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

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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.

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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.