Synthetic Peptide, a Fragment of the β-Subunit of Chorionic Gonadotropin, Inhibits Mitogen-Stimulated Proliferation of Human Lymphocytes *In Vitro*

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Similar to chorionic gonadotropin, synthetic fragment of the β-subunit of chorionic gonadotropin (128th-145th amino acid), inhibits mitogen-stimulated proliferation of human lymphocytes *in vitro* and binds to membrane receptors of human peripheral blood monocytes.

Key Words: *chorionic gonadotropin; peptide; lymphocytes; proliferation, inhibition*

Human chorionic gonadotropin (HCG), a gonadotropic glycoprotein, is a protein factor emerging in pregnancy [9]. It was reported that HCG not only fulfils the luteinizing function and stimulates production of steroid hormones but also possesses immunomodulating activity. However, there is controzversy over the effect of HCG on immunocompetent cells [6, 10], and the mechanisms of HCG action are unclear.

Since the C-terminal sequence of HCG β-subunit is unique for this hormone [2] it seems reasonable to study the contribution of this fragment to biological activity of HCG. Previously, it was shown that this fragment is not necessary for the interaction between HCG and the gonad cell receptors, intracellular cAMP increase, and induction of steroid hormone synthesis *in vitro* [7]. However, the significance of this fragment for immunomodulating activity of HCG is unclear.

The aim of the present study was to examine the *in vitro* effects of synthetic fragment of the C-terminal sequence of HCG β-subunit (128th-145th amino acid, C-terminal peptide) on mononuclear cells (MNC) isolated from human peripheral blood and compare it with the effect of native HCG. The ability of the C-terminal peptide (CTP) to inhibit mitogen-stimulated proliferation of lymphocytes and to bind to various blood cells was also assessed.

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MATERIALS AND METHODS

C-terminal peptide and its analog with an additional tyrosine residue in the N-terminal sequence were synthesized as described elsewhere [1]. The peptides were purified by reverse phase high-performance liquid chromatography. The resultant preparation contained not less than 95% peptide. The amino acid sequence of CTP is LPSPSRLPGPSDTPILPQ (a single-letter code).

The following reagents were used: HCG (Serono), RPMI-1640, fetal calf serum, L-glutamine (Sigma), and concanavalin A (ConA, Pharmacia). Other reagents were from Sigma and Serva; radioactive labels were from Amersham.

Peripheral blood MNC were isolated by gradient centrifugation [3]. Monocytes and T cells were isolated from the MNC fraction as described [4].

Blast transformation was performed in 96-well plates in a volume of 200 μl. Cells were cultured in

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RPMI-1640 with 5% heat-inactivated fetal calf serum at 37°C in a humidified atmosphere. Suspension of MNC (10^6 cells/ml) was incubated for 75 h in the presence of ConA (2 μg/ml) and HCG (2.5×10^{-7}-10^{-9} M) or CTP (10^{-4}-10^{-8} M). Intact and ConA-treated MNC served as the control.

Proliferation of MNC was assessed by ^{3}H-thymidine incorporation into DNA. The radiolabel was added 15 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).

The binding of CTP and HCG to blood cells was studied by the radioligand method. For this purpose tyrosyl-CTP and HCG were labeled with ^{125}I by the method [11]. Specific radioactivities of tyrosyl-CTP and HCG were 2×10^{18} cpm/mol and 10^{20} cpm/mol, respectively. Monocytes, MNC, or T cells (2×10^6 cells/point) were incubated with 1.2×10^{-7}-4.7×10^{-10} M ^{125}I-tyrosyl-CTP or 10^{-8}-10^{-13} M ^{125}I-HCG in 100 μl for 1 h at 4°C in phosphate-buffered saline with 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) [5]. The dissociation constants and the number of binding sites were determined using Scatchard plots [8].

Monocytes were incubated for 30 min at 37°C in RPMI-1640 with 5 μg/ml trypsin. Trypsin was blocked with 5% fetal calf serum; the cells were washed 2 times in medium 199 by centrifugation and used in the binding experiments.

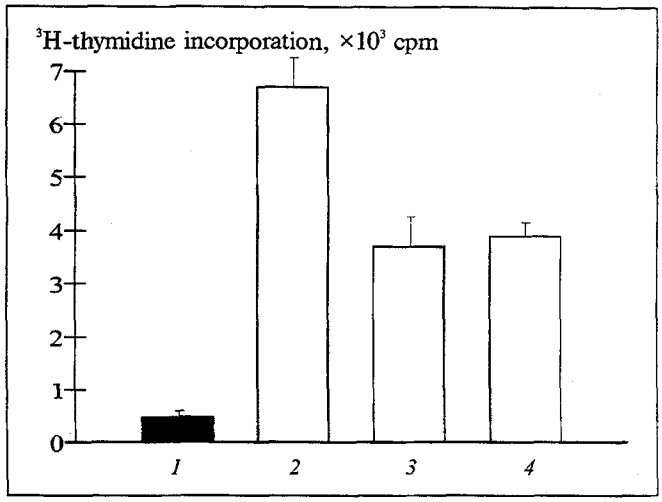

Fig. 1. Effect of human chorionic gonadotropin (*3*) and synthetic peptide CTP (*4*) on ConA-induced proliferation of human peripheral blood mononuclear cells *in vitro* (*2*). *1*) control; proliferation in the absence of ConA. The error of the mean is <10%.

The significance of differences was evaluated by Student's *t* test ($p<0.05$). At least 4 independent experiments were performed for each method; not less than 2 measurements were performed for each point.

RESULTS

The effect of synthetic CTP of the β-subunit of HCG on proliferation of human peripheral blood MNC was studied *in vitro*. Similar to HCG, CTP inhibited ConA-induced proliferation of MNC (Fig. 1). Nei-

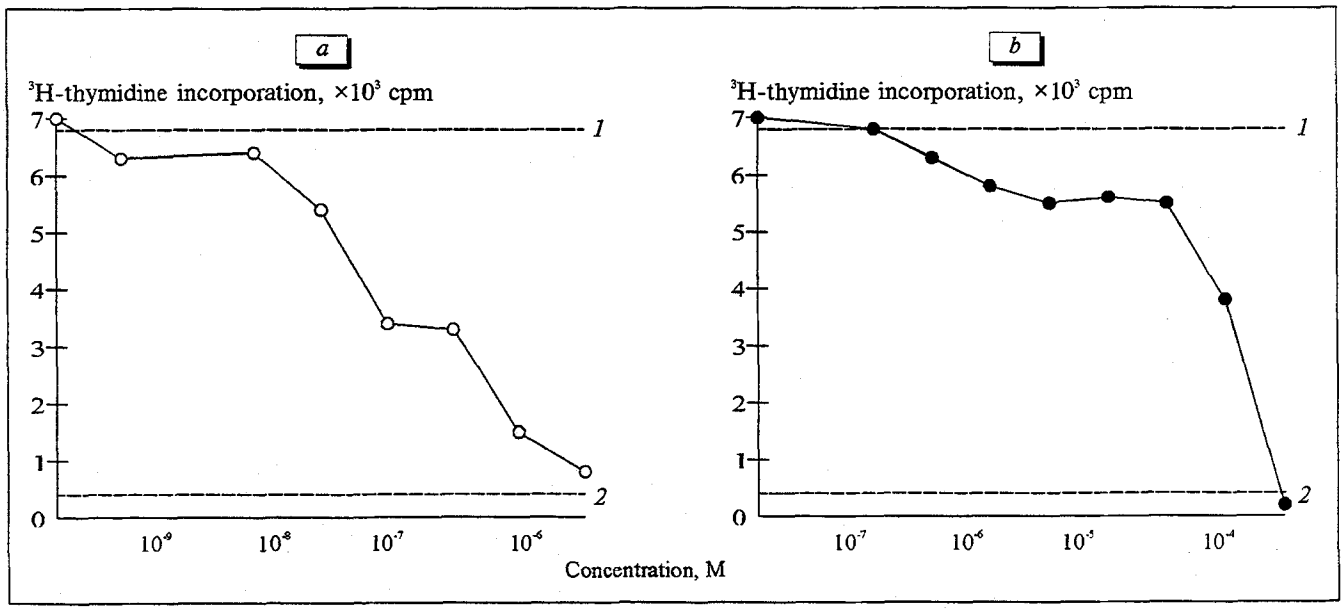

Fig. 2. Dependence of ConA-induced proliferation of human mononuclear cells on the concentration of HCG (*a*) and CTP (*b*). *1*) proliferation in the absence of HCG (*a*) and CTP (*b*); *2*) in the absence of ConA and HCG (*a*) and ConA and CTP (*b*).

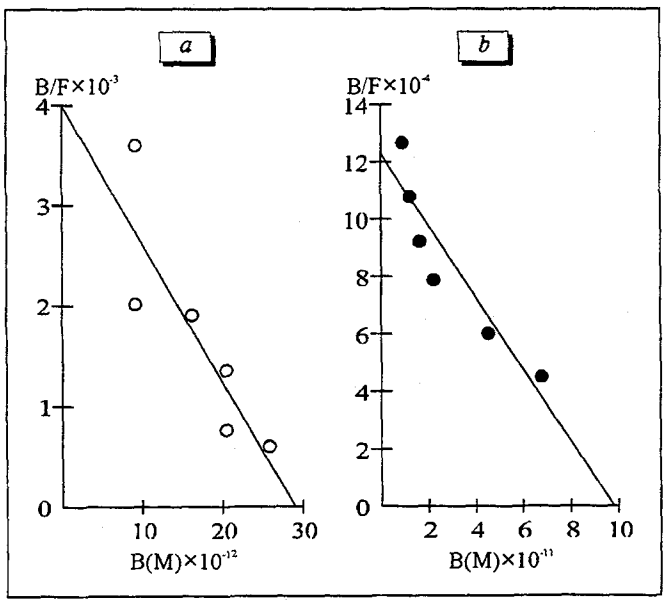

Fig. 3. Binding of ^{125}I-HCG (*a*) and ^{125}I-tyrosyl-CTP (*b*) to human peripheral blood monocytes. Scatchard plots. Dissociation constant: 6.3×10^{-9} M (*a*) and 10^{-8} M (*b*); number of binding sites per cell: 1600 (*a*) and 3500 (*b*).

ther CTP nor HCG produced any direct cytotoxic effect in the studied concentration range. The effects of HCG and CTP were dose-dependent. A 50% inhibition of lymphocyte proliferation was observed at 4×10^{-8} M HCG (Fig. 2, *a*) and 5×10^{-5} M CTP (Fig. 2, *b*).

These findings agree with the data of other researchers on 50% inhibition of mitogen-stimulating proliferation of lymphocytes of different donors treated with 2×10^{-8}-10^{-7} M HCG [10]. Unfortunately, there are no data on the antiproliferating effect of CTP.

Specific binding of CTP and HCG to various peripheral blood cells was studied by the radioligand method. Radioactive label (^{125}I, Iodogen) was inserted in the tyrosine aromatic ring [11] of CTP analog with additional tyrosine residue. This analog exhibited the same proliferative activity as CTP.

We failed to identify binding sites for CTP, tyrosyl-CTP, or HCG on the surface of T cells. However, HCG and tyrosyl-CTP reacted with monocytes (Fig. 3). For HCG, the dissociation constant was 6.3×10^{-9} M and the number of binding sites was 1600 per cell. For tyrosyl-CTP, the dissociation constant was 10^{-8} M and the number of binding sites was 3500 per cell. This finding suggests that antiproliferative effects of HCG and CTP on peripheral blood MNC are mediated by monocytes.

It was demonstrated that trypsinization completely abolishes the binding of HCG and tyrosyl-CTP to monocytes, indicating that monocyte receptors for HCG and tyrosyl-CTP are trypsin-sensitive.

Our results show that synthetic fragment of the β-subunit of HCG inhibits mitogen-stimulated proliferation of MNC. However, it is unclear whether the 18-amino acid-long fragment (128-145) is a functionally important sequence that mediates the effect of HCG on the immune system. Further investigations of biological properties of the synthetic fragment of the HCG β-subunit will be helpful both for elucidation of structural and functional peculiarities of HCG at the molecular level and for the development of new selective immunomodulating drugs.

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