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ROLE OF CARBOHYDRATE IN HUMAN CHORIONIC GONADOTROPIN: DEGLYCOSYLATION UNCOUPLES HORMONE-RECEPTOR COMPLEX AND ADENYLATE CYCLASE SYSTEM

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SUMMARY: Previous work has shown that deglycosylation of human chorionic gonadotropin (hCG) does not affect its receptor binding characteristics, but its ability to stimulate intracellular cyclic AMP accumulation and steroidogenesis in ovarian cells is abolished. To identify the site at which carbohydrate of hCG is involved in the mechanism of action of the hormone, we have studied adenylate cyclase activity in ovarian membrane preparations in response to deglycosylated and native hCG. The deglycosylated hCG does not stimulate adenylate cyclase of ovarian membrane preparation and also it acts as an inhibitor of hCG action. Data are presented to show that both hCG- and catecholamine receptors are coupled to the same adenylate cyclase complex. Since adenylate cyclase activity in the presence of deglycosylated hCG remains still responsive maximally to catecholamines, it indicates that the adenylate cyclase complex is functional and is unaffected by the interaction of deglycosylated hCG to its receptor. This is further supported by the fact that the deglycosylated hCG does not impair the maximal stimulation of adenylate cyclase by guanine nucleotides. Thus, the site of action of the carbohydrate of hCG is prior to the coupling of the hormone-receptor complex and the adenylate cyclase system.

HCG is a glycoprotein hormone with about 30-33% carbohydrate (1). Although the detailed structures of the carbohydrate units have recently been elucidated (2,3), their specific role in the mechanism of hCG action is still unresolved. Previous work from this laboratory has shown that the sequential removal of carbohydrate from the hormone by specific glycosidases and the subsequent chemical modification of the exposed sugar residues (4-8) or complete deglycosylation (9) results in a progressive or complete

Abbreviations: hCG, human chorionic gonadotropin; GPP(NH)P, guanylyl (β,γ-imido)diphosphate.

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loss of its biological activity. However, partial or complete removal of carbohydrate from hCG does not reduce its receptor binding activity (6-8, 10). These data indicate that the carbohydrate moiety of hCG is not involved in hormone-receptor interaction, but is essential for the expression of cellular response. As presently understood (9), the mechanism of hCG action involves its initial binding to a specific receptor site on the target cell membrane, followed by aggregation of the resulting hormone-receptor complex, leading to the activation of adenylate cyclase through a guanine nucleotide binding protein (9). To pinpoint the step at which the carbohydrate of hCG is involved in the above sequence, we have studied in rat ovarian membranes, adenylate cyclate response to hCG and isoproterenol, the latter activating the same adenylate cyclase system, but through β-adrenoceptor, also found in ovarian membranes (11,12). Our results indicate that the carbohydrate moiety of hCG is involved at a step prior to the hormone-receptor complex interaction with the adenylate cyclase complex.

MATERIALS AND METHODS

Deglycosylation of hCG was done as described (10). Creatine phosphate and creatine kinase (E.C.2.7.3.2., 35 U/mg) were from P.L. Biochemicals. ATP, GPP(NH)P, L-(-)-isoproterenol and DL-propranolol-HCl were from Sigma. [^{3}H]-cyclic AMP was from New England Nuclear and Norit SGX from Baker. Cyclic AMP binding protein was prepared from rabbit muscle by following the reported procedure (13) through the ammonium sulphate precipitation step.

Ovarian Membrane Preparation: Immature female rats (21-24 days old, from Harlan Sprague Dawley) were superovulated by injecting 50 IU/rat of pregnant mare serum gonadotropin and 25 IU/rat of hCG 56-65 hours later. Ovaries were removed 6-8 days after hCG injection, cleaned of adhering fat and connective tissues and were homogenized in 50 mM Tris-HCl, 1 mM $CaCl_2$ (pH 7.2) buffer (10 mg ovary/ml buffer). The homogenate was centrifuged at 10,000 g for 10 min at 4°C and the pellet was washed once with the same buffer. The final membrane pellet was resuspended in 1/5th the volume of the original homogenate and was used as the source of adenylate cyclase.

Adenylate Cyclase Assay: Ovarian membrans (20-30 µg protein), preincubated for 10 min at 30°C were incubated in 50 mM Tris-HCl (pH 7.4) containing 5 mM $MgCl_2$, 2 mM ATP, 20 mM creatine phosphate, 50 U/ml creatine kinase, 2 mg/ml bovine serum albumin, 10 µM GPP (NH)P and the indicated amounts of hormones and/or agents in a final volume of 100 µl at 30°C for 10 min. The reaction was

stopped by adding 250 µl of 50 mM Tris-HCl, 4 mM EDTA (pH 7.4) buffer and immediately boiling the tubes at 100°C for 5 min. Cyclic AMP produced was assayed in an aliquot of the supernatant by the protein binding method (14).

RESULTS AND DISCUSSION

Recent reports from this laboratory (9,10), have shown that in the superovulated rat ovary, deglycosylated hCG binds to hCG receptor in a manner identical to that of the native hormone, but does not stimulate intracellular cyclic AMP accumulation or steroidogenesis. Moreover, it acts as an inhibitor of hCG action. In the present study we have attempted in ovarian membranes to identify the step where the carbohydrate moiety of hCG is involved in its action.

Effect on Adenylate Cyclase Activity: Fig. 1A shows that in superovulated rat ovarian membrane preparation, native hCG stimulates adenylate cyclase activity in a dose-dependent manner, whereas the deglycosylated hCG fails to stimulate the enzyme above basal level even at a concentration of 10 µg/tube (33 µM). It can be seen from Fig. 1B that the deglycosylated hCG inhibits hCG stimulation of adenylate cyclase activity in a dose-dependent manner. These findings support the data on the lack of cyclic AMP accumulation in luteal cells by the deglycosylated hCG (9) and taken together with its unaltered receptor binding characteristics, show that the carbohydrate moiety of hCG plays a key role in the transmission of the signal from the hormone-receptor complex to the interior of the cell.

It is now established that a guanine nucleotide-dependent regulatory protein (G protein) couples the hormone-receptor binding to the catalytic subunit of adenylate cyclase (15). To see if the impairment of adenylate cyclase stimulation by hCG after deglycosylation is due to an inhibition of the guanine nucleotide binding activity of the G protein, we have studied the dose-response relationship of adenylate cyclase activity to GPP(NH)P, a non-hydrolysable

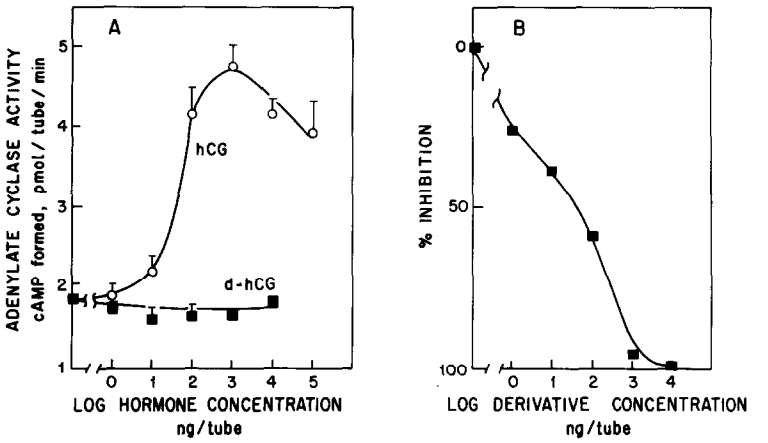

Fig. 1. Effect of hCG and deglycosylated hCG on the adenylate cyclase activity. A. Superovulated rat ovarian membranes were assayed in the presence of hCG (o) or deglycosylated hCG (■) as described under Methods. Values are mean ± S.D. of three determinations. B. Enzyme was assayed in the presence of 31.6 ng of hCG and the indicated concentrations of deglycosylated hCG. Basal enzyme activity was 0.82 ± 0.05 and with hCG alone was 1.98 ± 0.18 pmoles/min/tube.

analog of GTP, in the presence of hCG or deglycosylated hCG (Fig. 2). It can be seen that the magnitude of stimulation of adenylate cyclase caused by 200 μM GPP(NH)P is identical either in the absence or the presence of hCG or its deglycosylated derivative, showing that the guanine nucleotide binding to G protein is unaffected by deglycosylated hCG. It is clear from Fig. 2B that hCG causes a reduction in the ED_{50} (concentration required to stimulate the enzyme half maximally) of GPP(NH)P compared to the control value. This is due to the enhancement of the guanine nucleotide binding to G protein brought about by its interaction with the hormone-receptor complex (15). The deglycosylated hCG on the other hand, does not affect the sensitivity of adenylate cyclase response indicating that the deglycosylated hCG-receptor complex is unable to interact with G protein.

Further, to show that G protein, catalytic unit of adenylate cyclase and their interactions remain unaffected in the presence of deglycosylated hCG, we have taken advantage of the presence of β-adrenoceptors on ovarian plasma membrane, which also mediate the stimulation of adenylate cyclase. Results presented in Fig. 3 confirm the earlier observations (16) that stimulation of adenylate cyclase by saturating concen-

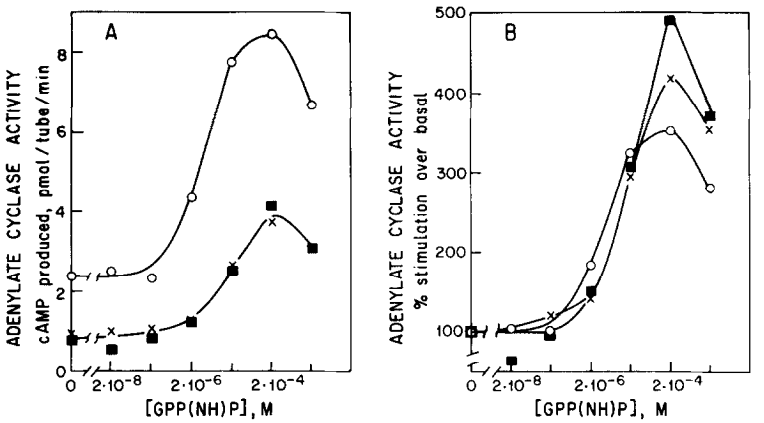

Fig. 2. Effect of GPP(NH)P on adenylate cyclase activity. A. Membranes were incubated with hCG, 1 μg (o), deglycosylated hCG, 1 μg (■) or no hormone (x) in the presence of the indicated concentrations of GPP(NH)P. B. Data in A are represented taking the basal value of each curve as 100. Symbols as in A.

trations of hCG and isoproterenol, a β-adrenoceptor agonist is not additive. This shows that both β-adrenoceptors and hCG receptors are coupled to the same adenylate cyclase system. It is further shown that in the presence of propranolol, a β-adrenoceptor antagonist, hCG can still stimulate adenylate cyclase activity showing that hCG receptor is independent of the β-adrenoceptor and that G protein and catalytic unit of adenylate cyclase remain unaffected and functional in the presence of a β-antagonist. Conversely, in the presence of deglycosylated hCG, an antagonist to hCG action, isoproterenol can still stimulate adenylate cyclase maximally (Fig. 3). This clearly shows that blocking hCG action by its deglycosylation does not derange the guanine nucleotide binding protein or the catalytic subunit of adenylate cyclase.

From the above data it is clear that the carbohydrate in hCG plays a role in coupling the hormone-receptor complex to its adenylate cyclase system, i.e. to the G protein and the catalytic subunit of adenylate cyclase. Although, this work for the first time establishes that the site of carbohydrate action in hCG is prior to the interaction of the hormone-receptor complex to adenylate cyclase system, the precise moelcular mechanism of coupling remains to be elucidated. It is conceivable that a coupling factor which recognizes the carbohydrate is involved in the interaction of the hormone-

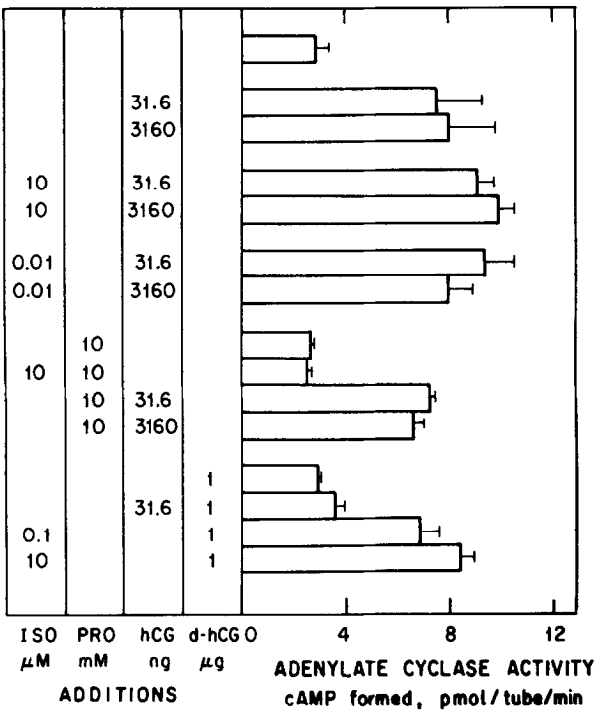

Fig. 3. Effect of β-adrenergenic drugs on hCG stimulated adenylate cyclase activity. Superovulated rat ovarian membranes were assayed in the presence of hCG or its deglycosylated derivative and/or β-adrenergenic agonist (L-(-)-isoproterenol) and antagonist (DL-propranolol) at indicated concentrations. Values are mean ± S.D. of three determinations. ISO, isoproterenol; PRO, propranolol; d-hCG, deglycosylated hCG.

receptor complex to the G protein. Other possibilities can also be considered: e.g., the deglycosylated hCG may not induce the appropriate conformation in the hormone-receptor complex necessary for its interaction with the G protein. The removal of carbohydrate may also affect the ability of the hormone-receptor complex to diffuse laterally in the membrane or to form microaggregates (17) or to stimulate membrane phospholipid transmethylation (18), which are thought to be involved in the mechanism of action of polypeptide hormones. Any of the above defects may again result in a failure to activate the G protein and thereby the stimulation of adenylate cyclase.

In summary, the present work clearly shows that the deglycosylation of hCG renders it antagonistic to the native hormone. It also establishes that the hCG receptor and β-adrenoceptor are coupled to the same adenylate cyclase system. Last, but not least, the carbohydrate of hCG is involved in coupling the hormone-receptor complex to the adenylate cyclase system.

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