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Human Chorionic Gonadotropin Stimulates Thyroid Hormone Secretion, Iodide Uptake, Organification, and Adenosine 3′,5′-Monophosphate Formation in Cultured Human Thyrocytes*

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ABSTRACT

Despite extensive studies, the issue of whether hCG possesses intrinsic thyrotropic activity remains unresolved. This is mainly because in the experimental systems used so far, the parameters measured did not include the thyroid-specific functions of iodine organification and the hormonal end-point response, T_3 secretion, and cells of nonhuman origin were employed, constituting a major drawback in view of the wide variation in sensitivity of thyroid responsiveness to hCG in different species. We investigated the thyrotropic activity of hCG, using for this purpose a novel homologous assay system consisting of human thyroid follicles cultured suspended in collagen gel in serum-free medium. Under these conditions, the cells are organized as follicular three-dimensional structures with normal polarity, enabling enhanced responsiveness to hormonal stimulation. The parameters measured were the thyroid-specific functions of iodide uptake, organification, and T_3 secretion, as well as formation of the second messenger, cAMP. Purified hCG (biological potency, 21,700 IU/mg; with no detectable TSH by immunoradiometric TSH assay) did indeed exhibit thyroid stimulatory activity. At doses ranging from 10–400 mg/L, hCG induced a dose-dependent increase in the parameters measured. The rise from basal to maximal levels achieved after hCG stimulation was 1.3 to 3.6 pmol/well for cAMP formation, 34 to 21,408 cpm/well for iodide uptake, 261 to 20,167 cpm/well for iodide organification, and 40 to 927 fmol/well for T_3 secretion. Maximal levels elicited by hCG (200 mg/L) relative to maximal values achieved with bovine TSH were 49%, 56%, and 42% for iodide uptake, organification, and T_3 secretion, respectively, and only 5% for cAMP. Iodide uptake proved to be the most sensitive indicator of the thyrotropic activity of hCG, with increases occurring at a concentration of 10 mg/L. Acting as a partial agonist, hCG was also capable of dose-dependently inhibiting TSH-stimulated cAMP formation. The free α- and β- subunits of hCG, at doses as high as 200 mg/L, had no thyroid-stimulating effect.

The present data thus clearly demonstrate that hCG is a human thyroid stimulator. Moreover, hCG managed to elicit substantial biological cell responses in human thyrocytes while evoking minimal amounts of cAMP, illustrating the concept of cAMP superfluity and highlighting the potential pitfalls of using cAMP as a reliable measure of hormonal bioactivity. (*J Clin Endocrinol Metab* **79:** 595–599, 1994)

WHETHER hCG possesses intrinsic stimulatory activity for the human thyroid has been controversial. On the one hand, the concept that hCG can express thyrotropic activity is supported by numerous clinical and experimental observations (reviewed in Refs. 1 and 2). Both biochemical and clinically overt hyperthyroidism have been observed in patients with trophoblastic tumors that secrete large amounts of hCG. Moreover, numerous studies have demonstrated that purified hCG stimulates cAMP generation and iodide uptake by animal thyroid cells. On the other hand, several workers have judged hCG to be a negligible human thyroid stimulator because it has little or no measurable stimulating effect on adenylate cyclase in human thyroid membranes (3) or human monolayer cell culture systems (4). It would appear, therefore, that despite extensive studies, the issue of whether hCG possesses intrinsic thyrotropic activity in humans remains unresolved.

The experimental systems used so far to examine the human thyroid-stimulating activity of hCG have had their limitations. First of all, the parameters measured in previous human systems have included neither thyroid-specific functions, such as iodine organification, nor a hormonal end-point response, *e.g.* T_3 secretion. Second, as the magnitude of the thyroid response to a given stimulator, including hCG, varies greatly in different species (4–7), it is obviously desirable that assessment of the human thyroid-stimulating activity of hCG be conducted in an assay system that uses human thyroid tissue; however, this has not always been the case. To circumvent these limitations, we have examined the human thyroid-stimulating activity of hCG using our novel homologous assay system, consisting of human thyrocytes cultured suspended in collagen gel in serum-free medium (8). Under these conditions, the cells become organized as follicular three-dimensional structures with normal cell polarity. Most importantly, cultured in this manner, human

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thyrocytes exhibit enhanced responsiveness to TSH. Furthermore, multiple critical steps involved in thyroid hormone biosynthesis can be readily assessed: iodide uptake, organification, and T_3 secretion, as well as formation of the second messenger, cAMP.

Materials and Methods

Cell culture

The method of preparation of human thyroid follicles has been previously described in detail (8). Essentially, the method consists of the following steps: 1) preparation of human thyroid cells in monolayer culture from colloid goiter tissue obtained at thyroidectomy from nodular goiter patients, 2) trypsinization and storage of the cells preserved in liquid nitrogen, 3) aggregation of thawed cells on agarose-coated dishes, 4) suspension of cell aggregates in collagen, 5) plating of one 20-μL drop (containing 200 × 10^3 cells) of the collagen suspension per well in 24-well microtiter plates, 6) gelling of the collagen suspension in an incubator at 37 C for 15–30 min, and 7) addition of 0.5 mL serum-free medium and antibiotics in the presence and absence of the agent(s) to be tested and culture at 37 C in an atmosphere of 5% CO_2-95% air in a water-saturated incubator. For T_3 measurements, KI (0.1 μmol/L) was also added to the medium; for cAMP measurements, 1-methyl-3-isobutylxanthine (0.1 mmol/L) was added to the medium.

At the end of the culture period, T_3 and cAMP secreted into the medium (the concentrations remaining in the cells were negligible) were measured by RIA, as described previously (8). [^{125}I]Iodide uptake and [^{125}I]iodine organification were determined as described previously (8).

Each experiment was repeated at least three times, using cell preparations obtained from separate patients, with good agreement between the results of individual experiments. The data shown are those from a representative experiment. Statistical analysis of the data was performed using Student's t test; $P < 0.05$ was considered significant.

Materials

Highly purified hCG was prepared as described previously (9). The biological potency of the purified preparation was 21,700 IU/mg, using CR 123-hCG (12,780 IU/mg) as the reference preparation in the rat Leydig cell testosterone bioassay. The hCG preparation was tested for possible TSH contamination by the TSH MAIA-clone Serono kit (Serono, Milan, Italy). At a concentration of 200 mg/L, there was no detectable TSH in the hCG preparation. The sensitivity of the TSH assay was less than 0.1 mU/L. Highly purified α- and β-subunits of hCG (batch CR 119, from the Center for Population Research. NICHHD, NIH) had interstitial cell-stimulating activities of 14.5 and 5.2 IU/mg, respectively, relative to the Second International Standard of hCG. Bovine (b) TSH (containing LH, 1–3%; FSH, <1%; GH, <1%; PRL, <1%; ACTH, <0.05%) was obtained from Sigma Chemical Co. (St. Louis, MO). All of the materials needed for the cell culture and measurements of T_3, cAMP, [^{125}I]iodide uptake, and organification were obtained as described previously (8).

Results

Human thyrocytes cultured in collagen responded to increasing doses of hCG or bTSH with elevations in cAMP formation, iodide uptake, iodine organification, and T_3 secretion (Fig. 1 and Table 1). A dose-dependent response was observed for all of the parameters tested, including hCG-stimulated cAMP, which rose from a basal level of 1.3 to 2.3 pmol/well ($P < 0.01$) with 50 mg/L hCG and to 3.6 pmol/well with 200 mg/L hCG. The most sensitive parameter of the thyroid-stimulating activity of hCG was observed with iodide uptake (Table 1). The free α- and β-subunits of hCG, at doses as high as 200 mg/L, had no stimulatory effect on any of the parameters (data not shown).

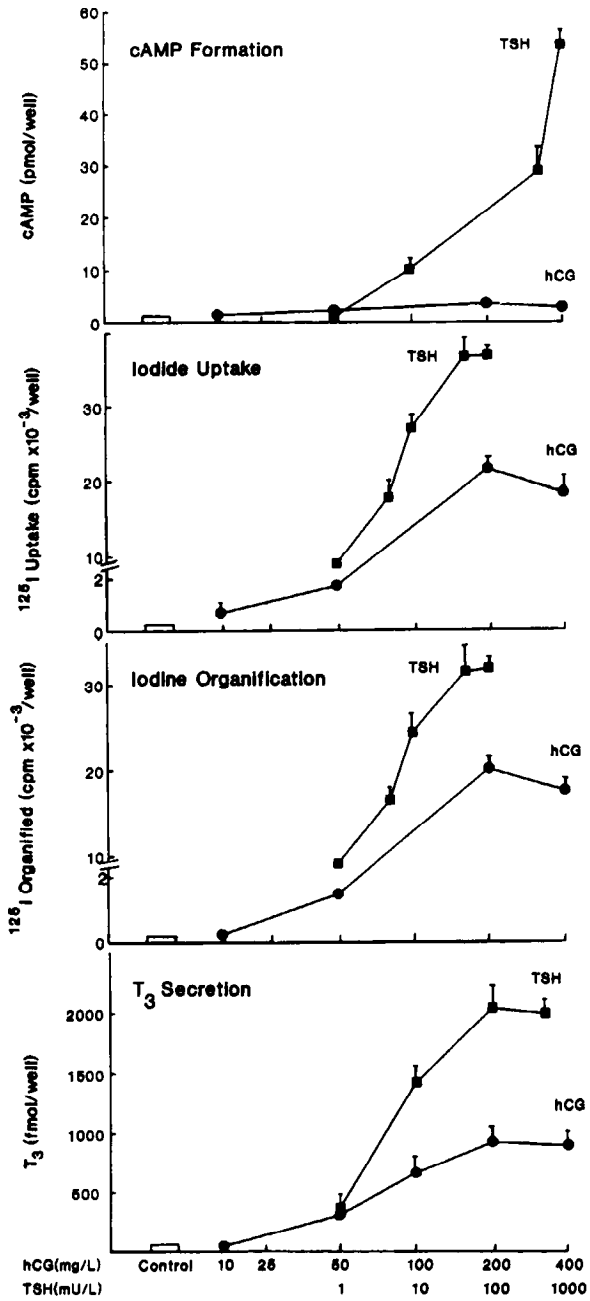

FIG. 1. Thyroid-stimulating activity of highly purified hCG in human thyrocytes cultured in collagen. After incubation with various concentrations of hCG for 7 days, formation of cAMP, iodide uptake, organification, and T_3 secretion were measured. Each *point* represents the mean ± SE of triplicate culture wells.

The maximal responses elicited by hCG differed from those caused by TSH (Fig. 2). This was especially evident with respect to cAMP formation; the maximal stimulation of cAMP by hCG was 5% of the maximal induced by TSH. In contrast, the maximal stimulations of iodide uptake, organification, and T_3 secretion were 49%, 56%, and 42%, respectively, of the maximal elicited by TSH. hCG exerted a dose-

TABLE 1. Responses of human thyrocytes cultured in collagen to the lowest concentrations of hCG used in this study

	cAMP (pmol/well)	^{125}I uptake (cpm × 10^3/well)	^{125}I organification (cpm × 10^3/well)	T_3 secretion (fmol/well)
Basal	1.3 ± 0.1	34.2 ± 2	261 ± 14	40 ± 3
hCG (10 mg/L)	1.5 ± 0.2	838 ± 270[a]	280 ± 20	42 ± 2
hCG (50 mg/L)	2.3 ± 0.2[a]	1552 ± 128[a]	1510 ± 112[a]	310 ± 8[a]

Values are the mean ± SE.
[a] $P < 0.01$ compared to basal.

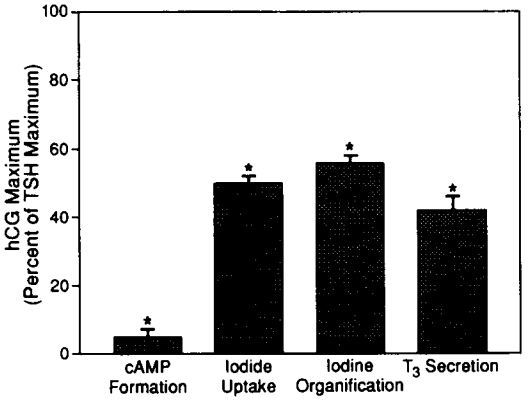

FIG. 2. Maximal levels of cAMP formation, iodide uptake, organification, and T_3 secretion elicited by hCG (200 mg/L), expressed as a percentage of the maximal levels achieved with bTSH (100 mU/L for iodide uptake, organification, and T_3 secretion and 1000 mU/L for cAMP formation) after exposure of human thyrocytes cultured in collagen to hCG or bTSH for 7 days. The maximum responses of the various dose-response curves were obtained using the computer program Allfit (10). Each *bar* represents the mean ± SE. ★, $P < 0.01$ compared to bTSH maximum (*i.e.* 100%).

dependent inhibition of TSH-stimulated cAMP formation (Fig. 3).

Discussion

The present data clearly demonstrate that hCG is a human thyroid stimulator. A highly purified preparation of hCG dose dependently stimulated T_3 secretion, iodide uptake, organification, and cAMP formation in human thyrocytes cultured in collagen. The experimental thyroid system used has several advantages compared to those previously employed to address the issue of whether hCG possesses intrinsic human thyroid-stimulating activity. With this system, it is possible to measure a range of parameters that span the complete thyroid hormone biosynthetic pathway: cAMP formation, iodide uptake, iodine organification, and T_3 secretion. A second advantage is the use of thyrocytes of human origin, which assumes great importance in view of the reported wide spectrum of sensitivity of thyroid responsiveness to hCG in different species (4–7). A third advantage is the use of serum-free medium, which 1) allows enhanced cell responsiveness to hormone stimulation (8), 2) avoids reversal in cell polarity (11, 12), 3) eliminates factors present in serum that can affect cell function and thus obscure cell responsiveness to test agents (13), and 4) avoids batch to batch variability in serum composition (14). A final advantage is

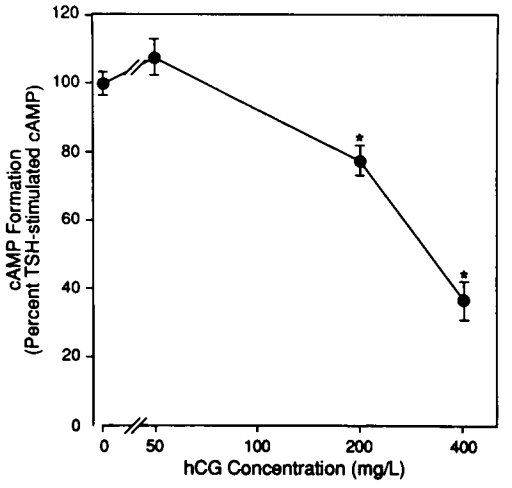

FIG. 3. Inhibition of TSH-stimulated cAMP by hCG. Human thyrocytes cultured in collagen were exposed to bTSH alone (50 mU/L) or with a submaximal concentration of bTSH (50 mU/L) together with various hCG concentrations for 7 days. Each *point* represents the mean ± SE of triplicate culture wells. ★, $P < 0.01$ compared to bTSH alone (*i.e.* 100%).

the improved functional responses achieved by culturing on collagen, which enables thyrocytes to be organized in follicular structure rather than in monolayer (8).

Our finding that hCG elicits a minute cAMP response from human thyrocytes confirms similar findings reported in a number of previous studies (reviewed in Refs. 1 and 2). This characteristic of the thyroid-stimulating activity intrinsic to hCG has been the basis of the controversy over whether hCG is a biologically important human thyroid stimulator (1, 2, 7). The present observations seem to resolve this controversy. hCG stimulated iodide uptake, iodine organification, and T_3 secretion to levels approximately 50% as high as the maximal levels stimulated with TSH, but it stimulated cAMP formation only 5% compared to the effect of TSH. Thus, although hCG marginally stimulated cAMP formation, it markedly enhanced post-cAMP steps in the thyroid hormone biosynthetic pathway.

That hCG is able to elicit substantial biological cell responses in thyrocytes while evoking minimal amounts of cAMP could imply that the hormone is acting through a non-cAMP intracellular signal system or could be another manifestation of cAMP superfluity that typifies glycoprotein hormone signal transduction systems (15). Indeed, this phenomenon of high cell secretory responses achieved in response to a minute fraction of the cAMP that the cell is capable of producing has been previously demonstrated in thyroid, luteal, Leydig, and Sertoli cells with the whole family of glycoprotein hormones, including TSH, LH, hCG, and FSH (15–18). The present study extends this concept to human thyrocytes cultured in collagen and highlights the potential pitfalls of using cAMP as a reliable measure of hormonal bioactivity.

By stimulation with hCG Chinese hamster ovary cells transfected with the human TSH receptor, a mechanism for the thyrotropic activity of hCG was recently demonstrated to involve its direct interaction with the TSH receptor (19,

20), probably as a consequence of the known homology between the hCG and TSH molecules as well as between their respective receptors (21, 22). These observations are consistent with the data from the present study, in which hCG was found, as also noted by others (3, 23), to inhibit TSH-stimulated cAMP. Such inhibition would be expected of a partial agonist on cAMP formation, which works via the TSH receptor to modulate cAMP formation.

Iodide uptake proved to be the most sensitive indicator of the thyrotropic activity of hCG, with increases occurring at a concentration of 10 mg/L, *i.e.* near the peak concentrations of hCG during normal early pregnancy (24–26). In this context, it is noteworthy that there is a slight increase in thyroid function coupled with a modest suppression of serum TSH levels in those normal pregnant women with hCG levels in the upper normal range (27–29). Furthermore, in patients with hyperthyroidism associated with hCG-secreting tissues, such as choriocarcinoma (30), hydatidiform mole (31), and hyperemesis gravidarum (32), hCG levels have nearly always exceeded those typical of normal pregnancy, in some cases reaching levels higher than 1000 mg/L (30). Thus, our observations support a role for hCG as an *in vivo* human thyroid stimulator (33). In this context, it is relevant to recognize that hCG is not a single molecule, but, rather, a family of isoforms that vary mainly in carbohydrate structure (34, 35) and to some extent in polypeptide structure as well (20, 36). Furthermore, the family of isoforms produced in pregnancy differs from that in neoplasms (37). It seems likely that these isoforms would display variability in their thyroid-stimulating activities.

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