Effect of Arachidonic Acid and Hydrocortisone on the Intracellular Concentration of Free Ca^{2+} Ions in JW Mouse Plasmacytoma Cells

E. I. Astashkin, A. B. Khodorova, O. A. Tumanova, V. E. Samoilova, and A. S. Dukhanin

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Arachidonic acid (AA) and its metabolites are involved in the regulation of various physiological processes, including the activation and division of lymphocytes [3]. AA is released from phospholipid components of the cell membrane due either to cleavage by phospholipase A_2 or to the sequential action of phospholipase C and diacylglycerin lipase [2,8], the second pathway probably being predominant in lymphocytes. Mitogens stimulate AA release from thymocytes and human peripheral blood lymphocytes into the incubation medium [4,5].

Glucocorticoid hormones suppress different types of immune responses mediated by T and B cells [7]. Both AA and glucocorticoids exhibit pronounced membranotropic properties owing to their high hydrophobicity.

The aim of the present study was to investigate the effect of exogenous AA and hydrocortisone on the cytoplasmic concentration of free calcium ions ($[Ca^{2+}]_i$) in IgE-producing murine JW cells [6], which are a convenient model of activated B lymphocytes.

MATERIALS AND METHODS

Murine IgE-producing JW-8 plasmacytoma cells were grown in 75 cm^2 flasks (Flow) in an incubator at 37°C and 5% CO_2. The culture medium was RPMI 1640, supplemented with 0.03% glutamine, 10% fetal calf serum, 50 μg/ml penicillin, and 100 IU/ml streptomycin. The cells were harvested 3 days after reculturing. Cells after the 3rd passage were used in the experiments.

The intracellular concentration of free Ca^{2+} ions was measured using a Fura 2-AM fluorescent probe, as described elsewhere [1].

RESULTS

The addition of AA (3 μM) to the JW cells suspended in Hanks solution containing 1.2 mM Ca^{2+} caused a rapid increase of $[Ca^{2+}]_i$ from the basal level of 120±18 nM to 240±25 nM (n=4) (Fig. 1, *a*). The rise of $[Ca^{2+}]_i$ was maximal 1.5-2 min after the addition of AA, after which the concentration of Ca^{2+} dropped to a certain steady level. A marked dose-dependent response was observed within the concentration range of 1-3 μM AA.

When hydrocortisone (HC) was added to the cell suspension against the background of AA, no subsequent drop of $[Ca^{2+}]_i$ was observed and the level of $[Ca^{2+}]_i$ remained stable (Fig. 1, *b*).

In order to determine from where Ca^{2+} enters the cytoplasm in response to AA, we carried out experiments in a Ca^{2+}-free medium. The calcium ions were bound with EGTA (final concentration 3 mM). The addition of EGTA to the cell suspension resulted in a sharp drop

Laboratory of Antitoxin Drugs, Biotekhnologiya Research Plant; Department of Molecular Pharmacology and Radiobiology, Russian State Medical University, Moscow. (Presented by P. V. Sergeev, Member of the Russian Academy of Medical Sciences)

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of the basal level of fluorescence, which was probably due to competition for Ca^{2+} ions between EGTA and the Fura-2, which leaked from dye-loaded cells (Fig. 2, *a*). AA (3 μM) added to the EGTA-pretreated cells increased the intracellular Ca^{2+} concentration by approximately 55±12 nM (n=4).

It is noteworthy that the typical dynamics of $[Ca^{2+}]_i$ was transitory in nature: the calcium concentration in the cytoplasm peaked after 1 min and during the subsequent 2-2.5 min dropped to the initial value, the magnitude of the entire response being approximately half that with Ca^{2+}-containing medium. This fact, together with the characteristic dynamics of the Ca^{2+} response to AA in the Ca^{2+}-free medium, suggests that AA induces Ca^{2+} release from the intracellular stores of JW cells. Hence, the Ca^{2+} response to AA in plasmacytoma cells consists of

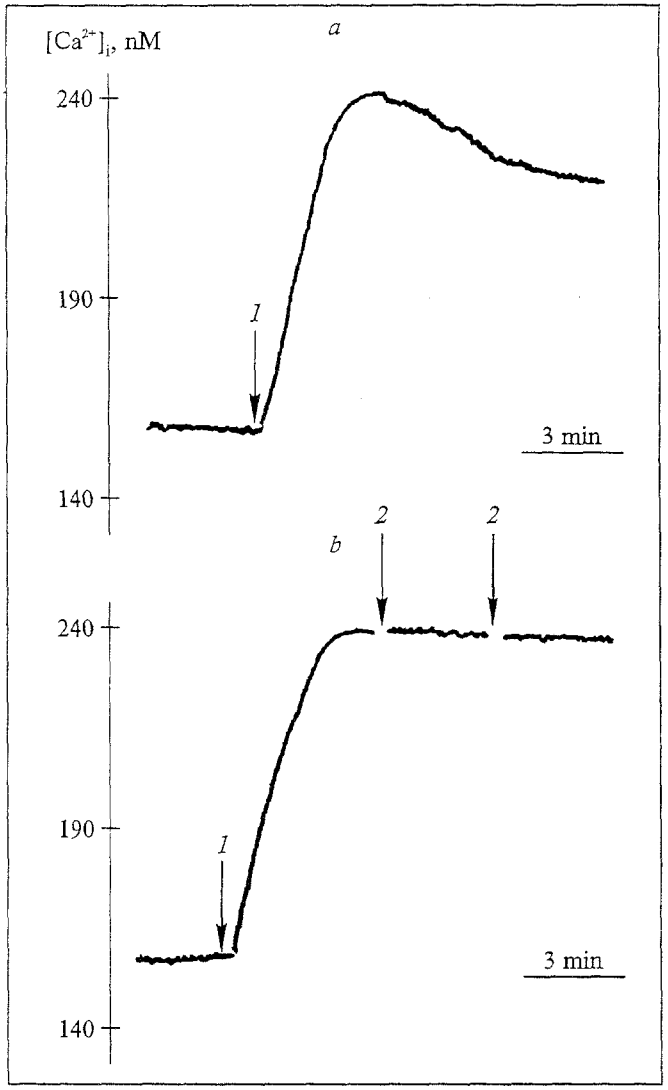

Fig. 1. Effect of AA (*a*) and HC against the background of AA (*b*) on $[Ca^{2+}]_i$ in JW cells. *1*) AA (3 μM); *2*) HC (5 μM).

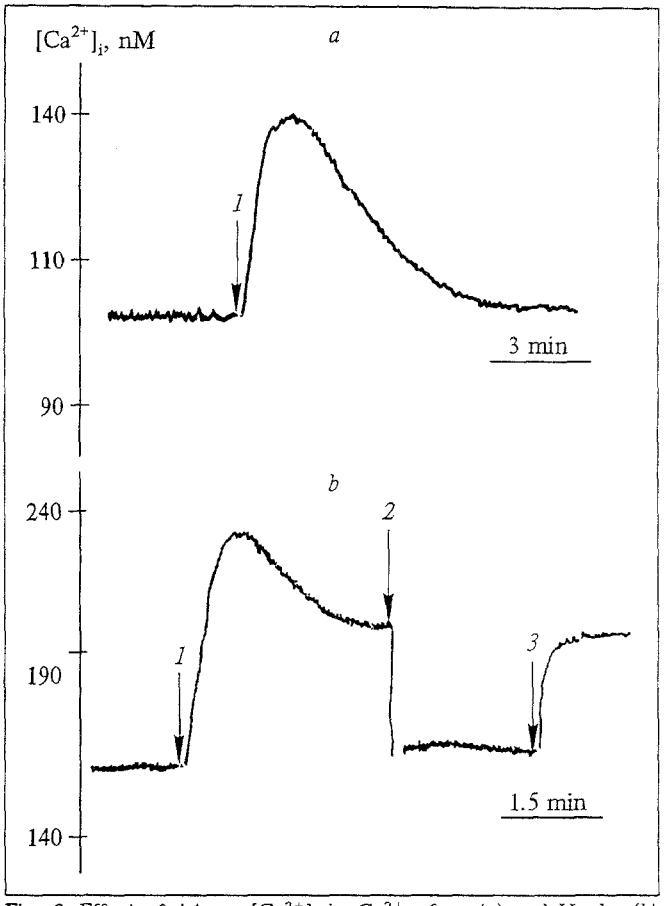

Fig. 2. Effect of AA on $[Ca^{2+}]_i$ in Ca^{2+} – free (*a*) and Hanks (*b*) medium. *1*) AA (3 μM); *2*) EGTA (3 mM); *3*) CaCl2 (4.5 mM).

2 superimposed processes: Ca2+ transport from the incubation medium and Ca^{2+} release from the intracellular stores (Fig. 2, *a*).

For confirmation of this assumption the cell suspension in Ca^{2+}-containing medium with AA added was treated subsequently with EGTA (3 mM) and then $CaCl_2$ (4.5 mM). Figure 2, *b* demonstrates that the EGTA-evoked drastic drop of $[Ca^{2+}]_i$ was followed by its restoration after the

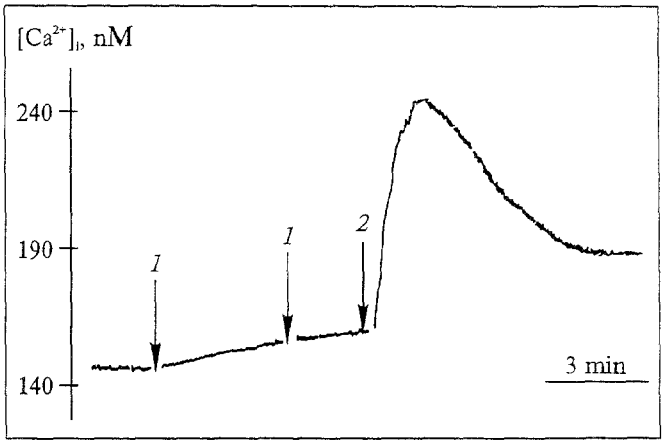

Fig. 3. Effect of AA and HC on $[Ca^{2+}]_i$ in JW cells in normal Hanks medium. *1*) HC (2.5 μM); *2*) AA (3 μM).

addition of Ca^{2+} and due to the Ca^{2+} influx through the membrane Ca^{2+} channels.

The addition of different concentrations of HC (0.5-5.0 μM) to the cell suspension showed that the glucocorticoid exerted a weak but reliable effect on the basal $[Ca^{2+}]_i$ (Fig. 3). The subsequent addition of AA (3 μM) resulted in a rise of $[Ca^{2+}]_i$ by 102±30 nM, which was similar to the Ca^{2+} response in the Ca^{2+}-free medium (Figs. 1, 2).

The data suggest that hydrocortisone in a therapeutic range of concentrations blocks the membrane Ca^{2+} channels and inhibits Ca^{2+} influx into JW cells. At the same time, it has virtually no effect on AA-induced Ca^{2+} release from the intracellular stores.

On the basis of these data we concluded that both arachidonic acid and hydrocortisone possess membranotropic activity in JW plasmacytoma cells. However, their effects are oppositely directed: arachidonic acid increases, while hydrocortisone decreases the permeability of the plasma membranes.

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Effect of Nitrobenzene and its Chloro-Substituted Derivatives on Parameters of Antioxidant Homeostasis in Rat Tissues

L. I. Paranich, A. V. Paranich, N. M. Vasilenko, E. V. Bugai, and L. Ortiz

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Nitrobenzene and its chloro-substituted derivatives are widely used in industry for the manufacture of dyes, pharmacological drugs, and other compounds. These substances are very harmful for workers coming in contact with them due to their volatility and ready ability to penetrate into the organism. The toxic properties of these substances are well known [3,12], but their metabolic pathways in the organism are little understood. General systems of xenobiotic decontamination [1,2,5,13] as well as lipid peroxidation (LPO) processes [6-8] are known to participate in nitrobenzene detoxication. However, there are practically no data on the involvement of antioxidation (AO) systems and lipid-soluble vitamins in the maintenance of the normal functioning of the organism's systems un-

Department of Molecular and Applied Biophysics, Khar'kov State University, Ukraine. (Presented by S. S. Debov, Member of the Russian Academy of Medical Sciences)

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