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Preferential Ligand Selectivity of the Monkey Type-II Gonadotropin-Releasing Hormone (GnRH) Receptor for GnRH-2 and Its Analogs

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Gonadotropin-releasing hormone (GnRH) regulates the reproductive system through the cognate GnRH receptor (GnRHR) in vertebrates. In this study, we cloned a cDNA encoding the full-length open reading frame sequence for green monkey type-II GnRHR (gmGnRHR-2) from the genomic DNA of CV-1 cells. Transient transfection study showed that gmGnRHR-2 was able to induce both c-fos promoter- and cAMP responsive element-driven transcriptional activities, indicating that gmGnRHR-2 couples to both Gs- and Gq/11-linked signaling pathways. gmGnRHR-2 responded better to GnRH-2 ([His5, Trp7, Tyr8]GnRH) than GnRH-1 ([Tyr5, Leu7, Arg8]GnRH). Substitutions of His5, Trp7, and/or Tyr8in GnRH-1 increased the potency to activate gmGnRHR-2, suggesting that individual His5, Trp7, and Tyr8 in GnRH-2 contributed to differential ligand sensitivity of gmGnRHR-2. Substitution of D-Ala for Gly6 in GnRH-2 increased the potency to activate the receptor, suggesting that GnRH-2 has a constrained conformation when it binds to the receptor. GnRH-induced gmGnRHR-2 activation was specifically inhibited by GnRH-2 antagonists, Trptorelix-1 and -2, but not by a GnRH-1 antagonist, Cetrorelix. In conclusion, gmGnRHR-2 revealed preferential ligand selectivity for GnRH-2 and its analogs, suggesting that gmGnRHR-2 has a functional activity that is different from mammalian type-I GnRHRs but similar to nonmammalian GnRHRs.

E106

Mutations of the Ser-Glu-Pro Motif in the Extracellular Loop3 of the Rat Gonadotropin-Releasing Hormone (GnRH) Receptor Reverse Ligand Selectivity for Mammalian GnRH and Chicken GnRH-II

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A Glu/Asp7.32residue in the extracellular loop 3 (ECL3) of the mammalian gonadotropin-releasing hormone receptor (GnRHR) is known to interact with Arg8 of mammalian GnRH (mGnRH), which may confer preferential ligand selectivity for mGnRH than for chicken GnRH-II (cGnRH-II). However, some nonmammalian GnRHRs also have the Glu/Asp residue at the same position, yet respond better to cGnRH-II than mGnRH. Amino acids neighboring Glu/Asp7.32are differentially arranged such that mammalian and nonmammalian GnRHRs have an S-E/D-P motif and P-X-S/Y motif, respectively. We presumed the position of Ser7.31 or Pro7.33 of rat GnRHR as a potential determinant for ligand selectivity. Either placing Pro prior to Glu7.32 or placing Ser after Glu7.32 significantly decreased the sensitivity and/or efficacy for mGnRH, but slightly increased that for cGnRH-II in several mutant receptors. Among them, those with a PEV, PES, or SES motif exhibited a marked decrease in sensitivity for mGnRH such that cGnRH-II had a higher potency than mGnRH, showing a reversed preferential ligand selectivity. Chimeric mGnRHs in which positions 5, 7, and/or 8 were replaced by those of cGnRH-II revealed a greater ability to activate these mutant receptors than mGnRH, while they were less potent to activate wild-type rat GnRHR than mGnRH. Interestingly, a mutant bullfrog type-I receptor with the SEP motif exhibited an increased sensitivity for cGnRH-II but a decreased sensitivity for mGnRH. These results indicate that the position of Pro and Ser near Glu7.32 in ECL3 is critical for the differential ligand selectivity between mammalian and nonmammalian GnRHRs.

E107

Identification of Frog Vasotocin and Mesotocin Receptors with Differential Pharmacological Activities and Tissue Distribution

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The neurohypophysial nonapeptides, mesotocin (MT) and vasotocin (VT) are the amphibian counterparts of oxytocin (OT) and arginine vasopressin (AVP). Here, we report the cloning and functional characterization of the receptors for mesotocin (MTR) and vasotocin (VTR) in two species of frog, *Rana catesbeiana* and *Rana esculenta*. The frog MTR and VTR cDNAs encode proteins of 384- and 419-amino acids, respectively. The frog MTR exhibits a high degree of sequence identity with the mammalian OT receptor (OTR) while the frog VTR possesses a strong homology to the mammalian AVP-1a receptor (V1aR). Activation of the MTR induced c-fos promoter-driven transcriptional activity but failed to induce cAMP-responsive element (CRE)-driven transcriptional activity, while activation of the VTR triggered both c-fos promoter- and CRE-driven transcriptional activities, suggesting differential G protein coupling between the MTR and VTR. The MTR exhibited the highest sensitivity for MT followed by OT>VT>AVP, whereas the VTR showed preferential ligand sensitivity for VT>OT>MT>AVP, respectively. A V1a agonist but not V2 and OT agonists substantially activated both MTR and VTR with a similar sensitivity. V1a, V2, and OT antagonists inhibited MT-induced MTR activation but not VT-induced VTR activation. In the frog brain, MTR and VTR mRNAs were found to be widely expressed in the telencephalon, diencephalon and mesencephalon, and exhibited very similar regional distribution. In the pituitary, MTR andVTR were expressed in the distal and intermediate lobes but were virtually absent in the neural lobe.

E108

Comparison of Phenotypic Differences and Signaling Pathways in Stromal Cells Isolated from Abrasion and Ablation Wound Cornea.

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In normal fibroblasts from uninjured stroma, collagenase-1 is only induced by treatment of IL-1. This induction is strongly inhibited by MEK or p38 MAP kinase inhibitors. In contrast, repair fibroblasts constitutively synthesized IL-1 as well as collagenase-1. However, IL-1 receptor antagonist partially blocked the constitutive collagenase-1 synthesis, suggesting other regulatory factors/cytokines might be involved in constitutive expression of collagenase-1 in activated fibroblasts besides IL-1. Interestingly, a PD98059 or SB203580 treatment did not inhibit synthesis of collagenase-1. However, treatment of both MAP kinase inhibitors was required for inhibition of collagenase synthesis. It is therefore likely that the balance between the activity of ERK and p38 MAPK pathways is crucial in the regulation of collagenase-1 expression in wound fibroblasts, and suggesting MAPK pathways are a target for selective inhibition of degradation of collagenous ECM in chronic corneal scar formation. PMA known as ERK activators induces collagenase-1 expression in NF due to cell shape change possibly via reorganization of the cytoskeleton. In contrast, WF expresses collagenase-1 without cell shape changes upon PMA treatment. These results suggesting that collagenase-1 expression is depend on unique combinations of signaling pathways that are cell type-specific genetic programs.