Role of Potassium Ions in Monocyte-Regulating Effects of Chorionic Gonadotropin

S. V. Shirshev, O. G. Lyalina, and S. A. Zamorina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 11, pp. 566-569, November, 2000
Original article submitted July 3, 2000

We studied the effects of chorionic gonadotropin on intracellular K^+ content and phagocytic activity of human peripheral blood monocytes. Since the immunomodulating properties of chorionic gonadotropin are known to depend on female sex steroids, the phase of the menstrual cycle was taken into account. Chorionic gonadotropic modulated the content of intracellular K^+ and phagocytic activity of monocytes. These changes were most pronounced under the effect of chorionic gonadotropin in high physiological doses (100 U/ml) and depended on the phase of the menstrual cycle. A direct correlation was found between elevation of K^+ content in monocytes and activation of phagocytosis during the luteal phase.

Key Words: *chorionic gonadotropin; potassium; fractionated monocytes; phagocytosis; menstrual cycle*

The major placental hormone chorionic gonadotropic (CG) possesses immunomodulating properties [5,6] and plays an important role in the regulation of gestation. The immune response depends on functional activity of monocytes/macrophages, the main target cells for CG. This hormone modulates phagocytotic activity and proliferation of macrophages [14], whose content in the uterus sharply increases during pregnancy [8]. CG-dependent immune suppression estimated by the reaction of lymphoblastic transformation is realized via monocytes [15], the source of apoptotic signal from T cells [9].

Female sex steroids can antagonize or potentiate immunomodulating activity of CG depending on the cell type, differentiation stage, and duration of exposure [6].

Functional activity of immunocompetent cells and macrophages is closely related to the ion transport system, *e.g.*, intracellular K^+ concentration ($[K^+]_i$) [3]. Potassium ions maintain intracellular pH, membrane potential, and cell volume and initiate proliferation and clonal expansion of immunocompetent cells [4].

Institute of Ecology and Genetics of Microorganisms, Ural Division of the Russian Academy of Sciences, Perm'

Here we studied the role of $[K^+]_i$ in monocyte-regulating effects of CG in various phases of the menstrual cycle.

MATERIALS AND METHODS

Peripheral blood leukocytes were obtained from healthy women in the follicular or luteal phase of the menstrual cycle. Peripheral blood monocytes were isolated by the standard method [12] with some modifications. Mononuclear cells were centrifuged in a Ficoll-Verografin density gradient (1.077 g/cm^3, Sigma, Spofa). The suspension was washed 2 times with medium 199 (Biomed) and incubated with 5% fetal bovine serum (Sigma) at 37°C for 45 min in petri dishes (Anumbra). Adherent monocytes were collected, washed 2 times, and incubated at 4°C for 45 min to stabilize functional activity. The purity estimated by immunofluorescence assay with monoclonal CD14 antibodies (Primary Anti-Human CD14, clone 2D-15C, ICN) was 78-85%. Cell viability determined by eosin incorporation (vital stain, Sigma) was 93-98%.

Fractionated monocytes were concentrated to 2×10^6/ml and incubated with CG (Serano) in doses of 10, 50, and 100 U/ml [1] at 37°C for 10, 30, and

0007-4888/00/0011-1099$25.00 ©2000 Plenum Publishing Corporation

60 min. Phagocytic activity was estimated and $[K^+]_i$ was measured.

Phagocytic activity of monocytes was evaluated by absorption of formalinized sheep erythrocytes (10^7/ml) added into the suspension of fractionated monocytes and incubated at 37°C for 20 min. After incubation, smears for microscopic examination were stained by the Romanovsky—Giemsa method. The phagocytic percent (mean number of cells phagocytizing 1 and more objects per 100 phagocytes, true phagocytes), phagocytic number (number of phagocytized objects per 1 of 100 phagocytes), and phagocytic index (number of phagocytized objects per 1 true phagocyte) were calculated [2].

$[K^+]_i$ in cells was measured after washout with medium 199 and cold isotonic $MgCl_2$ (95 mM) [11]. Monocytes were permeabilized with 1% Triton X-100 and precipitated by centrifugation [7]. $[K^+]_i$ in the supernatant was estimated on a FPL-1 flame photometer. Medium 199 served as the control.

The results were analyzed by paired and unpaired Student's *t* tests. The differences were significant at $p<0.05$.

RESULTS

During culturing of intact fractionated monocytes, $[K^+]_i$ decreased in cells from follicular phase women (Table 1), but remained unchanged in cells from luteal phase women (Table 2). This was probably related to various effects of sex steroids during these phases of the menstrual cycle. Incubation of monocytes with CG showed that phagocytotic activity and changes in $[K^+]_i$ depended on the dose of this hormone and duration of exposure. After 10-min incubation, CG did not change $[K^+]_i$ in monocytes from women in the luteal (progesterone-dependent) phase; in doses of 50 and 100 U/ml, this hormone decreased the content of phagocytic cells (Table 2). By contrast, CG in a dose of 50 U/ml decreased $[K^+]_i$, but did not modulate phagocytotic activity of monocytes from women in the follicular (estrogen-dependent) phase (Table 1). Thus, on the 10th min of culturing CG in doses similar to blood concentrations in the 2nd and 3rd trimesters of pregnancy suppressed progesterone-dependent phagocytic activity of peripheral blood monocytes.

On the 30th min of culturing CG did not change phagocytic activity of monocytes and $[K^+]_i$.

After 60-min incubation, CG in a high dose (100 U/ml) markedly increased $[K^+]_i$ in monocytes irrespective of the phase of the menstrual cycle. At the same time, the influence of CG on phagocytic activity depended on the cycle phase. During the follicular phase CG inhibited phagocytic activity of monocytes (phagocytic index), while in the luteal phase it increased this parameter. CG in doses of 50 and 100 U/ml stimulated total phagocytic activity (phagocytic number) in the luteal phase; in a dose of 100 U/ml this hormone increased the number of true phagocytes (Table 2).

Thus, we revealed a correlation between $[K^+]_i$ increase and changes in phagocytic activity of monocytes induced by CG in a dose similar to its blood concentration in the 1st trimester of pregnancy (100 U/ml). The hormone caused codirected or opposite

TABLE 1. Effects of CG on $[K^+]_i$ and Phagocytic Activity of Monocytes from Women in the Follicular Phase of the Menstrual Cycle ($M\pm m$, $n=12$)

Parameter, incubation, min		Control (solvent)	CG dose, U/ml		
			10	50	100
Phagocytic percent	10	49.17±5.41	55.240±4.878	53.58±7.76	53.860±7.955
	30	43.940±9.979	59.170±4.837	43.910±6.537	32.910±7.544
	60	55.61±5.00	64.270±4.465	59.790±4.854	49.90±5.84
Phagocytic number	10	1.190±0.386	0.95±0.16	1.030±0.231	1.020±0.251
	30	0.940±0.304	1.370±0.148	0.79±0.15	0.670±0.231
	60	1.41±0.08	1.040±0.186	1.41±0.07	0.860±0.238
Phagocytic index	10	2.230±0.554	1.710±0.201	1.810±0.225	1.750±0.224
	30	1.860±0.253	2.310±0.152	1.610±0.145	1.860±0.218
	60	2.610±0.246	1.680±0.302*	1.930±0.123*	1.670±0.351*
$[K^+]_i$, μmol/10^6 cells	10	19.050±2.262	18.660±1.356	15.660±2.386*	16.820±2.538
	30	10.74±1.54	10.610±1.785	8.200±1.401	9.550±2.566
	60	8.66±1.04+	8.660±1.404	11.110±1.494	10.000±1.312°

Note. $p<0.05$: compared to the control: *unpaired and °paired Student's *t* tests; +compared to 10-min incubation (*unpaired and °paired Student's *t* tests).

TABLE 2. Effects of CG on $[K^+]_i$ and Phagocytic Activity of Monocytes from Women in the Luteal Phase of the Menstrual Cycle ($M\pm m$, n=12)

Parameter, incubation, min		Control (solvent)	CG dose, U/ml		
			10	50	100
Phagocytic percent	10	62.43±6.82	49.98±7.21	42.35±5.56*	39.66±4.52*+
	30	55.03±4.48	55.19±4.98	49.63±5.37	54.70±6.65
	60	48.98±7.43	49.80±6.37	57.79±5.63	73.14±3.13*+
Phagocytic number	10	1.14±0.19	0.9±0.2	0.72±0.11	0.70±0.15
	30	1.01±0.20	1.05±0.15	0.88±0.16	0.98±0.21
	60	0.92±0.24	0.88±0.14	1.06±0.10*+	1.40±0.17*+
Phagocytic index	10	1.78±0.13	1.74±0.14	1.68±0.05	1.68±0.19
	30	1.78±0.18	1.86±0.12	1.72±0.14	1.72±0.19
	60	1.78±0.20	1.75±0.16	1.83±0.02	1.89±0.17
$[K^+]_i$, μmol/10^6 cells	10	5.500±0.468	6.500±1.151	8.11±1.88	8.030±1.898
	30	7.600±1.602	8.480±1.778	8.06±1.91	9.030±2.053
	60	7.430±1.801	7.49±1.68	7.880±1.808	9.31±1.74*

Note. $p<0.05$: compared to the control: *unpaired and +paired Student's t tests.

changes depending on the phase of the menstrual cycle and manifested only after 60-min incubation.

It is known that $[K^+]_i$ affects cell metabolism and generation of membrane potential thereby modulating phagocytic activity [3]. Previous studies showed that progesterone directly blocks potential-dependent and Ca^{2+}-activated K^+ channels in immunocompetent cells, which causes depolarization of the cytoplasmic membrane and $[K^+]_i$ increase [13]. It can not be excluded that CG-induced activation of phagocytosis during the progesterone-dependent phase is related to an increase in intracellular $[K^+]_i$. Progesterone probably potentiates CG-dependent mechanisms underlying the interrelation between $[K^+]_i$ and functional activity of monocytes. These data suggest that the rise of $[K^+]_i$ contributes to CG-induced modulation of monocyte activity. The directionality and degree of changes (*e.g.*, absorption capacity and number of activated phagocytes) depend on permissive effects of estrogens and progestins, respectively.

REFERENCES

1. D. Ya. Dimitrov, *Human Chorionic Gonadotropin* [in Russian], Moscow (1979).
2. V. N. Kaplin, *Nontraditional Immunology of Infection* [in Russian], Perm (1996).
3. Z. I. Krutetskaya and O. E. Lebedev, *Role of Tyrosine Phosphorylation in the Regulation of Activity of Cell Membrane Ion Channels* [in Russian], St. Petersburg (1998).
4. I. I. Marakhova, *Tsitologiya*, **33**, No. 11, 67-77 (1991).
5. S. V. Shirshev, *Biokhimiya*, **62**, No. 5, 514-522 (1997).
6. S. V. Shirshev, *Usp. Sovr. Biol.*, **118**, No. 1, 69-85 (1998).
7. J. C. Edberg, C.-T. Lin, D. Lau, *et al.*, *J. Biol. Chem*, **270**, No. 38, 22 301-22 307 (1995).
8. A. Bergh, J. E. Damber, N. van Rooijen, *J. Endocrinol.*, **135**, No. 3, 415-420 (1993).
9. Z. Katsuhiro, M. Junichi, K. Chiya, *et al.*, *J. Cell. Biochem.*, **19**, Suppl. B, 279 (1995).
10. S. W. Kuffler and J. G. Nicholls, *From Neuron to Brain: a Cellular Approach to the Function of the Nervous System*, Suderland (1977), pp. 88-98.
11. I. I. Marakhova, A. A. Vereninov, F. V. Toropova, and T. A. Vinogradova, *Biochem. Biophys. Acta*, **1368**, 61-72 (1997).
12. M. Rola-Pleszczynski, and H. W. Churchill, *J. Immunol. Methods*, **20**, 255-262 (1978).
13. S. K. Mujais, N. A. Nora, and Y. Chen, *J. Am. Soc. Nephrol.*, **3**, No. 8, 1488-1495 (1993).
14. E. Pernicheva-Rostaing, J. Foutagne, M. Adolbne, *et al.*, *Acta Endocrinol.*, **92**, No. 1, 187-192 (1979).
15. R. M. Ricketts and D. E. Jones, *J. Reprod. Immunol.*, **7**, No. 3, 225-232 (1985).