Role of Cyclooxygenase in the Chorionic Gonadotropin Regulation of Human Neutrophil Activity

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The involvement of cyclooxygenase in the regulation of the main functions of human neutrophils by chorionic gonadotropin was studied. The inhibitory effect of the hormone on phagocytosis and oxidative activity of neutrophils is completely or partially canceled under conditions of cyclooxygenase blocking. In addition, gonadotropin directly induces the expression of cyclooxygenase inducible form mRNA in these cells. These data indicate that realization of the suppressive effects of chorionic gonadotropin in the neutrophils is partially linked with activation of cyclooxygenase (both the constitutive and inducible forms of the enzyme).

Key Words: *neutrophils; oxidative activity; phagocytosis; cyclooxygenase; chorionic gonadotropin*

Study of immunomodulatory effects of reproduction hormones, for example, chorionic gonadotropin (CG; a major pregnancy hormone) is essential for understanding the mechanisms determining the changes in the female immune status in pregnancy. We have shown previously that CG effectively regulates human neutrophil functions [1], but the molecular mechanisms of this regulation remain largely unknown.

Many data indicate the probable involvement of cyclooxygenase (Cox) and its products in the realization of CG effect. First, it is known that CG in normal concentrations activates Cox [7] and induces the production of prostaglandin E_2 (PGE_2) by endometrial stromal cells [7] and the synthesis of $F_2\alpha$ prostaglandin by T-lymphocytes [2]. Second, we showed previously that some suppressive effects of gonadotropin in mouse splenocyte culture are realized only in the presence of macrophages and depend on Cox activity in these cells [3], while CG-dependent inhibition of human T-lymphocyte proliferative response to mitogen is more intense in the presence of autologous neutrophils, this effect also being inhibited during Cox blocking [5]. These two studies evaluated the effect of CG on the adaptive component of the immunity (lymphocyte reactions). It is therefore interesting to clear out the role of Cox in CG-dependent regulation of the functions of phagocytic cells (macrophages and neutrophils).

As Cox is present in the neutrophils and these cells produce PGE_2 [9,15], we studied the relationship between CG effects in neutrophil culture and the activity of this enzyme.

MATERIALS AND METHODS

Chorionic gonadotropin (Profasi, I.F.Serono S.p.A.) was used in physiological concentrations, corresponding to the hormone levels in the peripheral blood during the first and third trimesters of gestation, critical for pregnancy: 100 and 10 U/ml [1]. Sodium diclophenac (sodium 0-[(2,6-dichlorofunyl)-amino]-phenylacetate; Voltaren, Pliva) was used in a concentration of 0.015 mg/ml, most effectively inhibiting Cox [3].

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Neutrophils were isolated from the peripheral blood of nonpregnant women of reproductive age by centrifugation in ficoll-verograffin double density gradient (Serva; Spofa). The upper and lower gradient densities were 1.077 and 1.113 g/cm^3, respectively [1]. The cells were collected from the lower interphase. According to histological estimation, the purity of isolation was 96% and viability (evaluated by 0.1% Trypan Blur exclusion) was 97%.

Fractionated cells in a concentration of 5×10^6/ml were incubated with CG during 1 h for functional tests or 6 h for evaluating mRNA synthesis (for stimulated variant: 1-h preincubation with the hormone, addition of the stimulant, and subsequent incubation during 5 h). Incubation was carried out at 37°C and 5% CO_2 in complete nutrient medium based on Eagle's medium (Sigma) with 300 μg/ml L-glutamine (Serva), 0.01 M HEPES (Serva), 100 μg/ml gentamicin (Pharmacia), and 10% FCS (Serva). The Cox inhibitor was added into the culture simultaneously with CG. Native neutrophils (without preparations) served as the control.

The neutrophil phagocytic activity was evaluated by absorption of formalin-treated sheep erythrocytes (neutrophil/erythrocyte proportion 1:100) by analyzing the smears, stained after Romanowskii—Giemsa, under an optic microscope. Two parameters were evaluated: phagocytosis percentage (number of cells absorbing phagocytosis objects per 100 neutrophils) and phagocytic index (mean number of phagocytosis objects per one of 100 neutrophils capturing the phagocytosis objects).

The neutrophil oxidative activity was evaluated by the intensity of luminol-dependent chemiluminescence (LDCL) of the cell culture on a BLM-8703M bioluminometer (Nauka). Luminol (Sigma) was used in a concentration of 5×10^{-4} M, cell count per test being 10^5. The level of spontaneous LDCL of neutrophils was measured, after which the stimulator (opsonized zymosan) was added into the luminometer cuvette, and the LDCL curve was plotted by registration at equal intervals till the values passed the maximum and reduced by $^2/_3$.

Isolation of RNA, reverse transcription, and amplification were carried out using appropriate kits (Isogen Laboratories): for isolation of total RNA (Trizol RNA Prep 100), for reverse transcription (GenePak RT Core), and for amplification (GenePak PCR Core).

Specific primers and respective amplification protocols were used. For Cox-2: 5'-TTGTTCCA GACAAGCAGGC-3' and 5'-CATTCCTACCACCA GCAACC-3' [12]; 30 sec at 94°C, 60 sec at 60°C, and 60 sec at 72°C, 30 cycles. For HPRT (hypoxanthine phosphoribosyltransferase; mRNA positive control): 5'-TGGCGTCGTGATTAGTGATG-3' and 5'-CTGCATTGTTTTGCCAGTGT-3' (selected using Primer 3 software: http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi); 30 sec at 94°C, 60 sec at 60°C, and 60 sec at 72°C, 35 cycles.

Electrophoresis was carried out by the standard method in 1.5% agarose gel (Serva).

The results were processed using Student's *t* test.

RESULTS

Evaluation of the neutrophil phagocytic activity has shown a suppressive effect of CG high dose (Table 1), which is in line with our previous data on CG-dependent regulation of phagocytosis and oxidative activity of neutrophils [1] and, presumably, contributes to reduction of nonspecific resistance of the body in pregnancy. Sodium diclophenac exhibited no independent modulatory activity, but partially canceled the suppressive effect of CG (Table 1), this indicating the involvement of Cox in the realization of the hormone effect. Spontaneous oxidative activity of neutrophils was also inhibited by CG (100 U/ml), the hormone effect (similarly as in phagocytosis) not manifesting in the presence of Cox inhibitor (Fig. 1). Diclophenac sodium inhibited the oxidative activity of the cells along with CG (10 U/ml) under conditions of neutrophil stimulation by opsonized zymosan, this impeding the interpretation of the results (Fig. 2). However, interestingly, that despite independent negative effects of both substances, their combination was ineffective in regulation of LDCL, whose values were close to the control (Fig. 2).

TABLE 1. CG-Dependent Regulation of Neutrophil Phagocytosis under Conditions of Cox Blocking (*M*±*m*; *n*=10)

Experimental treatment	Phagocytosis parameter	
	phagocytosis percentage	phagocytic index
Control	28.30±2.77	1.900±0.255
CG, 10 U/ml	24.90±2.21	1.640±0.146
CG, 100 U/ml	19.10±2.67*	1.610±0.204
Sodium diclophenac, 0.015 mg/ml	22.90±2.29	2.080±0.432
CG, 10 U/ml+ sodium diclophenac, 0.015 mg/ml	25.60±3.56	1.860±0.312
CG, 100 U/ml+ sodium diclophenac, 0.015 mg/ml	23.20±4.58	1.760±0.211

Note. *$p<0.05$ *vs.* control.

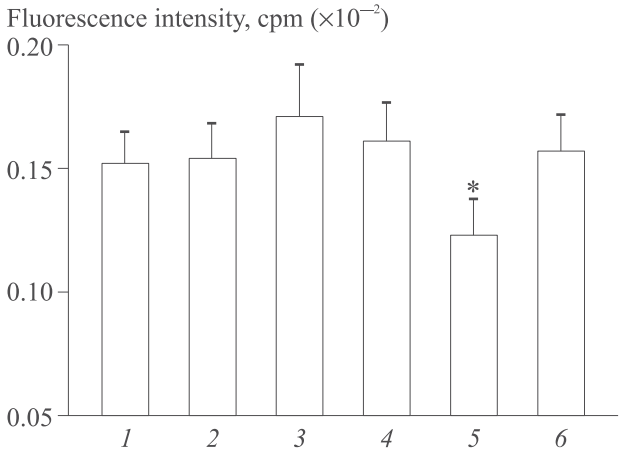

Fig. 1. Effect of CG on spontaneous LDCL of neutrophils under conditions of Cox activity blocking. *1*) control; *2*) sodium diclophenac; *3*) CG, 10 U/ml; *4*) CG, 10 U/ml+sodium diclophenac; *5*) CG, 100 U/ml; *6*) CG, 100 U/ml+sodium diclophenac. Here and in Fig. 2: **p*<0.05 *vs.* control; *n*=10.

Hence, the inhibitory effect of CG on the studied neutrophil functions were completely or partially canceled under conditions of Cox blocking, indicating that the hormone effects were presumably mediated through prostaglandins, for example, PGE_2. It is known that this prostaglandin is secreted by neutrophils in response to stimulation by opsonized zymosan or FMLP [9] and, at the same time, suppresses the neutrophils [6,13] binding to them via specific membrane receptors [6,13]. Both PGE_2 and CG make use of the cAMP-dependent intracellular mechanisms for realization of their effects [13,14]. This fact suggests that PGE_2, acting as a paracrine or autocrine factor, modulates the neutrophil functions along with and sometimes instead of CG. On the other hand, Cox inhibition can lead to accumulation of arachidonic acid in the cell, released during zymosan activation of phospholipase A_2 [11], and of its lipoxygenase products, for example, leukotriene B_4. Arachidonic acid and leukotriene B_4 activate cell functions by increasing the level of cytoplasmatic calcium and by directly stimulating NADPH oxidase [8,10]. However in our experiments Cox inhibitor has exhibited no independent stimulatory effect on the neutrophil function, including LDCL, and hence, it seems that its role in the realization of CG effects is determined by blocking of prostaglandin synthesis.

As the functional activity of neutrophils was evaluated in a short-term culture, we can speak here only about hormone activation of the constitutive Cox form (Cox-1). On the other hand, we know that neutrophils also express the inducible form of this enzyme (Cox-2), playing an important role in the realization of these cells' functions [9]. In order to evaluate the role of CG in activation of inducible Cox, we studied the expression of Cox-2 mRNA in a 6-hour neutrophil culture with the hormone. Chorionic gonadotropin in a high concentration induced a pronounced expression of this transcript in intact neutrophils (Fig. 3, *a*). On the other hand, the expression of this factor was not detected in tests with the hormone after cell stimulation by opsonized zymosan (Fig. 3, *b*), while Cox-2 mRNA was present in the control; this is justified and in line with published data [9]. The absence of Cox-2 mRNA in the neutrophils stimulated in the presence of CG can be explained by overlapping of zymosan-induced and hormone sig-

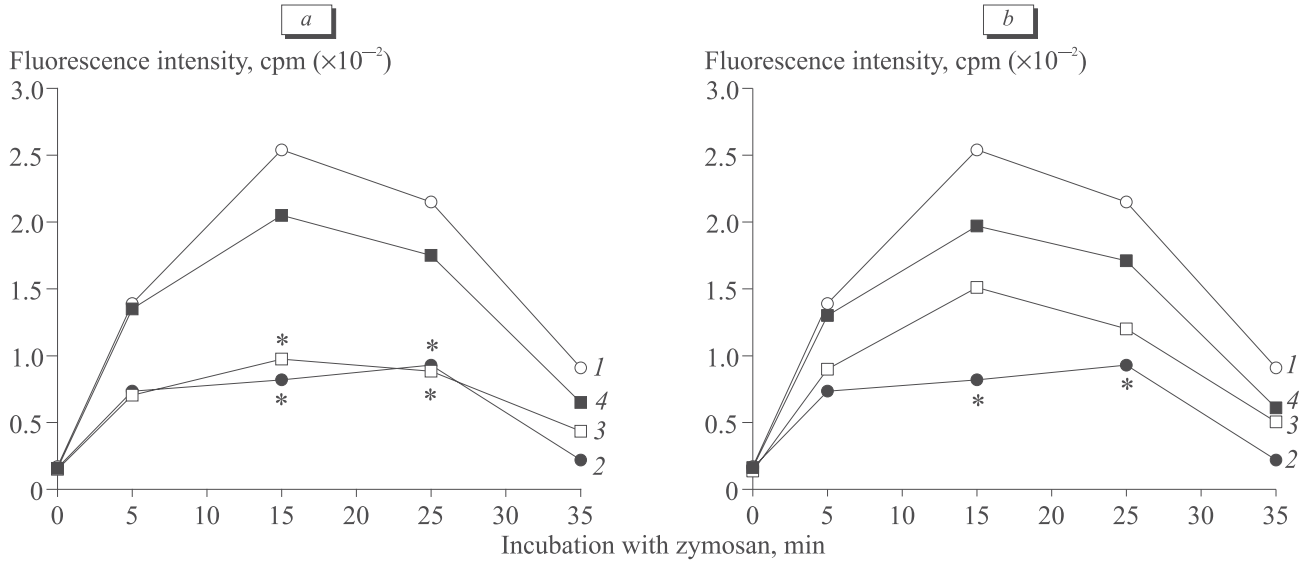

Fig. 2. Modulatory effect of CG in doses of 10 U/ml (*a*) and 100 U/ml (*b*) on stimulated neutrophil LDCL under conditions of Cox activity blocking. *1*) control; *2*) sodium diclophenac; *3*) CG; *4*) CG+sodium diclophenac.

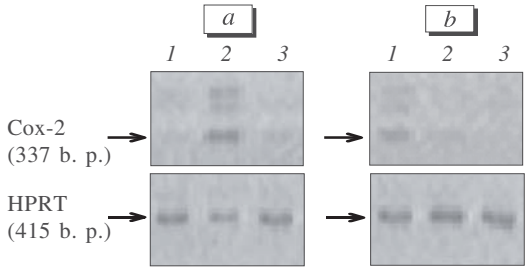

Fig. 3. Expression of Cox-2 mRNA by intact (*a*) and stimulated neutrophils (*b*) under the effect of CG. *1*) control; *2*) CG, 100 U/ml; *3*) CG, 10 U/ml. Arrows show the location of products corresponding to amplified sites of Cox-2 mRNA (337 b. p.) and HPRT (415 b. p.) The data of one of 5 similar experiments are presented.

nals at the level of second messengers and by the synthesis of new factors, blocking Cox-2 expression. It is most likely that successive activation of this enzyme through different receptors triggers the so-called retroinhibition in the cell through PGE_2 (regulation by the negative feedback mechanism) [4], as a result of which the synthesis of new Cox-2 molecules is arrested.

On the whole, our findings indicate that CG activates Cox expression in human neutrophils and this expression is involved in the realization of suppressive effects of the hormone in these cells, presumably via induction of PGE_2 synthesis. And as PGE_2 possesses immunomodulating activity of its own, forming conditions for survival of the semiallogenic fetus during gestation [4], the role of CG-dependent regulation of Cox expression seems to be even greater.

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