MICROBIOLOGY AND IMMUNOLOGY

Biological Effects of Human Chorionic Gonadotropin and Its Synthetic Peptide Fragment on HL-60 Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 4, pp. 424-426, April, 1997
Original article submitted October 20, 1996

Human chorionic gonadotropin and synthetic fragment of its β-subunit consisting of 18 amino acids (from the 128th to 145th residue) inhibit the growth of promyeloid HL-60 cell line and compete for the common binding sites on the plasma membrane of HL-60 cells.

Key Words: *chorionic gonadotropin; peptide; HL-60 cells; inhibition; receptor*

Human chorionic gonadotropin (HCG) is a polyfunctional glycoprotein hormone with immunoregulatory activity [5,7]. Although more attention has been focused on HCG as a prospective candidate for new reversible contraceptives, structural and functional organization of this hormone remains obscure.

The unique C-terminal sequence of the HCG β-subunit consisting of 24 hydrophilic amino acids is of special interest [3].

Previously we showed that similar to native HCG synthetic C-terminal peptide of the HCG β-subunit (from the 128th to 145th amino acid residue, CTP) inhibits proliferation of peripheral blood lymphocytes induced by a polyclonal activator and binds to the surface receptors of monocytes but not of T lymphocytes [1]. Based on these results, we have suggested that antiproliferative activities of HCG and CTP are mediated by monocytes.

The aim of the present study was to examine biological effects of CTP on promyeloid cell line HL-60. We assessed the ability of HCG and CTP to inhibit the growth of HL-60 cells and studied the binding of HCG and CTP membrane receptors of HL-60 cells.

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MATERIALS AND METHODS

C-terminal peptide and its analog with tyrosine residues in the amino end were synthesized as described elsewhere [2]. The peptides were purified by high-performance liquid chromatography on a reverse phase carrier, which provided at least 95% purity of the resultant peptide preparation. The amino acid sequence of CTP is LPSPSRLPGPSDTPILPQ (a single-letter code).

Human chorionic gonadotropin was from Serono; RPMI-1640 culture medium, fetal calf serum, and L-glutamine were from Sigma. Other reagents were from Sigma and Serva. Radioactive labels were from Amersham.

HL-60 cells were kindly provided by Dr. A. A. Kushch (D. I. Ivanovskii Institute of Virology, Russian Academy of Medical Sciences). Cells were grown in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum, and 2 mM L-glutamine at 37°C in a humidified atmosphere with 5% CO_2. Cultures were maintained in the logarithmic growth phase.

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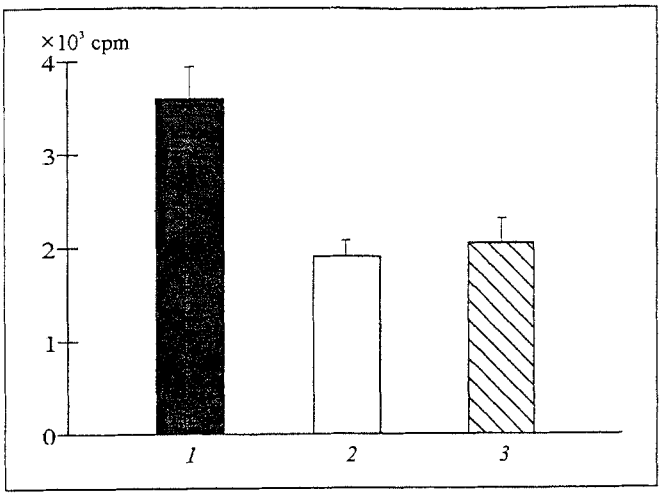

Fig. 1. Effect of HCG (2) and synthetic CTP (3) on the growth of HL-60 cells. Cell growth was assessed by incorporation of ^{3}H-thymidine (×10^3 cpm). Intact cells served as control (1).

The proliferative response of HL-60 cells was assessed by ^{3}H-thymidine incorporation into DNA. Cell suspension (1 ml, 2×10^4 cells/ml) was incubated in 24-well plates in the presence of HCG (3×10^{-8} M) or CTP (10^{-5} M) for 72 h. The radiolabel (1 μCi/well) was added 4 h before the end of culturing. Cells were then transferred onto GF-C fiberglass filters (Whatman), and radioactivity was measured in a Rack-beta liquid scintillation counter (LKB-Wallac).

The binding of CTP and HCG to HL-60 cells was studied by the radioligand method. For this purpose tyrosyl-CTP and HCG were labeled with ^{125}I by the method [8]. Specific radioactivities of tyrosyl-CTP and HCG were 2×10^{18} cpm/mol and 10^{20} cpm/mol, respectively.

The cells (10^6 per point) were incubated with ^{125}I-tyrosyl-CTP (1.2×10^{-7}-4.7×10^{-10} M) or ^{125}I-HCG (10^{-8}-10^{-13} M) for 1 h at 4°C in phosphate-buffered saline (100 μl) containing 0.02% sodium azide and 1% bovine serum albumin. After incubation cells were centrifuged in 10% sucrose gradient, and their radioactivity was measured in a γ-counter (LKB) [4]. The dissociation constants and the number of binding sites were determined using Scatchard plots [6].

In the competitive binding experiments HL-60 cells were incubated with ^{125}I-tyrosyl-CTP (4×10^{-9} M) in the presence of varied concentrations of HCG (10^{-4}-10^{-13} M).

RESULTS

Both CTP and HCG inhibited proliferation of HL-60 cells (Fig. 1) without producing cytotoxic effect in the studied concentration range.

The parameters of CTP and HCG binding to HL-60 cells were determined by the radioligand method. Radioactive label (^{125}I, Iodogen) was inserted in the tyrosine aromatic ring [8]. Since CTP contains no tyrosine residues, the peptide analog with tyrosine in the amino end was synthesized. This analog exhibited antiproliferative activity.

Both HCG and tyrosyl-CTP specifically reacted with surface receptors of HL-60 cells (Fig. 2). For HCG the dissociation constant was 1.5×10^{-9} M and the number of binding sites was 5000. For tyrosyl-CTP the dissociation constant was 1.9×10^{-9} M and the number of binding sites was 4600. It should be noted that the binding parameters for HCG and

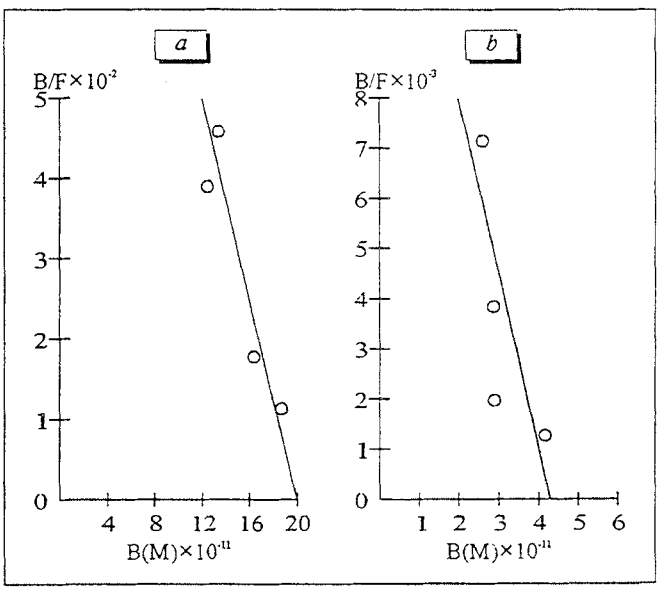

Fig. 2. Binding of ^{125}I-labeled human HCG (a) and tyrosyl-CTP (b) to HL-60 cells. Scatchard plots. a) dissociation constant is 1.5×10^{-9} M, number of binding sites per cell is 5000; b) 1.9×10^{-9} M and the number of binding sites per cell is 4600.

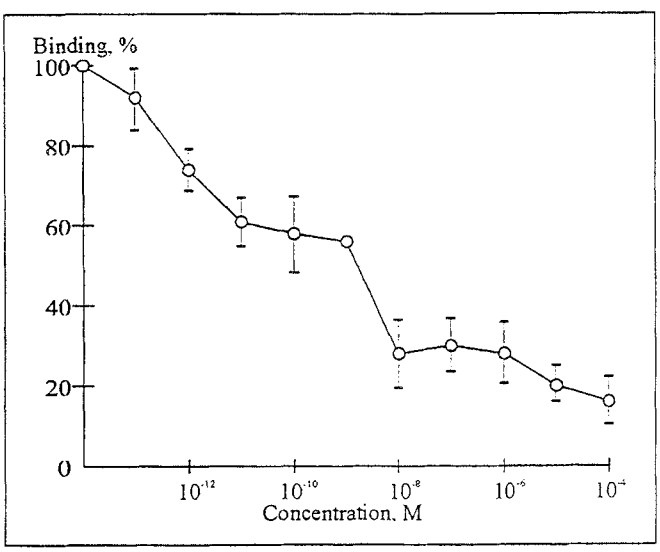

Fig. 3. Replacement of ^{125}I-tyrosyl-CTP bound to HL-60 cell receptors by HCG. Binding of ^{125}I-tyrosyl-CTP is the absence of unlabeled HCG in taken as 100%.

tyrosyl-CTP are similar and comparable to those of the binding to human peripheral blood monocytes [1].

In the competitive binding experiments, cells were incubated with iodinated tyrosyl-CTP in a constant concentration close to the dissociation constant and varied concentrations of unlabeled HCG. It was found that HCG replaced tyrosyl-CTP on specific receptors (Fig. 3). This indicates that HCG and synthetic tyrosyl-CTP compete for the common binding sites.

Our results suggest that C-terminal fragment of the HCG β-subunit is functionally active and mediates the immunoregulatory effects of HCG. Further investigation of biological activity of this fragment may contribute to the understanding of the mechanisms responsible for the effects of HCG and regulation of various immunocompetent cells.

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