INABILITY OF HIGHLY PURIFIED PREPARATIONS OF HUMAN CHORIONIC GONADOTROPIN TO INHIBIT THE PHYTOHEMAGGLUTININ-INDUCED STIMULATION OF LYMPHOCYTES

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1. Introduction

Several investigations, as reviewed by Anderson [1] and Billingham [2], were inspired by the fact that the foetus, though possessing different genetic features, is not rejected by the maternal organism like an allograft. Indeed it could be shown, that the maternal organism may specifically be sensitized against placental antigens mediated by the cellular immunity system [3]. An altered reactivity of this system might be one reason, why a rejection does not occur [4,5]. This idea is supported by the observation that the lymphocytes of pregnant women have a diminished non-specific reactivity, as measured by the phytohemagglutinin (PHA) stimulation test [6,7]. One possible explanation for this phenomenon was proposed by different reports. They dealt with an inhibitory effect of human chorionic gonadotropin (hCG) [8–10] and human chorionic somatomammotropin (hCS) [9], respectively, on the response of lymphocytes to PHA. These observations might explain the mechanism of acception of the foetus by the maternal organism in connection with the high formation rate of hCG (up to 1×10^6 IU/day) during the first trimester of pregnancy. The investigations reported in the literature [9,10] were performed with crude hCG preparations. Therefore, we have studied the effect of highly purified hCG as well as of fractions obtained in the course of hCG purification on lymphocyte stimulation.

2. Methods and materials

Crude hCG (4300 IU/mg) was purchased from Schering AG, Berlin, GFR. Highly purified hCG with a biological activity of 13 000 IU/mg was prepared according to Canfield et al. [11] with slight modifications [12]. The purification was achieved by a batch absorption of the crude hormone on SP-Sephadex C50 followed by chromatography on DEAE-Sephadex A50 and by a final chromatography on Sephadex G150. Biological activity was assayed by the rat prostate test [12].

Lymphocyte cultures: Freshly drawn heparinized human blood was allowed to sediment at 37°C for about 2 hr. The supernatant leukocyte-containing plasma was collected and passed through a column packed with nylon fibres (Leuko-Pak®, Fenwal Lab., Morton Grove, I11.) at 37°C. Cells were centrifuged at 160 *g* for 10 min and washed once with culture medium (Eagles MEM with 10 mM HEPES-buffer, pH 7.4, 200 IU/ml Penicillin, 200 μg/ml Streptomycin and 10% inactivated autologous serum). Lymphocyte cultures were prepared in test tubes (Falcon Plastics No. 2006, Los Angeles, Calif.), each tube containing 1 million mononucleated cells in 1 ml culture medium. The lymphocytes were preincubated 24 hr in the presence of the protein fraction to be tested for inhibition. Thereafter, PHA stimulation was started by adding 10 μl PHA-M (Difco Labs., Detroit, Michigan).

46 hr later, 2 hr before the end of incubation, 0.5 μCi of [^{3}H] thymidine (spec. act. 2 Ci/mmol, The Radiochemical Centre, Amersham) were added to each culture. Cells were collected on membrane filters (Millipore, PHWP 02500), washed with 5 ml saline, 3×5 ml 5% TCA and 5 ml 0.1 N HCl. The incorporated radioactivity was measured in a liquid scintillation spectrometer (Packard Instruments). The uninhibited PHA stimulation of lymphocytes, determined as

incorporation of [^{3}H] thymidine, in the mean yielded 35 000 cpm per culture. Cultures which were incubated in the absence of PHA displayed a blank value of 330 cpm per culture. In the case of purified hCG some assays were performed at one fifth the scale. Cell viability was tested by Trypan Blue exclusion before the addition of the labelled thymidine.

Anti-hCG antiserum was obtained from goats using highly purified hCG (13 000 IU/mg) for immunization as described elsewhere [13].

Anti-hCG-β subunit antiserum was obtained from rabbits using a β subunit preparation prepared as described by Merz et al. [12]. Per animal 250 μg of β subunit dissolved in complete Freund adjuvant and saline, respectively, were applicated three times, at intervals of one month.

3. Results

The influence of hCG fractions of different purity and biological activity on the PHA-dependent transformation of lympocytes was investigated. Unlike the commercially available crude hormone (see fig.1a) highly purified hCG (13 000 IU/mg) is not able to inhibit this reaction up to a dose of 13 000 IU/ml. This result is not confined to a certain batch of highly purified hormone. With one exception, the inhibitory properties of the fractions which are separated from hCG during the different purification steps do not exceed the inhibition caused by the crude hormone. The fraction which is separated in the first step of hCG purification, however, has a higher inhibitory potency. This purification step consists in a batch adsorption of crude hCG on SP-Sephadex. About half of the crude hormone is not adsorbed. This material (fraction I) has no detectable biological activity in the rat prostate assay for hCG [12] whereas the adsorbed material (fraction II) which is used for further purification is biologically active (6000 IU/mg). A comparison of the inhibition properties between highly purified hCG, crude hCG and fraction I is presented in fig.1b and in table 1, respectively. Even at the highest concentrations of the investigated substances cell viability is not diminished. In contrast to the stimulated cultures the inhibited ones show almost no agglutination of the cells.

4. Discussion

The results presented in this paper clearly indicate that the ability to inhibit the PHA induced transformation of lymphocytes is not correlated with the enrichment of the biological activity during the purification of hCG. To the contrary, this property is lost during the purification procedure. This may by the conse-

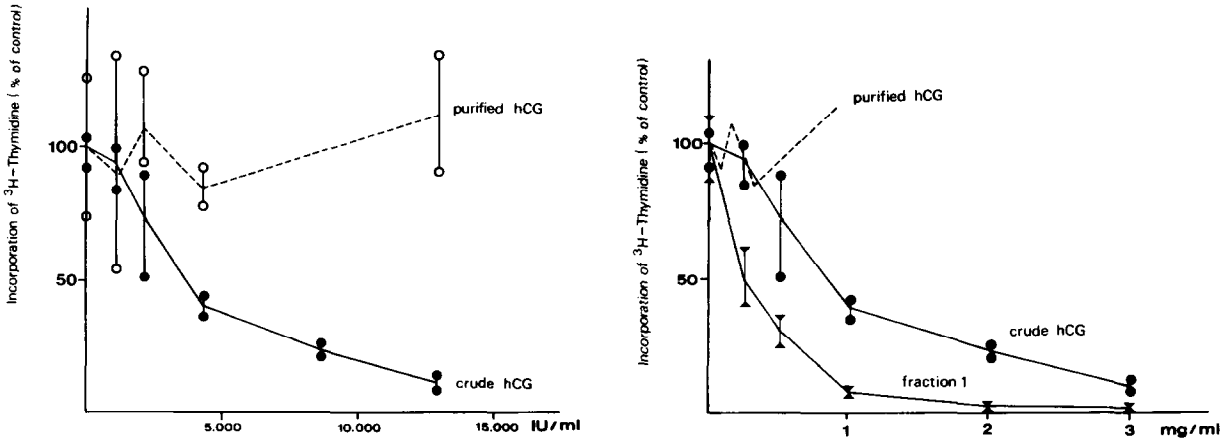

Fig.1. Influence of purified hCG, crude hCG and fraction I on the PHA-induced [^{3}H] thymidine incorporation into human peripheral lymphocytes. The result of one representative assay is shown. In fig.1a purified hCG is compared with crude hCG on the basis of hormonal activity. Fig.1b compares fraction I, crude hCG and purified hCG on the basis of weight. The mean values of triplicate assays are expressed as percent of the PHA-stimulated control cultures. The vertical bars represent the range.

Table 1
PHA stimulation of lymphocytes in presence of highly purified hCG, crude hCG and fraction I at a concentration of 1 mg/ml and 4300 IU/ml; respectively.

	Highly purified hCG, 4300 IU/ml	Crude hCG 4300 IU/ml = 1 mg/ml	Fraction I 1 mg/ml
Number of experiments	11	9	5
PHA-stimulation in % of uninhibited control cultures	95.3 ± 20.1%	44.8 ± 18.8%	20.5 ± 12.4%

The difference between the results for highly purified hCG and crude hCG is significant ($p<0.001$), as well as the difference between crude hormone and fraction I ($p<0.025$) as determined with the Mann-Whitney test [21].
The PHA stimulated control cultures yielded in the mean 35 000 cpm, the blank values displayed 330 cpm.

quence of the loss of a definite biological property of the hormone which possibly cannot be determined by means of the stimulatory effect on the gonades. On the other hand a substance distinct from hCG possessing this inhibitory ability might be separated during the purification. The above results favour the latter possibility because the inhibitory property is enriched in a fraction which shows no hCG activity. The idea that this substance might consist of either desialylated hormone or the isolated β subunit seems to be unlikely for the following reasons: First, fraction I reacts very weakly with an antiserum against hCG, contrary to desialylated hCG which shows a very good reaction. Furthermore, desialylated hCG should not be a part of fraction I because of its isoelectric point of pH 9.5. Secondly, no reaction could be observed between fraction I and an antiserum which is merely specific for the isolated β subunit of hCG.

A possible relation of the inhibiting substance to various pregnancy-correlated factors [4,15–17,19] which show an influence in different tests for cell mediated immunity has to be considered. Furthermore, it remains to be clarified whether this substance merely acts as a specific competing reactant for PHA. This conception is supported by the observation that fraction I also inhibits the agglutination of cells by PHA. On the other hand, Teasdale et al. [20] have found that their crude hCG (3700 IU/mg) also inhibits lymphocyte stimulation in the mixed lymphocyte culture test, indicating a change of the properties of the lymphocytes.

The differences between the results reported in the literature [8–10] can possibly be explained by hCG preparations of different purity. The crude hCG which was used in our laboratory shows a similar inhibitory activity to that reported by Adcock et al. [10]. The hCG preparation used by Contractor et al. [9] has a higher inhibitory activity, but a lower biological activity.

On the basis of the results presented in literature and in this paper, it is not at all clear, whether hCG is involved in a mechanism which prevents a rejection of the foetus.

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