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EFFECT OF β-HUMAN CHORIONIC GONADOTROPIN (β-HCG) ON NEUTROPHIL FUNCTIONS

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ABSTRACT

Many findings point to the presence of a close relationship between the immune, endocrine and neurologic systems. For example the suppressive effects of β-human chorionic gonadotropin (β-HCG) on IL-1 production, the potentiative effect of enkephalins on lymphocyte functions and the suppressive effect of leuteinizing hormone (LH) on natural killer (NK) cell activities have been clearly established. In this regard we have studied the effects of β-HCG on neutrophil function *in vitro*. This study was performed on 28 peripheral blood neutrophil samples (14 as test and 14 as control), by incubating neutrophil preparations with bacteria in the presence or absence of the hormone for one hour at 37°C. The results indicated that with 20 IU/mL of hormone, the total and intracellular bactericidal activities of neutrophils were significantly increased ($p<0.05$), but the phagocytic activity remained unchanged.

Keywords: Gonadotropin, human chorionic; neutrophil function

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INTRODUCTION

The results of recent research indicate that there is a close relationship between the immune and endocrine systems.[1] Recent findings indicate that lymphocytes and other immunocytes contain receptors for some hormones, and affect the production and secretion of some peptide hormones with regulatory functions in the immune system.[2,3] Allogeneic stimulation of lymphocytes induces the production of HCG,[4] which is very similar to that produced by the human chorion. It was also found that mouse lymphocytes secrete HCG, while production of this hormone by mouse chorion has not been confirmed yet.[4] It has been shown that β-HCG has some effects on phagocytes, especially neutrophils,[4,5] and the chemiluminescence response of HCG-treated phagocytes is significantly increased.[6] The present study was performed to evaluate the effects of β-HCG on the phagocytic activity of human neutrophils.

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MATERIALS AND METHODS

A. Preparation of neutrophils

Blood samples (10 mL) were withdrawn in heparinized 20 mL acutainers containing 5 mL dextran 6% (110) and vortexed, the needle of the syringe bent and incubated at 37°C in an upright position for an hour, followed by an additional 15 minutes incubation at room temperature. By this method, erythrocytes sedimented in the bottom of the acutainer and neutrophils remained in the supernatant fluid. The supernatant fluid, containing neutrophils, was transferred to a capped tube, diluted with an equal volume of phosphate buffer solution (PBS) and centrifuged at 1300 rpm at 37°C. This treatment was repeated twice. The resulting pellet

containing neutrophils was saved. The prepared neutrophils were suspended in 1 mL of RPMI 1640, shaken gently and counted and adjusted to 1×10^6 cells/mL. A smear of the neutrophil suspension was stained by Evans blue, to find the percentage of viable cells and for measurement of biological activity.

B. Preparation of bacterial suspension

The source of *Staphylococcus aureus* was stocks of this microorganism in agar slabs kept at 4°C. One colony of this stock was transferred to nutrient broth, and kept overnight at 37°C. A new generation of the bacteria grew in the tube. Then the tube was centrifuged at 3000 rpm and after discarding the supernatant, PBS was added and the centrifuge procedure was repeated twice. The bacterial number/mL was estimated spectrophotometrically by measurement at 620 nm. As claimed by Defernandez,[7] an absorbance of 0.05 at 620 nm corresponds to approximately 2×10^6 bacteria/mL.

C. Measurement of neutrophil activity

Two capped tubes were labeled as test and control. A 0.45 mL aliquot of neutrophil suspension, 0.45 mL of the bacterial suspension, and 0.1 mL of AB serum was added to each tube and mixed. Then 20μL equivalent to 20 IU of β-HCG in saline was added to each and mixed. The contents of each tube was used in the following experiments (steps).

1. Determination of total and viable bacterial count (S_1)

Each tube was vortexed and a 0.1 mL aliquot was transferred to a tube containing 9.9 mL of distilled sterile water to lyse neutrophils. Then 0.1 mL of this solution was transferred to another tube containing 9.9 mL PBS and vortexed. 20 μL of this suspension was added to each of three nutrient agar plates to cultivate bacteria at 37°C. We designated these test and control plates as S_1.

2. Determination of live bacteria inside and outside of neutrophils after incubation (S_2)

The bacteria and neutrophil suspension, both control and test tubes, were incubated for an hour at 37°C on a low speed shaker, then step 1 experiments were carried out and these plates designated S_2.

3. Determination of live extracellular non-phagocytosed bacteria (S_3)

Medium (1.6 mL of RPMI 1640) was added to each last step tube and centrifuged for 7 minutes at 900 RPM at 4°C. Then 0.1 mL of supernatant fluid was removed and treated as described and designated as S_3 plates.

4. Determination of live intracellular bacteria (S_4)

The supernatant fluid was discarded and 1 mL of RPMI was added to the pellet and vortexed. 0.1 mL of this suspension was used to inoculate new plates and designated as S_4.

5. Bacterial count method

All of the cultivated plates were transferred and incubated for 24 hours at 37°C. Then the colonies were counted. The mean value in each case was considered as the bacterial number in each step. The indices of neutrophil activities were determined by equations of Defernandez.[7]

$$\text{Total bactericidal capacity of neutrophils} = \frac{\text{Count step 1} - \text{Count step 2}}{\text{Count step 1}} \times 100$$

$$\text{Neutrophil phagocytosis capacity} = \frac{\text{Count step 1} - \text{Count step 3}}{\text{Count step 1}} \times 100$$

$$\text{Intracellular bactericidal capacity} = \frac{\text{Count step 4}}{\text{Count step 2} - \text{Count step 3}} \times 100$$

The results were evaluated with Student's t-test (paired two tail) and P values less than 0.05 were considered significant.

RESULTS

In this study fourteen healthy volunteers 21 to 45 years of age were selected for evaluation of the effect of β-HCG on their neutrophil function.

Total bactericidal capacity of test and control groups is shown in Fig. 1. As can be seen in the test group the bactericidal capacity of neutrophils has increased significantly ($p<0.05$).

According to Fig. 2, β-HCG did not have any significant effect on the phagocytosis capacity of neutrophils.

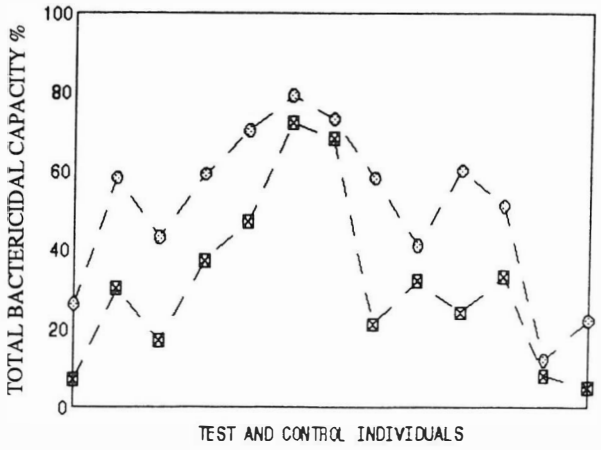

Fig. 1. Total bactericidal capacity of neutrophils.

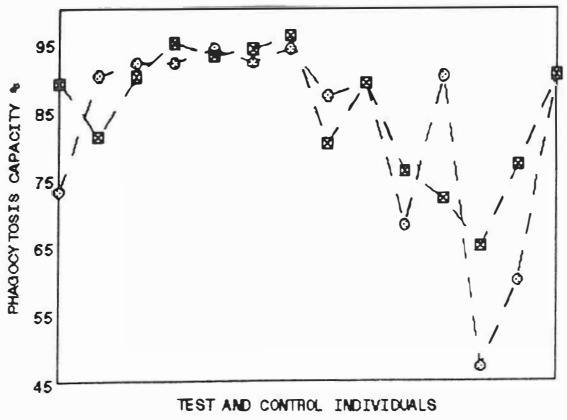

Fig 2. Phagocytosis capacity of neutrophils.

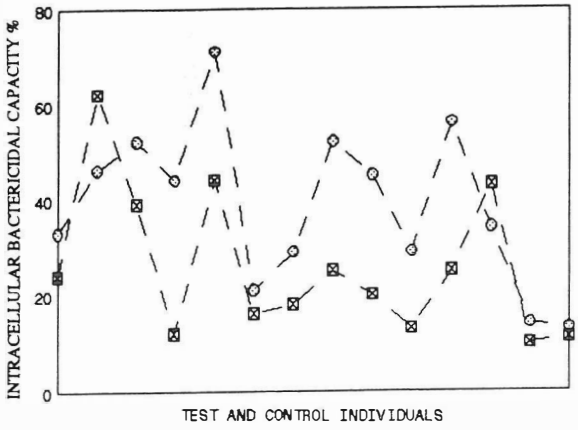

Fig 3. Intracellular bactericidal capacity of neutrophils.

As seen in Fig. 3, intracellular bactericidal capacity of neutrophils in response to β-HCG treatment was increased in nearly all members of the test group, which was significantly higher than controls ($p<0.05$)

DISCUSSION

The relationship of some aspects of the endocrine and immune systems have been elucidated. Endocrine hormones have different effects on the immune response. HCG is an important hormone that has different effects on specific and non-specific immunity. Recent studies on the effect of HCG on monocyte cultures indicate that this hormone augments the secretion of IL-6, IL-1, TNFα/β,[3] and also has chemotactic effects on neutrophils.[5] In rat testis, HCG causes an increase in blood vessel permeability and neutrophil aggregation.[8] There are some reports indicating that HCG significantly increases the chemiluminescence response of male, female and pregnant women's phagocytes.[6] In this study, the total bactericidal activity, intracellular bactericidal activity and phagocytic function of neutrophils were evaluated. As can be seen in Figures 1, 2 and 3, incubation of neutrophils for an hour at 37°C with 20 IU/mL of HCG significantly increased the total bactericidal and intracellular bactericidal activity of neutrophils as compared to control groups. Our findings also showed that after an hour of treatment with HCG, there was no significant difference between neutrophil phagocytic activity in test and control groups. According to Shibuya's findings, treatment of neutrophils with HCG for 48 hours augments the expression of Fc and C3b receptors on the surface of these cells.[6] Therefore the absence of any significant difference in phagocytic activity of neutrophils in our study is probably due to the short duration of HCG treatment. Further research is required to evaluate the effect of incubation time on these findings. These results suggest that an increase in bactericidal and probably phagocytic activity of neutrophils during pregnancy upregulates non-specific immunity in pregnant women.

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