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Hormone glycosylation required for lutropin receptor recognition in sheep testis

M.R. Sairam, G.N. Bhargavi and T.A. Yarney

Reproduction Research Laboratory, Clinical Research Institute of Montreal, Montreal, Québec, Canada

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The high affinity binding sites for ovine pituitary lutropin (oLH) present in DLS-1 sheep testis recognized only the fully glycosylated ovine or bovine hormone (bLH) in receptor binding assays using ^{125}I-labeled oLH. Chemically deglycosylated (DG-) oLH or bLH which were fully active with other lutropin receptors (rat/pig) were completely inert in the DLS-1 receptor assay. In the same membranes, the FSH (follitropin) receptor reacted well with both glycosylated FSH and DG-oFSH. In recombination studies, lutropin formed by glycosylated native α- and β-subunits of the hormone was fully active but when one of the subunits was in the deglycosylated form, receptor binding activity was greatly reduced. The presence of glycosylated α-subunit in the recombined hormone gave rise to 5× more activity than DG-$\alpha+\beta$. All these preparations were fully active in the rat/pig receptor assays for LH. These results demonstrate that lutropin hormone glycosylation is essential for optimum receptor recognition in the sheep testis, further emphasizing the importance of correct glycosylation in oLHα hormone function.

Gonadotropin; Lutropin receptor; Sheep testis; Glycosylation

1. INTRODUCTION

The gonadotropins, lutropin (luteinizing hormone; LH), follitropin (follicle stimulating hormone; FSH) of the pituitary and human/equine choriogonadotropin (hCG, eCG) of the placenta, regulate ovarian or testicular functions by specific interaction(s) with receptors on the cell membrane [1]. LH and hCG which bear similar structural and functional characteristics are believed to interact with the same receptor in many species [1]. Thus, both hormones can be interchangeably used to study the LH receptor in many species. However, while studying homologous hormone receptor interaction in sheep testis, we recently discovered that this testicular receptor was unique in being able to recognize only the homologous pituitary hormone (sheep LH) but not the placental hCG or eCG [2]. Recombination experiments using the α- and β-subunits of these hormones revealed that the presence of an ovine or bovine α- or β-subunit in the $\alpha\beta$ complex was required to generate an effective conformation capable of receptor recognition in sheep testis.

In characterizing this receptor we now report here that hormone glycosylation is essential for optimal lutropin receptor recognition but not for follitropin receptor interaction in the same tissue. This is the first instance in which hormone glycosylation has been shown to be important for its receptor recognition, whereas in all other instances investigated thus far [3], hormone glycosylation especially in the α-subunit has been implicated to play a key role in post-receptor binding events of intracellular signal transduction mechanisms of gonadotropins.

Correspondence address: M.R. Sairam, Reproduction Research Laboratory, Clinical Research Institute of Montreal, 110 Pine Ave. West, Montreal, Québec, Canada H2W 1R7

2. MATERIALS AND METHODS

2.1. *Hormones*

Ovine and bovine pituitary LH and their α- and β-subunits as well as their chemically deglycosylated variants were prepared as described earlier (see [2]). Ovine FSH and chemically deglycosylated hormone was prepared by established procedures [4]. Hormone recombinants were formed by incubating the subunits (α, β, native or deglycosylated) at 1 mg/ml concentration in 0.05 M phosphate, pH 7.5, at room temperature for 16–24 h. Assuming 1:1 recombination, the dimerized hormone complex was diluted appropriately with assay buffer and used for receptor binding assays.

2.2. *Sheep testicular receptor membranes and other gonadal preparations*

All experiments were performed with tissues of DLS strain of sheep supplied by Agriculture Canada (Lapocatière, Québec, Canada) (courtesy of Dr F.A. Fahmy). The tissues from 6-month-old animals were collected and processed as described [5]. The same receptor membrane preparation was used for performing both lutropin and follitropin receptor binding assays using respective ^{125}I-labeled hormones. In comparative studies, when necessary, either a rat testicular [2] and/or porcine ovarian granulosa cell receptor [2] was used.

2.3. *Labeled hormone preparation and binding assays*

Our highly purified oLH and oFSH and deglycosylated oFSH were labeled with carrier free Na^{125}I (Amersham Corp., Chicago, IL, USA) by the lactoperoxidase method as performed in our laboratory [2] and separated on Sephadex G-50 or Biogel P6-DG columns. In order to obtain labeled deglycosylated oLH (DG-oLH) with reduced nonspecific binding, we have now adopted the iodogen method of labeling performed essentially according to the manufacturer's instructions (Pierce Co., Rockford, IL, USA) with few modifications.

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Table I

Displacement of ^{125}I-oLH from DLS-1 lutropin receptor and rat/pig receptor

Hormone	% activity	
	DLS-1 sheep receptor	Rat/pig receptor
oLH (ovine)	100	100
bLH (bovine)	100	100
hLH (human)	8–14	100
rLH (rat)	4	50
hCG/eCG	0	100

All hormone preparations tested here were of the highest purity available. % activity calculations are based on displacement curves assessing the amount of hormone required for 50% displacement of the labeled hormone (^{125}I-oLH) under identical assay conditions. The assay with DLS-1 testicular receptor was carried out at 4°C and the experiments with rat testicular or pig ovarian receptor were done at 22°C. Same hormones were used in both types of assays.

The labeled hormone was separated from free Na^{125}I by adsorption onto a small column (0.5 ml) of SP-Sephadex C_{50} in 0.033 M sodium acetate, pH 5.4, and subsequent elution with 0.01 M Na_2HPO_4 containing 0.5 M NaCl. The specific activity of all labeled hormones was calculated to be in the range of 40–80 μCi/μg based on incorporation as well as self-displacement analysis [6]. They were used within 3 weeks of preparation.

All receptor binding assays were performed in polystyrene or glass tubes (12 × 75 mm) in duplicates or triplicates. The labeled hormone (0.5–1 ng, ≈70000 cpm), test samples, sheep testis membrane were incubated in assay buffer (total volume 0.5 ml) consisting of 25 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$ and 1 mg/ml bovine serum albumin (Sigma, St. Louis, MO, USA) at 4°C for lutropin receptor binding and at room temperature for follitropin binding. Both incubations were for about 16 h. These conditions had been established as optimal for evaluating the hormone/receptor interactions [5] in this species.

Table II

Hormone glycosylation and receptor recognition

	% activity			
	LH-R		FSH-R	
	DLS-1	Rat/Pig	DLS-1	Rat/Pig
oLH	100	100		
DG-oLH	0	150–200		
bLH	100	100		
DG-bLH	0	150–200		
oLHα	0	0		
oLHβ	0	0		
oLHα + oLHβ	100	100		
oLHα + DG-oLHβ	12	100		
DG-oLHα + oLHβ	2	150		
DG-oLHα + DG-oLHβ	0	200		
oFSH			100	100
DG-oFSH			150	150–200
oLHα + oFSHβ			100	100
DG-oLHα + oFSHβ			150	150

LH and FSH receptor binding assays were performed using respective ^{125}I-labeled ovine hormones and the indicated receptor (R) under optimal conditions (see section 2). For % activity calculations see note to Table I. When two subunits were mixed to form the recombinant hormone, dilutions were made assuming a 1:1 recombination and testing the same solution in respective assays. When no significant displacement was observed at concentrations 5–10 μg/ml, activity was deemed to be zero.

When performing binding assays for LH or FSH with the rat or pig receptors the incubations were done at room temperature (22°C). In all cases, after the incubation, the reaction was stopped by addition of 2 ml of assay buffer followed by centrifugation at 3000 × g for 20 min at 4°C. The radioactivity bound to the pellet was counted in an LKB-rackgamma counter. Nonspecific binding in each experiment was determined in presence of 1 μg of unlabeled hormone.

3. RESULTS

3.1. *Specificity of the sheep lutropin receptor*

Membrane preparations obtained from all DLS-1 animals of about 6 months of age and collected at different times of the year could distinguish between homologous (oLH/bLH) and heterologous hormones (hLH, rLH) and placental hCG/eCG (Table I) in a binding assay using ^{125}I-oLH, confirming previous observations. The same heterologous hormones (lutropins, hCG, eCG) were all equally active in a binding assay using rat/pig gonadal membrane, indicating the uniqueness of the sheep lutropin receptor. However, in the same sheep testis preparations, the follitropin receptor was nondiscriminatory (data not

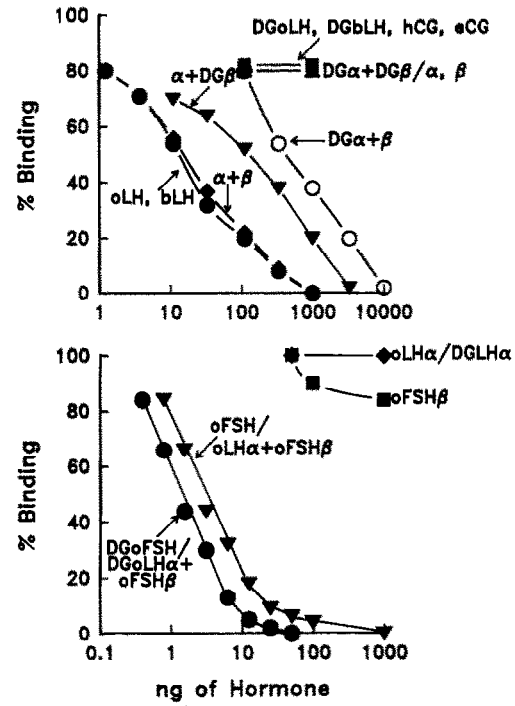

Figure 1. (Top) Lutropin receptor binding assay with DLS-1 sheep testicular receptor. In the experiment shown here 20% of total added ^{125}I-oLH was bound to the receptor and 70% of this was displaced by 1 μg unlabeled oLH. This figure is normalized to represent 100% binding for the calculations (see Table II). Displacement curves for the rat/pig receptor are not shown but represented in Table II. (Bottom) Follitropin receptor binding assay with DLS-1 receptor. The total specific binding was 18% of the added ^{125}I-oFSH under conditions of the assay. For activity calculations see Table 2. As oLHα is structurally identical to oFSHα it can be used in place of the latter for recombinations. The oLHα and DG-oLHα were the same preparations as used in the experiment at the top.

shown) and could react equally well with purified ovine, human, porcine or rat FSH preparations.

3.2. *Activity of deglycosylated hormones*

Deglycosylated hormones prepared by anhydrous HF treatment of ovine and bovine LH, which were reactive in the rat/pig receptors (Table II) were completely inert in the lutropin assays with DLS-1 receptor (Fig. 1, top), suggesting differences in receptor characteristics. However, in the follitropin receptor assay using ^{125}I-oFSH and the same DLS-1 membranes, DG-oFSH was slightly more active than oFSH (Fig. 1, bottom). Also labeled DG-oFSH bound to the DLS-1 receptor was displaced by both unlabeled oFSH and DG-FSH (not shown).

^{125}I-Labeled DG-oLH which could bind to the rat/pig receptor membranes showed no binding to the DLS-1 membrane, confirming the lack of the displacement noted in Fig. 1 (top). Considering the well documented observation that glycosylation of the hormone was not required for receptor binding of glycoprotein hormones in a number of systems [3], the observations with DLS-1 lutropin receptor were rather surprising. In order to explore this further, we prepared recombinants in which one of the two subunits of the hormone lutropin was selectively deglycosylated. As expected, the native (glycosylated) α- and β-subunits of oLH and bLH did not compete in the binding assay (Fig. 1, top). Their noncovalent recombination led to full activity as shown by complete displacement of the labeled hormone. Deglycosylation of one of the subunits and recombination with the glycosylated counterpart (DG-α + β or α + DG-β) produced a complex that showed some but not full activity in the DLS-1 lutropin receptor assay. The activity (2%) of the recombinant in which the common α-subunit was deglycosylated (e.g. DG-α + β was much less than that noted for the α + DG-β recombinant (12%) (Table II). All of these recombinants were fully active in the rat/pig lutropin receptor assay using the same ^{125}I-oLH as the ligand. In the FSH receptor assay, the recombinant of DG-LHα + oFSHβ had full activity, exhibiting the same potency as DG-FSH.

4. DISCUSSION

Effective receptor recognition on the cell membrane is a primary event in the initiation of polypeptide hormone action. For hormones such as LH, FSH, TSH (thyrotropin) of the pituitary and hCG and eCG of the placenta, which are large dimeric glycoproteins, removal of 75–80% of the accessible sugars in the oligosaccharide chain has been shown to compromise signal transduction without affecting receptor recognition [3]. On the other hand, receptor binding activity increased in many instances following deglycosylation of the hormone [3,8,9]. In the present investigation we demonstrate that with the DLS-1 sheep testicular lutropin receptor, hormone glycosylation is necessary even for the first event of hormone action. The fact that deglycosylated oLH and bLH preparations which were effective in the rat/pig gonadal systems were inert in the DLS-1 lutropin receptor (Table II, Fig. 1) suggest that this receptor could have some unique structural features that discriminate an active hormone conformation from an inactive hormone conformation. In this regard, it may be noted that the subtle changes in conformation following hormone deglycosylation not discernable by many biophysical studies [3,7,8] or studies with other receptors [3,8] could be detected by immunological techniques. These latter studies showed that certain antisera could discriminate glycosylated (active) and deglycosylated (inactive) forms of the hormone [10–12].

Glycosylation of both the α- and β-subunits of lutropin is clearly necessary for optimal receptor interaction as shown by data in Table II and Fig. 1 because only the α + β complex was fully active in the DLS-1 lutropin receptor. No such requirement is apparent for the FSH receptor in the same tissue but located on a different cell (Sertoli cell) in the testis. If we are to ascribe the relative importance of the subunits, it would be tempting to suggest that glycosylation of the α-subunit may be more critical for DLS-1 lutropin receptor recognition because of the significant decrease in activity seen for the DG-LHα + β complex. However, it may be noted that this recombinant as well as others were fully active in the rat or pig LH receptor assay (Table II). This speculation is particularly interesting in view of the observation that glycosylation of the α-subunit in glycoprotein hormones plays a critical role in signal transduction [13,14].

The DLS-1 lutropin receptor studied here is different from other lutropin receptors in several respects. First in its ability to distinguish between a pituitary and placental hormone (Table I, Fig. 1 (top) and [2]), second in requiring hormone glycosylation for binding and third in having a larger molecular weight as revealed by affinity cross-linking studies (Yarney et al., unpublished observations). The lutropin receptor present in this tissue is also different from the follitropin receptor as the latter does not require hormone glycosylation for effective interaction. As in many other receptor systems, it is becoming apparent that the lutropin receptor [15–17] and thyrotropin receptor [18] may also exist in different forms and this has been deduced by cloning data which have suggested alternate splicing patterns. Our cloning studies in progress for the gonadotropin receptors in DLS-1 sheep testis should cast additional light on the differences between the lutropin and follitropin receptors in this tissue.

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