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Endocrinology

POSSIBLE INVOLVEMENT OF ARGININE VASOTOCIN REPRODUCTIVE EVENTS OF THE MALE NEWT, CYNOPS **PYRRHOGASTER**

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In the breeding season, the male red-bellied newts, Cynops pyrrhogaster, vibrates his tail in front of the female. Following this behavior, the male parades in front of the female and deposits spermatophores. In the sexually mature male newts, injection of arginine vasotocin (AVT) induced the deposition of spermatophores. AVT enhanced both incidence and frequency of courtship behavior in the androgen-primed castrated newts. Administration of a V1 receptor antagonist but not of a V2 receptor antagonist suppressed courtship behavior as well as spermatophore deposition in sexually developed intact newts. AVT induced spermatophore deposition independently of the courtship. AVT also caused the decrease of content of a female attracting pheromone (sodefrin) in the abdominal gland, suggesting that the neurohypophyseal hormone induces the release of sodefrin. It was concluded that endogenous AVT is involved in the induction of courtship behavior and of deposition of spermatophore through V1 (vasopressor) receptor and that AVT may stimulate the discharge of sodefrin from the cloacal orifice.

EFFECT OF ACTIVIN.INHIBIN AND FOLLISTATIN ON THE RELEASE OF GONADOTROPIC HORMONE FROM THE BULLFROG PITUITARY.

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On the basis of the observation that gonadotrophs in the bullfrog pituitary gland contain activin/inhibin B_Bimmunoreactive substances, attempt was mede to study the effects of activin B and inhibin B on the release of LH and FSH from the enzymatically dispersed anterior pituitary cells. Activin B enhanced the release of both LH and FSH concentration-dependently. Inhibin B did not show a noticeable effect on the basal amount of gonadotropins released in this system. Inhibin, however, nurified the effect of activin. Similarly, follistatin also blocked the activin-induced enhancement of LH and FSH release. Involvement of activin B and/or inhibin B in the release of gonadotropins in an autocrine fashon was suggested.

EFFECTS OF ENVIRONMENTAL FACTORS ON THE GONADOTROPIN-RELEASING HOR-MONE NEURONAL SYSTEM IN THE HAMSTER.

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Dep. of Biol. Sci., Fac. of Sci., Toyama Univ., Toyama and ¹Exp. Animal Center, Miyazaki Med. College, Miyazaki. It is well known that in seasonal breeders like the hamster, gonadal function

depends on the photoperiod. The present study examined the effects of different photoperiods and ambient temperatures on the gonadotropin-releasing hormone (GnRH) neuronal system in the male hamster, Tscherskia triton. Hamsters were kept from birth under long photoperiods (LP: 16h light and 8h dark) and at 25 $^{\circ}$ until the experiment began. Animals were kept for 30 days under various environmental conditions. After autopsy, immmunohistochemical and quantitative RT-PCR analyses were performed in the hamster forebrain. Exposure of hamsters to short photoperiods (SP: 8h light and 16h dark) at 25 °C induced a functional decline in the GnRH neuronal system (increased number and stainability in neuronal perikarya). LP with cold ambient temperatuers (5-8 ℃) did not influence the GnRH neuronal system. In contrast, SP with cold ambient temperatures enhanced the SP-induced functional decline in GnRH neurons. No significant differences in the expression of the GnRH gene, however, were detected among the groups. These findings suggest that the functional decline of the GnRH neuronal sytem triggered by SP or SP and cold temperatures is due to the decreased secretory activity of GnRH neurons and not to the decreased synthesis of GnRH.

ROLE OF IMMUNE CELLS ON PROLIFERATION OF PORCINE GRANULOSA CELLS IN CULTURE K. Yasuda, M. Hiroshima and M. Inagaki

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In the process of follicular development, granulosa cells proliferate actively. We carried out our experiment on the assumption that the role of FSH on granulosa cell proliferation may be mediated by immune cells. Mononuclear cells (lymphocytes + monocytes) were isolated from porcine blood, and cultured for 3 days with FSH(100ng/ml) in DME supplemented 10% FCS. After 3 days, the conditioned medium (CM) was collected. Porcine granulosa cells were harvested from small(1-2mm) follicles. The cells were suspended in DME supplemented with 10% FCS and inoculated at low density (5X104 cells/35mm dish). One day after, the medium was replaced with 1% FCS DME. The cells were then cultured for 6 days with CM. At the end of culture, cell numbers were counted. The conditioned medium of mononuclear cells prominently enhanced granulosa cell proliferation. On the contrary, FSH failed to enhance the granulosa cell proliferation directly in our cell culture. The immune cells may act as mediators between FSH and granulosa cells.

Characterization and developmental changes of ecdysone 20monooxygenase during embryogenesis in the Silkworm, Bombyx mori.

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The amount of 20-hydroxyecdysone increase sharply as embryogenesis proceeded,but remained at a low level in diapause eggs. We investigated whether the ecdysone 20-monooxygenase participates in the developmental changes in the amount of 20-hydroxyecdysone in the diapause eggs and non-diapause eggs.

By using differential centrifugation and marker enzymes, most 20monooxygenase was found to be associated with microsomes. The cytocrome P450 inhibitor, metyrapone and imidazole compound (KK-42), and the NADPH-cytocrome P-450 reductase antibodies inhibited the ecdysone 20-monooxygenase activity very effectively, thus confirming the microsomal cytocrome P-450 nature of this enzyme. The specific activity of the enzyme increased sharply on parallel with the amount of 20-hydroxyecdysone in non-diapause eggs, but little or no activity of the enzyme was detected in diapause eggs. The activity observed in nondiapause eggs was affected by the treatment of actinomycin D and α amanitin. These results suggest that ecdysone 20-monooxygenase, which is synthesized de novo, may function as a rate-limitting enzyme in the developmental changes of 20-hydroxyecdysone.

IMMUNOELECTRON MICROSCOPIC STUDIES IN THE PROLACTIN AND GROWTH HORMONE CELLS OF GUPPY, POECILIA RETICULATA, ALONG WITH THE CHANGE OF ENVIRONMENTAL SALINITY T.Oda¹, S.Tanaka¹, M.Nozaki² and Y.Oota¹. Dept. of Biology, Fac. of Science, Shizuoka Univ., Shizuoka ²Sado Marine Biological Station, Niigata Univ., Sado

It is a well established fact that the prolactin (PRL) and growth hormone (GH) play an important role in osmoregulation of teleosts. Although they are members of the same hormone family, antagonistic actions are detectable in the osmoregulatory mechanism. In this study, histological and ultrastructural morphometric analysis using immunocytochemical techniques were carried out on the PRL and GH cells of guppy, Poecilia reticulata. The gupples were divided into three groups, with one group adapted to fresh water (FW), another to 50% seawater (50%SW) and the third group adapted to 100% seawater (SW) for 30 days respectively. The PRL cells in the FW adapted fish have a well developed rough endoplasmic reticulum, Golgi apparatus, and numerous secretory granules, compared with those in the 50%SW and SW adapted fish. However, there was no significant morphological difference in the GH cells among the three groups. These results suggest that PRL plays a main role in osmoregulation in the FW adapted guppy.