COMBINED ACTION OF CORTISOL AND CONCANAVALIN A ON CALCIUM ION CONCENTRATION IN THYMIC LYMPHOCYTES

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UDC 616.132-018.61-008.924.1-02:615.22]-092.9

KEY WORDS: glucocorticoids; hormone-resistance; lymphocytes; concanavalin A

Glucocorticoid hormones (GC) have found wide application in medicine on account of their antiinflammatory and immunosuppressive action. However, their prolonged administration may lead to the appearance of glucocorticoid resistance – absence of a therapeutic effect from hormone therapy. Lymphocytes are an adequate model reflecting sensitivity of the body to GC, for they have a considerable complement of receptors, similar in many respects to tissue receptors of the internal organs. Sensitivity of lymphocytes to the action of hormones, and consequent prediction of the efficacy of hormonal therapy, can be estimated in various ways. These include: determination of the viability of lymphocytes on incubation with GC [5]; a method based on determination of inhibition by GC of the lymphocyte blast transformation reaction [2], and radioreceptor analysis [4]. The methods listed above enable the sensitivity of cells to hormones to be established comparatively precisely, but they are somewhat laborious and may take from 12 to 72 h.

The aim of this investigation was to study the effect of cortisol on the mitogen-induced Ca^{2+} level in the cytoplasm of hormone-sensitive and hormone-resistant thymic lymphocytes, and on that basis to develop a rapid method of determining the sensitivity of target cells to GC.

EXPERIMENTAL METHOD

To obtain a suspension of thymocytes the thymus was removed from rats weighing 150-170 g, decapitated under superficial ether anesthesia, and placed in Hanks' solution (pH 7.4, t = 4°C). The tissue of the organ was transferred to a homogenizer (glass–glass) and thymocytes expressed until the stroma was completely empty. The resulting suspension was filtered through nylon gauze and centrifuged at 800*g* for 10 min. The cells were washed twice in medium 199 and in HEPES 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, buffer made up at room temperature [6]), and they were counted in a Goryaev's chamber. The viability of the thymocytes was determined by intravital staining with trypan blue. After intraperitoneal injection of an aqueous-alcoholic suspension of dexamethasone (from "Sigma)," into the rats, the animals were sacrificed 24 h after the last injection of the preparation. Incorporation of a fluorescent indicator of Ca^{2+} ions ("Calbiochem") into the cells was carried out by the method in [6]. After incubation of the cells 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 added to the measuring cell of a "Hitachi MPF-4" spectrofluorometer, kept at a constant temperature of 37°C. Wavelengths of excitation were 350 and 385 nm, and of recording 500 nm. The intracellular Ca^{2+} ion concentration was calculated by the equation:

$$[Ca^{2+}]_{in} = K_d \cdot (R - R_0)/(R_1 - R) \text{ [3]},$$

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 612-614, June, 1992. Original article submitted November 25, 1991.

0007-4888/92/0006-0818$12.50

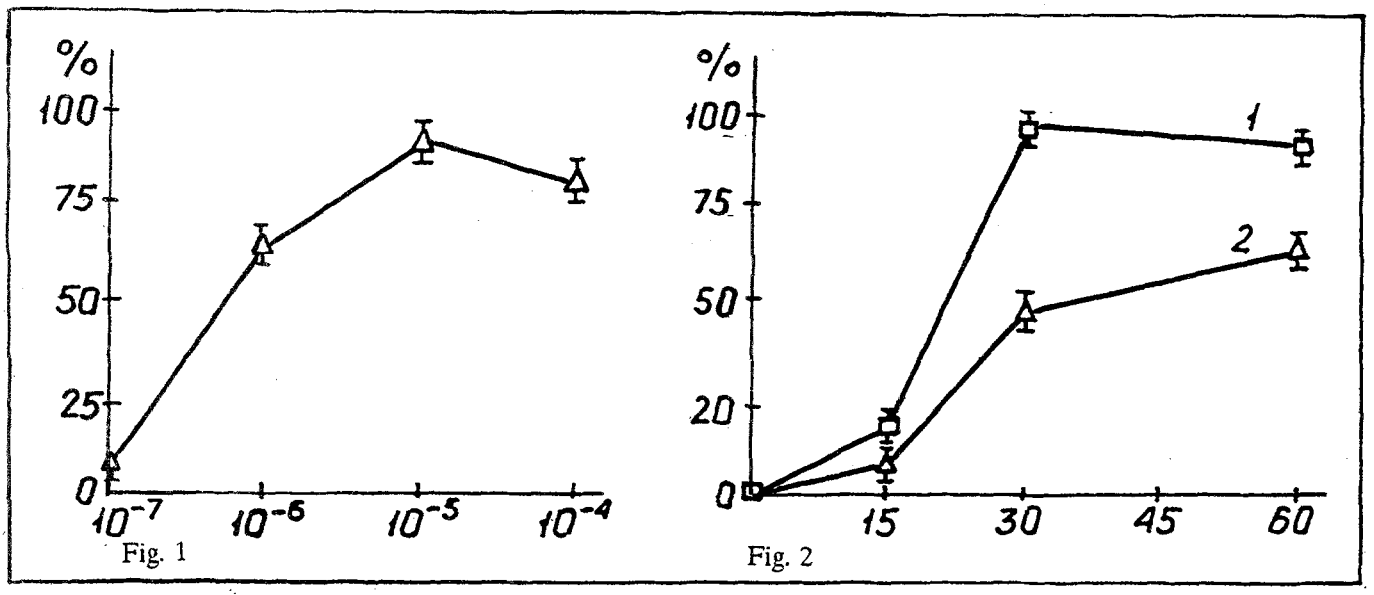

Fig. 1. Dose dependence of inhibitory effect of cortisolon mitogen-induced increase in calcium concentration in thymocytes. Abscissa, cortisol concentration (in M); ordinate, inhibitory effect (in %). Maximal effect with cortisol concentration at 10 μM taken as 100%. Incubation time 30 min. Conditions: incubation temperature 37°C, duration of preincubation with hormone 30 min, duration of incubation with concanavalin A (25 μg/ml) 2 min; concentration of cells $3 \cdot 10^6$/ml). Calcium concentration was determined 2 min after addition of mitogen.

Fig. 2. Dynamics of inhibitory effect of cortisol on Ca^{2+}-response of thymocytes. Abscissa, time (in min); ordinate, inhibitory effect (in %). 1) Cortisol in concentration of 10 μM, 2) of 1 μM. Conditions as in Fig. 1.

where R is the ratio F350/F385 for the test sample (F350 and F385 indicate the intensity of fluorescence at excitation wavelengths of 350 and 385 nm respectively); R_0 denotes the ratio F350/F385-determined after addition of 5 mM $MnCl_2$, when the Ca^{2+} concentration is minimal; R_1 denotes the ratio F350/F385 during saturation of the probe with calcium, and which was determined after destruction of the cells with digitonin ("Sigma," 40 μM), K_d the equilibrium constant of formation of the FURA-2 complex with Ca^{2+} ions, and equal to 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 free Ca^{2+} ion concentration, determined in resting thymocytes loaded with FURA-2, was 105 ± 5 nM (n = 16). Incidentally, in some series of experiments the resting calcium concentration was rather higher or lower, for example: 140 nM or 90 nM. On addition of the mitogen con A to the cell suspension, the Ca^{2+} ion concentration in the cytoplasm increased after 10-20 sec. It reached a maximum of about 370 ± 30 nM after 1.5-2 min, i.e., 3 or 4 times higher than the basal level). These results are in agreement with data in the literature [1].

The total lymphocyte fraction of the thymus included 95% of hormone-sensitive cells and only 5% of hormone-resistant thymocytes [5]. The total thymocyte fraction of intact rats can therefore be regarded as a model of hormone-sensitive cells.

Preliminary incubation (30 min at 37°C) of the cell suspension with cortisol in a final concentration of 1 μM significantly lowered the mitogen-dependent rise of the intracellular calcium level in the thymocytes, to 180 ± 20 nM. Under these experimental conditions cortisol itself had no significant ($p > 0.05$) effect on the calcium concentration in the thymocytes.

Dependence of the inhibitory effect of cortisol on dose and time was studied (Figs. 1 and 2). The action of the glucocorticoid began to appear with a concentration of 0.5 μM, and with an increase in dose of cortisol to 10 μM the inhibitory action increased. A further increase in cortisol concentration did not significantly change the effect.

TABLE 1. Effect of Different Steroid Hormones on Mitogen-Induced Increase in Calcium Ion Concentration (nM)

Control	Con A	1	2	3	4	5
106±5	370±30	170±20*	190±20*	280±35	350±40	260±30

Legend. Con A) concanavalin A, 1) con A + cortisol (10 μM), 2) con A + dexamethasone (10 μM), 3) con A + progesterone (10 μM), 4) con A + estradiol (10 μM), 5) con A + testosterone (10 μM). Results (M ± m) of 4-6 independent experiments. Asterisk indicates significant differences in groups compared with effect of con A.

TABLE 2. Effect of Cortisol (10 μM) on con A-Stimulated Increase in Ca^{2+} in Rat Thymocytes

Group of animals	Intracellular Ca^{2+} level, nM		
	basic level	con A	con A + cortisol
Control	106±5	370±30	170±10**
Single injection of:			
dexamethasone, 2 mg/kg	160±8	420±35	270±20*
dexamethasone, 5 mg/kg	150±7	360±27	280±20
5-day administration of dexamethasone, 1 mg/kg	110±5	300±20	310±25

Legend as to Table 1. *$p < 0.05$, **$p < 0.01$.

For cortisol to exert its calcium-blocking action, preliminary incubation of the hormone with the cells was shown to be necessary; an optimal incubation time is 30 min.

The observed effect of inhibition of Ca^{2+}-response of the cells to stimulation by mitogens is specific for glucocorticoids. Other steroid hormones (progesterone, testosterone, estradiol), used in the same concentration (10 μM) had a much weaker effect on the calcium level in thymocytes (Table 1).

In an experiment to obtain a glucocorticoid-resistant lymphocyte population, a pharmacologic dose of the hormone was administered; at the height of action of the hormone (after 12-24 h) mainly fractions resistant to glucocorticoids remained in the thymus, lymph nodes, and blood. Their response to the mitogens was unchanged [6].

Thymocytes from animals receiving single injections of dexamethasone (in doses of 2 and 5 mg/kg body weight) and daily injections for 5 days in a dose of 1 mg/kg were used as models of hormone-resistant cells in our investigation.

The ability of cortisol to inhibit the Ca-response of thymocytes isolated from these animals was appreciably less (Table 2). Repeated injections of dexamethasone into the animals led to reduction of the weight of the thymus on average by 80%. Incidentally, the basal calcium level in the remaining thymocytes was higher than in the thymocytes of intact rats (180 ± 32 nM and 103 ± 18 nM respectively). Cortisol had virtually no effect on mitogenic stimulation of hormone-resistant thymocytes. The results are evidence that the method we have developed can be used to assess the sensitivity of cells to the action of glucocorticoids in vitro.

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SELECTIVE ANALYZERS OF D_2-DOPAMINE RECEPTORS MODULATE SEROTONIN METABOLISM IN THE STRIATUM AND NUCLEUS ACCUMBENS AFTER DOPAMINERGIC NEURON BLOCKADE

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UDC 612.825.015:577.175.823].06

KEY WORDS: dopamine agonists; serotonin synthesis; gamma-butyrolactone

To assess the contribution of the presynaptic component of regulation of dopamine (DA) biosynthesis in the brain, a gamma-butyrolactone model has been suggested [11, 13]. Gamma-butyrolactone (GBL) can selectively block the spike discharge of dopaminergic neurons [3], as a result of which the release of DA from the corresponding nerve endings is reduced [6, 11], as also is the inhibitory effect mediated by terminal DA autoreceptors, belonging to the D_2 subtype. In this way DA biosynthesis is disinhibited on the feedback principle. Replacement of the endogenous ligand by a D_2-receptor agonist reduces the rate of DA biosynthesis and, in turn, this can be reversed by administration of a dopamine receptor antagonist [11, 13].

The state of the serotoninergic systems of the brain under the conditions of this model remains virtually unstudied. Yet this is problem of great interest, bearing in mind the important role of serotoninergic systems in brain functions. Accordingly, the aim of the investigation described below was to assess the possible influence of selective agonists and antagonists of D_2-dopamine receptors on serotonin biosynthesis and metabolism in the striatum and nucleus accumbens of the rat brain, when the dopaminergic spike discharge is blocked, so that effects of pharmacologically modified activity of dopamine neurons can be excluded.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-300 g. The state of DA and serotonin biosynthesis in the striatum and nucleus accumbens of the rat brain was assessed by measuring accumulation of L-3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) respectively after inhibition of the decarboxylase of L-aromatic amino acids with 3-hydroxybenzylhydrazine (3-HBH). The schedule of administration of these

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Laboratory of Neurochemical Pharmacology, Research Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 614-616, June, 1992. Original article submitted October 28, 1991.

0007-4888/92/0006-0821$12.50