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Amino-terminal leucine-rich repeats in gonadotropin receptors determine hormone selectivity

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Recombinant expression of truncated receptors for luteinizing hormone/chorionic gonadotropin (LH/CG) revealed that the amino-terminal leucine-rich repeats 1-8 of the extracellular receptor domain bind human chorionic gonadotropin (hCG) with an affinity (K_d = 0.72 ± 0.2 nM) similar to that of the native LH/CG receptor (K_d = 0.48 ± 0.05 nM). LH/CG receptor leucine-rich repeats 1-8 were used to replace homologous sequences in the closely related receptor for follicle stimulating hormone (FSH). Cells expressing such chimeric LH/CG-FSH receptors bind hCG and show elevated cylic AMP levels when stimulated by hCG but not by recombinant human FSH (rhFSH). Similarly, a chimeric LH/CG receptor in which leucine-rich repeats 1-11 originated from the FSH receptor is activated by rhFSH but not by hCG. For this chimera, no residual [^{125}I] hCG binding was observed in a range of 2 pM to 10 nM. Our results demonstrate that specificity of gonadotropin receptors is determined by a high affinity hormone binding site formed by the amino-terminal leucine-rich receptor repeats.

Key words: glycoprotein hormone receptors/gonadotropins/G protein-coupled receptors/hormone binding/leucine-rich glycoproteins

Introduction

Chorionic gonadotropin (CG), luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) are heterodimeric glycoproteins (relative mol. wt, M_r 34 kd) composed of a specific β subunit non-covalently bound to a common α subunit (Pierce and Parsons, 1981; Strickland *et al.*, 1985). They interact selectively with members of the large family of G protein-coupled cell surface receptors which display seven transmembrane spanning segments (Libert *et al.*, 1989; Loosfelt *et al.*, 1989; McFarland *et al.*,1989; Nagayama *et al.*, 1989; Parmentier *et al.*, 1989; Frazier *et al.*, 1990; Sprengel *et al.*, 1990), and whose hormone-dependent activation leads to the stimulation of adenylyl cyclase (Hunzicker-Dunn and Birnbaumer, 1985; Reichert and Dattatreyamurty, 1989).

Different from other G protein-coupled receptors, the glycoprotein hormone receptors contain a large extracellular domain. This domain displays sequence repeats built on a motif similar to other leucine-rich glycoproteins (LRG) (Patthy, 1987; McFarland *et al.*, 1989) which appear to form amphipathic peptide surfaces involved in protein-protein interaction (Field *et al.*, 1989; Krantz and Zipursky 1990). As suggested by us (McFarland *et al.*, 1989; Sprengel *et al.*, 1990) and experimentally indicated (Tsai-Morris *et al.*, 1990; Xie *et al.*, 1990), this large extracellular receptor domain mediates hormone binding. Ligand binding to the extracellular domain of a G protein-coupled receptor is exceptional since in most other members the ligand binding pocket appears to lie within the plane of the lipid bilayer and hence involves particular amino acid residues in the transmembrane regions (for review see Strader *et al.*, 1989). To probe the specificity of hormone binding and hormone-mediated receptor activation in glycoprotein hormone receptors, we expressed truncated LH/CG receptor molecules in cell culture and identified leucine-rich repeats 1-8 of the extracellular LH/CG receptor domain as high affinity hCG binding site. Subsequently, we exchanged hormone binding sites between the LH/CG and the FSH receptors and analyzed these chimeric receptors for hormone specificity and signal transduction.

Results

Hormone binding of truncated LH/CG receptor molecules

By subcloning cDNA segments encoding extracellular receptor parts, we constructed several receptor mutants designed for the expression of the entire extracellular domain (LH_{364}) and of several carboxy(C)-terminal deletions within this domain (LH_{336}, LH_{299} and LH_{232}; Figure 1). Mutant LH_{364} ends at position 338 of the mature receptor (McFarland *et al.*, 1989), directly preceding the first membrane spanning segment. In mutant LH_{336}, the extracellular domain is truncated at repeat 14. Mutant LH_{299} contains at its C-terminus 10 residues which are highly conserved between the three glycoprotein hormone receptors (Figure 4). The residues include two adjacent cysteines and correspond to the amino(N)-terminal end of repeat 11. The shortest mutant, LH_{232}, spans the first 8 repeats and lacks a region with sequence similarity to soybean lectin (McFarland *et al.*, 1989). Hormone binding properties of these truncated receptors were analyzed in transiently or stably expressing cultured mammalian cells. Concentration dependent and saturable binding of [^{125}I]hCG revealed that all truncated receptors retain hormone binding with no significant change in affinity (Figures 1 and 2), demonstrating that in LH/CG receptors, hCG binding occurs in the N-terminal eight repeats of the extracellular domain.

This was further demonstrated by cross-linking of [^{125}I]hCG/receptor complexes. Solubilized LH/CG receptor as well as the extracellular domain and its truncated versions were incubated with [^{125}I]hCG in the absence or presence

TRUNCATED LH/CG - RECEPTORS		K_d [nM] A/B	B_{max} [fmol/mg protein] A	B
1 2 3 4 5 6 7 8 9 10 11 12 13 14 I II VII 674	LHR *	0.48 ± 0.05	16472 ± 774	103 ± 6
338	LH_{364}*	0.52 ± 0.07	1970 ± 171	21571 ± 795
310	LH_{336}	0.50 ± 0.02	311 ± 19	2112 ± 82
273	LH_{299}	0.71 ± 0.1	160 ± 14	1048 ± 68
206	LH_{232}*	0.72 ± 0.2	13 ± 2.2	

Fig. 1. Hormone binding properties of truncated LH/CG receptors. The complete receptor and truncated receptor versions are schematically depicted in the left panel. Leucine-rich repeats 1–14 of the extracellular domain are numbered in arabic, and membrane spanning segments in roman numerals (I–VII). Potential N-linked glycosylation sites in the extracellular domain are marked by black boxes. The C-terminal amino acids in the truncated LH/CG receptor proteins are indicated (alanine 338, asparagine 310, serine 273 and asparagine 206) . The columns at the right give the apparent dissociation constant K_d (nM) and the maximal binding values B_{max} (fmol/mg protein) for [^{125}I]hCG as measured in crude membranes (**A**) and soluble cell extracts (**B**) after one step separation of homogenized cells expressing the truncated LH/CG receptors. To determine the K_d and B_{max} values for [^{125}I]hCG in cells containing LH/CG receptor mutant LH_{232}, crude membranes and soluble cell extracts were not separated. The substantial decrease in the amount of binding observed relative to LH/CG receptor, particularly for the shortest receptor version (LH_{232}), might reflect a reduced efficiency in trapping the [^{125}I]hCG-complexes of smaller molecular weight. Data indicated by * were obtained with stably expressing cells. The numbers are representative of experiments that were performed 3–5 times with essentially identical results.

of a molar excess of unlabeled hormone. Analysis of hormone-bound, glutaraldehyde cross-linked complexes by autoradiography of gel-resolved products revealed all complexes in the predicted molecular weight range and in similar amounts, including the most truncated receptor version, LH_{232} (Figure 3).

A substantial difference between complete and truncated receptors was observed in the cellular distribution of the hormone binding sites. After a one-step purification of soluble proteins and crude membranes, >70% of the binding sites for [^{125}I]hCG were detected in the soluble protein fraction (Figure 1). Since the expression of all truncated receptors was designed to utilize the leader peptide of the native receptor, we expected to find secreted, soluble binding sites. However, in multiple experiments (n = 6), no [^{125}I]hCG binding was detected in the media of transfected cells for any of the receptor mutants. Furthermore, concentrated media did not compete in [^{125}I]hCG binding assays (not shown). These results indicate that, contrary to another report (Tsai-Morris *et al.*, 1990), receptor molecules synthesized without membrane spanning segments remain within the cells.

Hormone specificity of chimeric gonadotropin receptors

To investigate if glycoprotein hormone receptors can be activated by extracellularly bound heterologous hormone, we re-engineered the FSH receptor to bind hCG. Taking advantage of the sequence similarity between the gonadotropin receptors (Sprengel *et al.*, 1990), the whole extracellular domain (LFSHR-Sa) and also leucine-rich repeats 1–6 (LFSHR-X) of the FSH receptor were replaced by the homologous segment of the LH/CG receptor (Figure 4). Cultured cells transiently expressing these chimeric receptors were analyzed for hormone binding and hormone dependent adenylyl cyclase activation. As shown in Figure 4, both chimeric LH/CG-FSH receptors bind [^{125}I]hCG with K_d values similar to those displayed by the full length LH/CG receptor, and can be activated by hCG. Hence, while hormone binding is selective, a gonadotropin once bound can activate the related receptor. The hormone needs to be bound for receptor activation since chimeras LFSHR-Sa and -X which both lack a binding site for FSH do not mediate

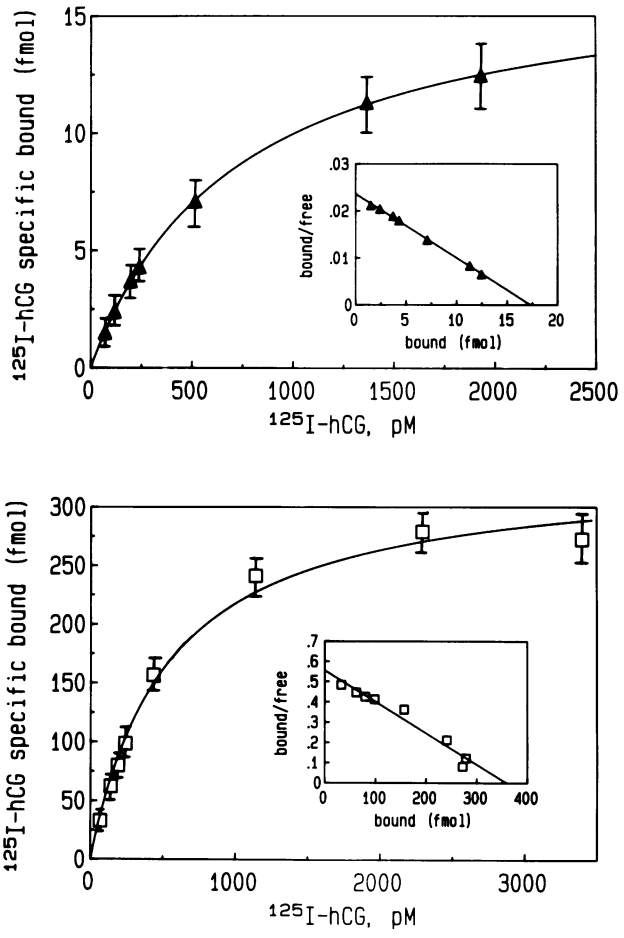


Fig. 2. Saturation isotherms and Scatchard analyses of [^{125}I]hCG binding to total cell extracts prepared from 293 cells expressing the LH/CG receptor (bottom) and the deletion mutant, LH_{232} (top). The K_d and B_{max} values are as listed in Figure 1. The data presented are from a representative experiment where each point is the average of triplicate determination.

stimulation of adenylyl cyclase by biologically active rhFSH (Figure 4).

Confirmative results were obtained for reciprocal chimeras having the hCG binding site of the LH/CG receptor replaced

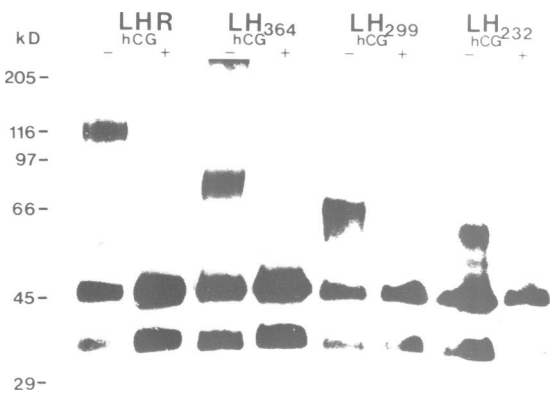


Fig. 3. Analysis of cross-linked receptor hormone complexes. Total cell extracts of cells expressing full length LH/CG receptor (LHR) or C-terminal LH/CG receptor mutants LH_{364}, LH_{299} and LH_{232} were incubated with [^{125}I]hCG in the absence (−) or presence (+) of excess unlabeled hCG and were cross-linked by glutaraldehyde as detailed in Materials and methods. Products were analyzed on an 8% polyacrylamide gel under non-reducing conditions followed by autoradiography. Molecular weight standards (Sigma) are indicated on the left. The labelled 45 kd and 35 kd complexes represent hormonal $\alpha\beta$ and α subunits respectively.

by homologous FSH receptor sequences. In these chimeras, FLHR-X carried FSH receptor leucine-rich repeats 1−6, FLHR-B repeats 1−7 and FLHR-A repeats 1−11 (Figure 4). Cells expressing these constructs showed no hCG binding nor hCG dependent adenylyl cyclase stimulation when treated with hCG in concentrations ranging from 20−2600 pM.

By analogy to the LH/CG-FSH receptors, we expected that rhFSH would activate the chimeras FLHR-X, FLHR-B and FLHR-A since all three receptors contain the N-terminal extracellular part of the FSH receptor. However, only cells expressing FLHR-A showed elevated cAMP levels after stimulation with rhFSH. Thus, different from the LH/CG receptor, extracellular repeats 1−11 of the FSH receptor seem necessary for receptor activation and hence, presumably for receptor binding.

Discussion

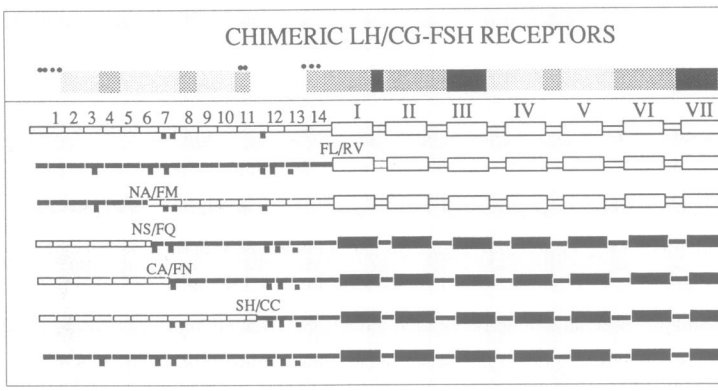
Our experiments identified the N-terminal part of the large extracellular receptor domain as containing a high affinity hormone binding site. This binding site consists of leucine-rich repeat sequences and comprises eight repeats in case of the hCG binding site. Leucine-rich repeats have been identified in several other proteins from different species, including the human leucine-rich α2 glycoprotein (Takahashi *et al.*, 1985) , α and β chains of the human platelet glycoprotein IB (Lopez *et al.*, 1987 and 1988; Titani *et al.*, 1987), the porcine ribonuclease inhibitor (Hofsteenge *et al.*, 1988), the Chaeoptin (Reinke *et al.*, 1988) and Toll (Hashimoto *et al.*, 1988) gene products from *Drosophila*, and the adenylyl cyclase from *Saccharomyces cerevisiae* (Kataoka *et al.*, 1986; Yamawaki-Kataoka *et al.*, 1989). For most of these proteins, the function of the leucine-rich motifs remains speculative but for yeast adenylyl cyclase there is direct evidence that this sequence promotes the interaction between enzyme and RAS protein (Field *et al.*, 1989; Suzuki *et al.*, 1990). Thus it is likely that similar to the leucine-zipper (Landschulz *et al.*, 1988), leucine-rich repeats favor the formation of peptide surfaces predisposed for specific protein−protein interactions.

In the LH/CG receptor, leucine-rich repeats 8−14 are not required for the high affinity hCG binding site. Repeats 12 and 13 are highly variable in length and amino acid composition (Figure 4), and their leucine-rich motif is rudimentary (Sprengel *et al.*, 1990). These can be considered as hinge region separating hormone binding site and membrane spanning domains. Indeed, it was shown recently for the TSH receptor that this region can be deleted without loss of receptor function (Wadsworth *et al.*, 1990). Other dispensable repeats for hormone binding, repeats 9, 10, 11 and 14, contain amino acids highly conserved in all glycoprotein hormone receptors, including several common cysteines. These amino acids may be important for the conformational integrity of the receptor. We observed that LH/CG receptor molecules lacking repeats 10 and 11 bind hCG. However, these receptors are rapidly degraded within the cell and do not appear on the cell surface (Braun and Sprengel, 1991).

No difference in hormone affinity was seen for the truncated and full length LH/CG receptors. Therefore, hormone affinity of glycoprotein hormone receptors appears not to be affected by association of the receptor with G_s protein, as descibed for the β-adrenergic receptor (for review see Limbird, 1981). In this case, a physical interaction of the G protein and intracellular receptor regions seems to alter the structure of the ligand binding pocket, formed by the arrangement of membrane spanning helices (Dixon *et al.*, 1987; Strader *et al.*, 1987; Kobilka *et al.*, 1988). The gonadotropin receptors have the ligand binding site in a separate protein domain, the structure of which is apparently not influenced by conformational shifts of the membrane spanning segments.

The functional analysis of the hybrid LH/CG-FSH receptors showed that the hormone binding site can be exchanged between LH/CG and FSH receptor without loss of receptor function. In these hybrid receptors, the hormone specificity is determined by the hormone binding site present. Leucine-rich repeats 1−6 of the LH/CG receptor mediate hCG responses, and FSH receptor repeats 1−11 confer FSH responses to the heterologous receptor. Hybrid FSH-LH/CG receptors containing LH/CG receptor repeats 1−6 and FSH receptor repeats 6−14 cannot be activated by FSH. This indicates that FSH specificity is determined by amino acid residues of leucine-rich repeats around repeat 6. Binding studies with truncated FSH receptor molecules will show whether the same repeats form the FSH binding site.

The construction of hybrid receptors demonstrates that glycoprotein hormone receptors can be activated by any gonadotropin bound to their extracellular domain. Generally, G protein-coupled receptors seem to perform signal transduction by a conformational shift within the membrane spanning segments, induced by ligand binding. Two models can be envisaged for the signal transduction mechanism in glycoprotein hormone receptors (Figure 5). In one model, hormone binding leads to a conformational change of the entire receptor. A thioredoxin-like catalytic property of gonadotropins (Boniface and Reichert, 1990) might shuffle disulfide bridges inducing and stabilizing an activated state of the membrane spanning segments. In a second model, hormone binding triggers receptor stimulation by the correct



	K_d [nM]	cAMP[fmol/10^3cells]		
	hCG	-	+ hCG	+ rhFSH
FSHR	n.d.	55	20	1100
LFSHR-Sa	0.52 ± 0.08	12	1350	32
LFSHR-X	0.62 ± 0.18	3	600	4
FLHR-X	n.d.	13	3	6
FLHR-B	n.d.	5	5	5
FLHR-A	n.d.	12	32	980
LHR	0.48 ± 0.08	34	1200	20

Fig. 4. Hormone binding and hormone-mediated activation of chimeric LH/CG-FSH receptors. The stippled diagram at the top of the left column represents conserved (darkly shaded) and variable (lightly shaded) regions among LH/CG-, FSH- and TSH-receptors. Positions of conserved cysteine residues in the extracellular domain of all glycoprotein hormone receptors are indicated by filled circles. The individual receptor constructs are schematically outlined. The extracellular domains of the FSH receptor (open structures) and the LH/CG receptor (solid structures) are subdivided into 14 imperfectly duplicated units. Potential N-linked glycosylation sites in the extracellular domains are indicated by filled squares. The amino acid sequence of the LH/CG−FSH receptor junctions are given in one letter code (L = leucine, A = alanine, R= arginine, F = phenylalanine, S = serine, H = histidine and C = cysteine). The columns on the right show ability of the receptor molecules to bind hCG and to stimulate adenylyl cyclase in transiently expressing cultured cells. The dissociation constant K_d (nM) for [^{125}I]hCG was measured in crude extracts of homogenized cells expressing the different receptor molecules, as detailed in Materials and methods. Intracellular cAMP levels were determined 30 min after incubating cells with hCG (2.6 nM), rhFSH (2.6 nM), or in the absence (−) of hormones. The data represent the average of three independent experiments with errors comprising ~20% for all determined cyclic AMP values. n.d. = not detectable.

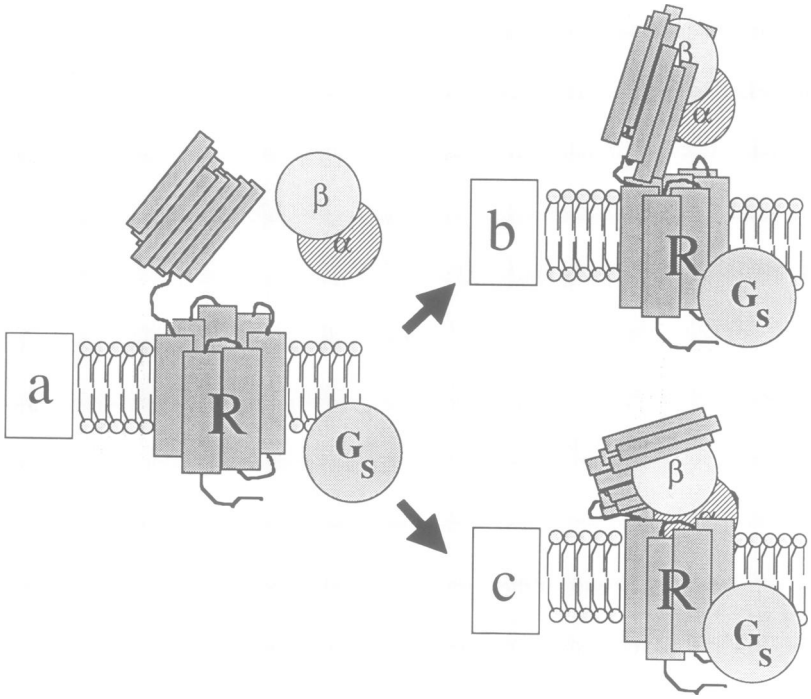


Fig. 5. Models for gonadotropin receptor activation. (**a**) Hormone binds and (**b**) binding leads to a conformational change of the entire receptor molecule, or (**c**) bound hormone is exposed and interacts with a receptor activation site defined by the arrangement of the membrane spanning segments. The activated receptor stimulates G_s protein. R = gonadotropin receptor; G_s = G protein; α/β = glycoprotein hormone.

positioning of the hormone into a receptor activation site defined by the arrangement of the membrane spanning segments. In this case, hormone binding and receptor activation are determined by different sequences. Binding might be mediated by sequences of the hormone specific β-chain and receptor activation by residues of the common α-chain. This would be compatible with the notion that the receptor is activated by amino acid residues and carbohydrate side chains of the a subunit. (Dahl *et al.*, 1988; Matzuk *et al.*, 1989; Sairam 1989 and 1990). The second model proposes a receptor activation site analogous to the ligand binding pocket of other G protein-coupled receptors. The identification of such a site for receptor activation would allow the design of potential receptor agonists and antagonists of a nonpeptidic nature acting at this site. Such compounds would open new opportunities in treating reproductive malfunctions, and could be employed to induce temporary infertility.

Materials and methods

Materials

Highly purified hCG (CR-123; 12 780 IU/mg) was a gift from the National Hormone and Pituitary Program of the NIDDK (NIH). For competition experiments, hCG (5000 IU/mg) was from Sigma. Recombinant human FSH (~10 000 IU/mg) was synthesized and purified by Pacific Biotechnology Ltd., Sydney. Hormones were diluted in phosphate buffered saline (PBS) containing 0.1% bovine serum albumin. Oligonucleotides LH_{336}, LH_{299}, LH_{232} were synthsized with the use of an Applied Biosystems DNA synthesizer under the direction of Dr Michael Nassal.

Plasmids encoding truncated LH/CG receptors

For the construction of expression plasmids encoding truncated LH/CG receptor molecules, unique *Eco*RV restriction sites were introduced at different positions within the LH/CG receptor cDNA by oligonucleotide-directed mutagenesis (Sayers *et al.*, 1988). As template for the mutagenesis we used pBS-LHR, created by subcloning a 2667 bp *Cla*I–*Xba*I restriction fragment (containing the the full-length coding region of the rat LH/CG receptor plus 5′ and 3′ untranslated regions) from pCLHR into pBS/sk$^-$ (Statagene). pCLHR has been previously described as expression vector for the rat luteal LH/CG receptor cDNA (McFarland *et al.*, 1989). A 45mer olignucleotide, LH_{336} (5′-CACATTGGAGTGTCTTGTTTGAACAGT-TGCCGATATCATAATCCCA-3′) was used to introduce an *Eco*RV restriction site (underlined) at nucleotide position 1009. With the 33mer oligonucleotide LH_{299} (5′-CACATTGGAGTGTCTTGTTTGAACAGTT-GCCGATATCATAATCCCA-3′) the same restriction site was introduced at nucleotide position 898, and with the 27mer oligonucleotide, LH_{232} (5′-CAATTTGGTGGAAGAGATATCCAGGAT-3′) at nucleotide position 697. Nucleotide positions are according to McFarland *et al.*, (1989). The *Eco*RI restriction site at the 5′ end of the cDNA and the mutagenesis-derived *Eco*RV sites were used for isolating cDNA fragments encoding part of the rat LH/CG receptor extracellular domain. Those fragments were ligated into a eukaryotic expression vector (Gorman *et al.*, 1988; Gorman *et al.*, 1990), except that stop codons were introduced in all three frames at the 3′ end of the cDNA insertion site. The deletion construct LH_{364} was obtained by cloning an *Eco*RI–*Eco*NI (nucleotides −43 to 1096) cDNA restriction fragment into the same expression vector. The ensuing plasmids pCLH336, pCLH299, pCLH232 and pCLH364 encode rat luteal LH/CG receptors truncated after asparagine 310, serine 273, asparagine 206 and alanine 338 respectively, of the mature receptor molecule. Numbers in the plasmid name give the length of the truncated receptor molecules in amino acids including the signal peptide.

Plasmids encoding chimeric FSH-LH/CG receptors

LH/CG-FSH receptor hybrids were constructed using the expression plasmids pRFSHR-3, and pRLHR-3. In pRFSHR-3, the entire coding region of the testicular rat FSHR cDNA is inserted as *Eco*RI–*Bam*HI restriction fragment (nucleotides −71 to 2095; Sprengel *et al.*, 1990) into a cytomegalovirus promoter based expression vector (Gorman *et al.*, 1988 and 1990). For plasmid pRLHR-3, a LH/CG receptor cDNA fragment (nucleotides −43 to 2173; *Eco*RI–*Ssp*I fragment) from pBS-LHR (see above) was introduced into the same expression vector. Plasmids containing chimeric receptors were constructed by replacing internal FSHR cDNA fragments from pRFSHR-3 with equivalent fragments from the LH/CG receptor cDNA. As LH/CG-FSH cDNA junctions, common *Sau*I (pLFSHR-Sa), *Bsp*MI (pFLHR-B), *Alw*NI (pFLHR-A), and *Xmn*I restriction sites (pLFSHR-X and pFLHR-X) were selected. The chimeric receptors are joined between amino acids leucine 340/arginine 348 (LFSHR-Sa), alanine 146/phenylalanine 148 (LFSHR-X), serine 147/phenylalanine 147 (FLHR-X), alanine 172/phenylalanine 172 (FLHR-B) and histidine 257/cysteine 257 (FLHR-A). Amino acids of the putative native receptors are numbered according to Sprengel *et al.* (1990).

Transfections

Human embryonic kidney cells 293 (ATCC CRL 1573) were maintained in minimum essential medium (MEM) with Earle's salts (Gibco) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and 10% heat inactivated fetal calf serum in a humidified atmosphere containing 5% CO_2. Cells 50–70% confluent were transfected as described (Chen and Okayama, 1986). Growth medium was replaced 18 h after transfection. To select stably transfected cells, plasmid pMC1neo (Stratagene) was added to the transfection cocktail and cells were selected by Geneticin (G418, 800 μg/ml). Resistant foci were isolated and receptor expression monitored by hCG binding assays.

Binding assays

Embryonic kidney cells were transfected with recombinant expression vector encoding truncated and chimeric receptor sequences (10 μg plasmid DNA/10 cm plate). 48 h after transfection, cells were washed twice in PBS (37°C) and were harvested by intense rinsing using ice cold PBS. Cells were spun (5000 *g*, 10 min), resuspended in buffer containing 125 mM Tris/Cl pH 7.5, 5 mM EDTA, and homogenized using and ultraturrax (level 7, 30 s). After a one-step separation (100 000 *g*; 1h), supernatant and crude membranes were diluted in 125 mM Tris/Cl pH 7.5 EDTA, 0.1% BSA. Samples from each fraction were incubated in a final volume of 300 μl for 12 h at 4°C with [^{125}I]hCG. Iodination of hCG was as described (McFarland *et al.*, 1989). Thereafter, 3 ml of ice-cold PBS containing 0.1% BSA was added to each tube and receptor-bound and free hormone were separated by filtration over GF/C filters (Schleicher and Schüll, no.34) presoaked in 0.3% polyethylenimine. Filters were washed twice with 3 ml PBS, 0.1% BSA. Nonspecific binding was determined in the presence of 1 μg of hCG per assay and was generally 1–3% of total radioactivity added. The binding data were used to construct a Scatchard plot to obtain the equilibrium dissociation constant (K_d) and maximal binding capacity (B_{max}). All assays were done in triplicates.

Cyclic AMP assays

To determine hormone mediated cAMP production exponentially-growing human embryonic kidney cells 293 were transfected (see above) with recombinant expression vectors encoding native and chimeric receptor sequences. 48 h after transfection, cells were washed twice in PBS and harvested by rinsing. 2.5×10^5 viable cells per assay were pre-incubated for 10 min in 0.5 ml PBS containing 0.1 mM IBMX (3-isobutyl-1-methylxanthine, Sigma) to inhibit cyclic AMP phosphodiesterase. Stimulation was performed for 30 min at 37°C in the presence of hCG (2.6 nM), rhFSH (2.6 nM) and in the absence of hormones (BSA; 1 μg/ml) Hormone treatment was stopped by adding 100 μl 3.9 M perchloric acid. Cell debris were removed by centrifugation and 250 μl of the supernatant was neutralized by adding potassium carbonate to a final concentration of 0.2 M. After 30 min incubation on ice, precipitated salt was removed by centrifugation and 200 μl of supernatant was equilibrated using 50 μl, 1 M sodium acetate buffer (pH 6.0) and 1 and 5 μl were assayed for cAMP using an Amersham kit (RPA.508). Cyclic AMP levels were calculated using a calibration curve determined in parallel by the addition of cylic nucleotide in the range between 0 and 1600 fmoles.

Hormone cross-linking

Transfected cells were homogenized in PBS ane crude membranes were solubilized in PBS, 1% Triton X-100. Aliquots were incubated with 100 000 c.p.m. of [^{125}I]hCG in the absence or presence of 3 μg of unlabeled hCG at 4°C overnight. The receptor hormone complexes were cross-linked by adding 5 μl of 0.1 M glutaraldehyde to the incubation mixture and the incubation was continued for 1 h at room temperature. The reaction was stopped by the addition of 1 M Tris/HCl, pH 7.5 to a final concentration of 50 mM.

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References

Boniface,J.J. and Reichert,L.E.Jr (1990) *Science*, **247**, 61–64.

Braun,T. and Sprengel,R. (1991) In Hunzicker-Dunn,M. and Schwartz,N. (eds) *Regulation and Actions of Follicle Stimulating Hormone*, Serono Symposia, USA, in press.

Chen,C. and Okayama,H. (1987) *Mol. Cell. Biol.*, **7**, 2745–2751.

Dahl,K.D., Bicsak,T.A. and Hsueh,A.J.W. (1988) *Science*, **239**, 72–74.

Dixon,R.A.F., Sigal,J.S., Rands,E., Register,R.B., Candelore,M.R., Blake,A.D. and Strader,C.D. (1987) *Nature*, **326**, 73–77.

Field,J., Xu,H.P., Michaeli,T., Ballester,R., Sass,P., Wiggler,M. and Collicelli,J. (1989) *Science*, **247**, 464–467.

Frazier,A.L., Robbins,L.S., Stork,P.J., Sprengel,R., Segaloff,D.L. and Cone,R.D. (1990) *Mol. Endocrinol.*, **4**, 1264–1276.

Gorman,C.M., Gies,D.R., McCray,G. and Huang,M. (1989) *Virology*, **171**, 377–387.

Gorman,C.M., Gies,D.R. and McCray,G. (1990) *DNA Prot. Engin. Technol.*, **2**, 3–10.
Hashimoto,C., Hudson,K.L. and Anderson,K.V. (1988) *Cell*, **52**, 269–279.
Hofsteenge,J., Kieffer,B., Marthies,R., Hemmings,B.A. and Stone,S.R. (1988) *Biochemistry*, **27**, 8537–8544.
Hunzicker-Dunn,M. and Birnbaumer,L. (1985) In Ascoli,M. (ed.) *Luteinizing Hormone Actions and Receptors*. CRC Press, Boca Raton, p. 57.
Kataoka,T., Broek,D. and Wigler,M. (1986) *Cell*, **43**, 493–505.
Kobilka,B.K., Kobilka,T.S., Daniel,K., Regan,J.W., Caron,M.G. and Lefkowitz,R.J. (1988) *Science*, **240**, 1310–1316.
Krantz,D.E. and Zipursky,S.L. (1990) *EMBO J.*, **9**, 1969–1977.
Landschulz,W.H., Johnson,P.F. and McKnight,S.L. (1988) *Science*, **240**, 1759–1764.
Libert,F., Lefort,A., Gerard,C., Parmentier,M., Perret,J., Ludgate,M., Dumont,I.B. and Vassart,G. (1989) *Biochem. Biophys. Res. Comm.*, **165**, 1250–1255.
Limbird,L. (1981) *Biochem. J.*, **195**, 1–13.
Loosfelt,H., Misrahi,M., Atger,M., Salesse,R., Thi,M.T.V., Jolivet,A., Guiochon-Mantel,A., Sar,S., Jallal,B., Garnier,I. and Milgrom,E. (1989) *Science*, **245**, 525–528.
Lopez,J.A., Chung,D.W., Fujikawa,K., Hagen,F.S., Papayanopoulou,T. and Roth G.J. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 5615–5619.
Lopez,J.A., Chung,D.W., Fujikawa,K., Hagen,F.S., Davie,E.W. and Roth G.J. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 2135–2139.
Matzuk,M.M., Keene,J.L. and Boime,I. (1989) *J. Biol. Chem.*, **264**, 2409–2414.
McFarland,K.C., Sprengel,R., Phillips,H., Köhler,M., Rosemblit,N., Nikolics,K., Segaloff,D. and Seeburg,P.H. (1989) *Science*, **245**, 494–499,
Nagayama,Y., Kaufman,K.D., Seto,P. and Rapaport,B. (1989) *Biochem. Biophys. Res. Comm.*, **165**, 1184–1190.
Parmentier,M., Libert,F., Maenhaut,C., Lefort,A., Gérard,C., Perret,I., van Sande,I., Dumont,I.E. and Vassart,G. (1989) *Science*, **245**, 1620–1622.
Patthy,L. (1987) *J. Mol. Biol.*, **198**, 567–577.
Pierce,J.G. and Parsons,T.F. (1981) *Annu. Rev. Biochem.*, **50**, 465–495.
Reichert,L.E.Jr and Dattatreyamurty,B. (1989) *Biol. Reprod.*, **40**, 13–26.
Reinke,R., Krantz,D.E., Yen,D. and Zipursky,S.L. (1988) *Cell*, **52**, 291–301.
Sairam,M.R. (1989) *FASEB J.*, **3**, 1915–1926.
Sairam,M.R., (1990) *Biochem. J.*, **265**, 667–674.
Sayers,J.R., Schmidt,W. and Eckstein,F. (1988) *Nucleic Acids Res.*, **16**, 791–802.
Sprengel,R., Braun,T., Nikolics,K., Segaloff,D.L. and Seeburg,P.H. (1990) *Mol. Endocrinol.*, **4**, 525–530.
Strader,C.D., Sigal,J.S., Register,R.B., Candelore,M.R., Rands,E. and Dixon,R.A.F. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 4384–4388.
Strader,C.D., Sigal,I.S. and Dixon,R.A.F. (1989) *FASEB J.*, **3**, 1825–1832.
Strickland,T.W., Parsons,T.F. and Pierce,J.G. (1985) In Ascoli,M. (ed.) *Luteinizing Hormone Action and Receptors*. CRC Press, Boca Raton, FL. p. 1; Roche,P.C. and Ryan,R.J., ibid., p. 17.
Suzuki,N., Choe,H.-R., Nishida,Y., Yamawaki-Kataoka,Y., Ohnishi,S., Tamaoki,T. and Kataoka,T. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 8711–8715.
Takahashi,N., Takahashi,Y. and Putnam,F.W. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 1906–1910.
Titani,K., Takio,K., Handa,M. and Ruggeri,Z.M. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 5610–5614.
Tsai-Morris,C.H., Buczko,E., Wang,W. and Dufau,M.L. (1990) *J. Biol. Chem.* **265**, 19385–19388.
Wadsworth,H.L., Chazenbalk,G.D., Nagayama,Y., Russo,D. and Rapoport,B. (1990) *Science*, **249**, 1423–1425.
Xie,Y.-B., Wang,H. and Segaloff,D.L. (1990) *J. Biol. Chem.*, **265**, 21411–21414.
Yamawaki-Kataoka,A., Tamaoki,T., Choe,H.-R., Tanaka,H., and Kataoka,T. (1989) *Proc. Natl. Acad. Sci.*, **86**, 5693–5697.

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