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# A COMPETITIVE ANTAGONIST OF THYROTROPIN: ASIALO-CHORIOGONADOTROPIN

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SUMMARY: Characterization of the interaction between human thyroid membranes and asialo-choriogonadotropin, obtained by neuraminidase treatment of highly purified human choriogonadotropin, was undertaken using thyrotropin binding assays and adenylate cyclase experiments. The results show that asialo-choriogonadotropin acts as a typical competitive antagonist of thyrotropin action on its hormone receptor-adenylate cyclase system.

INTRODUCTION: A wide variety of hormones are known to interact with specific cell surface receptors and thereby to activate the membrane bound adenylate cyclase (ATP pyrophosphate lyase EC 4.6.1.1) (1). In several instances, substances capable of competitively antagonizing the effect of the hormones on adenylate cyclase activity have been identified (2,3). Such antagonists have been useful in studies directed toward elucidating the molecular mechanisms of hormone action (3-7). In many respects, the β-adrenergic receptor-adenylate cyclase system has served as a model for adenylate cyclase-coupled hormone receptors in general due to the availability of β-adrenergic antagonists (4-6). In the case of glycoprotein hormones, such antagonists, which could have analogous applications, have not yet been described. In this paper we show that asialo-choriogonadotropin (as-hCG)[1], obtained by neuraminidase treatment of highly purified human choriogonadotropin (hCG), is a competitive antagonist of thyrotropin (TSH) action on its hormone receptor-adenylate cyclase system.

[1]Abbreviations: as-hCG, asialo-choriogonadotropin; hCG, human choriogonadotropin; TSH, thyrotropin; GPP(NH)P, guanyl-5'-yl imidodiphosphate; GTP, guanosine 5'-triphosphate; EDTA, ethylene diamine-tetraacetic acid.

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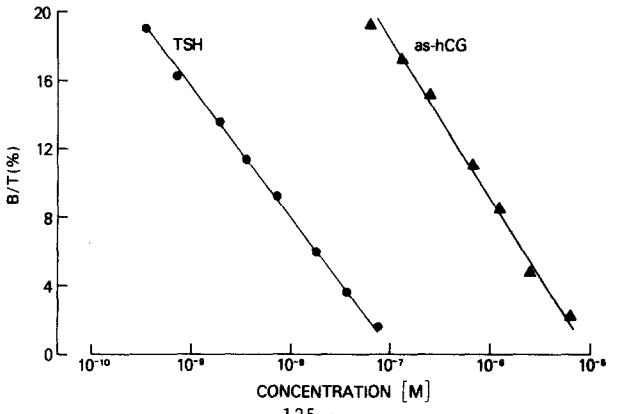


Fig. 1. Fig. 1 Inhibition of [$^{125}$I]TSH binding to human thyroid membranes by TSH(●) and as-hCG (▲).

MATERIALS AND METHODS: Human thyroid tissue was obtained from patients who underwent thyroidectomy during surgery for laryngeal cancer. The membranes were prepared as previously detailed (8,9). The radioligand receptor assay for TSH was performed as previously described (8), using highly purified bovine TSH, a generous gift of Dr. J.G. Pierce, Los Angeles, Ca. TSH labeled by the lactoperoxidase method was incubated in 20 mM Tris-maleate pH 7.8 containing 50 mM NaCl, 1 mM EDTA and 0.1% bovine serum albumin for 30 min at 34°C with 0.5 mg/ml of membrane protein in the presence of varying amounts of as-hCG and bovine TSH. Adenylate cyclase activity was determined as the production of cyclic AMP by thyroid membranes incubated in the conditions previously described (8). Cyclic AMP was measured by radioimmunoassay using reagents purchased from Schwarz-Mann, Orangeburg, N.Y. In one set of experiments, pre-treatment of thyroid membranes was carried out at 34°C for 20 min in 20 mM Tris-maleate pH 7.4 containing 1mM EDTA in the absence or presence of TSH and/or as-hCG. The preincubation was stopped by 100-fold dilution with ice cold buffer. The membranes were then centrifuged at 30,000 x g for 20 min at 4°C. The washing procedure was repeated thrice and the membranes were resuspended for adenylate cyclase assay. Preparations of as-hCG were obtained by neuraminidase treatment of highly purified hCG (10,11) and kindly provided by Drs. S. Birken and R. Canfield, New York. GPP(NH)P and GTP were purchased from Sigma, St. Louis, Mo. All other reagents were of the highest purity commercially available.

RESULTS: The effects of the TSH and as-hCG preparations on the binding of $^{125}$I-TSH to human thyroid membranes were compared (Fig. 1). Half-maximum inhibition of binding was achieved by TSH at a concentration of $5.6 \times 10^{-9}$ M and as-hCG at a concentration of $7.9 \times 10^{-7}$ M. Thus, the as-hCG displayed about 0.7% of the displacing activity of TSH.

The as-hCG preparation was incubated with thyroid membranes at concentrations ranging from $10^{-8}$ to $10^{-4}$ M. Neither stimulation nor inhibition of the adenylate cyclase was observed. In contrast, as-hCG inhibited, in a dose-dependent manner, the enzyme stimulated by varying

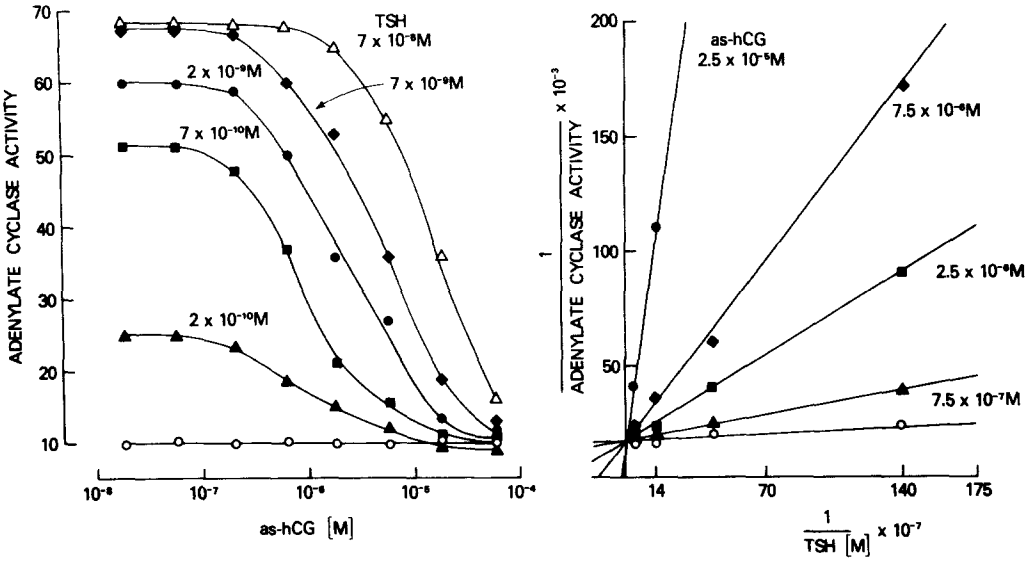


Fig. 2. Effect of as-hCG on TSH-stimulated adenylate cyclase activity. Dose response curves of as-hCG inhibition of adenylate cyclase activity assayed in the absence (o) or the presence of varying TSH concentrations as indicated (left panel). Double reciprocal plots of TSH stimulation of the adenylate cyclase activity in the absence (o) or the presence of varying as-hCG concentrations as indicated (right panel).

amounts of TSH (Fig. 2, left panel). Analysis of the data by double-reciprocal plots of the TSH concentration versus the adenylate cyclase activity showed that as-hCG acted typically as a competitive inhibitor of the TSH-stimulated adenylate cyclase activity (Fig. 2, right panel).

Stimulation of the adenylate cyclase activity of thyroid membranes by NaF, GPP(NH)P and GTP was not significantly altered by the addition of as-hCG in an amount that was sufficient to completely inhibit TSH stimulation of enzyme activity (Table I).

Preincubation of thyroid membranes in the presence of TSH induced an increase in the ability of the guanine nucleotides to subsequently stimulate the adenylate cyclase: membranes preincubated in the absence of TSH and subsequently reacted with GPP(NH)P and GTP showed increases over the basal level of the adenylate cyclase activity of 14.7 and 2.7 fold, respectively; when membranes were preincubated in the presence of TSH, the increases over the basal level of the enzyme activity elicited

TABLE I

Effect of as-hCG ($2.5 \times 10^{-5}$ M) on the adenylate cyclase activity of human thyroid membranes incubated in the absence (basal) or presence of NaF ($10^{-2}$ M), GPP(NH)P ($10^{-4}$ M) or GTP ($10^{-4}$ M).

| | Adenylate Cyclase Activity[a] | | | |
|---|---|---|---|---|
| | basal | NaF | GPP(NH)P | GTP |
| Control | 4.3 ± 0.4[b] | 99.2 ± 10.2 | 64.7 ± 5.8 | 12.3 ± 1.3 |
| as-hCG | 3.8 ± 0.4 | 98.0 ± 11.4 | 69.3 ± 7.1 | 11.9 ± 1.1 |

[a] pmol cAMP x $min^{-1}$ x mg membrane $protein^{-1}$

[b] mean ± SD of 4 determinations

by GPP(NH)P and GTP were 28.8 and 4.4 fold, respectively. Regardless of the addition to preincubation, no change was found in the basal and NaF-stimulated adenylate cyclase activity (Table II). Preincubation of the membranes with both as-hCG and TSH resulted in inhibition of the TSH effect on stimulation by GPP(NH)P and GTP, while as-hCG itself was without effect (Table II).

TABLE II

Adenylate cyclase activity of human thyroid membranes previously incubated in the absence or presence of TSH ($2.6 \times 10^{-9}$ M) and/or as-hCG ($2.5 \times 10^{-5}$ M). Concentrations of NaF, GPP(NH)P and GTP were the same as those given in Table I.

| | Adenylate Cyclase Activity[a] | | | |
|---|---|---|---|---|
| Addition to preincubation | basal | NaF | GPP(NH)P | GTP |
| None | 1.4 ± 0.1[b] | 31.2 ± 3.0 | 20.6 ± 1.8 | 3.8 ± 0.3 |
| TSH | 1.8 ± 0.2 | 32.4 ± 3.2 | 51.8 ± 4.3 | 7.9 ± 0.6 |
| as-hCG | 1.4 ± 0.1 | 30.4 ± 2.9 | 21.8 ± 2.0 | 4.1 ± 0.3 |
| TSH + as-hCG | 1.6 ± 0.2 | 30.2 ± 3.1 | 23.8 ± 2.2 | 4.3 ± 0.4 |

[a] pmol cAMP x $min^{-1}$ x mg membrane $protein^{-1}$

[b] mean ± SD of 4 determinations

DISCUSSION: This study demonstrates that as-hCG is a competitive antagonist of TSH action on its hormone receptor-adenylate cyclase system. The as-hCG preparation inhibits TSH binding to thyroid membranes and competitively inhibits TSH stimulation of adenylate cyclase activity. We find no evidence for as-hCG stimulation of thyroidal adenylate cyclase whereas such evidence has been previously reported (8) for the native hCG preparations from which the as-hCG is derived by neuraminidase treatment.

The molecular mechanisms of transduction of the hormonal signal into stimulation of cAMP synthesis are not yet completely understood. Nevertheless, there is a general notion that, at least three molecular species, namely receptor, guanine nucleotide dependent regulatory subunit and adenylate cyclase catalytic moiety, are involved in hormonal stimulation of the adenylate cyclase (4-7). Whether preincubated with the membranes or added directly to the adenylate cyclase assay, the as-hCG was found devoid of any effect on the catalytic activity of the enzyme. A possible interaction between as-hCG and the guanine nucleotide dependent subunit is ruled out by the observation that as-hCG does not elicit any change in the ability of GPP(NH)P and GTP to stimulate the adenylate cyclase. These observations, taken together with the fact that as-hCG is able to inhibit the TSH-induced increase in the subsequent guanine nucleotide stimulation of the adenylate cyclase, further argue that as-hCG antagonizes TSH action only by inhibiting binding to the receptor. As a typical competitive antagonist of TSH, as-hCG offers a useful tool for studies dealing with the molecular mechanisms of action of TSH at the cell membrane level.

Preparations of hCG have been shown to inhibit TSH binding (8,12) and to stimulate the adenylate cyclase (8) in human thyroid membranes, giving evidence that hCG is an agonist of TSH. Neuraminidase treatment of hCG increases the thyrotropin displacing activity of the molecule

(13) but completely abolishes its ability to stimulate the adenylate cylase of human thyroid membranes. This property seems not to be a characteristic of desialylated glycoproteins in general as the asialo-β subunit of hCG assayed in the same concentration range as the as-hCG, does not exhibit any thyrotropin displacing activity (data not shown). Considered together with previous work indicating that carboxypeptidase digestion of hCG increases the thyrotropic activity of the hormone (14), these observations emphasize that subtle modifications in the structure of the hCG can lead to important changes in the biological activity of the hormone.

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