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SEX REVERSAL IN THE MEDAKA TREATED *IN VITRO* 17α-METHYLDIHYDROTESTOSTERONE DURING OOCYTE MATURATION

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To examine the effect of androgen on sex determination, intrafollicular immature oocytes of the S-rR strain(X^rX^r, X^rY^R) medaka, *Oryzias latipes*, were isolated before breakdown of the germinal vesicle and incubated in the presence of a non-aromatizable 17α-methyldihydrotestosterone(MDHT) for about 10 hr during their maturational period. At the end of incubation, mature oocytes were rinsed and then artificially inseminated in regular saline. The fertilized eggs were then allowed to develop in tap water, and the fry were reared on a regular powdered diet until sexual maturation. By subsequent examination of sex, sex reversal of genotypic females to phenotypic males was found in a manner dependent on the dose of MDHT. In the solvent control group in which intrafollicular oocytes were matured in medium containing no exogenous sex steroids, no sex reversal was observed. These results suggest the existence in the oocyte of a sex determinant sensitive to androgen.

GENE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN THE RAT PITUITARY DURING FORMATION OF THE VASCULAR SYSTEM IN THE HYPOTHALAMIC-PITUITARY AXIS

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The present study using immunohistochemistry and FITC-conjugated to gelatin injection method showed a close relationship between hormone-producing cells and microvessels in the hypothalamic-pituitary axis. The in situ RT-PCR method demonstrated expression of vascular endothelial growth factor (VEGF) mRNA-expressing cells in the pars tuberalis (PT) and in the pars distalis (PD) at E 15.5 and in the later stages. The primary capillaries grew well along the developing PT. On the other hand, portal vessels penetrated into the PD at E15.5 and subsequently expanded into the entire lobe to form the secondary capillary plexus. These results suggest that VEGF is involved in the vascularization of the PD, but not in the formation of portal vessels, because the latter begins at E13.5 which is before the appearance of VEGF in the rostral region of the PT. Some VEGF-positive cells were also stained with anti-ACTH, suggesting that some ACTH cells have the potential to produce VEGF. In conclusion, the present study suggests that VEGF is involved in the development of the primary capillaries and in the vascularization of the PD, but not in the development of portal vessels.

HORMONAL CONTROL OF SEASONAL MORPH DEVELOPMENT IN THE NYMPHALID BUTTERFLY, *ARASCHNIA BUREJANA*

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The nymphalid butterfly, *Araschnia burejana* Bermer, displays seasonal color polyphenism, in the pigmentation of its dorsal wing. Larvae reared under long-day conditions (LD) develop into summer-morph butterflies with black and white pigmentation, whereas larvae reared under short-day conditions (SD) become diapause pupae and emerge as spring-morph butterflies with red. Seasonal morph development in this butterfly is controlled by the secretion timing of 20-hydroxyecdysone after pupation. In this study, we attempted to clarify the presence and secretion timing of summer-morph-producing hormone (SMPH) as a factor controlling seasonal-morph development except for 20-hydroxyecdysone. Therefore, we investigated the regulation mechanism secreting SMPH to seasonal-morph development using the experiments of the brain extirpations and injection of 20-hydroxyecdysone, and of the neck ligation and injection of 20-hydroxyecdysone, respectively. When LD-pupae were removed brain and injected 20-hydroxyecdysone within 4hr after pupation, they developed into spring-morph butterflies. This result suggested that a SMPH of *A. burejana* might be secreted into hemolymph at early LD-pupal stage.

IMPACT OF *bek* SIGNALING IN DEVELOPMENT OF FEMALE REPRODUCTIVE TRACT

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Stratified epithelium of the vagina becomes thick, squamous and cornified in response to estrogen. Exposure of mice to estradiol (E2) or FGF7 during perinatal periods leads vagina to possess abnormal epithelium that is permanently and estrogen-independently hyperplasic and cornified. Treatments of neonatal mouse vagina with neutralizing antibodies against FGF7 and FGFR2IIIb in combination with E2 *in vitro* resulted in absence of development of the stratified flattened epithelium which is observed in the vagina treated with E2 alone. In addition, neutralization of FGF2IIIc, another splicing variant of *bek* gene, attenuated the E2 effects. Interestingly, the vagina exposed to FGF2, a ligand of FGF2IIIc, did not possess squamous epithelium but papillary epithelium with winding outline of the lumen. Furthermore, treatment of neonatal mouse vagina with a caspase inhibitor caused development of papillary epithelial structure, suggesting prevention of programmed cell death mediates the FGF2 effect in developing vagina. Thus, FGFs and *bek* signaling are largely involved in morphogenesis of the female reproductive tracts, and their imbalance may cause abnormalities in reproduction.

SEX DIFFERENCE IN THE EXPRESSION OF Ad4BP/SF-1 IN THE QUAIL BRAIN

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Ad4BP/SF-1 was originally identified as the steroidogenic tissue-specific transcription factor that regulates the steroidogenic cytochrome P450 genes. Ad4BP/SF-1 was detected in peripheral steroidogenic glands. Based on the sexually dimorphic expression of Ad4BP/SF-1 in fetal gonads, Ad4BP/SF-1 is considered to be involved in gonadal sex differentiation. Because the avian brain actively produces neurosteroids around hatch, Ad4BP/SF-1 may regulate the formation of neurosteroids. In this study, we therefore investigated the expression of Ad4BP/SF-1 mRNA in the quail brain. Ad4BP/SF-1 mRNA was highly expressed in the diencephalon unlike other brain regions. The expression of Ad4BP/SF-1 mRNA in the diencephalon around hatch was higher than that in the adult. Around hatch, there was a clear sex difference in the Ad4BP/SF-1 mRNA expression. Newly hatched males showed a higher expression of Ad4BP/SF-1 mRNA in the diencephalon compared with newly hatched females. Because the diencephalon expresses cytochrome P450 genes, Ad4BP/SF-1 may induce sex difference in the formation of neurosteroids aroud hatch which may be involved in sexually dimorphic characters of the avian diencephalon.

SPATIAL AND TEMPORAL CHANGES OF FACTORS INVOLVED IN RATHKE'S POUCH FORMATION IN THE CHICKEN EMBRYO

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With the aim of elucidating mechanisms involved in the early stage of pituitary organogenesis, we studied by using chicken embryos the spatial and temporal expression patterns of some factors involved in the formation of the adenohypophyseal primordium and Rathke's pouch.In the chicken embryo, sonic hedgehog (shh) mRNA was initially expressed in the posterior region from the invaginated part of the oral ectoderm, and another shh-expressing region appeared in the most rostral part of the recess on embryonic day 3.0 (E3.0).LIM homeobox protein 3 (Lhx3) mRNA expression first appeared in a large anterior area of the invaginated part of the oral ectoderm and then the expression region shifted caudally from a more rostral position.At E4.0, α-GSU mRNA-expressing cells were observed either end of the Lhx3-expressing region, and these expression areas also subsequently shifted caudally and then fused together to make the cephalic region of the pituitary gland.Considering the expression patterns of these factors in the early chicken embryo, the presumptive adenohypophyseal primordium may migrate from the rostral region toward the invaginated area of the oral ectoderm.

THE DIFFERENTIATION OF THE PARS INTERMEDIA IN *XENOPUS LAEVIS* WITHOUT PARTICIPATION OF THE HYPOTHALAMUS

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At present most textbooks describe the indispensable role of the hypothalamus in the development of the pars intermedia of the pituitary.I presented the data, however, that the pars intermedia arose in about 40% of hypothalectomized *Rana japonica* larvae (Gen. Comp. Endocrinol. 119: 37-42, 2000). Such contradictory data prompted me to investigate whether the differentiating ability of the pars intermedia differs in different amphibian species. In the present study, hypothalectomy of *Xenopus laevis* embryos was performed. The results of microsurgery, however, was disappointing since the hypothalamus of operated *Xenopus* larvae was regenerated in most cases (110/116). In the course of this experiment, many congenitally dark colored tadpoles were obtained from an adult female toad of an inbred strain. These congenitally hyperpigmented larvae as well as surgically hypothalectomized ones possessed the pars intermedia in spite of the absence of the posterior hypothalamus. Hyperpigmentation of these abnormal larvae may be due to an augmented secretion of αMSH from the pars intermedia as already suggested in *Rana japonica*.

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