H3 Relaxin Is a Specific Ligand for LGR7 and Activates the Receptor by Interacting with Both the Ectodomain and the Exoloop 2*

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Leucine-rich repeat-containing, G protein-coupled receptors (LGRs) represent a unique subgroup of G protein-coupled receptors with a large ectodomain. Recent studies demonstrated that relaxin activates two orphan LGRs, LGR7 and LGR8, whereas INS3/Leydig insulin-like peptide specifically activates LGR8. Human relaxin 3 (H3 relaxin) was recently discovered as a novel ligand for relaxin receptors. Here, we demonstrate that H3 relaxin activates LGR7 but not LGR8. Taking advantage of the overlapping specificity of these three ligands for the two related LGRs, chimeric receptors were generated to elucidate the mechanism of ligand activation of LGR7. Chimeric receptor LGR7/8 with the ectodomain from LGR7 but the transmembrane region from LGR8 maintains responsiveness to relaxin but was less responsive to H3 relaxin based on ligand stimulation of cAMP production. The decreased ligand signaling was accompanied by decreases in the ability of H3 relaxin to compete for 33P-relaxin binding to the chimeric receptor. However, replacement of the exoloop 2, but not exoloop 1 or 3, of LGR7 to the chimeric LGR7/8 restored ligand binding and receptor-mediated cAMP production. These results suggested that activation of LGR7 by H3 relaxin involves specific binding of the ligand to both the ectodomain and the exoloop 2, thus providing a model with which to understand the molecular basis of ligand signaling for this unique subgroup of G protein-coupled receptors.

Relaxin and Leydig insulin-like peptide/relaxin-like factor (INS3) are peptide hormones with a two-chain structure similar to that of insulin (1, 2). Relaxin is important for the function of reproductive tissues, heart, kidney, and brain (3), whereas INS3 is essential for testis descent (4, 5). We have recently demonstrated that two orphan leucine-rich repeat-containing, G protein-coupled receptors (LGRs) with homology to gonadotropin and thyrotropin receptors, are capable of mediating the action of relaxin through a cAMP-dependent pathway (6). These two receptors, LGR7 and LGR8, share 50% sequence identity to each other, and contain a unique low density lipoprotein receptor-like cysteine-rich motif at the amino terminus. However, LGR7 and LGR8 do not have the consensus hinge region found in gonadotropin and thyrotropin receptors. In contrast to relaxin, INS3 activates LGR8 but not LGR7, interactions between INS3 and LGR8 were demonstrated by ligand-receptor cross-linking (7).

In addition to the two known human relaxin genes, H1 (8) and H2 (9), another related gene, designated H3 relaxin (H3), was identified recently. A synthetic peptide with a design based on this gene was found to possess relaxin activity in bioassays using the human monocyte cell line, THP-1 (10). Here, we demonstrate that H3 relaxin activates recombinant LGR7 but not LGR8. Taking advantage of the structural similarity of LGR7 and LGR8, and the differential specificity of relaxin-related peptides to these receptors, we designed chimeric LGR7/LGR8 receptors to identify the domains in the receptor that are important for their ligand specificity. We demonstrate that both the ectodomain and exoloop 2 of LGR7 are important for ligand receptor binding and signaling.

MATERIALS AND METHODS

Hormones and Reagents—Porcine relaxin was purchased from the National Hormone and Peptide Program (Torrance, CA). Recombinant human H2 relaxin was a gift from Dr. Elaine Unemori (Connectics Co., Palo Alto, CA). H3 relaxin and human INS3 were chemically synthesized and characterized as described previously (10, 11). Anti-FLAG M1 monoclonal antibody and the FLAG peptide were purchased from Sigma Chemical Co. (St. Louis, MO). The soluble ectodomain of LGR7 was prepared as described previously (6). Briefly, cDNA for the ectodomain of human LGR7, named as 7BP, was fused in-frame with the prolactin signal peptide and the FLAG epitope at the 5'-end, the ectodomain was connected to the single transmembrane junctional amino acid sequences for different chimeric receptors are

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The abbreviations used are: INS3, insulin-like peptide 3/Leydig insulin-like factor; LGR, leucine-rich repeat-containing, G protein-coupled receptor; PBS, phosphate-buffered saline; 7BP, ectodomain of LGR7.

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Table I
Junctional amino acid sequences for different chimeric receptors

| Receptor         | Amino Acid Sequence          |
|------------------|------------------------------|
| LGR7/8(EL3)      | SSLEN/LGR7 401/LGR8 412/LLANN |
| LGR7/8(EL2)      | SSFED/LGR7 413/LGR8 402/LLASI |
| LGR7/8(EL1)      | GIFDF/LGR7 467/LGR8 470/KFREG |
| LGR7/8(EL1)      | PVFVV/LGR8 657/LGR7 648/KPDSL |
| LGR7/8(EL2)      | VIPEW/LGR8 558/LGR7 549/KNEFF |
| LGR7/8(EL3)      | EIPGT/LGR8 590/LGR7 580/LGIFL |

* Numbers behind LGR7 or LGR8 denote the amino acid position for each receptor.
* The junction (LGR7 401) of the ectodomain and transmembrane region for these receptors is the same as chimeric receptor LGR7/8.

Table II
Cell surface expression of LGR7, LGR8, and different chimeric receptors

| Receptor         | cpm/10⁶ cells |
|------------------|--------------|
| Vector           | 2,261 ± 463  |
| LGR7             | 16,015 ± 271 |
| LGR8             | 16,314 ± 576 |
| LGR7/8           | 21,694 ± 1,458 |
| LGR7/7           | 17,242 ± 228 |
| LGR7/8(EL1)      | 18,660 ± 952 |
| LGR7/8(EL2)      | 16,961 ± 973 |
| LGR7/8(EL3)      | 19,427 ± 359 |

Fig. 1. LGR7 is activated by porcine relaxin and H2 relaxin as well as H3 relaxin, whereas LGR8 is activated by porcine relaxin, H2 relaxin, and INS3. A, ligand-stimulated cAMP production mediated by LGR7. B, ligand-mediated cAMP production mediated by LGR8. Purified porcine relaxin, recombinant H2 relaxin, synthetic H3 relaxin, and synthetic INS3 were used.

Listed in Table I, Polymerase chain reaction was performed using Vent DNA polymerase (New England BioLabs, Inc., Beverly, MA) in accordance with the manufacturer’s instructions. All cDNAs were subcloned into the expression vector pcDNA3.1/Zeo (Invitrogen Co.). To allow efficient targeting of receptors to the cell surface, a lead cDNA sequence containing a prolactin signal peptide for secretion (MNIKGSP-WKGSLLLLLLVSNLLLCQSVAP) and an M1 FLAG (DYKDDDDK) epitope were added to the amino terminus of all receptors (15). The expression constructs were purified using the Plasmid Maxi kit (Qiagen, Inc., Valencia, CA). Fidelity of the PCR products before use in expression studies.

Transfection of Cells and Analysis of Signal Transduction—Human 293T cells derived from human embryonic kidney fibroblast were maintained in Dulbecco’s modified Eagle’s medium/Ham’s F-12 (Invitrogen Co.) supplemented with 10% fetal bovine serum, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Before transfection, 2 × 10⁶ cells were seeded in 10-cm dishes (Becton Dickinson, Franklin Lakes, NJ). When cells were 70–80% confluent, transient transfection was performed using 10 μg of plasmid by the calcium phosphate precipitation method. Cells (2 × 10⁶/ml) were plated in 24-well tissue culture plates (Corning, Corning, NY) and preincubated at 37 °C for 30 min in the presence of 0.25 mM 3-isobutyl-1-methyl xanthine (Sigma Chemical Co.) before treatment with or without hormones for 18 h. At the end of incubation, cells and medium were frozen. After thawing, samples were heated to 95 °C for 3 min to inactivate phosphodiesterase activity, and total cAMP was measured in triplicate by a specific radioimmunoassay as described previously (16). All experiments were repeated at least three times using cells from independent transfections. To monitor transfection efficiency, 0.5 μg of β-galactosidase plasmid (17) was routinely included in the transfection mixture, and β-galactosidase activity in the cell lysate was measured as described previously (18). Statistical analysis was performed using Student’s t test.

Determination of FLAG Epitope-tagged Receptors on the Cell Surface (M1 Binding)—The levels of cAMP production were normalized by correcting for varying expressions of receptors to monitor the levels of their tagged epitope. Transfected cells were washed twice with Dulbecco’s PBS, and resuspended cells (2 × 10⁶/tube) were incubated with the FLAG M1 antibody (Sigma Chemical Co.) in Tris-buffered saline (pH 7.4) containing 5 mg/ml bovine serum albumin and 2 mM CaCl₂ (assay buffer) for 4 h at room temperature in siliconized tubes. Cells were then washed twice with 1 ml of assay buffer after centrifugation at 14,000 × g for 15 s. The 125I-labeled secondary antibody (anti-mouse IgG from sheep; ~400,000 cpm/tube) was added to the resuspended cell pellet and incubated for 1 h at room temperature. Cells were washed twice with 1 ml of assay buffer by repeated centrifugation before determination of radioactivity in cell pellets. Background binding was determined by adding excess amounts of the synthetic FLAG peptide (Sigma Chemical Co.) at a concentration of 100 μg/ml.

Ligand Binding Analysis—Transiently transfected 293T cells were plated in 24-well plates for whole cell binding assays. Media were removed, and cells were washed with PBS before preincubation in 300 μl of binding buffer (20 mM HEPES, 50 mM NaCl, 1.5 mM CaCl₂, 1%
Fig. 2. Direct binding of 32P-labeled H2 relaxin to LGR7 and LGR8 and competition by relaxin-related peptides. A. Scatchard plot analyses of 32P-labeled H2 binding to LGR7 and LGR8. B, competition of 32P-labeled H2 relaxin binding to LGR7 by relaxin-related peptides. C, competition of 32P-labeled H2 relaxin binding to LGR8 by relaxin-related peptides. D, the soluble ectodomain of LGR7 (7BP) blocks H3 relaxin stimulation of cAMP production by LGR7-expressing cells.

TABLE III

Kinetic parameters for the hormonal stimulation of cAMP production by wild type and mutant receptors and the competition for labeled H2 binding to the same receptors by different peptides

Data represent the mean ± S.E. of three to four separate experiments with triplicate cultures for each experiment. Estimations of EC50 values for all peptides were based on maximal cAMP production (Max) induced by H2. EC50 and IC50 values are in nM.

|       | H2   | H3   | INSL3 |
|-------|------|------|-------|
|       | EC50 | IC50 |       |
| LGR7  | 0.62 ± 0.05 | 0.09 ± 0.12 | 8.9 ± 0.4 |
| Max   | 407.3 ± 18.4 | 258.4 ± 7.9 | 1576 ± 592 |
| LGR8  | 9.02 ± 0.51 | 0.86 ± 0.48 | 4.21 ± 0.29 |
| Max   | 141.9 ± 5.9  | 238.4 ± 12.3 | ND |
| IC50  | 0.25 ± 0.00  | 215.9 ± 44.50 | ND |
| LGR7/8| 1.09 ± 0.07  | 66.2 ± 4.9   | ND |
| Max   | 441.9 ± 5.9  | 334.4 ± 12.5 | ND |
| IC50  | 0.27 ± 0.00  | 90.3 ± 44.50 | ND |
| LGR8/7| 7.08 ± 0.38  | ND           | 2.32 ± 0.24 |
| Max   | 456.1 ± 16.0 | ND           | 482.5 ± 27.5 |
| IC50  | 0.61 ± 0.31  | 680.3 ± 678.6 | 0.07 ± 0.03 |
| LGR7/8(EL1)| 1.23 ± 0.07 | 69.7 ± 3.0  | ND |
| Max   | 398.1 ± 6.6  | 355.8 ± 6.6  | ND |
| IC50  | 0.38 ± 0.13  | 167.2 ± 33.2 | ND |
| LGR7/8(EL2)| 0.86 ± 0.06 | 22.6 ± 0.7   | ND |
| Max   | 482.1 ± 0.0  | 406.8 ± 9.1  | ND |
| IC50  | 0.21 ± 0.15  | 12.4 ± 15.5  | ND |
| LGR7/8(EL3)| 1.65 ± 0.15 | 71.8 ± 2.3   | ND |
| Max   | 366.8 ± 24.7 | 239.1 ± 9.2  | ND |
| IC50  | 0.77 ± 0.33  | 314.5 ± 17.7 | ND |

a Max, maximal levels of cAMP production (pmol/2 × 10^5 cells).
b ND, not determined due to nonsaturation.
bovine serum albumin, 0.1 mg/ml lysine, 0.01% NaN₄, pH 7.5) (19). Binding studies were performed with 100 μl of 33P-labeled H2 relaxin, labeled as previously described (20), and 100 μl of competitor or blank in binding buffer at 25 °C for 60 min. Saturation binding was performed using increasing concentrations of 33P-labeled H2 relaxin, whereas competition experiments were performed with 100 nM 33P-labeled H2 relaxin in the absence or presence of increasing concentrations of unlabeled peptides. Nonspecific binding was determined by an excess of H2 relaxin (1 μM). After incubation, the cells were washed with PBS followed by their recovery from the plates using 500 μl of 1 N NaOH before transfer to scintillation vials. Liquid scintillation mixture (Ultima Gold, Packard, Meriden, CT) was added to these vials for counting in a liquid scintillation analyzer (Packard 1900 TR). Data are expressed as mean ± S.E. of the percentage of specific binding of triplicate determinations performed at least three times. Furthermore, data were analyzed using the LIGAND program (21). All Scatchard plots were linear, and the best fit to the data, given by LIGAND, was obtained with a one-binding site model. IC₅₀ values, determined from the competition curves, were analyzed by one-way analysis of variance followed by a Newman-Keuls multiple comparison test.

RESULTS

LGR7 Is Activated by Porcine Relaxin, H2 Relaxin, and H3 Relaxin, Whereas LGR8 Is Activated by Porcine Relaxin, H2 Relaxin, and INSL3—Earlier studies indicated that porcine relaxin activates both LGR7 and LGR8, whereas different INSL3 preparations activate only LGR8 (6, 7). Furthermore, H3 relaxin, like H2 relaxin, stimulates cAMP production in the THP-1 cells and competes for 33P-relaxin binding to its receptors (10). Based on these results, we tested the ability of these peptides in the activation of human LGR7 and LGR8. 293T cells were transiently transfected with LGR7 and LGR8 plasmids and ligand signaling was estimated based on total cAMP production (Fig. 1). To correct for varying receptor expression, all data for this and subsequent experiments were normalized based on cell surface M1 antibody binding to the tagged FLAG epitope of the recombinant receptors (Table II). The EC₅₀ values and maximal levels of cAMP production for different dose-response curves are shown in Table III.

In cells expressing LGR7, treatment with porcine relaxin and H2 relaxin led to dose-dependent increases in cAMP production. Although with lower efficacy, treatment with H3 relaxin also stimulated a dose-dependent increase in cAMP levels, whereas treatment with INSL3 was ineffective (Fig. 1A). In contrast, cells expressing LGR8 responded to treatment with INSL3, porcine relaxin, and H2 relaxin with increases in cAMP production (Fig. 1B). Moreover, treatment with H3 relaxin was ineffective in activating LGR8.

To demonstrate the receptor binding of relaxin-related peptides, cells expressing LGR7 and LGR8 were incubated with 33P-labeled H2 relaxin with or without increasing competing ligands. As shown in Fig. 2A, Scatchard plot analyses of saturation binding studies indicated that 33P-labeled H2 relaxin shows a higher affinity for LGR7 (Kᵣ: 0.209 ± 0.025, n = 4) than LGR8 (1.062 ± 0.127; n = 4) (p < 0.05). Consistent with these results and the observed potencies in stimulating cAMP production, H2 relaxin was most potent in competing for 33P-H2 relaxin binding to LGR7/8 (Fig. 2B). The IC₅₀ values for different peptides are shown...
These results demonstrate that H3 relaxin binds to LGR7, but not LGR8, and stimulates cAMP production mediated by LGR7. Thus, H3 relaxin is a specific ligand for LGR7, but not LGR8. In contrast, INSL3 binds to LGR8 and activates cAMP production, acting as a specific ligand for LGR8.

We have used an anchored receptor approach to generate soluble ectodomains of the gonadotropin and thyrotropin receptors (15), as well as the ectodomain of LGR7. The soluble ectodomain of LGR7, designated as 7BP, was able to block relaxin actions in vitro and in vivo (6). To test whether the ectodomain of LGR7 is capable of interacting with H3 relaxin, we treated 293T cells expressing LGR7 with H3 relaxin and H2 relaxin together with 7BP. As shown in Fig. 2D, co-treatment with 7BP completely blocked the stimulatory effects of H3 and H2 relaxin in a dose-dependent manner, thus demonstrating the ability of the ectodomain of LGR7 to bind these ligands.

**H3 Activates Chimeric Receptor LGR7/8, Whereas INSL3 Activates Chimeric Receptor LGR8/7**—To further confirm the importance of the ectodomain of LGR7 and LGR8 for ligand binding, we constructed chimeric receptors with their extracellular regions switched. LGR7/8 is comprised of the extracellular region from LGR7 and the transmembrane region to the carboxyl terminus from LGR8. In contrast, LGR8/7 has the extracellular region from LGR8 and the transmembrane region and C-tail from LGR7. As shown in Fig. 3, H2 relaxin stimulated cAMP production in transfected 293T cells expressing LGR7/8 (Fig. 3A) or LGR8/7 (Fig. 3C), whereas treatment with INSL3 resulted in a dose-dependent cAMP increase only in cells expressing LGR8/7 (Fig. 3C). Even though H3 relaxin stimulates cells expressing LGR7/8 to produce cAMP in a dose-dependent manner (Fig. 3A), the efficacy of cAMP production is lower compared with the H3 relaxin stimulation of wild type LGR7 (Table III). H3 was 30-fold less potent than H2 in stimulating wild type LGR7 (Table III). In contrast, H3 was 60-fold less potent than H2 in activating the chimeric LGR7/8. Receptor binding analyses also showed that H3 relaxin exhibited a...
decreased ability to compete for $^{33}$P-H2 relaxin binding to the chimeric receptor LGR7/8 (Fig. 3B) as compared with the H3 relaxin competition for $^{33}$P-labeled H2 relaxin binding to different receptors is compared with binding in the absence of competing ligands (set at 100%).

Although the ability of H3 relaxin to bind and stimulate the LGR7 receptor could be blocked by the soluble ectodomain of LGR7, these results suggested that additional regions of LGR7 might participate in receptor signaling by the H3 relaxin. In contrast, the chimeric receptor LGR8/7 responded to INSL3 and H2 relaxin but was not stimulated by H3 relaxin. Furthermore, INSL3 and H2 relaxin competed effectively for $^{33}$P-H2 relaxin binding to LGR7 (Fig. 3D).

Replacement of Exoloop 2, but Not Exoloop 1 or 3, of LGR7 in the Chimeric Receptor LGR7/8 Restores Ligand Binding and Signaling by H3 Relaxin—We hypothesized that exoloops in LGR7, in addition to the ectodomain, are important for interaction with H3 relaxin and designed additional chimeric constructs by replacing the individual exoloop of LGR7 into the chimeric receptor LGR7/8. The chimeric receptor LGR7/8 with exoloop 2 and the ectodomain from LGR7 responded to H3 relaxin treatment with an EC$_{50}$ value comparable to the H3 stimulation of wild type LGR7 (Figs. 1A versus 4B and Table III). In contrast, LGR7/8(EL1) and LGR7/8(EL2), chimeric receptors with the other exoloops replaced, responded to H3 relaxin treatment with an EC$_{50}$ similar to the H3 stimulation of LGR7/8 (Figs. 4A and 4C versus Fig. 3A and Table III). Likewise, replacement of exoloop 2, but not exoloop 1 or 3, in the chimeric receptor LGR7/8 restores receptor binding by H3 relaxin (Fig. 5).

When H3-stimulated maximal cAMP production mediated by wild type and mutant receptors was normalized as 100% (Fig. 6A), it became apparent that LGR7/8(EL2) is capable of responding to H3 relaxin treatment with cAMP production comparably to that of the wild type LGR7 (Table III). In contrast, cAMP production in cells expressing LGR7/8(EL1) or LGR7/8(EL3) showed higher EC$_{50}$ values similar to that of LGR7/8 (EC$_{50}$: LGR7/8, 66.2 nM; LGR7/8(EL1), 69.7 nM; LGR7/8(EL3), 71.8 nM; $p < 0.01$ versus LGR7 or LGR7/8(EL2)). Likewise, the competition of $^{33}$P-labeled H2 relaxin binding to LGR7 and LGR7/8(EL2) by relaxin H3 (IC$_{50}$: 33 and 12 nM, respectively) displayed a >10-fold reduction in the IC$_{50}$ value as compared with those for LGR7/8, LGR7/8(EL1), and LGR7/8(EL3) (IC$_{50}$: 557, 167, and 315 nM, respectively; $p < 0.01$ versus LGR7 or LGR7/8(EL2)) (Fig. 6B). These data demonstrate the importance of exoloop 2 in the H3 relaxin binding and activation of LGR7.

**DISCUSSION**

Recent studies demonstrated that porcine relaxin activates LGR7, and, with lower efficacy, LGR8. In addition, INSL3 is a specific and more potent ligand for LGR8 than relaxin (6, 7). The present data indicate that relaxin H3 is a specific ligand for LGR7 but not LGR8. However, relaxin H3 is less potent than porcine relaxin or H2 relaxin in activating LGR7.

Taking advantage of the structural similarity between LGR7 and LGR8, and because leucine-rich repeats in the
Mechanisms of LGR7 Activation by H3 Relaxin

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Introduction

The LGRs (ligand-receptor-family of orphan receptors) are a family of orphan receptors that are thought to play a role in the regulation of various biological processes, including embryonic development, metabolism, and hormone regulation. The LGRs are divided into three subfamilies: the relaxin family, the leutropin/thyroid-stimulating hormone (L/TSH) family, and the carciinoembryonic antigen-related antigen (CEA) family. The relaxin family receptors (LGR1-16) are members of the CEA family and are involved in the regulation of hormone signaling.

The relaxin family receptors are involved in the regulation of hormone signaling and are found in a variety of tissues, including the ovary, prostate, and placenta. The relaxin family receptors are activated by relaxin, a peptide hormone that is produced by the ovary and placenta. Relaxin binds to the receptor and activates a signal transduction pathway that leads to various biological effects, including the stimulation of cAMP production and the activation of downstream effectors.

The LGR7 receptor is a member of the relaxin family and is involved in the regulation of hormone signaling. The LGR7 receptor is activated by relaxin, a peptide hormone that is produced by the ovary and placenta. Relaxin binds to the receptor and activates a signal transduction pathway that leads to various biological effects, including the stimulation of cAMP production and the activation of downstream effectors.

At least three steps are involved in the ligand signaling of glycoprotein hormone receptors, each probably requiring unique but overlapping domains (31–34). First, the heterodimeric ligands interact with the ectodomain of the receptor, consisting of leucine-rich repeats that could form a 1/3 donut structure important for ligand-interaction. Second, ligand binding leads to the disruption of the constraint on the transmembrane region exerted by the interactions between the ectodomain (likely the hinge region) and exoloop 2. Third, the relaxed transmembrane region, as the result of ligand binding, interacts with the Gs protein to activate the adenylyl cyclase. In this model, it is likely that the common α-subunit of the glycoprotein hormones interacts with the leucine-rich repeats of the receptor ectodomains, whereas the unique β-subunits of these ligands stabilize the ligand-receptor complex by binding to the exoloops (33). Due to the lack of a hinge region in LGR7 and LGR8 compared to those found in glycoprotein hormone receptors, it is unclear whether the ectodomains of these relaxin receptors are capable of constraining their transmembrane region similar to glycoprotein hormone receptors.

Although the present studies using chimeric receptors and the soluble ectodomain of LGR7 suggest an important role for the ectodomain in ligand-receptor binding and signal transduction, our data demonstrate that H3 relaxin binds to both the ectodomain and exoloop 2 of LGR7 to induce maximal signal transduction. We propose a model for the activation of LGR7 by H3 relaxin (Fig. 7). First, H3 relaxin binds to the ectodomain of LGR7 through the putative contact motif RXRXRX(R)UV (Fig. 7A). This interaction could be blocked by the soluble ectodomain of LGR7. Subsequently, H3 relaxin also binds to exoloop 2 of LGR7 to stabilize the ligand-receptor complexes (Fig. 7B). Binding of H3 relaxin to both regions of LGR7 evokes efficient receptor activation by interacting with the Gs protein and stimulating cAMP production (Fig. 7C). Because LGR7 and LGR8 show 59% homology in exoloop 2, H3 relaxin could interact with the consensus sequence of these receptors and the present model could apply to the H2 relaxin.

In conclusion, we demonstrate that H3 relaxin is a specific ligand for LGR7, and using chimeric receptors, H3 relaxin is shown to bind both the ectodomain and the exoloop 2 for the activation of its receptor. Further studies using chimeric LGR7 and LGR8 receptors could provide useful information regarding the mechanism of receptor activation by H2 relaxin and INS3 and aid in the understanding the structural-functional relationship between ligands and receptors for this unique group of G protein-coupled receptors.

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