Mammalian receptors for gonadotropin-releasing hormone (GnRH) have over 85% sequence homology and similar ligand selectivity. Biological studies indicated that the chicken GnRH receptor has a distinct pharmacology, and certain antagonists of mammalian GnRH receptors function as agonists. To explore the structural determinants of this, we have cloned a chicken pituitary GnRH receptor and demonstrated that it has marked differences in primary amino acid sequence (59% homology) and in its interactions with GnRH analogs. The chicken GnRH receptor had high affinity for mammalian GnRH ($K_i$ 4.1 ± 1.2 nM), similar to the human receptor ($K_i$ 4.8 ± 1.2 nM). But, in contrast to the human receptor, it also had high affinity for chicken GnRH ([Gln$^8$]GnRH) and GnRH II ([His$^5$,Trp$^7$,Tyr$^8$]GnRH) ($K_i$ 5.3 ± 0.5 and 0.6 ± 0.01 nM). Three mammalian receptor antagonists were also pure antagonists in the chicken GnRH receptor. Another three, characterized by D-Lys$^6$ or D-isopropyl-Lys$^6$ moieties, functioned as pure antagonists in the human receptor but were full or partial agonists in the chicken receptor. This suggests that the Lys side chain interacts with functional groups of the chicken GnRH receptor to stabilize it in the active conformation and that these groups are not available in the chicken GnRH receptor to stabilize it in the active conformation.

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lectivity compared with mammalian GnRH receptors (16). In particular, some antagonists of mammalian GnRH receptors act as agonists of the chicken GnRH receptor, stimulating luteinizing hormone (LH) release from chicken pituitary cells (20). Cloning and characterization of the chicken gonadotrope GnRH receptor would, therefore, potentially provide the means for identifying domains and residues involved in ligand selectivity and underlying receptor activation.

We report the cloning of a novel GnRH receptor from chicken pituitary that differs from the mammalian GnRH receptor in its primary structure, ligand selectivity, and in the agonistic behavior of certain mammalian GnRH receptor antagonists. Analysis of the functional properties of a range of antagonists of the mammalian GnRH receptors in the chicken GnRH receptor shows that agonism in the chicken receptor is conferred by a basic δ-Lys or δ-Ipr-Lys in position 6. Chicken-human chimeric receptors identified the receptor determinant of the agonist activity of the "antagonist" peptides as extracellular loop 2 (EC2) of the chicken GnRH receptor.

**EXPERIMENTAL PROCEDURES**

**Reagents and Peptides—**The sequences of GnRH analogs used in the study are shown in Table I. Agonists were synthesized by conventional solid-phase methodology and purified to more than 95% homogeneity by preparative C-18 reversed-phase chromatography (16). Antagonist 26 was a gift from Dr. D. H. Coy (Tulane University Medical Center, New Orleans, LA) and antagonists 27, 135-18, 135-25, 134-53, and 134-46 were from Dr. R. W. Roeke (Indiana University, Indianapolis, IN).

**Receptor Amino Acid Residue Numbering—**A numbering scheme, in which amino acids of GnRH receptors are numbered relative to the most conserved residues in the TM segments of the rhodopsin-like GPCRs, is used to facilitate comparison among different receptors (21). The amino acid identifier, which follows the name of the amino acid, consists of the TM number followed by the position of the amino acid relative to the most conserved residue, in parentheses. For example, the Asp residue that is located immediately amino-terminal to the most conserved residue, Pro7.50, in TM7 is designated Asp7.49(319) in the human receptor and Asp7.49(310) in the chicken GnRH receptor (see Fig. 2 for alignment of these receptor sequences).

**Cloning of the Chicken GnRH Receptor**—A 120-base pair product encoding EC3 was amplified from 1 μg of chicken genomic DNA using the degenerate primer pairs (JH5S/JH6A) to conserved regions in TM6 and TM7 of GnRH receptors (22) (Fig. 1). The 120-base pair product was cloned into the pMOS-blue vector (Amersham Pharmacia TM6 and TM7 of GnRH receptors (22) (Fig. 1). The 120-base pair product was cloned into the pMOS-blue vector (Amersham Pharmacia TM6 and TM7 of GnRH receptors (22) (Fig. 1). The 120-base pair product was cloned into the pMOS-blue vector (Amersham Pharmacia TM6 and TM7 of GnRH receptors (22) (Fig. 1). The 120-base pair product was cloned into the pMOS-blue vector (Amersham Pharmacia TM6 and TM7 of GnRH receptors (22) (Fig. 1). The 120-base pair product was cloned into the pMOS-blue vector (Amersham Pharmacia TM6 and TM7 of GnRH receptors (22) (Fig. 1). The 120-base pair product was cloned into the pMOS-blue vector (Amersham Pharmacia TM6 and TM7 of GnRH receptors (22) (Fig. 1). The 120-base pair product was cloned into the pMOS-blue vector (Amersham Pharmacia TM6 and TM7 of GnRH receptors (22) (Fig. 1). The 120-base pair product was cloned into the pMOS-blue vector (Amersham Pharmacia TM6 and TM7 of GnRH receptors (22) (Fig. 1). The 120-base pair product was cloned into the pMOS-blue vector (Amersham Pharmacia TM6 and TM7 of GnRH receptors (22) (Fig. 1). The 120-base pair product was cloned into the pMOS-blue vector (Amersham Pharmacia TM6 and TM7 of GnRH receptors (22) (Fig. 1).

**Radioligand Binding Assay—**Radioligand binding assays were performed on intact cells 48 h after transfection. Transfected cells in 12-well culture plates were washed and incubated for 3 h at 4°C with 125I-GnRH-A (100,000 cpm) and various concentrations of unlabeled GnRH agonist or antagonist in buffer A (0.1% fetal bovine serum, 10% dialyzed FCS, 0.1% bovine serum albumin, and 8 mM d-glucose, pH 7.4) and incubated for 1 h at 37°C with 10 mM LiCl and appropriate concentrations of GnRH agonists or antagonists in the presence and absence of 10 nm [Gln8]GnRH (chicken GnRH receptor) or 1 nm mammalian GnRH (human GnRH receptor). Experiments were performed in duplicate and repeated at least three times.

**Cell Culture and Receptor Transfection—**COS-1 cells, grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, were seeded into 12-well plates (Corning Glass) (0.6–1 × 10^5) cells/25-mm well, pre-coated with poly-D-lysine (10 μg/ml). Cells were transfected for 3.5 h with plasmid DNA (0.5 to 1 μg/well), using a modified DEAE-dextran method (25).

**Inositol Phosphate (IP) Assay—**IP production was measured as described previously (26). Briefly, cells were labeled with myo-[3H]inositol (1 μCi/ml, Amersham Pharmacia Biotech) in Medium 199, supplemented with 2% fetal bovine serum, penicillin, and streptomycin for 16–22 h. Cells were washed twice with buffer A (140 mM NaCl, 4 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 20 mM Hepes, 0.1% bovine serum albumin, and 8 mM d-glucose, pH 7.4) and incubated for 1 h at 37°C with 10 mM LiCl and appropriate concentrations of GnRH agonist or antagonist in the presence and absence of 10 nM [Gln8]GnRH (chicken GnRH receptor) or 1 nM mammalian GnRH (human GnRH receptor). Experiments were performed in duplicate and repeated at least three times.
Chicken GnRH Receptor EC2 Confers Agonism to Antagonists

RESULTS

Cloning of Chicken GnRH Receptor cDNA—Screening of chicken pituitary cDNA libraries failed to identify any GnRH receptor clones. As the chicken thyrotropin-releasing hormone receptor was cloned from the same libraries (27), it appears that GnRH receptor mRNA is expressed at low levels in chicken pituitaries, as in some mammalian species (25, 29). The full-length chicken GnRH receptor cDNA was therefore isolated by the combined strategies of genomic library screening, 5′-rapid amplification of cDNA ends, and PCR of total RNA prepared from pituitaries of castrated chickens (see under “Experimental Procedures”). Sequencing of genomic clones revealed that the cloned chicken GnRH receptor has introns located in the amino-terminal domain and the carboxyl-terminal tail that is absent from mammalian GnRH receptors (17–19). The carboxyl-terminal domain has been shown to regulate GnRH receptor desensitization and internalization (32, 33). Also similar to other nonmammalian GnRH receptors, the chicken GnRH receptor has Asp in both loci of the conserved helix 2/helix 7 functional microdomain. This microdomain consists of Asn².50/Asp⁷.49 (9) in the human GnRH receptor and Asp².50/Asn⁷.49 in most other GPCRs, and it regulates GPCR coupling and expression (7, 8). The presence of two Asp residues in the chicken GnRH receptor suggests that the nonmammalian GnRH receptors may represent an evolutionary intermediate between the Asp².50/Asn⁷.49 arrangement of the microdomain found in most GPCRs and the Asn².50/Asp⁷.49 of the mammalian GnRH receptors. The presence of Asp².50/Asn⁷.49 in the Drosophila melanogaster homolog of the GnRH receptor (34) supports this conclusion. Other residues that are important for coupling of mammalian GnRH receptors to coty- solic signal transduction, the Arg cage motif (DRXXK) at the cytosolic end of TM3 (15) and Ala².29/30/31 in IC3 (14) are conserved in the chicken GnRH receptor. All of the residues previously shown to have a role in ligand binding of mammalian GnRH receptors, Asp².50/Asn⁷.49 (12), Asn².50/Asp⁷.49 (9), Lys³.32/121 (11), and Glu⁷.32/301 (10), are conserved in the chicken receptor (Fig. 2).

GnRH Agonist Interactions—The cloned chicken GnRH receptor exhibited high affinity binding to a series of GnRH agonists (Table II). It had high affinity for both mammalian GnRH and the native chicken ligand [Gln⁸]GnRH (Kᵢ 4.1 ± 1.2 and 5.3 ± 0.5 nM, respectively), in contrast to the human GnRH receptor, which had similar high affinity for mammalian GnRH (Kᵢ 4.8 ± 1.2 nM) but low affinity for [Gln⁸]GnRH (Kᵢ 174 ± 69 nM) (Table II). The chicken GnRH receptor had much higher affinity for GnRH II (0.60 ± 0.01 nM), also contrasting with low affinity in the human GnRH receptor (Kᵢ 39 ± 8.5 nM). The substitution of a D-amino acid for Gly⁶ in GnRH is thought to constrain the peptide in the biologically active β-II-turn conformation and enhance binding affinity for mammalian GnRH receptors (see Ref. 4). This is reflected in the 3-fold enhancement of the affinities of [D-Arg⁶]GnRH II and GnRH-A compared with unconstrained ligands in the human GnRH receptor (Table II). In contrast, there was little or no enhancement in binding affinity of the D-amino acid-containing ligands for the chicken GnRH receptor (Table II). These direct receptor binding studies confirm previous bioassay data and support the suggestion that incorporation of D-amino acids in position 6 of GnRH does not enhance binding affinity for the chicken GnRH receptor and that GnRH binds the chicken GnRH receptor differently from the mammalian receptor (16).

The cloned chicken GnRH receptor stimulated IP accumulation in response to GnRH agonists (Table II). The rank order of agonist ED₅₀ values was the same as the rank order of Kᵢ values determined in ligand binding assays (Table II). The Kᵢ values for binding of these agonists to the cloned chicken GnRH receptor and the ED₅₀ values for agonist-stimulated IP accumulation are consistent with the ED₅₀ values previously reported for stimulation of LH release from cultured chicken pituitary cells (16, 35).

Ligand-independent IP accumulation
was not detected in cells transfected with the chicken GnRH receptor.

**Agonism of Mammalian Receptor Antagonists**—We have previously reported that some antagonists of mammalian GnRH receptors behave as agonists, stimulating LH release from cultured chicken pituitary cells (20). A series of mammalian receptor antagonists was used to define the structural basis of agonism in the cloned chicken pituitary. All of the analogs functioned as full antagonists with high binding affinity in the human GnRH receptor (Table II and Fig. 3). The analogs had much lower binding affinity for the chimeric chicken GnRH receptor (Table II). Furthermore, three analogs exhibited distinct agonist activity, stimulating IP accumulation in cells expressing the chimeric chicken GnRH receptor (Table II and Fig. 3), whereas the other three analogs functioned as pure antagonists (Table II). Antagonist 135-18 was a full agonist, and antagonists 135-25 and 26 were partial agonists (Fig. 3). All of the analogs that showed agonist activity contained a basic D-amino acid (D-Lys or D-Ipr-Lys) in position 6, and all peptides that were antagonists in the chicken GnRH receptor had uncharged side chains in this position (Table I).

The agonist behavior of three antagonists in the chicken receptor may arise from interactions of these peptides with amino acid residues that are unique to the chicken receptor. We attempted to identify the domains of the chicken receptor that are involved using chimeric receptors in which EC domains of the human receptor were substituted with EC domains of the chicken receptor. Since EC1 is highly conserved (Fig. 2), exchanges were confined to EC2 and EC3. The chimeric receptor containing EC3 of the chicken GnRH receptor did not bind GnRH or stimulate IP accumulation in response to GnRH, suggesting that the EC3 chimera was poorly expressed or uncoupled from activation of phospholipase C (data not shown). However, the chimera containing the chicken receptor EC2 substituted in the human receptor exhibited high affinity binding and IP accumulation. The EC2-containing chimera bound antagonists 26, 135-25, and 135-18 with high affinities (Kᵢ, 7.8 ± 2.9 nM; and 18 ± 5.3 nM, respectively), which were similar to their affinities for the wild type human receptor and higher than those for the chicken receptor (Table II), indicating that the NH₂-terminal domain, EC1, EC3, and superficial regions of the TM domains of the human receptor are major contributors to high affinity building of the antagonists (Tables II). Antagonists 26 and 132-25, which were partial agonists in the chicken GnRH receptor, behaved as antagonists in the chimera (Fig. 4), similar to the wild type human receptor. Antagonist 135-18, which was a full antagonist in the human GnRH receptor and a full agonist in the chicken GnRH receptor, exhibited partial agonist behavior in the chimera (Fig. 4). As was found for the chicken GnRH receptor, no constitutive activity was detectable in the chimeric receptor.

**DISCUSSION**

The definitive molecular delineation of ligand binding, signal propagation, and G protein coupling of the human GnRH receptor and the development of GnRH analogs is a major goal in reproductive medicine. Progress in this regard has been made through the cloning of GnRH receptors and a combination of molecular modeling and mutagenesis studies (4). Since the various mammalian receptors have close sequence homology and similar ligand selectivity (4), information on the primary sequence of a related, but pharmacologically distinct, nonmammalian receptor would potentially contribute in these endeavors. The chicken GnRH receptor that we have cloned exhibits marked pharmacological differences in its interaction with GnRH agonist and antagonist analogs and has sequence differences from the mammalian receptors that may be used to identify functional residues.

**Agonist-binding Site Differs in Chicken and Mammalian GnRH Receptors**—The conservation of the Asp², Lys⁶, and Lys² residues in the chicken GnRH receptor is expected, as these residues are believed to interact with the amino- (Glu⁴⁻His⁸) and carboxyl-terminal (Gly¹⁰·NH₂) residues of the GnRH ligands, which are conserved in the native mammalian and chicken forms of GnRH (9, 11, 12). As expected from biological assays, the chicken GnRH receptor does not distinguish between its cognate native ligand, [Gln⁶·GnRH (K, 5.3 ± 0.5 nM), and mammalian GnRH (K, 4.1 ± 1.2 nM). Surprisingly, the affinity of the chicken receptor for both ligands was as high as the affinity of the human GnRH
receptor which is selective for mammalian GnRH (K_i 4.8 nM) and binds [Gln8]GnRH with low affinity (K_i 174 nM). The high affinity of the chicken receptor is unexpected because high affinity binding of mammalian GnRH depends on an interaction of Arg8 with an acidic residue in the EC3 domain (Glu3.32(301) in the mouse and Asp 3.32(302) in the human) (10). The equally high affinity of mammalian GnRH and [Gln8]GnRH binding to the chicken receptor suggests, therefore, that the Arg8-Glu3.32(301)/Asp3.32(302) interaction does not occur but is compensated by alternative interaction(s) in the chicken receptor. Thus, it appears that, although there are interactions that are common to the mammalian and chicken receptors, the binding sites differ in unique interactions.

This suggestion that [Gln8]GnRH utilizes a different binding site in the chicken GnRH receptor is not unexpected. [Gln8]GnRH is not configured in the folded b2II-turn conformation characteristic of mammalian GnRH (36, 37), and substitution of a d-amino acid for Gly8, which enhances the folded conformation, does not enhance binding affinity for the chicken receptor as it does in the human receptor (Table II). The amino- and carboxyl-terminal residues, Glu(P)1, His2, and Gly10NH2, of the ligand and their cognate mammalian receptor binding residues, Asp 2.61(98), Asn 2.65(102), and Lys 3.32(121), are all conserved in species of fish, amphibians, birds, and mammals (4, 17–19). We therefore propose that the interactions between these residues are also conserved. Since the receptors have different requirements for ligand conformation, we further propose that the spatial arrangement of the receptor-binding sites differs between the chicken and mammalian receptors. This allows the accommodation of binding of the configured ligand in mammalian receptors and the nonconfigured ligands in the chicken GnRH receptor, through the same interactions. This proposal suggesting that the receptors are configured differently is supported by the presence of two Asp residues.
TABLE II
Receptor binding and peptide-regulated IP production of GnRH analogs in COS-1 cells expressing chicken and human GnRH receptors

| Analog                | Ligand binding | IP production |
|-----------------------|----------------|---------------|
|                       |                | Chicken GnRH receptor | Human GnRH receptor | chicken GnRH receptor | Human GnRH receptor |
|                       | $K_c$ (nM) | $E_D_{50}$ (nM)$^b$ | $I_C_{50}$ (nM)$^c$ | $E_D_{50}$ (nM)$^b$ | $I_C_{50}$ (nM)$^c$ |
| Agonists              |               |               |               |               |               |
| | [Glu]GnRH          | 5.3 ± 0.5$^a$ (1.0) | 174 ± 69 (0.03) | 2.7 ± 0.6 (1.0) | 0.9 ± 0.1 (0.02) |
| | GnRH II            | 0.6 ± 0.01 (11.0) | 39 ± 8.5 (0.1) | 0.04 ± 0.01 (6.9) | 0.5 ± 0.2 (0.04) |
| (d-Arg$^8$)GnRH II   | 0.6 ± 0.09 (11.0) | 16 ± 2.5 (0.3) | 0.9 ± 0.02 (3.0) | 0.02 ± 0.005 (1.0) |
| | GnRH-A             | 3.0 ± 0.3 (2.3) | 1.5 ± 0.4 (3.2) | 0.02 ± 0.0002 (126) | 0.3 ± 0.03 (0.07) |
| Antagonists           |               |               |               |               |               |
| 27                    | 61 ± 1.3 (0.09) | 4.1 ± 0.9 (1.2) | 177 ± 43 (0.02) | 5.9 ± 0.7 (0.005) |
| 134–46                | 310 ± 24 (0.02) | 7.0 ± 1.7 (0.7) | 27 ± 3.7 (0.1) | 1.7 ± 0.1 (0.01) |
| 134–53                | 310 ± 19 (0.02) | 8.1 ± 2.1 (0.6) | 28 ± 2.1 (0.1) | 1.8 ± 0.5 (0.01) |
| 26                    | 126 ± 2.4 (0.04) | 3.8 ± 0.7 (1.3) | 9.7 ± 0.2 (0.3) | 3.8 ± 0.2 (0.005) |
| 135–25                | 4900 ± 380 (0.001) | 56 ± 5.3 (0.09) | 1300 ± 71 (0.002) | 16 ± 1.2 (0.001) |
| 135–18                | 703 ± 153 (0.007) | 23 ± 7.4 (0.2) | 33 ± 2.1 (0.08) | 2.0 ± 0.5 (0.003) |

$^a$ Data are mean ± s.e. of two to four experiments. Relative potencies, calculated as the ratio of $K_c$ (binding) or $E_D_{50}$ values (IP production) of GnRH analog relative to that of the native ligand ([Glu]GnRH for chicken receptor and mammalian GnRH for the human receptor) are shown in parentheses.

$^b$ $E_D_{50}$ values for agonist activity of peptides.

$^c$ $I_C_{50}$ values for antagonism of IP production stimulated by 1 nM [Glu]GnRH (or 10 nM for antagonist 135–25) for the chicken GnRH receptor or 1 nM mammalian GnRH for the human GnRH receptor.

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Behavior of Antagonists—

Analysis of the sequences of the antagonist analogs revealed that a single feature is unique to those with agonist activity. This feature is the presence of a functional group, which is available only in the active conformation of the receptor. The absence of this feature in the inactive conformation prevents the antagonist from binding and stabilizing the receptor in the active conformation. Thus, although the receptor has a functional group, it is not available to the antagonist in the inactive conformation.

Another three pure mammalian antagonists, 26, 135–25, and 135–18, behaved as partial or full agonists in their interaction with the chicken GnRH receptor. There was no correlation of agonist/antagonist behavior and binding affinities at the mammalian receptor. Thus, although the chicken EC2 domain is a determinant of agonist activity of antagonist 135–18, which was a full agonist in the chicken receptor, antagonists 26, 135–25, and 135–18 behaved as partial or full agonists in their interaction with the mammalian receptor.

Proposed Mechanism of Agonist Activity of Antagonists—

Contemporary thinking proposes that agonist analogs bind and stabilize the receptor conformation that is active in the receptor. The interaction of the agonist with the receptor is sufficient to confer agonist activity. This is supported by the observation that the receptor conformation is maintained by the agonist.

Agonist formation of the agonist domain of the receptor drives the equilibrium between the active and inactive states of the receptor. This is consistent with the observation that the receptor conformation is maintained by the agonist.

Inverse agonists bind both the active and inactive states of the receptor. This is consistent with the observation that the receptor conformation is maintained by the agonist.

Insertion of the chicken GnRH receptor EC2 domain into the human receptor conferred partial agonist activity. This is consistent with the observation that the receptor conformation is maintained by the agonist.

The insertion of the chicken EC2 domain into the human receptor conferred partial agonist activity. This is consistent with the observation that the receptor conformation is maintained by the agonist.

There was no correlation of agonist/antagonist behavior and binding affinities at the mammalian receptor. Thus, although the chicken EC2 domain is a determinant of agonist activity of antagonist 135–18, which was a full agonist in the chicken receptor, antagonists 26, 135–25, and 135–18 behaved as partial or full agonists in their interaction with the mammalian receptor.
Since a D-Lys or D-Ipr-Lys residue in position 6 of the mammalian GnRH receptor antagonists is the unique feature associated with agonist activity, it presumably interacts with a receptor amino acid side chain that is accessible in the active conformation of the chicken receptor and is absent from, or inaccessible in, the active conformation of the human GnRH receptor. A candidate for a strong interaction with D-Lys would be an acidic residue in the receptor. Since substitution of the chicken EC2 in the human receptor conferred partial agonism to antagonist 135-18, the Glu5.34(200) and Glu5.35(201) residues in the chicken EC2 (His5.34(207) and Gln5.35(208) in mammalian receptors) are candidates. Glu5.35(201) is also present in the Xenopus GnRH receptor in which antagonist 135-18 also behaves as an agonist.2

An alternative proposal is that ligand binding induces the active state of the receptor (39). In the β-adrenergic (40) and 5HT2A (41) receptors, a different positioning of certain ligands alters their ability to induce the active state of the receptor. It may be conceived that the interaction of D-Lys or D-Ipr-Lys with extracellular EC2 of the chicken receptor changes positioning of these ligands to allow interaction with receptor-activating sites.

Mutations in a number of GPCRs have been described that confer agonism to antagonists. Mutations in TM3 of the AT1 angiotensin receptor (39, 42), rhodopsin (43), and the β-adrenergic receptor (40), in TM4 of μ- and β-opioid receptors (44), in TM6 of the μ-opioid receptor (45), in IC3 of the α2-δ-adrenergic receptor (46), and in IC2 of the V2 vasopressin receptor (47) conferred agonistic activity to antagonist ligands. The present study is the first description of a mutation in an extracellular domain that confers agonism to an antagonist. Receptor activation is believed to involve movement (e.g. rotation) of the TM domains and that this is propagated into structural changes in the connecting IC loop domains (reviewed in Refs. 48 and 49). Our demonstration that mutation of EC2 allows the interaction with a single residue in an antagonist to confer agonism indicates that a distinct relationship exists between EC and TM domains and that ligand interaction with EC domains can stabilize the receptor in the active conformation. Indirect evidence that molecular interactions with EC domains can lead to receptor activation has been obtained from antibody studies. Antibodies against EC2 of M1 and M2 muscarinic, AT1 angiotensin, and β1- and β2-adrenergic receptors caused receptor activation (50–53), pointing to a role for this domain in stabilizing receptor conformation.

In conclusion, our findings here identified a single amino acid side chain (D-Lys or D-Ipr-Lys) in mammalian GnRH receptor antagonists that confers agonist activities to these antagonists when interacting with the chicken GnRH receptor. This phenomenon is conferred to the human receptor with incorporation of EC2 of the chicken GnRH receptor. This suggests that an interaction of the D-Lys or D-Ipr-Lys side chain with a residue in EC2 of the chicken GnRH receptor stabilizes the active conformation of the receptor. Identification of other contact sites of this ligand will assist in delineating molecular distances of TM domains of the GnRH receptor in the active and inactive conformations and shed light on the molecular mechanism of ligand-mediated receptor activation.

REFERENCES
1. Millar, R. P., King, J. A., Davidson, J. S., and Milton, R. C. (1987) S. Afr. Med. J. 72, 748–755
2. Barbieri, R. L. (1992) Trends Endocrinol. Metab. 3, 30–34
3. Casper, R. F. (1991) Can. Med. Assoc. J. 144, 153–158
4. Sealton, S. C., Weinstein, H., and Millar, R. P. (1997) Endocr. Rev. 18, 180–205
5. Davidson, J. S., Flanagan, C. A., Zhou, W., Becker, I., Elario, R., Emeran, W., Sealton, S. C., and Millar, R. P. (1995) Mol. Cell. Endocrinol. 107, 241–245
6. Davidson, J. S., Assefa, D., Pawson, A., Davies, P., Hapgood, J., Becker, I., Flanagan, C., Roeske, R., and Millar, R. (1997) Biochemistry 36, 12881–12889
7. Flanagan, C. A., Zhou, W., Chi, L., Yuen, T., Rodic, V., Robertson, D., Johnson, M., Holland, P., Millar, R. P., Weinstein, H., Mitchell, R., and Sealton, S. C. (1999) J. Biol. Chem. 274, 28880–28886
Chicken GnRH Receptor EC2 Confers Agonism to Antagonists

8. Zhou, W., Flanagan, C., Ballesteros, J. A., Konvicka, K., Davidson, J. S., Weinstein, H., Millar, R. P., and Sealfon, S. C. (1994) Mol. Pharmacol. 45, 165–170.

9. Davidson, J. S., McArdle, C. A., Davies, P., Elario, R., Flanagan, C. A., and Millar, R. P. (1996) J. Biol. Chem. 271, 15510–15514.

10. Flanagan, C. A., Becker, I., Davidson, J. S., Wakefield, I. K., Zhou, W., Sealfon, S. C., and Millar, R. P. (1996) J. Biol. Chem. 270, 22920–22926.

11. Zhou, W., Rodic, V., Kitanovic, S., Flanagan, C. A., Chi, L., Weinstein, H., Maayani, S., Millar, R. P., and Sealfon, S. C. (1995) J. Biol. Chem. 270, 18855–18867.

12. Flanagan, C. A., Rodic, R., Konvicka, K., Yuen, T., Chi, L., Rivier, J. E., Millar, R. P., Weinstein, H., and Sealfon, S. C. (1999) J. Biol. Chem. 274, 1039–1046.

13. Arora, K. K., Sakai, A., and Catt, K. J. (1995) Endocrinology 136, 107–113.

14. Ballesteros, J., Kitanovic, S., Guarnieri, F., Davies, P., Fromme, B. J., Konvicka, K., Chabot, B., and Sealfon, S. C. (1999) J. Biol. Chem. 273, 173, 110–116.

15. Ballesteros, J., Kitanovic, S., Guarnieri, F., Davies, P., Fromme, B. J., Konvicka, K., Chabot, B., and Sealfon, S. C. (1999) J. Biol. Chem. 274, 1039–1046.

16. Millar, R. P., Flanagan, C. A., Milton, R. C., and King, J. A. (1989) J. Biol. Chem. 264, 21097–21013.

17. Illing, N., Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.

18. Jacobs, G. F., Flanagan, C. A., Roeske, R. W., and Millar, R. P. (1995) Mol. Cell. Endocrinol. 110, 107–113.

19. Ballesteros, J., and Weinstein, W. (1995) Methods Neurosci. 25, 366–428.

20. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 2526–2531.

21. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.

22. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.

23. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.

24. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.

25. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.

26. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.

27. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.

28. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.

29. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.

30. Troskie, B. E., Nahorniak, C. S., Hapgood, J. P., Peter, R. E., and Millar, R. P. (1999) J. Biol. Chem. 274, 1764–1771.