GHRELIN-INDUCED GH RELEASE FROM ISOLATED RAT ANTERIOR PITUITARY CELLS DEPENDS ON INTRA- AND EXTRA-CELLULAR Ca^{2+}

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Using an isolated cell perifusion system, we examined whether ghrelin-induced GH release from rat pituitary cells depends on intra- and extra-cellular Ca^{2+}. We found that reductions in the extracellular Ca^{2+} concentration resulted in decreases in ghrelin-stimulated GH release in a dose-dependent manner, and pretreatment with thapsigargin, an endoplasmic in reticulum Ca^{2+} ATPase inhibitor, reduced GH release by about 60 %. Next, we measured the ghrelin-stimulated GH concentration following treatment with nifedipine, an L-type channel inhibitor, and following replacement of extracellular Na^{+} in the medium with an impermeable molecule, and we found that the amounts of GH release were reduced by 44 % and 47 %, respectively. In this study, we demonstrated that the GH-stimulatory effect of ghrelin is achieved through both intra- and extra-cellular Ca^{2+} sources and that ghrelin-induced extracellular Ca^{2+} influx is involved in L-type Ca^{2+} channel and Na^{+} influx.

TEMPERATURE-DEPENDENT SEX DIFFERENTIATION IN *HYNOBIUS RETARDATUS*: EXPRESSION PATTERN OF P450-AROMATASE

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Sex determination in amphibians is believed to be controlled genetically as well as epigenetically or environmentally. When larvae of the salamander *Hynobius retardatus* were reared at defined temperatures from hatching to metamorphic stages, a high temperature induced exclusively female gonads (ovaries), whereas intermediate or lower temperatures produced a 1:1 sex ratio of the morphological gonads. The thermosensitive period was determined to be restricted from 15 to 30 days after hatching, just before or when sexual differentiation occurred. *Hynobius* P450 aromatase cDNA was isolated from adult gonads. In the normally developing larvae, strong expression of the aromatase was detected in the female gonads, but not in male ones, showing a typical sexual dimorphism. When larvae were reared at the female-producing temperature, strong expression was detected in all the temperature-treated larvae. Our results confirm the importance of aromatase regulation in female versus male differentiation of gonads and demonstrate that an up-regulation of aromatase expression is involved in the process of temperature-sensitive sex reversal in this species.

NEUROENDOCRINE REGULATION OF HAIR DEVELOPMENT IN THE VENTRAL HIND WING OF *PRECIS COENIA*

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The buckeye butterfly, *Precis coenia*, shows seasonal color polyphenism in the pigmentation of its ventral hind wing. Animals reared under long-day conditions (LD) develop a pale tan pigmentation (*linea* form), whereas animals reared under short-day conditions (SD) develop a dark reddish-brown pigmentation (*rosa* form). Seasonal form development is controlled by ecdysteroids during 28 and 48 h following pupation. In this study, we attempted to clarify the difference between both forms except for its pigmentation. By counting the number of hairs in six cells on the wing, it was found that the number of hairs differed among both forms. Therefore, we investigated the regulation mechanism pertaining to hair development. The number of hairs in SD-adults was much greater than that in LD-adults in each cell. When LD-pupae 4 h following pupation were chilled, the number of hairs increased several fold compared to the control. When decerebration or injection of brain extracts was performed on SD-pupae 4 h following pupation, the number of hairs decreased. These results suggested that a hormonal factor might play an important role in the hair development process at the early SD-pupal stage.

THE MODE OF ESTROGEN ACTION ON THE GROWTH OF PURKINJE CELLS

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The Purkinje cell, a typical cerebellar neuron, is a major site for neurosteroid formation in the brain. We have reported recently that the rat Purkinje cell activity produces estradiol de novo from cholesterol during neonatal life, when cerebellar neuronal circuit formation occurs dramatically. We have further demonstrated that estradiol promotes Purkinje dendritic growth, spinogenesis, and synaptogenesis via its receptor. On the other hand, there are some reports showing that neurotrophin-3 (NT-3) is involved in the development of Purkinje cells during neonatal life. To reveal the mode of estrogen actions on Purkinje dendritic growth, spinogenesis, and synaptogenesis, we therefore examined the effect of estradiol on the expression of NT-3 in the cerebellum during rat neonatal life. Estradiol administration increased the expression of NT-3 in the cerebellum. In contrast, tamoxifen, an estrogen receptor antagonist, suppressed the expression of NT-3 in the cerebellum. These results suggest that estrogen actions on dendritic growth, spinogenesis, and synaptogenesis of the developing Purkinje cell are mediated by NT-3.

POSSIBLE INVOLVEMENT OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) IN THE INNERVATION OF DOPAMINERGIC NEURONS INTO THE RAT PARS INTERMEDIA

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Synthesis and release of α-MSH in the pars intermedia (PI)are negatively regulated by dopaminergic neurons (DN) from the periventricular nucleus in the hypothalamus. However, the innervation mechanism of the DN into the PI remains unsolved. BDNF, one of the neurotrophins, has been identified as a factor for the survival of DN. We investigated whether BDNF is involved in the innervation of DN into the PI. We first observed the innervation process of DN by immunohistochemistry using an antibody against tyrosine hydroxylase (TH), a key enzyme in dopamine synthesis. Also, we examined the spatial and temporal expressions of BDNF mRNA during the development of the PI. TH-positive neurons were observed in the connective tissue between the PI and the pars nervosa in fetal rats aged 19.5 to 21.5 days. In 1.5-day-old neonatal rats, the neurons were distributed throughout the PI. On the other hand, BDNF mRNA was first detected in the melanotrophs of 18.5-day-old fetus, with an increase of its expression in 20.5-day-old fetus. Thereafter, no positive staining of BDNF mRNA was observed.

PHOTOPERIODIC REGULATION OF THE TERMINATION OF ADULT REPRODUCTIVE DIAPAUSE IN THE ASIAN COMMA BUTTERFLY, *POLYGONIA C-AUREUM* L.

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The Asian comma butterfly, *Polygonia c-aureum*, has seasonal (summer and autumn) morphs. They count the number of long (or short) days from the 2nd to 5th instar (or to the middle of pupal stage) for determining whether they develop into suumer or autumn morphs (whether they undergo adult reproductive diapause). Autumn morph development is not always associated with undergoing adult reproductive diapause in this butterfly.

CHARACTERIZATION OF *ATF-4* GENE DURING SEX DIFFERENTIATION IN *XENOPUS LAEVIS* EMBRYOS

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We had an attempt for investigating environmenrtal factors on which adults of P. c-aureum determine to maintain or to terminate adult reproductive diapause. Autumn morph adults obtained under 10L-14D (short-day conditions) at 20℃ were subjected to long-day and short-day conditions at 20℃ and each 10 female adults were dissected at 5- to 10-day intervals to observe whether eggs were produced in their ovaries.

ISOLATION AND CHARACTERIZATION OF GENES DURING SEX DIFFERENTIATION IN *XENOPUS* EMBRYOS

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The results indicated that autumn morph adults of P. c-aureum count the number of long-day (or short-day) after adult emergence to determine whether they should terminate adult reproductive diapause.