Turbinidae)

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Gonadal development, gametogenesis, reproductive cycle, gonad index, flesh weight rate, and first sexual maturity of the turban shell, *Lunella coronata coreensis* were investigated by histological observation. The materials used were collected monthly from the rocky intertidal zone of Daehang-ri, Buan-gun, Jeollabuk-do, on the west coast of Korea, from July 1998 to June 1999. Sexes of *L. coronata coreensis* were separate. The gonad was widely located in the spirals of the visceral mass buried in the digestive gland. The ovary and testis were composed of a number of oogenic lobules and spermatogenic tubules, respectively. Monthly variations in the gonad index increased from March (23.86±3.73) when the water temperature increased and reached the maximun in July (49.76±6.47). And then, the gonad index sharply decreased in September (15.58±2.33). The flesh weight rate ranged from 25.2% to 32.1%, and its variation showed a similar pattern to the gonad index. Individuals of 5.9 ㎜ and less in shell height could not take part in reproduction in both sexes. Percentages of first sexual maturity of female and male specimens ranging from 7.0~7.9 ㎜ in shell heights were 84.6% and 91.7%, respectively, and 100% in those over 8.0 ㎜ in shell height in both sexes took part in reproduction. By studying the monthly changes of the morphological features and sizes of germ cells during gametogenesis in the gonad, the reproductive cycle of this species could be devided into five successive stages: early active (December to April), late active (January to July), ripe (May to August), spawning (July to September), and recovery (September to March). The spawning period of this species was once a year between July and September, and the main spawning occurred in July when the seawater temperature reached above 24.8℃. The fully ripe eggs were 150~160 ㎛ in diameter.

D106

Multiple Signaling Pathways of Bullfrog GnRH Receptors

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It has been known that the agonist-bound gonadotropin-releasing hormone receptor (GnRHR) engages several distinct signaling pathways including activation of phosholipase C (PLC), followed by the activation of protein kinase C (PKC), and elevation of intracellular Ca^{2+} levels. However, it has been questioned whether the GnRHR activaton involves cAMP-mediated PKA activation, extracellular signal-related kinase (ERK), and Jun N-terminal kinase (JNK) activation. In the present study we demonstrated bullfrog (bf) GnRHR-mediated multiple signaling cascades, indicating that at least both PKA and PKC pathways play equally important roles in GnRHR-mediated signaling. The activation of bfGnRHRs triggered both inositol phosphate production and cAMP response element (CRE)-mediated luciferase activity in a dose-dependent manner. Treatment with GF109203X, a PKC inhibitor, U73122, a PLC inhibitor, or EGTA an extracellular calcium chelator could partially inhibit the GnRHR-mediated CRE activity, indicating the potential cross-talk between PKA and PKC pathway. Furthermore, we examined the involvement of ERK or JNK signaling by cotransfection of GAL4 response element driving luciferase construct (GAL-Luc) with GAL4-Jun or GAL4-Fos construct. Taken together, this study demonstrates that

bfGnRHRs can trigger multiple signaling cascades, suggesting the diverse roles of bfGnRHR in physiological conditions.

D107

Alternative Splice Variants of Bullfrog type III Gonadotropin-Releasing Hormone Receptor Inhibit the Wild Type Signal Transduction.

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Recently we characterized three types of GnRH receptor from bullfrog (bfGnRHR-1, bfGnRHR-2, and bfGnRHR-3). In the present study, we provide evidence that five different mRNA species were generated from the primary bfGnRHR-3 transcript by exon skipping (sv1), retention of intron (sv2 and sv3), and/or transcriptional slippage (sv4), apart from the constitutively spliced form (wt). PCR analysis of bullfrog genomic DNA revealed that the bfGnRHR-3 gene consisted of three exons separated by two introns. Immunoblotting demonstrated the presence of 48 kDa (wt), 27 kDa (sv1), and 34 kDa (sv2-4) bfGnRHR-3 proteins in transfected HeLa cells. GnRH-induced signal transduction was obtained in HeLa cells transfected with wt cDNA, but not with sv1-4 cDNAs. Co-transfection of wt with sv2-4, but not with sv1, cDNAs decreased the GnRH-induced, wt receptor-mediated signaling. Using. GFP constructs, a membrane-associated localization of the wt protein was observed. However, the sv1 protein was exclusively retained in the cytosol, whereas the sv2-4 proteins showed both membrane-associated and cytoplasmic localization. The expression levels of the wt versus sv2-4 transcripts significantly increased from hibernation to prebreeding season. Collectively, these results suggest that a regulated alternative splicing mechanism plays a role in the fine-tuning of GnRHR function in amphibians.

D108

Cloning and Identification of Three Distinct Types of Gonadotropin-Releasing Hormone Receptor in Rana Dybowskii.

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Recently, we have identified three distinct types of gonadotropin-releasing hormone (GnRH) receptor in the bullfrog and a variant of mammalian type GnRH, [Trp8] GnRHin *R. dybowskii*. In the present study, we isolated three complementary DNA (cDNA) clones, encoding three corresponding types of GnRHR (designated dyGnRHR-1, dyGnRHR-2 and dyGnRHR-3), from *R. dybowskii*, and examined structure-function relationships involved in ligand binding specificity. The sequence analysis revealed high homology of dyGnRHRs to the bullfrog GnRHRs of 96%. Northern blot analysis revealed a differential expression of each receptor in the pituitary (type I) and brain (type II and III), as well in liver (type III). Expression of each receptor in kidney and testis was also examined with RT-PCR. Transfection studies revealed that dyGnRHR-2 and -3 exhibited differential sensitivity to various ligands tested (cGnRH-II, mGnRH, [Trp8]GnRH), with a pharmacological profile resembled that of bfGnRHR. Interestingly, dyGnRHR-1 showed about 10 times lower sensitivity to GnRHs than that of bfGnRHR-1. The data obtained here indicates the presence of three types of the GnRHR in the amphibian species and important roles of these receptors in amphibian reproduction and behavior.