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A Role for Glycosylation of the α Subunit in Transduction of Biological Signal in Glycoprotein Hormones

Abstract. *The biological properties of recombinants of glycoprotein hormones in which the α and β subunits were differentially deglycosylated have been investigated. Specific deglycosylation of the α subunit generated a recombinant that had more receptor-binding activity but did not produce hormone response in the target cells. The deglycosylated α + β recombinant was also an antagonist of the action of the native hormone. Thus, the carbohydrates in the α subunit play a dominant role in the transduction of the hormone signal into the cell.*

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Glycoproteins are ubiquitous in nature, performing important functions as enzymes, immunoglobulins, hormones, and receptors. One or more carbohydrate moieties may be involved in stabilization of protein conformation, regulation of metabolic half-life, uptake by cells, recognition, differentiation, growth, and metabolism (*1*). The oligomeric glycoprotein hormones of the pituitary and placenta are unusual because of the high carbohydrate content in the noncovalently linked α and β subunits (*2*). The removal of terminal sialic acid residues in human choriogonadotropin (hCG), follicle-stimulating hormone (FSH), and human luteinizing hormone (hLH) destroys the in vivo biological activity of the hormones (*3*) by rapid elimination from circulation but in vitro biological response is retained (*4*). However, removal of additional sugars by exoglycosidases or a mixture of exo- and endoglycosidases as in hCG (*5*) or chemical treatment of all the glycoprotein hormones (*6, 7*) has shown that the carbohydrate residues are not essential for binding to the receptors (*7*). We have observed that the carbohydrate moiety in the α subunit (*8*) of the pituitary glycoprotein hormone plays a critical role in coupling the hormone-receptor complex to the adenylate cyclase and thus the transduction of the biological signal.

Appropriate recombination of the isolated α and β subunits of the glycoprotein hormones generates a product that is virtually identical to the native hormone in all respects including biological activity (receptor binding, adenylate cyclase activity, and cellular response) (*2*). The complete integrity of the carbohydrate units in ovine LH is not required for subunit interaction or receptor binding (*7, 9*). Specific chemical deglycosylation of the ovine LH-α (DG-LH-α) enhanced its ability to combine with the β subunit, as reflected by the increase in receptor-binding activity of the DG-LH-α + LH-β recombinant (Fig. 1). In less than 10 minutes after recombination of the subunits, full receptor-binding activity was regenerated in this incubation mixture; by comparison, about 24 hours were required to attain maximal activity in LH-α + LH-β (in which both subunits are fully glycosylated). Specific deglycosylation of the ovine LH-β did not significantly affect its ability to regener-

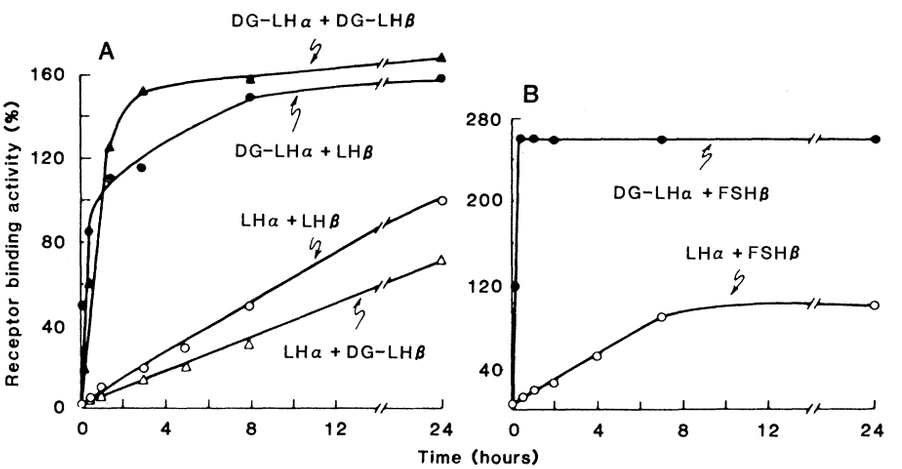

Fig. 1. Effect of deglycosylation of the α subunit on regeneration of receptor-binding activity. Native and deglycosylated subunits (1 mg/ml) were dissolved in 0.05*M* phosphate buffer, *p*H 7.5, and recombination was initiated by mixing equal volumes of these solutions. The solutions were incubated at 37°C, and at specified intervals aliquots were withdrawn and frozen at −20°C for receptor-binding assay. LH and FSH receptor-binding activities were determined with rat testicular homogenate (*7*) which contains different population of cells with separate and specific receptors for LH and FSH. In each receptor assay, about 75,000 count/min of ^{125}I-labeled ovine LH or ovine FSH was incubated with the homogenate for 2 hours at 37°C in a total incubation volume of 500 μl. The specific radioactivity bound to the membrane pellet after centrifugation was determined. Nonspecific binding was determined in the presence of 1 μg of unlabeled ovine LH or ovine FSH. The activity of the native hormone in each case was set as 100 percent for the calculation of relative receptor-binding activity. (A) LH receptor assay; (B) FSH receptor assay. Neither the isolated subunits nor their deglycosylated counterparts had receptor-binding activity. Similar data were obtained with pig ovarian preparations which also have both LH and FSH receptors. Deglycosylation of the α subunit did not affect the receptor-binding specificity of the complex. The same preparation of LH-α and DG-LH-α subunits was used for recombination in both assays as had been used in our previous work (*9*). The LH-β preparation was prepared by the salt precipitation method (*9*) but was also further purified by reversed-phase high-performance liquid chromatography. The major component, which had no intrinsic receptor-binding or biological activity, was selected for the recombination.

ate activity when combined with native ovine LH-α (Fig. 1A). Ovine LH-α can effectively substitute for FSH-α in combination with FSH-β to give full FSH receptor-binding activity (*10*), since the two α subunits have identical structure. Accordingly, the same preparation of DG ovine LH-α also recombined faster with the ovine FSH-β (Fig. 1B). Based on these data, we conclude that the specific chemical deglycosylation that resulted in the removal of about 75 percent of the carbohydrate moiety in the α subunit increased its binding affinity to the β subunit and of the complex to respective receptors.

When the ability of these recombinants (Fig. 1) to activate the cellular machinery was examined in vitro, a different pattern emerged (Fig. 2). The recombinant of ovine LH-α + ovine LH-β activated dispersed rat Leydig cells incubated in vitro, causing the accumulation of adenosine 3′,5′-monophosphate (cyclic AMP), an effect that was concentration-dependent (Fig. 2). When the LH-β was deglycosylated for recombination in LH-α + DG-LH-β, the activity of the recombinant was not substantially affected. However, specific deglycosylation of the α subunit, to form DG-LH-α + LH-β, produced a marked alteration in its biological profile. This recombinant, which presumably has greater affinity for the receptor (Fig. 1), was virtually inactive in stimulating adenylate cyclase (Fig. 2). The DG-LH-α + DG-LH-β recombinant had no effect in this system, which confirms previous observations (*9*). Recombination of the DG-LH-α with ovine FSH-β produced similar results in the cells of the rat testis that were responsive to FSH (Fig. 2B). In these cells, the LH-α + FSH-β recombinant (in which the α subunit is fully glycosylated) acquired the full complement of biological response. These data demonstrate that differential deglycosylation of the α subunit interferes with the ability of the recombinant to communicate the hormone signal to the cellular interior.

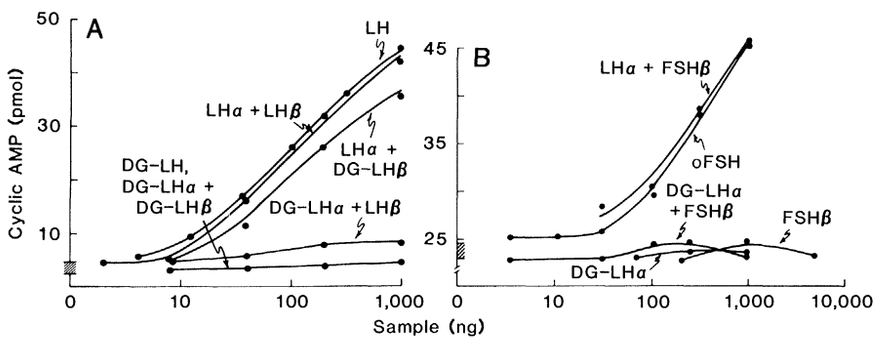

Fig. 2. Effect of recombinants (Fig. 1) on cyclic AMP accumulation in target cells. Hatched lines on the ordinates indicate basal levels. (A) LH action in adult rat Leydig cells; (B) FSH action in immature rat testicular cells. Collagenase-dispersed rat testicular Leydig cells (A) from adult male rats (150 to 180 g) or (B) seminiferous tubular cells from 19-day-old rats were incubated (0.6 ml) with the preparations for 30 minutes at 37°C (5 percent CO_2) in Dulbecco's modified Eagle's medium containing 0.1 percent bovine serum albumin. After 30 minutes, the mixture was transferred to a boiling water bath for 15 minutes to stop the reaction; cyclic AMP in the supernatant was determined by the protein-binding assay (*11*). The 24-hour recombinants (see Fig. 1) were used in this experiment. oFSH, native ovine FSH.

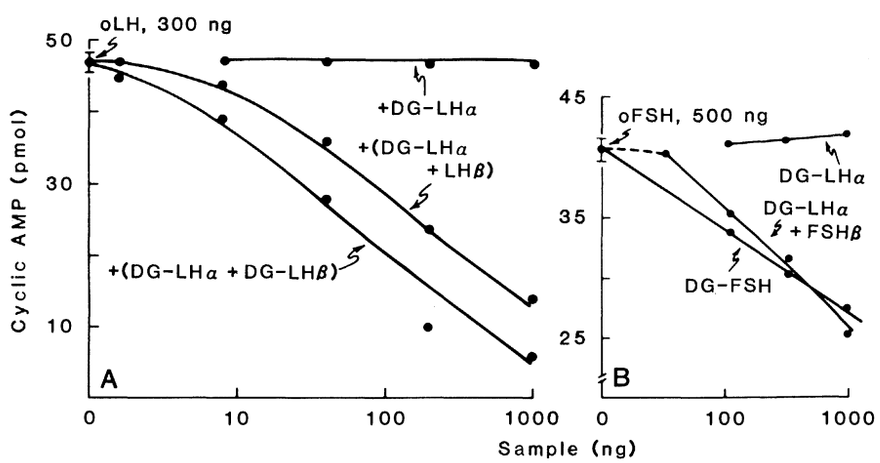

Fig. 3. Inhibitory activity of the recombinant in which the α subunit is deglycosylated. Hormone-responsive cells of the testis were prepared and incubated as described (Fig. 2, legend). (A) Leydig cells were challenged with 300 ng of ovine LH and different concentrations of the indicated samples were added. (B) The FSH-responsive seminiferous tubular cells were incubated with 500 ng of the native ovine FSH (oFSH) in the absence or presence of other samples; cyclic AMP accumulated after 30 minutes was measured by a protein-binding assay (*11*). The deglycosylated α subunit by itself had no effect on the hormone response in either cell system.

These data show that the carbohydrate units of the α subunit in the gonadotropic hormones are important in biological function. Their specific removal, without affecting the polypeptide structure (*6*), causes an uncoupling of the receptor–adenylate cyclase system in the target cells of the testis. We predict a similar behavior for the DG-LH-α + TSH-β recombinant in its interaction with thyroid cells (*12*). Numerous studies (*2*) concerning the modification of functional amino acid side chains in the α subunit of the glycoprotein hormones have established the importance of this subunit in receptor binding. We can now conclude that the integrity of the carbohydrate in the α subunit is directly or indirectly involved in the transduction of the biological signal in the glycoprotein hormones. This may be important in understanding the mechanisms for the biosynthesis, physiology, and pathology of the glycoprotein hormones.

Finally, the role of the glycosylated α subunit in signal transduction could be of evolutionary significance as there is a high degree of structural conservation of the α subunit among different species and an identical α subunit is used in the assembly of the three glycoprotein hormones in all species.

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Thyrotropin-Releasing Hormone Induces Rhythmic Bursting in Neurons of the Nucleus Tractus Solitarius

Abstract. *The nucleus tractus solitarius (NTS) contains neurons that are part of the central neuronal network controlling rhythmic breathing movements in mammals. Nerve terminals within the NTS show immunoreactivity to thyrotropin-releasing hormone (TRH), a neuropeptide that has potent stimulatory effects on respiration. By means of a brainstem slice preparation in vitro, TRH induced rhythmic bursting in neurons in the respiratory division of the NTS. The frequency of bursting was voltage-dependent and could be reset by short depolarizing current pulses. In the presence of tetrodotoxin, TRH produced rhythmic oscillations in membrane potential whose frequency was also voltage-dependent. These observations suggest that TRH modulates the membrane excitability of NTS neurons and allows them to express endogenous bursting activity.*

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In mammals, rhythmic breathing movements are controlled by a central neuronal network composed of several groups of neurons found in the brainstem (*1*). Most of the neurons in the dorsal respiratory group (DRG), which is located within the nucleus tractus solitarius (NTS), are active during inspiration (*2*). Several neuropeptides have been found within nerve terminals in the NTS by means of immunohistochemical techniques; one of these peptides is thyrotropin-releasing hormone (TRH) (*3*). The application of exogenous TRH to the brainstem increases respiratory rate and minute ventilation (*4*). In addition, respiratory depression caused by barbiturates is diminished by TRH (*5*). These observations suggest that TRH may play a central role in the neural control of respiration; however, the effects of TRH have not been studied at the cellular level. We used a brainstem slice preparation that allows stable intracellular recordings to be made in vitro from NTS neurons and found that TRH produces rhythmic bursting activity in neurons in the respiratory part of the NTS.

Brainstem slices were obtained from adult guinea pigs (350 to 500 g in body weight) by means of standard tissue slicing techniques (*6*). Three to four slices (400 μm thick) were cut in transverse section beginning at the level of the obex and continuing toward the rostrum. The use of transverse slices facilitated the identification of the tractus solitarius and its surrounding nuclear areas. In several experiments, parasagittal and longitudinal slices were studied to ensure that our observations did not depend on the presence or absence of a particular part of a neuron's dendritic field. No differences in neuron properties or responses were observed for the different slice orientations. After being cut, the slices were transferred immediately to a recording chamber and were subfused with oxygenated Ringer solution (*6*). All drugs were applied to the bath from separate reservoirs of Ringer solution. A mixture of 95 percent O_2 and 5 percent CO_2, warmed and humidified, was blown over the slices. All experiments were performed at 37°C.

Three criteria were used to determine whether or not a neuron was healthy: (i) a stable resting potential of at least −50 mV, (ii) a spike overshoot of at least +35 mV (spike amplitude, >85 mV), and (iii) an input resistance of at least 50 megohms. Only neurons meeting all three criteria were used. In general, we obtained stable recordings from NTS neurons for periods of 1 to 4 hours without deterioration of their membrane properties. Observations were based on recordings from 23 neurons in 22 slices.

The distribution of respiratory neurons in the brainstem of guinea pigs has been mapped in situ by means of extracellular recording techniques (*7*) (Fig. 1A). Because respiratory neurons as such could not be identified in the slice, we have referred to these cells simply as NTS neurons. However, these neurons could be distinguished from those in adjacent parts of the NTS and reticular formation by both their morphology and their membrane properties (*8*).

In the slice preparation, NTS neurons in the DRG did not show a pattern of activity suggestive of a respiratory rhythm. They did, however, show nonrhythmic activity at a rate of 1 to 3 spikes per second (Fig. 1B, upper trace). After TRH was added to the bath, a rhythmic bursting pattern was established in 70 percent of the neurons studied (Fig. 1B, lower trace) and was observed for concentrations of TRH between 0.1 and 10.0 μ*M* (*9*). The development of rhythmic bursting activity followed a stereotyped time course (Fig. 1C). Five to 10 minutes after the slice was exposed to TRH, the action potential of NTS neurons developed a depolarizing afterpotential (DAP). The size of the DAP increased with time and was associated with the appearance of rhythmic bursting activity. Exposure to TRH for 1 to 2 hours led to a decline in bursting activity that may have been due to a desensitization phenomenon (*9*). The action potential of NTS neurons, however, still showed a DAP.

Depolarizing afterpotentials are characteristic of endogenous pacemaker and bursting neurons in both invertebrates and mammals (*10*). The development of a DAP after exposure to TRH therefore suggests that rhythmic bursting activity