides of the PQRFamide peptide group in vertebrates.

PROCESSING OF MATURE GONADOTROPIN-INHIBITORY HORMONE IN THE QUAIL HYPOTHALAMUS

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We previously isolated a novel dodecapeptide from the quail brain. This quail peptide was shown to be located in the hypothalamo-hypophysial system and to decrease gonadotropin release from the cultured anterior pituitary. We therefore designated this peptide gonadotropin-inhibitory hormone (GnIH). We further characterized the GnIH cDNA from the quail brain and found that the deduced GnIH precursor polypeptide encoded one GnIH and two gene-related peptides (GnIH-RP-1 and GnIH-RP-2). These peptides possessed a LPXRFamide (X=L or Q) motif at their C-termini. Subsequently, GnIH-RP-1 and GnIH-RP-2 were identified as mature endogenous peptides using mass spectrometric analyses. In the present study we found that GnIH and GnIH-RP-2 were processed from their N-terminally extended forms in the quail hypothalamus by biochemical and immunohistochemical analyses. This result suggests that the unique N-terminal processing mechanism may be present in the precursor polypeptide of GnIH in the quail hypothalamus.

MINERALOCORTICOID RECEPTOR GENE EXPRESSION IN THE BRAIN OF THE 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, is involved in the male courtship behavior. We have recently demonstrated that a brain of the frog *Rana nigromaculata* expresses genes encoding cytochrome P45011beta,aldo, which catalyzes production of corticosterone in the amphibian adrenals, and glucocorticoid receptor. On the other hand, mineralocorticoid receptor (MR) is involved in glucocorticoid actions in other vertebrates. In the present study, we analyzed *MR* gene expression in the brain of the frog *R. nigromaculata*. Firstly, we isolated the *Rana MR* cDNA fragment, which showed 80% homology at the nucleic acid level with that of *Xenopus laevis MR* cDNA. RT-PCR analysis demonstrated that the *Rana* brain expressed *MR* mRNA, without a clear-cut sex difference. In addition, the *MR* gene expression was detected throughout the frog brain, such as the telencephalon, diencephalon, midbrain, and cerebellum. These results were similar to those obtained in *cytochrome P45011beta,aldo* and *GR* genes, and may suggest that MR together with GR is involved in corticosterone action in the frog brain.

REGULATORY MECHANISM OF PROLIFERATION OF PROLACTIN-SECRETING CELLS IN THE MOUSE PITUITARY

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Proliferation of mammotrophs (PRL cells) in the mouse pituitary gland is stimulated by transforming growth factor-α (TGF-α) and insulin-like growth factor-I (IGF-I). The present study was aimed at clarifying the signaling pathways of TGF-α- and IGF-I-induced DNA replication of PRL cells. Anterior pituitary cells were cultured in serum-free condition according to our previous report (Oomizu *et al.*, 1998). DNA replication was studied by monitoring bromodeoxyuridine (BrdU) uptake into the nucleus of PRL cells identified immunocytochemically. TGF-α (10 ng/ml) and IGF-I (75 ng/ml) treatment for 24 hr increased BrdU-uptake in PRL cells. PD098059, MAP kinase inhibitor, and LY294002, PI-3 kinase inhibitor, decreased TGF-α- or IGF-I-induced BrdU-uptake in PRL cells. These results suggest that MAP kinase- and PI3-kinase pathways were involved in TGF-α- or IGF-I-induced DNA replication in the mouse pituitary.

MECHANISM OF CILIATED AND SECRETORY CELL DIFFERENTIATION IN MOUSE OVIDUCTAL EPITHELIUM

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The mammalian oviductal epithelium consists of two major cell populations, ciliated cells and secretory cells. The ciliated cells and secretory cells are always present, and changes in relative numbers of the 2 types of cells are associated with the estrous cycle or menstrual cycle. However, the origin and lineage of the 2 types of cells is poorly understood.In the ciliogenesis during development of oviduct, foxj1 has critical role and are regulated by E_2 stimulation. To investigate the role of foxj1 in oviductal epithelium, foxj1 was overexpressed in ciliated and secretory cell lines derived from oviductal epithelium of p53 deficient mouse. Quantitative RT-PCR was performed to determine if foxj1 overexpression altered expression of other gene related the ciliogenesis of oviductal epithelium.Overexpression of foxj1 resulted that no alteration of gene expression was detected in both ciliated and secretory cell lines. On the other hand, overexpression of foxj1 in undifferentiated cell lines derived from neonatal oviductal epithelium down-regulated ER α expression. These results suggest that foxj1 regulated expression of ER α directly.

ESTROGEN ACTION ON PRL CELLS OF ANTERIOR PITUITARY IN IGF-I KO MICE

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E2 stimulates secretion of PRL and proliferation of the PRL-producing cells (PRL cells). In pituitary, E2 also increases IGF-I mRNA and VIP content. IGF-I or VIP stimulates cell proliferation of PRL cells in vitro. On the other hand, in IGF-I knockout (KO) mice, number of PRL cells is reduced and administration of IGF-I increases neither