STRUCTURE AND ACTIVITY OF ANDROGENIC GLAND HORMONE IN *ARMADILLIDIUM VULGARE*

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The androgenic gland hormone (AGH) is known to control sex differentiation in crustaceans. AGH was extracted and purified from androgenic glands of the male isopod *Armadillidium vulgare* by HPLC. Then N-terminal sequence information was obtained. A cDNA encoding AGH has been cloned by PCR and sequenced. AGH was found to be produced as a preprohormone consisting of a signal peptide (21 residues), B chain (44 residues), C peptide (46 residues) and A chain (29 residues). After processing, the A and B chains might form a heterodimer interlinked by disulfide bonds. The A chain possessed a putative N-linked glycosylation site. The prohormone was produced with the baculovirus expression system. The prohormone did not show activity, but lysylendopeptidase digestion yielded an active peptide, which lacked a part of C peptide. Digestion of this active peptide with glycopeptidase F resulted in complete loss of activity. These results indicated that the post-translational processing was essential for AGH activity.

EPIDERMAL GROWTH FACTOR-RECEPTOR (EGF-R) mRNA EXPRESSION IN MOUSE UTERINE STROMAL CELLS

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Several growth factors are produced in mouse uterine stromal cells, and their expression is regulated by ovarian sex steroidal hormones. This study was designed to determine whether EGF-R, epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) were produced in the mouse stromal cells. The effect of sex steroidal hormones on EGF-R, EGF, TGF-a mRNA expression was also studied. Total RNA was extracted from the stromal cells cultured in serum-free condition. Northern blot analysis of EGF-R mRNA using mouse EGF-R cDNA probe demonstrated EGF-R transcripts of 8.4 kb, 6.1 kb, 2.8 kb in the stromal cells. The 6.1 kb- and 2.8 kb-transcripts correspond to the full-length form and the truncated form of EGF-Rs, respectively. The 6.1 kb EGF-R mRNA was increased by treatment of 17β-estradiol (E_2, 10^{-9} M) for 24 hr, and the 2.8 kb EGF-R mRNA was decreased by treatment of E_2 and progesterone (P, 10^{-7} M) for 24 hr.EGF and TGF-α mRNA were also detected by Northern blot analysis using mouse EGF, TGF-α cDNA probe. E_2+P or P treatment for 24 hr increased TGF-α mRNA expression. E_2 or P tended to increase the EGF mRNA expression. These studies show that EGF, TGF-α and EGF-R are produced in the mouse uterine stromal cells,their expression is regulated by E_2 and P cooperatively.

MECHANISM OF MOLTING BY LOCALIZATION AND EXPRESSION OF THYROID HORMONE RECEPTORS

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Short day and low temperature terminate breeding activity and induce molting in Japanese quail. With these treatments, circulating levels of testosterone decrease and thyroid hormones increase. Many studies in literature indicate thyroid hormones control molting. However, actual mechanism of molting and a role of the thyroid hormone still remain unsolved.

To clarify the function of thyroid hormones, we studied a localization of thyroid hormone receptors in feather follicle of primaries immunohistochemically using an antibody to thyroid hormone receptor.

Results showed that the thyroid hormone receptor did not exist in the inner and outer root sheath of the feather before molting. The receptor appeared in the inner and outer root sheath at the base of the follicle during molting. The receptor disappeared when the new feather grew and collected melanin granules. These observations suggest that the thyroid hormones have a direct effect on loss of an old feather and emergence of a new feather in feather follicle.

EXTRAPITUITARY PROLACTIN IN THE GOLDFISH, FROG AND MOUSE: ITS ANCIENT ORIGIN AND DISTINCT EVOLUTION

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Various extrapituitary tissues in mammals are known to produce the pituitary hormone, prolactin. We examined the presence of prolactin transcripts in the organs of goldfish, African clawed frog and mouse by southern analysis of RT-PCR products and competitive RT-PCR. In the frog, the transcript was detected in some organs, in the following order of abundance: pituitary >> brain > testis > ovary. In the goldfish, unexpectedly, the transcript was detected in the ovary, testis, liver, kidney, spleen, gill, muscle and brain in slightly lower abundance than in the pituitary. In the mouse, the copy number of the transcript per μg of total RNA was estimated at ~10^{10} in the male pituitary, ~10^4 in the placenta, hypothalamus and testis, ~10^3 in the thymus and experimentally-induced deciduoma, and ~10^2 in the ovary. We also examined the expression of two PRL genes in the goldfish organs. These results suggest that the origin of extrapituitary prolactin goes back to the common ancestor of fishes and tetrapods, but that distinct evolution has occurred in each lineage.

N-TERMINAL HORMONOGENIC SITES OF BULLFROG THYROGLOBULIN

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Thyroglobulin, prothyroid hormone, is a major glycoprotein (660 kDa) of the thyroid gland and is composed of two identical subunits (330kDa). Thyroid hormones, thyroxine and triiodothyronine, are formed by iodination of tyrosyl residues and coupling of iodotyrosyls to form iodothyronines in the thyroglobulin molecule. Several hormonogenic sites of thyroglobulin have been clarified in mammalian species, but these sites have not yet been confirmed in amphibians. For this study we tried to isolate thyroxine-containing peptides by monitoring at 325nm, after reduction and alkylation of purified bullfrog (*Rana catesbeiana*) thyroglobulin and tryptic digest of a low molecular weight fragment. Purified thyroglobulin was first obtained by size-exclusion (Sephacryl S-300 HR) and ion-exchange (DEAE Sepharose FF) chromatography. A thyroglobulin fragment (ca.8kDa) derived from reduced and alkylated thyroglobulin was isolated by Sephacryl S-100 HR column chromatography. A partial amino acid sequence of the N-terminus of this fragment was the same sequence as the amino acid sequence surrounding a putative thyroxine generating site (Tyrosine-5) which we had already reported. Moreover, one of the tryptic peptides from this low moleacular fragment contained a partial amino acid sequence closely relating to another hormonogenic site (Tyrosine-130) which we had found in a lysyl endopeptidase digested peptide of the purified bullfrog thyroglobulin.

TRANSFORMNIG GROWTH FACTOR-α (TGF-α) STIMULATED THE DIFFERENTIATION OF MAMMOTROPHS IN THE RAT PITUITARY TUMOR GH_3 CELL.

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The rat pituitary tumor GH_3 cell secretes growth hormone (GH) and prolactin (PRL), and is suitable for the analysis of differentiation of mammotroph. A combination treatment of epidermal growth factor (EGF, 10 nM), insulin (300 nM) and 17β-estradiol (E_2, 1 nM) stimulated the differentiation of mammotroph in GH_3 cells. Our recent study showed that EGF was required for the differentiation of mammotrophs in GH_3 cells. TGF-α, which is known to bind EGF receptors, was detected in GH_3 cells by Western blot analysis. TGF-α mRNA was also detected by Northern blot analysis, indicating that GH_3 cells synthsized TGF-α. A combination treatment of EGF, insulin and E_2 increased the percentage of TGF-α-expressing cells. These observation suggested that endogenous TGF-α stimulated the differentiation of mammotroph in GH_3 cells in an autocrine or paracrine manner.