EVIDENCE THAT CHORIONIC GONADOTROPIN HAS INTRINSIC THYROTROPIC ACTIVITY

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SUMMARY

The thyrotropic and gonadotropic specific biologic activities of chorionic gonadotropin (hCG) as assessed by the mouse thyrotropin and prostate weight bioassays increased at least 5 fold during purification. The hCG generated from recombination of hCG subunits that were essentially devoid of both activities recovered thyrotropic as well as gonadotropic biologic activity. These data show that the substance with thyrotropic activity found in hCG preparations has physicochemical properties indistinguishable from those of hCG and therefore, these results strongly suggest that thyrotropic activity is an intrinsic property of hCG.

The observation that pregnancy urine contains a substance with thyrotropic activity was made over two decades ago (1). Subsequently, thyrotropic activity has been found in commercially available chorionic gonadotropin (hCG) preparations (2) from pregnancy urine, prompting consideration of the hypothesis that thyroid stimulating activity is an intrinsic property of hCG. It has been well established that when separate, the α and β subunits of hCG are essentially devoid of gonadotropic activity, and that the hCG formed by recombination of these subunits regains activity (3). If thyroid stimulating activity were also a property of hCG, then the thyrotropic biologic activity of highly purified hCG would be expected to behave in a similar fashion. Therefore, we assessed the relative thyrotropic activities of crude hCG, highly purified hCG, hCGα subunit, hCGβ subunit, and hCG generated by recombination of purified hCGα and hCGβ subunits.

MATERIALS AND METHODS

Highly purified hCG was derived from commercially available hCG (Organon, Oss, Netherlands) as previously described (4-5). There was a 4 to 5 fold increase in hCG specific biologic activity during purification (4). The present study was performed using purified hCG batches CR115 and CR119. The method for separation and purification of the hCGα and hCGβ subunits has been described (6). To generate recombined hCG, the purified subunits were mixed in equimolar amounts in 0.02 M Tris buffer, 0.15 M NaCl, pH 7.4, and incubated at 37 C for 6 hr. The buffer used to prepare solutions for bioassay in addition contained 1% bovine serum albumin.

The thyroid stimulating activity of the hormone preparations was determined by the mouse thyrotropin bioassay as described elsewhere (7). The response variable in blood samples obtained at 2, 9, and 22 hr after intraperitoneal injection of test solutions was calculated as percent of the counts in the initial sample. The interstitial cell stimulating acitivity of the hormone preparations was determined by Griff T. Ross, National Institute of Child Health and Human Development, NIH, using a ventral prostate weight bioassay as described elsewhere (8). For all assays, potency estimates were calculated by the method of Brownlee (9) and the 95% fiducial limits by the method of Finney (10).

RESULTS

As shown in Table 1, the thyrotropic activity as well as the gonadotropic activity of the hCG preparation increased markedly during purification. There was a 5 fold increase in the activity

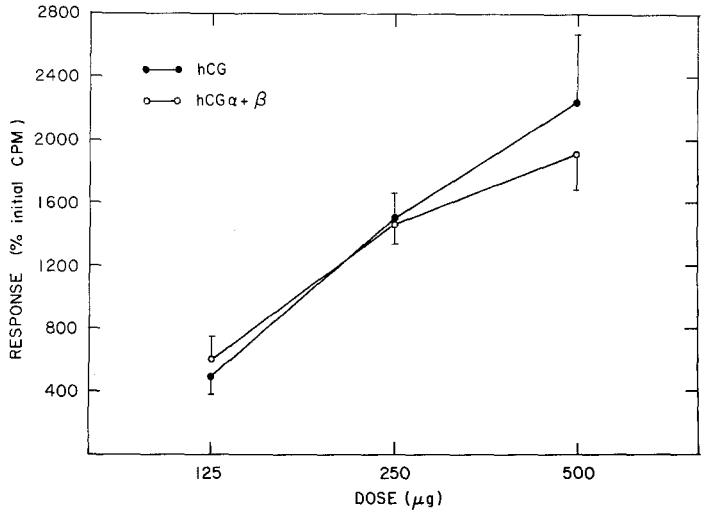

Figure 1. Dose-response curves of highly purified hCG (CR119) and recombined hCG (hCGα+β) in the mouse thyrotropin bioassay. Symbols and vertical bars indicate the mean and S.E. of the responses (9 hr) of 5 mice.

of purified hCG in the gonadotropin bioassay and about a 7 fold increase in the mouse thyrotropin bioassay. Dissociation of the highly purified hCG into subunits resulted in loss of virtually all biologic activity in the mouse thyrotropin bioassay, as well as in the gonadotropin bioassay (Table 1). Even doses as large as 1.0 mg of the subunits were devoid of biologic activity at 2, 9, and 22 hr in the mouse thyrotropin bioassay. However, as shown in Table 1, the hCG generated from recombination of these subunits regained both thyrotropic and gonadotropic biologic activities. Figure 1 depicts the dose-response curves obtained with highly purified hCG (CR119) and hCG generated from recombination of purified hCG (CR119) subunits. The maximum responses obtained with the hCG preparations were in excess of 2000% and thus greatly exceeded the non-specific responses reported for some substances (11). The slopes of the curves were not significantly different and the point estimate of the potency ratio was 0.96

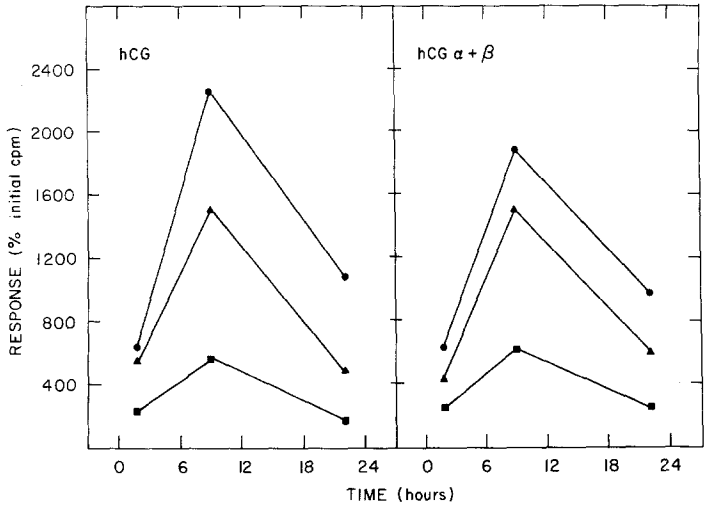

Figure 2. Time course of the response obtained with highly purified hCG and recombined hCG (hCGα+β) in the mouse thyrotropin bioassay. Each symbol indicates the mean of the responses of 5 mice given 125 μg (squares), 250 μg (triangles), or 500 μg (circles). The CR119 batch of hCG was used in this experiment.

(95% fiducial limits = 0.73-1.26). Furthermore, the shape of the time course of the response obtained with highly purified hCG was similar to that obtained with its recombinant (Figure 2). At all dose levels, the maximum response occurred at 9 hr.

DISCUSSION

The results of the present study strongly suggest that the thyrotropic activity found in hCG preparations resides in the same molecular species as the gonadotropic activity. That both gonadotropic and thyrotropic specific biologic activities increased by similar factors during purification supports this hypothesis. The hCGα and hCGβ subunits purified by both ion exchange chromatography and gel filtration were essentially devoid of both activities. Hence, the observation that recombined hCG regained both thyrotropic and gonadotropic

biologic activities in the same ratio as the native hCG cannot be accounted for on the basis of contamination of the subunits with thyrotropic activity. Not only the slope of the dose-response curve, but also the time course of the response obtained with recombined hCG was essentially the same as that obtained with native hCG. Our finding that the recombined hCG generated from biologically inactive subunits regained thyrotropic activity quantitatively and qualitatively similar to that in the highly purified hCG preparation strongly suggests that hCG has intrinsic thyrotropic activity.

Table 1. Thyrotropic and gonadotropic biologic activities in crude hCG, highly purified hCG (CR115), hCGα subunit, hCGβ subunit, and recombined hCG.

Preparation	Relative Potency* Mouse Thyrotropin Bioassay	Relative Potency* Ventral Prostate Weight Bioassay
Crude hCG	0.14	0.20
Purified hCG	1.00	1.00
hCGα	<0.02	<0.001
hCGβ	<0.02	<0.001
Recombined hCG	0.82	0.80

*Potencies calculated relative to purified hCG (CR115).

Since chorionic thyrotropin, the substance with thyrotropic activity extracted from placenta (12), has been reported to possess physicochemical properties different from those of the substance with thyrotropic activity in urinary hCG preparations (13), the possibility of chorionic thyrotropin copurifying with hCG is unlikely. Also, it has been shown that chorionic thyrotropin does not have an α-subunit structure identical to that of hCG since antisera generated to the hCGα subunit neutralized the biologic activities of hCG and human pituitary thyrotropin, but not chorionic thyrotropin (7). In contrast,

the data presented here indicate that the substance exhibiting thyrotropic activity in highly purified hCG has an α-subunit structure apparently indistinguishable from that of hCG. Thus, our results support the concept that the placenta secretes a substance with thyrotropic activity that is distinct from chorionic thyrotropin and has physicochemical properties indistinguishable from those of hCG.

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