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Immunohistochemical study of bullfrog gonadotropin in the course of sexual maturation.

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We immunohistochemically studied gonadotropin productibility in young and subadult frogs of Rana catesbeiana. Immunostaining was performed by the PAP method using monoclonal antibodies to bullfrog LHβ, FSHβ, and α-subunit. In this study, frogs which metamorphosed this year were termed 1-year, and 1- to 3-year male and female frogs were used as materials. Regardless of age,sex or season, the rates for each type of immunostained cell to all anterior pituitary cells were in the following order: α-subunit > FSHβ >> LHβ. In the course of sexual maturation, each type of subunit-producing cell increased in 2- and 3-year male and female frogs. Regardless of age or sex, no change was detected in the immunoreactive cells of each type during autumn.

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ANNUAL CHANGES OF PLASMA PROLACTIN LEVELS IN THE NEWT, CYNOPS PYRRHOGASTER

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The homologous radioimmunoassay (RIA) for newt prolactin (PRL) was applied to the determination of plasma PRL levels in newts captured in every month of the year. In the adult newts of both sexes, plasma PRL levels were relatively low after the breeding season in early spring and during summer and early autumn (5-70 ng/ml). In the male, the levels rose markedly in March (205.4 ± 49.4 ng/ml), while in the female, the levels were elevated moderately in February (95.5 ± 10.9 ng/ml) and November (107.8 ± 8.5 ng/ml). PRL in the male is presumed to be necessary for the development of the cloacal glands, tail fin, Mauthner's neuron, and courtship behavior. In the female, PRL may induce oviducal development. Immunohistochemical study revealed that pituitary PRL cells in the spring newt were stained weakly, while those in the summer animal were stained rather strongly. In the PRL cells of spring newt, but not of the summer animal, well-developed Golgi apparatus was observed, suggesting that the secretory activity is higher in spring than in summer.

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ESTABLISHMENT AND APPLICATION OF TIME-RESOLVED FLUOROIMMUNOASSAY (TR-FIA) FOR THE MEASUREMENT OF NEWT GONADOTROPIN (GTH)

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Plasma- and pituitary gonadotropin of newts was measured for the first time via cross-reaction with a TR-FIA system for bullfrog LH. TR-FIA is a newly developed, non-isotopic immunoassay, in which europium, a lanthanide element, is used as a label. The present assay system is based on the "sandwich-binding" principle; monoclonal anti-bullfrog LHß (BL4B11) immobilized in microtiter wells is used as a capturing antibody, and europium-labeled polyclonal anti-bullfrog LH as a detecting antibody. Dilution curves of newt pituitary homogenates and high GTH sera obtained from mammalian GnRH-treated newts were parallel to the standard curve below 4 ng/ml of bullfrog LH. To determine the electric nature of newt GTH measurable by this assay system, pituitary homogenate of male newts collected in July was subjected to an isoelectric focusing analysis. Nine components with pI's ranging from 9.8 to 5.2 were observed by TR-FIA. All of them were also detectable by RRA using *Xenopus* testicular homogenates as the receptor preparation and ^{125}I rat FSH as the radioligand. The RRA is thought to be specific for LH-like GTH. Pooled plasma of adult newts collected monthly throughout one year was assayed for GTH by TR-FIA. Significant increases were observed in June (0.39 ng/ml) for males and in May (0.79 ng/ml) for females, which correspond to the period of spermatogenetic recrudescence and ovulation, respectively, showing the usefulness of this assay system for the further investigation on reproduction of newts.

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HOMOLOGOUS RADIOIMMUNOASSAY OF BULLFROG JOINING PEPTIDE

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A double antibody RIA for bullfrog (Rana catesbeiana) joining peptide (fJP), which belongs to POMC-related peptides, was established. The antiserum was produced by immunizing a rabbit with synthetic fJP conjucated with BSA. Synthetic fJP was used for radioiodination and as a reference standard. The intra- and inter-assay coefficient of variations were 3.7% and 4.8%, respectively. Sensitivity of the assay was 0.054 ± 0.021 ng/ml. Several dilutions of plasma as well as homogenates of anterior (PD) and neurointermediate lobes (PIN) of bullfrog and R. ornativentris yielded dose response curves which pararelled to the standard curve. Plasma from totally hyopophysectmized bullfrogs contained no measurable JP, but from distalobectmized bullfrogs contained twice as much as those of intact ones. Plasma and homogenates of PD and PIN of Bufo japonicus and Xenopus laevis gave inhibition curves which did not pararell the standard. Purified frog NPP and several human POMC-related peptides did not react in this assay. Both PD and PIN cultured separately released JP considerably, the amounts of JP from PD being 1/2 of those from PIN. Co-cultured PD and PIN released JP more than the sum of JP from PD and PIN cultured separately.