Effect of Prednisolone on Secondary Messenger Metabolism in the Lymphocytes from Patients with Acantholysis Bullosa

N. P. Teplyuk, A. S. Dukhanin*, S. I. Ogurtsov*, and T. S. Belysheva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 9, pp. 308-310, September, 2005
Original article submitted February 4, 2005

Prednisolone in therapeutic concentrations blocks Ca^{2+} channels of lymphocyte plasma membranes and prevents arachidonic acid-induced Ca^{2+} entry into the cells. Glucocorticoid virtually did not modulate arachidonic acid-stimulated release of Ca^{2+} from intracellular stores. No appreciable effect of the hormone on mitogen-induced changes in the intracellular content of cAMP was detected.

Key Words: *glucocorticoids; calcium ions; cAMP; cell response; peripheral blood lymphocytes*

Acantholysis bullosa (AB) is an autoimmune disease; the production of autoantibodies is associated with both Th1 and Th2 lymphocytes [12]. The principal pathogenetic treatment consists in prescription of systemic glucocorticoid drugs [1,11]. The main drugs arresting clinical signs of this severe disease are dexamethasone, prednisolone, and 6-methylprednisolone [10].

New data on the molecular mechanisms underlying the effects of glucocorticoids (GC) on cell function are reported [3,8]. Secondary messengers play a certain role in the realization of early GC effects [4,5,9].

We studied the membranotropic effects of glucocorticoids on the second messenger system in lymphocytes from patients with AB.

MATERIALS AND METHODS

Blood (15 ml) was collected from the ulnar vein into a plastic tube, containing 3 ml anticoagulant (25 g hydrated sodium citrate). Blood (5 ml) was layered with a Pasteur pipette onto Isopaque-Ficoll gradient (3 ml) and centrifuged for 15 min at 1500*g* at ambient temperature. The upper layer containing mononuclear leukocytes (MNL) was collected. The cells were resuspended in Hanks medium and washed by centrifugation. The suspension of peripheral blood mononuclears isolated by the above method contains 90% lymphocytes and 10% monocytes [7]. About 70% MNL are T-lymphocytes. The cells were then placed into 10 ml HEPES buffer of the following composition (mM): 145 NaCl, 5 KCl, 1 Na_2HPO_4, 1 $CaCl_2$, 0.5 $MgSO_4$, 5 glucose, 10 Na-HEPES (pH 7.4) at 37°C.

Intracellular level of free Ca ions was evaluated using Fura-2/AM fluorescent dye [2]. In order to measure cAMP in lymphocytes, after 40 min preincubation at 37°C the substances (20 μl) or 20 μl Hanks solution (control samples) were added to the samples containing 1 ml cell suspension. The cell concentration was 10^6 cells/ml medium (Hanks solution, pH 7.4). After incubation the cells were destroyed in ice-cold Tris-EDTA buffer (pH 7.5), the samples were immediately placed into water bath (90°C) for 3 min, and then centrifuged for 10 min at 8000*g*. cAMP concentration in the supernatant was measured using standard Amersham kits. The results were statistically processed using Student's test.

I. M. Setchenov Moscow Medical Academy; *Russian State Medical University, Moscow

0007-4888/05/1403-0323

RESULTS

Antiproliferative effect of hormones (due to their capacity to inhibit early stages of lymphoid cell activation) is a marker of cell sensitivity to GC. These early stages include changes in the second messenger (Ca^{2+}, cAMP) system serving as the mechanisms triggering cell activation and division.

Intracellular level of Ca ions ($[Ca^{2+}]_{cyt}$) in intact cells did not exceed 110 nM (98±12 nM). Addition of 3 μM prednisolone (corresponding to the mean therapeutic concentration of prednisolone in blood plasma) into cell suspension did not change Ca^{2+} concentration.

The following compounds with different properties were used for stimulating lymphoid cell activation: exomitogens, Con A, dextrane sulfate, and arachidonic acid (AA, endomitogen). Addition of 0.1 mg/ml dextran sulfate (Dex S; B-lymphocyte mitogen) or 25 μg/ml Con A (mitogen modulating mainly T-component of the immunity) to lymphocytes increased intracellular calcium concentration to 189±12 (n=6) and 220±18 nM (n=6), respectively. A more pronounced response to T-mitogen is determined presumably by the predominant content of T-lymphocytes in the cell suspension.

The effects of mitogens on lymphoid cells are mediated by AA release from phospholipids into the incubation medium. AA and its metabolites participate in the regulation of various physiological processes, including activation and division of lymphocytes. The release of AA from phospholipid components of the plasma membrane is induced by phospholipase A_2 or successive effects of phospholipase C and diacylglycerine lipase. The latter pathway seems to predominate in lymphoid cells [6].

Studies of AA effects in a concentration of 3 μM on changes in the intracellular concentration of Ca^{2+} in lymphocytes showed a rapid increase of $[Ca^{2+}]_{cyt}$ to 240±25 nM (p<0.05). In order to determine the source of Ca^{2+} entering the cell cytoplasm under the effect of AA, experiments in a calcium-free medium were carried out. Ca^{2+} in incubation medium bound EGTA (final concentration of 1 μM), which led to a drop of the basal fluorescence level (Fig. 1). AA (3 μM) in the presence of EGTA caused an increase in intracellular Ca^{2+} concentration by 55±12 nM (p<0.05), which was about 2-fold lower than in incubation medium with normal content of Ca^{2+}. This fact and characteristic kinetics of Ca response to AA in Ca-free medium suggest that AA induces Ca^{2+} release from intracellular depots. If EGTA (1 mM) was added to cell suspension in medium with Ca^{2+} ions in the presence of AA, a drop of $[Ca^{2+}]_{cyt}$ was observed (Fig. 1), the level of Ca being restored after addition of $CaCl_2$ at the expense of Ca^{2+} entry through plasma membrane channels. Hence, Ca response under the effect of AA is a superposition of two processes: Ca^{2+} transport from incubation medium into the cytoplasm and Ca^{2+} release from intracellular depots.

Addition of AA (3 μM) in the presence of prednisolone (3 μM) increased $[Ca^{2+}]_{cyt}$ by 52±13 nM (p<0.02) compared to its basal level, which corresponded to increase of Ca^{2+} concentration in Ca-free medium (Fig. 2). These results suggest that prednisolone in therapeutic concentrations blocks Ca channels of plasma membranes and prevents AA-induced entry of Ca^{2+} into the cells. GC virtually did not modify AA-stimulated release of Ca^{2+} from intracellular depots.

The study of the adenylate cyclase component of lymphocyte response in patients with AB showed that the basal cAMP level was 2.3±0.6 pmol/10^7 cells (n=10). Evaluation of Con A effect on cAMP content in lymphocytes showed appreciable individual variations. Addition of Con A to lymphocyte suspension

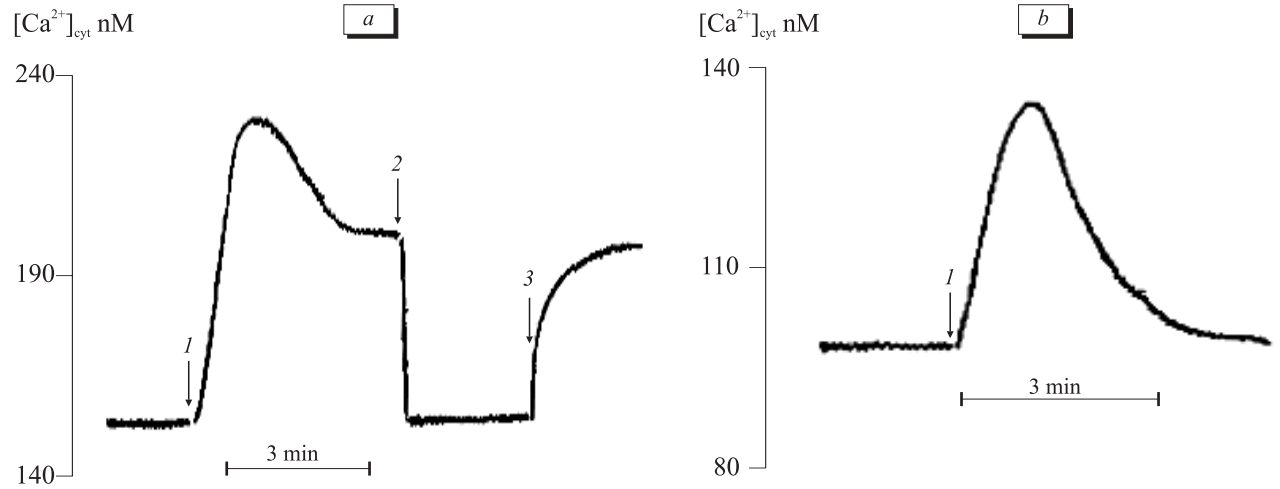

Fig. 1. EGTA effect on arachidonic acid (AA)-induced increase of $[Ca^{2+}]_{cyt}$ level in medium containing 1 mM $CaCl_2$ (*a*) and in Ca-free buffer (*b*). *1*) AA (3 μM); *2*) EGTA (1 mM); *3*) $CaCl_2$ (4.5 mM).

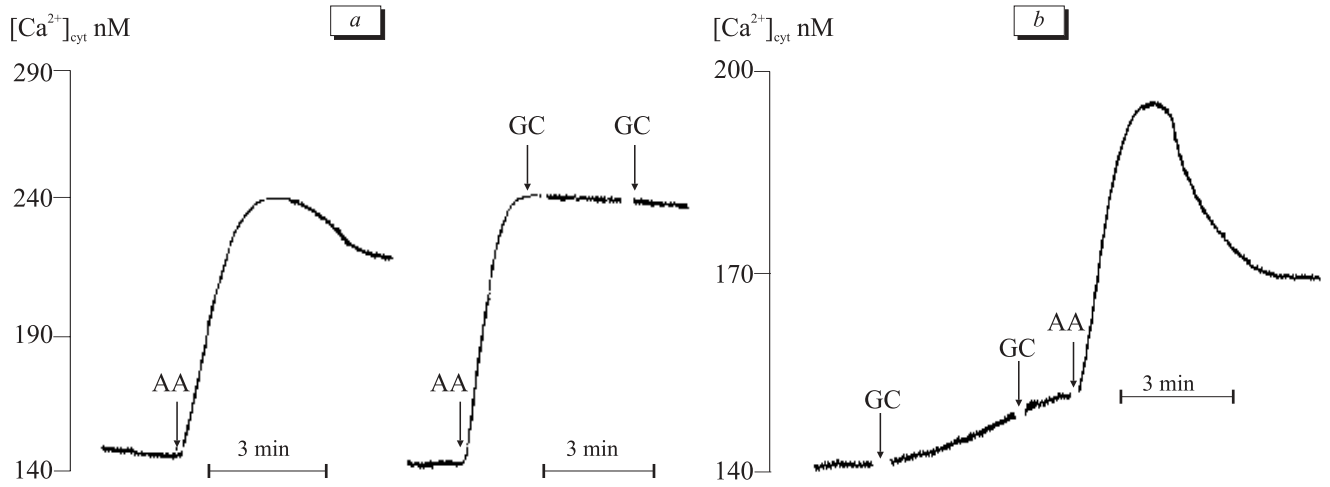

Fig. 2. AA (3 μM) effect on Ca^{2+} content in lymphocytes. *a*) prednisolone (GC) treatment in the presence of AA; *b*) AA treatment in the presence of GC.

(25 μg/ml) in some cases (n=7) led to a significant decrease in cAMP level (1.2±0.4 pmol/10^7 cells), in others (n=6) caused an increase in intracellular concentration of cAMP (3.1±0.4 pmol/10^7 cells). The direction of Con A effect on cAMP content did not correlate with sex, age, and disease form in the patients. AA (1-3 μM) virtually did not change the intracellular content of cAMP. Studies of prednisolone (3 μM) effect on cAMP response of cells failed to show any regularities.

Our findings suggest that prednisolone in a concentration of 3 μM suppressed stimulated elevation of $[Ca^{2+}]_{cyt}$ and did not change the basal levels of Ca^{2+} in lymphocytes from patients with AB. No latent period was needed for the manifestation of Ca-blocking effect of GC. Prednisolone in the studied dose had no effect on mitogen-induced changes in cAMP level. Hence, the inhibitory effect of GC on lymphocytes from patients with AB is mediated through inhibition of cellular Ca response. The mechanisms of lymphocyte activity regulation, including the adenylate cyclase system, do not seem to be the target of GC nongenomic effect.

REFERENCES

1. N. S. Potekaev, N. G. Kochergin, and N. P. Teplyuk, *Modern Problems in the Treatment of Acantholysis Bullosa* [in Russian], Moscow (2002), pp. 3-19.
2. P. V. Sergeev, P. A. Galenko-Yaroshevskii, A. I. Khankoeva, and A. S. Dukhanin, *Byull. Eksp. Biol. Med.*, **122**, No. 3, 288-291 (1996).
3. P. V. Sergeev and A. S. Dukhanin, *Ibid.*, **134**, No. 9, 244-253 (2002).
4. M. V. Andrade, T. Hiragun, and M. A. Beaven, *J. Immunol.*, **172**, No. 12, 7254-7262 (2004).
5. R. J. Borski, G. N. Hyde, and S. Fruchtman, *Steroids*, **67**, No. 6, 539-548 (2002).
6. P. C. Calder, P. Yagoob, F. Thies, *et al.*, *Br. J. Nutr.*, **87**, Suppl. 1, S31-S48 (2002).
7. S. Fukuda, H. Mitsuoka, and G. W. Schmidt-Schonbein, *J. Leukoc. Biol.*, **75**, No. 4, 664-670 (2004).
8. T. Kino, M. U. De Martino, E. Charmandari, *et al.*, *J. Steroid Biochem. Mol. Biol.*, **85**, Nos. 2-5, 457-467 (2003).
9. M. P. Nambiar, E. J. Enyedy, C. U. Fisher, *et al.*, *Cell Immunol.*, **208**, No. 1, 62-71 (2001).
10. J. R. Stanley, *Arch. Dermatol.*, **135**, No. 1, 76-78 (1999).
11. E. T. Stoopler, A. Pinto, and S. S. DeRossi, *NY State Dent. J.*, **69**, No. 7, 30-32 (2003).
12. C. Veldman, A. Stauber, R. Wassmuth, *et al.*, *J. Immunol.*, **170**, 635-642 (2003).