Significance of the carbohydrate moiety of the rat ovarian luteinizing-hormone/chorionic-gonadotropin receptor for ligand-binding specificity and signal transduction

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The contribution of the carbohydrate moiety of the rat ovarian luteinizing-hormone (LH)/chorionic-gonadotropin (CG) receptor to ligand-binding specificity and signal transduction was investigated by using glycosidases. Purified membranes from pseudo-pregnant rat ovaries were treated with neuraminidase or peptide N-glycosidase F, to remove terminal sialic acids and N-linked oligosaccharides of the receptor, respectively. Ligand blotting and densitometric scanning of the autoradiograms showed that 90–95% of the receptors were deglycosylated, and that desialylation was virtually complete. Neither the desialylated nor the deglycosylated receptors were able to bind human follicle-stimulating hormone or bovine thyroid-stimulating hormone, as revealed by competition binding experiments. The 50% effective dose of hCG for adenylate cyclase activation, as determined by

measuring the formation of cyclic [32 P]AMP from [α - 32 P]ATP for 15 min at 30 °C, was similar in the control and deglycosylated membranes: 10.2 ± 3.3 nM and 12.2 ± 3.8 nM respectively. The same was true for the time course of the basal, hCG- and forskolin-stimulated enzyme activity. In addition, removal of oligosaccharides from the receptor did not restore the ability of desialylated hCG, nor of the deglycosylated hormone, to stimulate adenylate cyclase. In conclusion, the carbohydrate moiety of the native membrane-inserted rat ovarian LH/CG receptor does not contribute to the ligand-binding specificity, and it is not required for the functional coupling of the occupied receptor and the adenylate cyclase system. These functions are associated with the polypeptide portion of the receptor.

INTRODUCTION

Luteinizing hormone (LH) and its agonist, human chorionic gonadotropin (hCG), regulate gonadal functions by binding to a common high-affinity receptor on the surface of target cells. This results in the G-protein-mediated activation of plasma-membrane adenylate cyclase, and the subsequent increase in intracellular cyclic AMP (cAMP) ultimately stimulates steroid biosynthesis and secretion. The LH/CG receptor belongs to the G-protein-coupled receptor family [1–3]. It is a transmembrane glycoprotein that contains six potential sites for N-linked glycosylation in its extracellular domain. However, not all of the potential sites are used [4]. By using exo- and endo-glycosidases, it has been shown that the receptor contains no O-linked oligosaccharides and the N-linked chains are sialylated and of the complex type [4–8].

The deglycosylated LH/CG receptor binds native hCG [4,5,9], as well as the desialylated and deglycosylated hormone [4] with the same affinity, but otherwise the functional role of the oligosaccharides has not yet been elucidated. In the present study, we have employed glycosidases to investigate the role of the N-linked oligosaccharides in the hormone recognition and binding specificity of the LH/CG receptor and in the transmission of the hormonal signal from the occupied receptor to adenylate cyclase. This approach provides a useful strategy for investigation of the structure and function of the oligosaccharides of the native receptor, once the latter has been inserted into the plasma membrane in a functionally mature conformation. Site-directed mutagenesis of the putative N-glycosylation sites, and the use of

glycosylation inhibitors, are alternative methods, but they are better suited to a study of the effects of glycosylation on the acquisition of hormone-binding ability, or on the transport and correct insertion of the receptor into the plasma membrane. The evidence presented here indicates that the oligosaccharides of the membrane-inserted LH/CG receptor have no functional significance for either the ligand-binding specificity or the hormone-stimulated adenylate cyclase activation. These functional properties are thus inherent to the polypeptide backbone of the receptor.

MATERIALS AND METHODS

Chemicals and hormone derivatives

Peptide N-glycosidase F (EC 3.2.2.18, from *Flavobacterium meningosepticum*) was obtained from Boehringer-Mannheim (Mannheim, Germany) and neuraminidase (EC 3.2.1.18, from *Vibrio cholerae*) from Calbiochem (San Diego, CA, U.S.A.). Pregnant-mare-serum gonadotropin (PMSG; 2820 i.u./mg) and hCG (5600 i.u./mg), which were used to induce pseudo-pregnancy, were obtained from Diosynth (Oss, Netherlands). Human follicle-stimulating hormone [hFSH; 7000 i.u./mg; LH contamination less than 2% and thyroid-stimulating hormone (TSH) contamination less than 1%] and hTSH (7 i.u./mg; LH and FSH contamination about 1%) were obtained from Sigma (St. Louis, MO, U.S.A.), and bovine TSH (bTSH; 20 i.u./mg; LH contamination less than 2% and FSH contamination less than 1%) was from UCB (Braine l'Alleud, Belgium). Na 125 I, [α - 32 P]ATP (30 Ci/mmol) and [3 H]cAMP (24 Ci/mmol) were

Abbreviations used: cAMP, cyclic AMP; CG, chorionic gonadotropin; FSH, follicle-stimulating hormone; G $_s$, G-protein that mediates stimulation of adenylate cyclase; LH, luteinizing hormone; PBS, phosphate-buffered saline (10 mM sodium phosphate, pH 7.4, containing 140 mM NaCl); PMSG, pregnant-mare-serum gonadotropin; TSH, thyroid-stimulating hormone; the prefixes b and h refer to bovine and human respectively.

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from Amersham International (Amersham, Bucks., U.K.). The equipment and reagents for electrophoresis and blotting were from Bio-Rad (Richmond, CA, U.S.A.). All other reagents were obtained from Sigma.

hCG (12000 i.u./mg) was purified from crude hCG (Schering, Berlin, Germany), and the hormone was desialylated and deglycosylated essentially as described previously [4]. hCG was radio-iodinated with Na^{125}I by the chloramine-T method [10], as described in [4]. The specific radioactivity of the preparations was 55–80 Ci/g, as determined by self-displacement assay [11]. The fraction of the labelled hormone capable of binding to an excess of ovarian membranes was 40–55 %.

Animals

Immature 27-day-old female Sprague–Dawley rats were rendered pseudo-pregnant by sequential injections of PMSG (40 i.u.) and hCG (25 i.u.), as described in [12]. The luteinized ovaries were collected 7–9 days after hCG injection.

Purification and enzymic digestions of ovarian membranes

The luteinized ovaries were freed from extraneous tissue and minced in a medium containing 1.0 mM NaHCO_3 and 0.2 mM MgCl_2 (250 mg of tissue/ml). The minced tissue was allowed to swell for 20 min and was then homogenized in a Dounce homogenizer (Kontes Glass Co., Vineland, NJ, U.S.A.) with 15 strokes of a loose pestle, and the homogenate was centrifuged at 800 *g* for 5 min. The homogenization and the centrifugation were repeated three times. The supernatants were combined and the crude plasma-membrane particles were purified by the flotation method, as described in [13]. This procedure gave an average 5-fold purification of the membranes, as determined by ^{125}I -hCG binding.

The purified membranes were treated with neuraminidase and peptide N-glycosidase F, as described previously [4,5], except that the incubation buffer, 0.1 M sodium phosphate (pH 7.0), contained 1.0 mM EDTA (peptide N-glycosidase F) or 0.2 mM phenylmethanesulphonyl fluoride (neuraminidase). The pelleted membranes were stored at -70°C , and they retained adenylate cyclase activity for at least 3 weeks.

To obtain the solubilized receptor for ligand-blotting analysis, 50 μg of the pelleted membranes was solubilized by incubating them at 20°C for 120 min, in 200 μl of SDS-sample buffer containing 2 % (w/v) SDS, 125 mM Tris/HCl (pH 6.8), 10 % (v/v) glycerol and 0.001 % (w/v) Bromophenol Blue. Before SDS/PAGE, the samples were centrifuged at 13000 *g* for 20 min at 20°C .

Hormone-binding assays

The hormone-binding characteristics of the membrane-bound receptor were determined by competition binding experiments. Membrane samples (5 μg of protein) were incubated with ^{125}I -hCG (4 ng) in the absence or presence of increasing concentrations of unlabelled hCG (0.04–400 ng/ml), hFSH (4–2000 ng/ml), bTSH (4–2000 ng/ml) or hTSH (4–2000 ng/ml), in a final volume of 250 μl of 10 mM phosphate-buffered saline (PBS, pH 7.4), containing 0.1 % (w/v) BSA (buffer A), for 16 h at 20°C . Thereafter, 1 ml of ice-cold buffer A was added and the tubes were centrifuged at 13000 *g* for 20 min. The pellets were resuspended in buffer A, centrifuged and counted for ^{125}I radioactivity. Non-specific binding was determined in the presence of an excess of unlabelled hCG (2 μg). The half-maximal inhibition of hCG binding was determined graphically from

competitive displacement curves. In order to determine the maximum binding capacities, membrane samples (5 μg of protein) were incubated with increasing amounts (0.5–25 ng) of ^{125}I -hCG in the absence or presence of an excess of unlabelled hormone (2 μg), and the binding data were transferred to Scatchard plots [14].

Adenylate cyclase assay

Adenylate cyclase activity was determined at 30°C in medium containing 1.0 mM ATP (with $(1-2) \times 10^6$ c.p.m. of $[\alpha\text{-}^{32}\text{P}]\text{ATP}$), 5 mM MgCl_2 , 1.0 mM EDTA, 0.25 mM isobutylmethylxanthine, 0.01 % BSA, 0.1 mM GTP, 1.0 mM cAMP, 10 mM phosphocreatine, 10 units of creatine kinase and 25 mM Tris/HCl (pH 7.6). When present, and unless indicated otherwise, the following concentrations of stimulators were used: hCG, 5 $\mu\text{g}/\text{ml}$; desialylated hCG, 5 $\mu\text{g}/\text{ml}$; deglycosylated hCG, 5 $\mu\text{g}/\text{ml}$; forskolin, 100 μM . Stock solutions of hormones were prepared by dissolving them in 25 mM Tris/HCl (pH 7.6)/0.01 % BSA (buffer B) to a concentration of 10 mg/ml, and they were diluted from frozen stock solutions in buffer B immediately before use for individual assays. Basal adenylate cyclase values were determined in the presence of buffer B alone. A stock solution of 20 mM forskolin was prepared by dissolving in 98 % ethanol.

The reaction was initiated by addition of the membrane suspension (5–20 μg of protein) to the incubation medium. Fixed-time assays were conducted in a final volume of 200 μl and were carried out for 15 min at 30°C . These assays were stopped by addition of 20 μl of stopping solution containing 10000 c.p.m. of $[\text{H}^3]\text{cAMP}$ in 2.2 M HCl. The tube contents were heated at 95°C for 4 min, and then cooled in an ice/water bath. Time courses of cAMP accumulation were carried out by incubating larger volumes, removing 200 μl samples at the indicated times, and stopping the reaction by addition to 20 μl of stopping solution. Assay blanks were prepared by omitting the membranes from the incubation medium. The $[\text{H}^3]\text{cAMP}$ formed and the $[\text{H}^3]\text{cAMP}$ added to monitor recovery were isolated by the method of Alvarez and Daniels [15], using alumina chromatography. cAMP recoveries averaged 80–95 %. The $[\text{H}^3]\text{cAMP}$ and $[\text{H}^3]\text{cAMP}$ present in the column eluates were quantified by radioactivity counting in a LKB 12009 Rackbeta liquid-scintillation counter (LKB Wallac, Sweden), and counts were converted into nucleotide concentrations. Conversion of ATP into cAMP in blank tubes ranged from 0.005 to 0.01 % of the added $[\alpha\text{-}^{32}\text{P}]\text{ATP}$. The half-maximal stimulation of the adenylate cyclase activity was determined graphically from dose-response curves.

SDS/PAGE and ligand blotting

SDS/PAGE was performed as described by Laemmli [16], using 4 % stacking gels and 7.5 % separating gels. Samples containing the solubilized LH/CG receptor were subjected to SDS/PAGE under non-reducing conditions and without prior heating. Prestained molecular-mass markers (SDS-7B; Sigma) were used to calibrate the gels. The proteins resolved by SDS/PAGE were electrophoretically transferred from the gels on to nitrocellulose sheets (Schleicher and Schuell, Dassel, Germany; 100 V, 60 min, Bio-Rad Mini-Trans Blot apparatus) and processed further as described previously [17]. For detection of radioactivity, the dried nitrocellulose sheets were exposed, at -70°C for 5–21 days, to Kodak XAR-5 film (Eastman Kodak, Rochester, NY, U.S.A.) with an intensifying screen (Kodak X-Omatic). The extents of desialylation and deglycosylation of all membrane preparations used were tested by ligand blotting. The relative

Figure 1 Identification of the rat ovarian LH/CG receptor by ligand blotting after neuraminidase and peptide N-glycosidase F treatment of the membranes

Purified membranes (0.5 mg of protein/ml) were incubated alone, with neuraminidase (60 m-units/ml), or with peptide N-glycosidase F (16 units/ml) for 120 min at 30 °C. After digestion, the membranes were washed and solubilized in SDS-sample buffer. The solubilized receptor was subjected to SDS/PAGE under non-reducing conditions (7.5%-acrylamide slab gel) and electroblotted on to a nitrocellulose sheet. The latter was incubated with ^{125}I -hCG (2 ng/ml) in the absence or presence of an excess of unlabelled hCG for 16 h at 20 °C. M_r markers are indicated on the right (from the top: α_2 -macroglobulin, β -galactosidase, 6-phosphofructokinase, pyruvate kinase and fumarase).

quantities of the labelled bands on the autoradiograms were analysed by densitometric scanning using an image analysis processor M2 (Imaging Research, Brock University, Ontario, Canada).

Miscellaneous

Protein was assayed by the method of Bradford [18] with BSA as standard. Statistical analyses were performed by Student's *t* test. Each experiment was performed in triplicate and repeated at least three times using at least two different membrane batches. Unless otherwise indicated, the results show a single representative experiment rather than the average of several experiments.

RESULTS

Desialylation and deglycosylation of the ovarian membranes

Purified rat ovarian membranes were treated with neuraminidase or peptide N-glycosidase F, to remove terminal sialic acids or N-linked oligosaccharides from the LH/CG receptor, respectively. Treatment of the membranes with neuraminidase decreased the M_r of the receptor from 90 000 to about 79 000, whereas peptide N-glycosidase F decreased the M_r to about 62 000 (Figure 1). Densitometric scanning of the autoradiograms showed that, in the different membrane preparations used, only 5–10% of the receptors were unresponsive to the peptide N-glycosidase F treatment, whereas removal of sialic acids by neuraminidase was virtually complete.

Effect of desialylation and deglycosylation of the LH/CG receptor on ligand-binding specificity

The contribution of the N-linked oligosaccharides of the LH/CG receptor to the hormone-binding specificity was assessed by

Figure 2 Competitive displacement of ^{125}I -hCG binding to control (a), desialylated (b) and deglycosylated (c) rat ovarian membranes by hCG, hFSH and bTSH

Membranes (5 μg of protein/250 μl) were incubated for 16 h at 20 °C with 4 ng of ^{125}I -hCG and the indicated concentrations of unlabelled hormone, as described in the Materials and methods section. Non-specific binding was determined in the presence of an excess (2 μg) of unlabelled hCG. Binding in the absence of unlabelled hormone was regarded as 100%. The maximum binding was 2.77 ± 0.41 , 2.78 ± 0.10 and 2.55 ± 0.17 fmol/ μg (mean $\pm$ S.D., determined from three experiments) for the control, desialylated and deglycosylated membranes respectively. Each point in this and all subsequent Figures represents the mean of triplicate determinations, and the experiments were repeated with similar results at least three times.

determining the ability of the membrane-bound desialylated and deglycosylated receptor to bind hCG, hFSH, bTSH and hTSH in competition binding experiments. As expected, hCG inhibited the binding of ^{125}I -hCG to the control, desialylated and deglycosylated membranes in similar dose-dependent manners (Figure 2). The half-maximal inhibition of binding was obtained at concentrations of 0.22 ± 0.03 nM, 0.21 ± 0.06 nM and 0.19 ± 0.08 nM respectively, assuming an M_r of 37 900 for hCG (the values are means $\pm$ S.D., derived from three experiments). hFSH and bTSH produced respectively about 30% and 40%

Figure 3 Effect of various hCG concentrations on the adenylate cyclase activity in control and deglycosylated rat ovarian membranes

Membranes (60 μ g of protein/ml) were incubated for 15 min at 30 °C with increasing concentrations of hCG (0.05–50 000 ng/ml), as described in the Materials and methods section. The basal activity was 1.32 and 1.36 nmol of cAMP/15 min per mg for the control and deglycosylated membranes respectively.

inhibition of 125 I-hCG binding at the highest concentrations tested (2 μ g/ml) (Figure 2), which probably resulted from LH contamination of the hormone preparations. No differences in binding inhibition with hFSH, bTSH or hTSH (results not shown) were detected in the membrane preparations tested, indicating that the carbohydrates of the LH/CG receptor are not required for the specificity of hormone recognition or binding. In addition, Scatchard analysis of the hCG-binding showed that the maximum binding capacities were similar for all membrane preparations used (results not shown), indicating that there were no differences in the number of receptors.

Effect of deglycosylation of the LH/CG receptor on adenylate cyclase activation

The role of carbohydrates in the functional coupling of the LH/CG receptor to the adenylate cyclase system was investigated by first determining the dose-responsiveness of the enzyme activity towards hCG. The adenylate cyclase was similarly stimulated by hCG in a concentration-dependent manner in the control and deglycosylated membranes (Figure 3). A significant increase above basal activity was observed at 0.05 μ g/ml, with a progressive dose-related increase that reached a maximum at 12.5 μ g/ml. The mean half-maximal stimulation of adenylate cyclase activity, determined from three separate experiments, was essentially similar in the control and deglycosylated membranes: 10.2 ± 3.3 nM and 12.2 ± 3.8 nM (mean $\pm$ S.D.) respectively.

Having determined that the dose-responsiveness of adenylate cyclase to hCG was similar in the control and deglycosylated membranes, we next examined whether there were differences in the time course of the enzyme activity. Adenylate cyclase activation was monitored for 60 min (Figure 4). The basal stimulation rate was low, but uniform, throughout the whole period. In the presence of 5 μ g/ml hCG, the stimulation was about 3–4-fold higher than that observed in the absence of hCG, but this rate was maintained for only about 20 min. Thereafter, the activity declined and approached the basal activity, suggesting that the enzyme system became desensitized to hCG stimulation

Figure 4 Time courses of activation of adenylate cyclase in control (a) and deglycosylated (b) rat ovarian membranes

Membranes (60 μ g of protein/ml) were incubated for various times at 30 °C, in the absence of stimulators (basal), or in the presence of either hCG (5 μ g/ml) or forskolin (100 μ M), as described in the Materials and methods section.

Table 1 Stimulation of the adenylate cyclase in the control and deglycosylated rat ovarian membranes by hCG, desialylated hCG and deglycosylated hCG

Membranes (60 μ g of protein/ml) were incubated for 15 min at 30 °C, in the absence or presence of hCG (5 μ g/ml), desialylated hCG (5 μ g/ml) or deglycosylated hCG (5 μ g/ml). The results presented are means $\pm$ S.D. of three experiments performed in triplicate. The fold stimulation of adenylate cyclase is shown in parentheses.

Membranes...	Adenylate cyclase activity (nmol of cAMP/15 min per mg)	
	Control	Deglycosylated
Basal activity	1.64 ± 0.08	1.60 ± 0.07
hCG (5 μ g/ml)	4.67 ± 0.53 (2.84)	4.76 ± 0.15 (2.97)
Desialylated hCG (5 μ g/ml)	2.79 ± 0.22 (1.70)	2.60 ± 0.26 (1.63)
Deglycosylated hCG (5 μ g/ml)	1.67 ± 0.19 (1.02)	1.46 ± 0.04 (0.91)

upon exposure to hCG. No differences were observed, however, in the time course of the hCG-stimulated adenylate cyclase activity in the control and deglycosylated membranes. Forskolin (100 μ M) also stimulated the activity in a similar manner in both membrane preparations. The stimulation was about 10-fold and remained constant throughout the incubation period. The NaF (10 mM)-stimulated activity was also linear for up to 60 min (results not shown). Since forskolin-stimulated activity was similar in the control and deglycosylated membranes, peptide N-glycosidase F treatment did not affect the function of the adenylate cyclase molecule itself. This is important, because it

has been shown previously that the enzyme may be glycosylated [19] and is probably also deglycosylated.

The ability of the desialylated and deglycosylated hCG to stimulate the adenylate cyclase activity was also examined (Table 1). At 5 µg/ml, the former gave 55–60% stimulation compared with native hCG, whereas the latter was completely unable to stimulate the enzyme, irrespective of the presence or absence of oligosaccharides on the receptor molecule.

DISCUSSION

The present results show that the polypeptide core of the membrane-bound rat ovarian LH/CG receptor not only binds the hormone [4], but also dictates the hormone-binding specificity, as hFSH and bTSH were not able to bind to the desialylated or deglycosylated receptor in competition binding experiments. Similarly, the removal of carbohydrates from the β_2 -adrenergic [20,21] and D_2 -dopaminergic [22] receptors has no effect on ligand recognition and binding. The lack of effect of glycosylation on the ligand-binding specificity has also been demonstrated for the β_2 -adrenergic receptor expressed in *Escherichia coli*, an organism in which post-translational modifications such as glycosylation do not take place [23]. Moreover, the non-glycosylated β_2 -adrenergic receptor synthesized in a cell-free system was able to bind agonists and antagonists in a normal manner [24]. On the other hand, the carbohydrates of the glycoprotein hormones may have some influence on binding specificity, as it has been shown that desialylated and deglycosylated forms of hCG can bind to the FSH [25] and TSH [26,27] receptors.

Our results also show that the carbohydrate moiety of the rat ovarian LH/CG receptor is not needed for the activation of the receptor–hormone complex, as the receptors on deglycosylated membranes were able to stimulate the hormone-sensitive adenylate cyclase. Both the concentration- and the time-dependent activation of the enzyme were essentially similar in the control and deglycosylated membranes. Thus the deglycosylated membrane-bound LH/CG receptor either retains its functional conformation, or permits the conformational changes that are required for the coupling and uncoupling of the occupied receptor and the stimulatory G-protein (G_s). This seems to be true also for the other G-protein-coupled receptors. Adenylate cyclase activation in membranes of S49 cells, containing the β_2 -adrenergic receptor, was not changed after treatment with the glycosylation inhibitors, tunicamycin and swainsonine [28]. Similarly, the purified deglycosylated β_2 -adrenergic receptors from hamster lung were able to couple normally with G_s proteins in a reconstituted system [21]. The non-glycosylated β -adrenergic and m_2 -muscarinic acetylcholine receptors obtained by site-directed mutagenesis also interact normally with their respective G-proteins, the former resulting in adenylate cyclase activation [29] and the latter in its inhibition [30]. However, results indicating the contrary have also been published. Boege et al. [31] discovered an impaired coupling of the β_2 -adrenergic receptor with the G_s protein and adenylate cyclase in a reconstituted system from tunicamycin-treated A431 cells.

Although the carbohydrate moiety of the LH/CG receptor does not seem to have any functional role in signal transduction, that of the hormone, particularly of its α -subunit, is needed for receptor–hormone complex activation, as revealed in numerous reports (for review, see [32]). Removal of carbohydrates from LH or hCG, by either enzymic or chemical means, lowers the biological activity. The relative potency of these modified hormones in activating the adenylate cyclase system and steroidogenesis is either decreased or abolished. This is also true

for the non-glycosylated hCG obtained by site-directed mutagenesis [33]. It has been suggested that the oligosaccharides of the hormone may either contribute to the active conformation of the molecule [34–37], or they may participate directly in the activation of adenylate cyclase by interacting with the receptor, or other plasma-membrane components [38]. In the present study, removal of the oligosaccharides from the LH/CG receptor did not restore the ability of the deglycosylated hCG to stimulate the adenylate cyclase, although it has been shown to interact normally with the deglycosylated receptor [4].

The present results indicate that the oligosaccharides of the LH/CG receptor have no functional role once the receptor molecules have been incorporated into the plasma membrane, as far as ligand binding and activation of the receptor–hormone complex are concerned. This does not, however, rule out the possibility that they may still have an important role in, for example, the intracellular transport, membrane insertion or recycling of the receptor. Indeed, blockade of N-glycosylation by tunicamycin has been shown to abolish ^{125}I -hCG binding to mouse Leydig tumour cells and rat granulosa cells [9,39]. Similarly, in a recent report by Zhang et al. [40], it was shown that the mutagenesis of three of the possible N-glycosylation sites of the rat LH/CG receptor resulted in either the complete or partial cessation of ^{125}I -hCG binding to transfected COS1 cells. Although blockade of N-glycosylation impairs the normal expression of many receptors [41–45], it is not a universally conserved feature. For example, the asialoglycoprotein [46] and interferon- γ [47] receptors are expressed normally in their non-glycosylated forms.

N-glycosylation may also be important in the biosynthetic process of LH/CG receptor activation and the acquisition of the correct ligand-binding conformation. This event is a necessary intracellular step in the acquisition of the ligand-binding ability of the insulin, epidermal growth factor and nicotinic acetylcholine receptors (for review, see [48]). It has also been suggested that this process might be related to a glycosylation-dependent formation of a correct disulphide-bonded structure in the cysteine-rich regions of the extracellular domain (for review, see [48]). As far as the LH/CG receptor is concerned, the late formation or rearrangement of improperly formed disulphide bonds is worth considering. The extracellular domains of the glycoprotein hormone receptors constituting the ligand-binding site have conserved cysteine residues at their N- and C-termini [1–3,49–54], and intact disulphide bonds are required for the ligand-binding activity of the LH/CG receptor [55]. However, carbohydrate removal does not change the ligand-binding conformation of the mature LH/CG receptor. This is true for the membrane-bound receptor, as well as for the solubilized receptor, as the deglycosylated receptor was able to bind hormone in ligand-blotting assays, even if the carbohydrates were removed after solubilization (U. E. Petäjä-Repo, unpublished work). Similarly, the oligosaccharides of the receptor are not needed for the renaturation of the protein, as long as the disulphide bonds are intact, because the deglycosylated receptor was able to bind hormone when blotted on to nitrocellulose after SDS/PAGE.

In conclusion, the carbohydrate portion of the rat ovarian LH/CG receptor does not contribute either to the specificity of ligand binding or to the functional coupling of the adenylate cyclase system. The ability to recognize and bind the correct hormone and to stimulate the adenylate cyclase are thus determined by the polypeptide portion of the receptor. Future work on the role of glycosylation in the biosynthesis and intracellular trafficking of the LH/CG receptor should provide further insight into the function of the oligosaccharide moiety of the receptor.

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