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SIALIC ACID RESIDUES OF THE α-SUBUNIT ARE REQUIRED FOR THE THYROTROPIC ACTIVITY OF HCG

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SUMMARY: The desialylated human chorionic gonadotropin α- and β-subunits were combined with their native complementary subunits and the thyrotropic activities of the recombinants were compared to those of native and desialylated human chorionic gonadotropin using human thyroid membranes. All the combined forms interacted with the thyrotropin receptor-adenylate cyclase system, but only those with sialic acid residues present on the α-subunit were able to activate the enzyme. These data support the concept that the α-subunit contains the domain through which this hormone activates adenylate cyclase.

INTRODUCTION: The combination of two dissimilar, noncovalently linked subunits, designated α and β, makes up the structure of the human chorionic gonadotropin (hCG)[1] molecule. Both subunits are glycosylated (1,2); the α-subunit contains two Asn-linked oligosaccharide chains, whereas the β-subunit contains two Asn-linked and four Ser-linked oligosaccharide chains, the latter being in the carboxyl terminal region. Sialic acid residues are present in both the asparaginyl and the seryl oligosaccharides. By interacting with membrane receptors, hCG stimulates not only gonadal function, but also thyrotropic function (3). Purified hCG inhibits the binding of thyrotropin (TSH) to thyroid membranes and stimulates the adenylate cyclase therein (4,5). While it is well established that combination of the two subunits is required for the several biological activities intrinsic to hCG (6-8), relatively little is known of the structural

[1]Abbreviations: hCG or α:β, human chorionic gonadotropin; TSH, thyrotropin; α, native hCG α-subunit; β, native hCG β-subunit; asα, desialylated hCG α-subunit; asβ, desialylated hCG β-subunit; asα:asβ, desialylated hCG; α:asβ, native α combined with desialylated β; asα:β, desialylated α combined with native β.

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domains that elicit its biological effects. Sialic acid residues are not essential for the gonadotropic activity of hCG (9); in contrast, their removal greatly enhances the affinity of this hormone for the TSH receptor (10) and leads to a molecule which behaves as a competitive antagonist of TSH at the TSH receptor-adenylate cyclase system in human thyroid membranes (11). Thus, while the intact hCG molecule (α:β) contains a structural domain(s) that activates thyrotropic function, the desialylated hCG molecule (asα:asβ) does not. This observation indicates that the carbohydrate moieties of hCG play a key functional role in the interactions yielding activation of thyroidal adenylate cyclase. We performed the present study to determine which of the hCG subunits contains the carbohydrate domain that determines the ability of hCG to activate thyroidal adenylate cyclase.

MATERIALS AND METHODS: Desialylated α (asα) and desialylated β (asβ), obtained by neuraminidase treatment (12) of the highly purified hCG subunits, were recombined with each native complementary subunit by incubation in 0.1 M NH_4HCO_3 (pH 7.9) for 24 hr at 4°C. The recombinant hCG molecules, (asα:β) and (α:asβ), were purified based on their testis receptor binding activity (13) by gel-HPLC using one TSK-SW 2000 and two TSK-SW 3000 columns (Beckman Instruments, CA) in series. Amino acid composition analyses of the recombinants were in close agreement with those of native hCG. Crude plasma membranes were prepared from human thyroid tissue(14) and adenylate cyclase activities were determined as previously described (15).

RESULTS AND DISCUSSION: The α:β molecule activated thyroidal adenylate cyclase in a dose-dependent manner, while the recombinant asα:β molecule produced no activation of the enzyme (Fig. 1). Similarly, α:asβ stimulated adenylate cyclase activity in a dose-dependent manner; while asα:asβ did not (Fig. 1). Hence, the presence of sialic acid residues on the α-subunit is necessary for thyrotropic activity whichever β-subunit is present. However, such activation requires combination of subunits since the uncombined α and β, either native or desialylated, were inactive (Fig. 1).

Like α:β, α:asβ activated thyroidal adenylate cyclase; whereas asα:β, like asα:asβ, did not; thus, the presence of sialic acid residues on the β-subunit is neither necessary nor sufficient for the activation of this enzyme. At a concentration of 2×10^{-5} M, α:asβ elicited 22% of the maximal stimulation observed with bovine TSH (7.1×10^{-9} M), while α:β

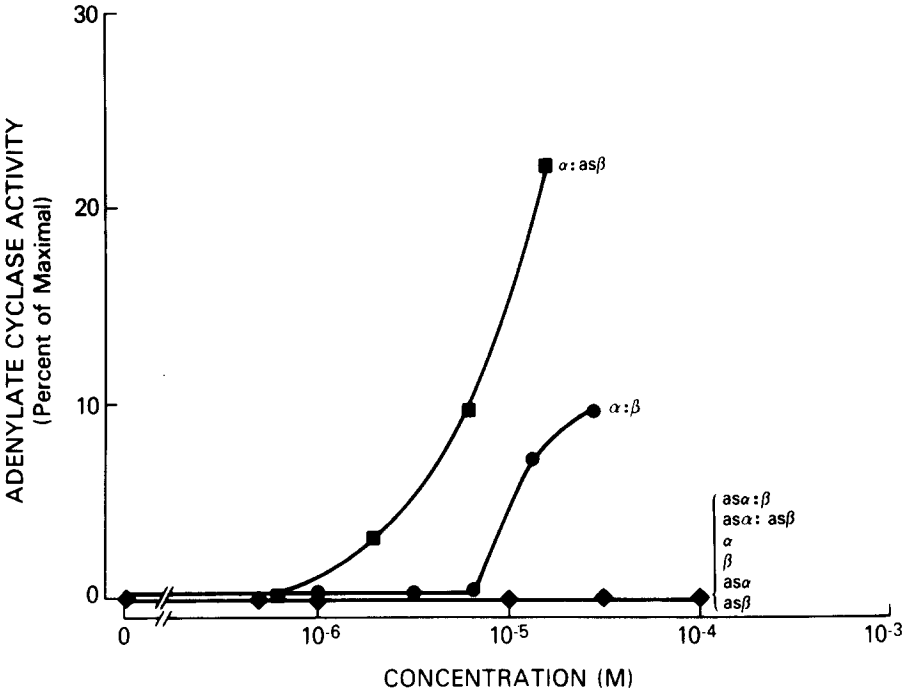

Fig. 1. Stimulation of adenylate cyclase activity by hCG and its derivatives. Enzyme activity was measured in the presence of various concentrations of intact hCG (α:β), desialylated hCG (asα:asβ), hCG α-subunit (α), hCG β-subunit (β), desialylated hCG α-subunit (asα) desialylated hCG β-subunit (asβ) and the recombinant molecule (asα:β) or (α:asβ). Each point indicates the mean of four replicates with an overall coefficient of variation of 7%. The data are expressed in terms of percent of maximal activity (7.1×10^{-9} M bovine TSH).

elicited 10% of maximal stimulation (Fig. 1). This suggests that α:asβ is intrinsically more active than α:β, and therefore that the removal of sialic acid residues from the β-subunit enhances the intrinsic thyrotropic activity of hCG. Removal of several amino acid residues from the carboxyl terminal region of the β-subunit is also associated with an enhancement of thyrotropic activity (16). These observations indicate that alterations in the β-subunit structure can bring about conformational changes in the dimer that modulate its function (17), but that neither the sialic acid nor the several amino acid residues of the carboxyl terminus are required for thyrotropic activity.

Our observation that the recombinant asα:β molecule did not stimulate adenylate cyclase activity does not imply a lack of interaction with the TSH

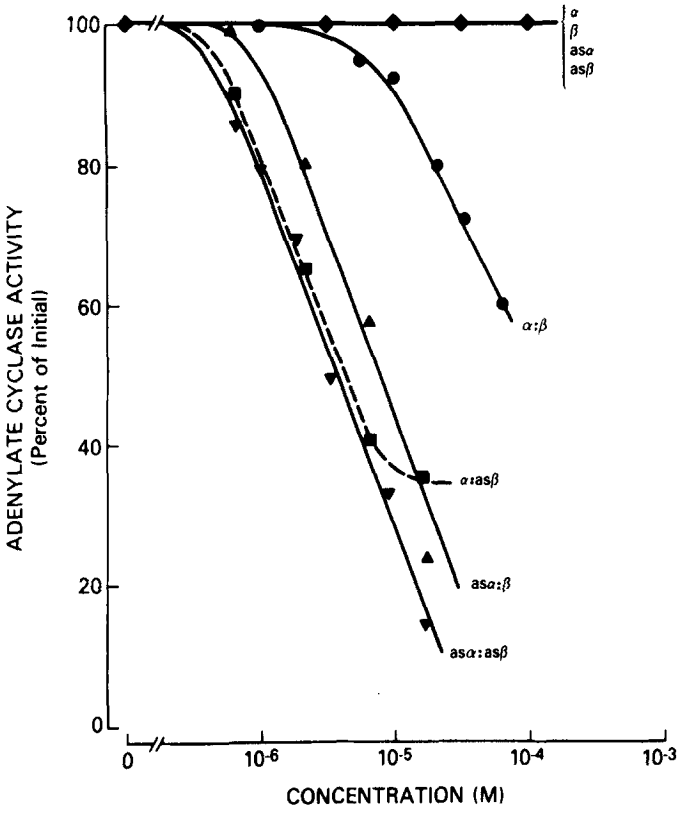

Fig. 2. Inhibition of TSH stimulated adenylate cyclase activity by hCG and its derivatives. Enzyme activity stimulated by a submaximal dose of bovine TSH (2.3×10^{-9} M) was measured in the presence of various concentrations of intact hCG (α:β), desialylated hCG (asα:asβ), hCG α-subunit (α), hCG β-subunit (β), desialylated hCG α-subunit (asα) desialylated hCG β-subunit (asβ) and the recombinant molecule (α:asβ) or (asα:β). Each point indicates the mean of four replicates with an overall coefficient of variation of 7%. The data are expressed in terms of percent of initial activity obtained with bovine TSH alone.

receptor-adenylate cyclase system. Indeed, asα:asβ does not stimulate adenylate cyclase, but nevertheless interacts with the TSH receptor and thereby produces competitive antagonism of adenylate cyclase activation by TSH (11). Accordingly, we examined the effects of increasing concentrations of hCG and its various derivatives on adenylate cyclase activity as stimulated with a submaximal dose of bovine TSH (2.3×10^{-9} M). We found that TSH stimulation of adenylate cyclase activity was inhibited in a dose-dependent manner by the combined subunits, regardless of the presence or absence of sialic acid (Fig. 2). Thus, all of the combined forms of hCG, but none of the uncombined subunits interacted with the TSH receptor-adenylate cyclase system, and those combined

forms lacking sialic acid residues on the α-subunit exhibited no intrinsic activity. The fact that the agonist molecules, α:β and α:asβ, are capable of inhibiting TSH stimulation of adenylate cyclase activity is anticipated from the relatively low intrinsic activities exhibited by these two hormones (Fig. 1); that is, they behave as typical partial agonists (18).

The asα:asβ, asα:β and α:asβ molecules were more potent than the α:β molecule in inhibiting TSH stimulated adenylate cyclase (Fig. 2), suggesting that the removal of sialic acid residues from either subunit actually enhances the affinity of the interaction of hCG with the TSH receptor. On the other hand, the uncombined subunits, either native or desialylated, did not inhibit TSH stimulation of adenylate cyclase (Fig. 2). Thus, the ability of the hormones to interact with the TSH receptor-adenylate cyclase system is conferred by combination of the two subunits, and does not require sialylation; while the ability of the hormones, once interacting with the receptor, to bring about the conformational change in the receptor that results in activation of adenylate cyclase is dependent on sialylation of the α-subunit.

Previous modifications of the α-subunit have resulted in a loss of thyrotropic activity, but the effect has been attributable to a decrease in binding of hormone to receptor (19,20). Our data clearly show, for the first time, a modification of the α-subunit which affects the intrinsic activity without decreasing the binding to the TSH receptor. That the effect is brought about by such a subtle change in the α-subunit structure argues for the concept that the α-subunit rather than the β-subunit contains the domain through which hCG activates thyroidal adenylate cyclase. Bovine TSH and human luteinizing hormone, both of which stimulate thyroidal adenylate cyclase (5,15), have been reported to contain contain terminal sulfate, rather than sialic acid, on their α-subunit carbohydrate side chains (21); Comparison of the structures of these various agonists suggests the hypothesis that activation of thyroidal adenylate cyclase by glycoprotein hormones might involve negative charges and that these can be carried by either sialic acid or sulfate groups in the carbohydrate chains of the α-subunit.

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