EFFECT OF CORTISOL ON CALCIUM CONCENTRATION IN LYMPHOCYTES OF BRONCHIAL ASTHMATICS

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The long-term use of glucocorticoids (GC) for the treatment of patients with bronchial asthma (BA) can cause hormone-dependence and hormone-resistance. Resistance to GC may exist in the patient even before their use, i.e., it may be genetically determined. As a rule, however, it develops gradually and is a type of drug tolerance [5]. The problem of preliminary assessment of the patient's response to GC is thus highly topical. Lymphocytes constitute an adequate experimental model, reflecting the sensitivity of the body to GC, i.e., they have a broad set of receptors similar in many respects to the tissue receptors of internal organs. There are various ways of estimating sensitivity of lymphocytes to the action of hormones, and thereby predicting the efficacy of hormone therapy. One of the most widely used of these methods is that based on determination of suppression by GC of the blast transformation reaction of lymphocytes induced by mitogens [2]. However, determination of the sensitivity of cells to hormones by this method is quite laborious, and takes 72 h. With the creation of a new generation of fluorescent Ca indicators it is now possible to record initial changes in the concentration of this ion arising through the action of mitogens on lymphocytes after only 10-20 sec [7]. The maximal rise in the level of calcium ions is observed 1.5-2 min after addition of the preparations [1]. This does away with the need to observe the whole process of blast transformation in its entirety.

The aim of this investigation was to study the effect of cortisol on the mitogen-induced rise of the Ca^{2+} level in the cytoplasm of peripheral blood lymphocytes from healthy blood donors and patients with hormone-resistant and hormone-sensitive forms of bronchial asthma.

EXPERIMENTAL METHOD

Altogether 10 patients with the infectious-allergic and atopic form of bronchial asthma were studied. The control group consisted of six persons (healthy donors aged from 25 to 50 years). To obtain a lymphocyte suspension from human peripheral blood (HPB) blood (30 ml) was taken from the cubital vein into a plastic test tube containing 6 ml of anticoagulant (25 g of sodium dihydrogen citrate). The blood (5 ml) was layered from a Pasteur pipet on to an Isopaque-Ficoll gradient (3 ml). The sample was centrifuged at 1500*g* for 15 min at room temperature, after which the uppermost translucent layer located above the gradient was removed. It contains mononuclear leukocytes, which were resuspended in Hanks' medium and washed by centriiugation [3]. The cells were transferred into 10 ml of HFPES-buffer (140 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 5 mM Glu, 1 mM Na_2HPO_4, 1 mM $CaCl_2$, 10 mM HEPES), pH of the medium 7.35, the buffer made up at room temperature [7]. Incorporation of the fluorescent Ca ion indicator (from "Calblochem") into the cells was carried out by the method in [8]. After incubation of the cells

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TABLE 1. Effect of Cortisol (10 μM) on Mitogen-Dependent Increase in Calcium Concentration in Peripheral Blood Lymphocytes from Healthy Donors (n = 6)

Calcium ion concentration in cytoplasm, nM		
basal level	stimulation by con A	con A + cortisol
105	346	170
112	327	163
109	317	155
134	289	176
119	305	180
98	418	204
108	392	245
166	285	194
152	316	180
81	365	154
73	408	139
92	411	162
163	298	184
152	307	170

for 20 min at 37°C with a solution of FURA-2/AM in a final concentration of 3 μm the cells were washed and resuspended in HEPES buffer. Next, 2 ml of the cell suspension ($3 \cdot 10^6$ cells/ml) was transferred into the cell of a Hitachi MPF-3 spectrofluorometer, thermostated at 37°C. The wavelengths of excitation were 350 and 385 nm, and of recording 500 nm. The intracellular concentration of calcium ions was calculated by the equation:

$$Ca = K_d \cdot (R - R_0)/(R_1 - R), \qquad [7]$$

where R denotes the ratio F350/F385 for the test sample (F350 and F385 indicate the intensity of fluorescence at excitation wavelengths at 350 and 385 nm respectively), R_0 is the ratio F350/F385 determined after addition of 5 mM $MnCl_2$, when the Ca^{2+} concentration is minimal, R_1 is the ratio F350/F385 on saturation of the probe with calcium which was determined after disintegration of the cells with digitonin ("Sigma," 40 μM), kD the equilibrium constant of formation of a complex of FURA-2 with Ca^{2+} + ions, with the value of 225 nM at 37°C The mitogen concanavalin A (con A, from "Sigma") was used in a concentration of 25 μg/ml [1].

EXPERIMENTAL RESULTS

The basal calcium level in resting lymphocytes was 106 ± 5 nM. The study of the effect of con A (25 μg/ml) on the free Ca^{2+} level in HPB lymphocytes showed an increase in Ca^{2+} concentration after 10-20 sec, to reach a maximum (360 ± 20 nM) 1-1.5 min after addition of the preparation. Cortisol inhibits the mitogen-induced rise of the intracellular Ca level. The writers showed previously that the inhibitory effect of cortisol is dose- and time-dependent [6]. For the glucocorticoid to exhibit its action, free incubation with the cells for 30 min was essential. The optimal concentration of the hormone is 10 μM (Table 1).

Data on the combined action of con A and cortisol on the calcium concentration in HPB lymphocytes are summarized in Table 1. Significant individual differences in the response of the lymphocytes to the preparations (within the range 20-50%) will be noted.

Meanwhile, on repeated determination of the Ca^{2+} response of cells of the same subject the differences did not exceed 10-15%, evidence of the good reproducibility of the results.

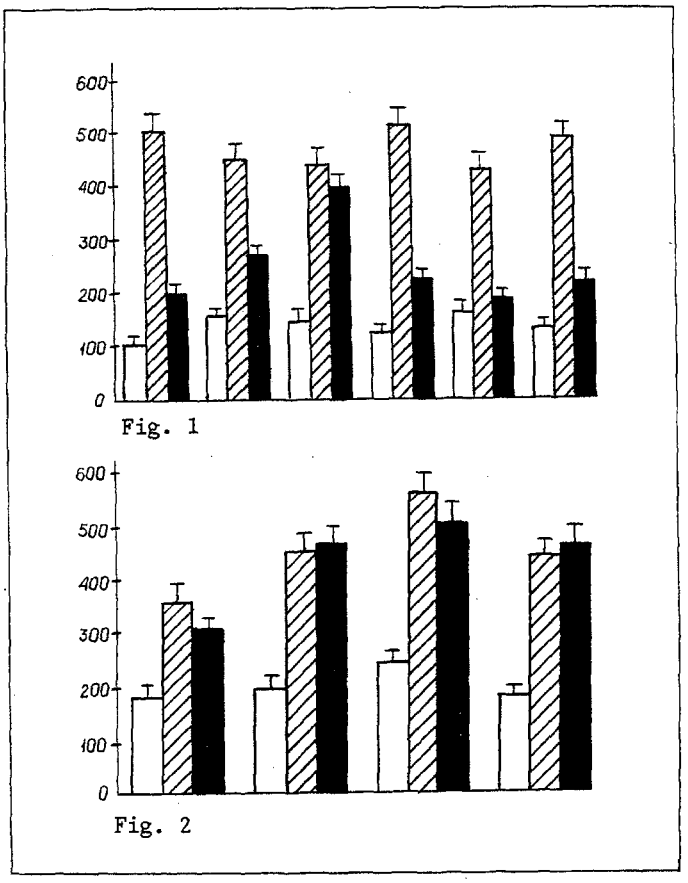

Fig. 1. Effect of cortisol on concanavalin-induced rise of calcium concentration in lymphocytes of patients with hormone-dependent form of BA Ordinate, calcium concentration (in mM); unshaded columns – control, obliquely shaded – concanavalin A, black columns – concanavalln A + cortisol.

Fig. 2. Effect of cortisol on concanavalin-induced rise of calcium concentration in lymphocytes of patients with hormone-resistant form of BA. Ordinate, calcium concentration (in mM). Legend as to Fig. 1.

In patients with the hormone-dependent form of BA, induction of lymphocytes by the mitogen caused a greater increase in the calcium concentration in the cytoplasm (4-5 times). The inhibitory action of cortisol (10 μM) was preserved, and in only one of the six patients tested was the suppressor effect significantly reduced (Fig. 1).

In patients with the hormone-resistant form of asthma and receiving the glucocorticoid preparations for 6 months or more (Fig. 2), the effect of cortisol on the Ca-response of the lymphocytes was virtually undetectable (four patients were tested).

The results suggest that the method we have developed for assessing the effectiveness of hormone therapy of BA can be used before glucocorticoid therapy is commenced.

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EFFECT OF SYSTEMIC ADMINISTRATION OF BETA-CASOMORPHINE-7 ON NOCICEPTION IN RATS

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The casomorphines were first isolated from commercial casein peptone [2, 3]. Tests of its various fractions showed that they contain a compound with high opioid activity, It was established that this compound is the hepta-peptide Tyr-Pro-Phe-Pro-Gly-Pro-Ile, and that it corresponds to the beta-casein fragment (60-66). Accordingly, this compound was called beta-casomorphine-7 (β-CM7). The presence of a large number of proline residues in its composition determines its high resistance to the action of proteolytic enzymes [6]. The ability of β-CM7 to be formed in the intestine [7], absorbed into the blood stream [10], and to act on peripheral mu-receptors [5], also has been demonstrated. Interest in compounds of this class has recently increased greatly in connection with the quest for new therapeutic substances of natural origin. Meanwhile, research with the casomorphines is still only on a small scale, and it is not mentioned in the USSR/CIS literature The analgesic properties of β-CM7 have been described mainly after intragastric injection or on various models (but never by systemic administration). Accordingly, the aim of the present investigation was to study dependence of the analgesic activity of β-CM7 on dose and on the initial pain sensitivity of experimental animals when injected intraperitoneally.

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