MECHANISMS OF REALIZATION OF THE COMBINED ACTION OF CORTISOL AND ADENOSINE ON PERIPHERAL BLOOD LYMPHOCYTES IN BRONCHIAL ASTHMA

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One reason why glucocorticoid hormones are so effective in the treatment of patients with bronchial asthma is their ability to act directly on immunocompetent cells (lymphocytes, monocytes), to inhibit lymphokine release and cytotoxicity, and to activate suppressor activity [3]. The mechanism of action of glucocorticoids on target cells involves interaction of the hormones with specific membrane and intracellular receptors [4]. The process of formation of hormone-receptor complexes (HRC) may act as the point of application of the various regulatory agents, whether biologically active substances of different chemical nature (catecholamines, purines, prostaglandins) or pharmacological preparations [1]. The modifying influence of these compounds on function of the receptor apparatus leads to changes in sensitivity of the cells to the action of steroids, and this has led to a search for new and optimal treatment and dose schedules for hormonal therapy. However, the mechanisms of realization of heterospecific regulation of glucocorticoid reception by target cells in bronchial asthma have not yet been explained.

According to ideas held at the present time, exacerbation of the course of bronchial asthma is accompanied by a three-fourfold increase in the blood level of the purine compound adenosine in the patients (to 0.5-10 μM) [7]. We showed previously that adenosine, as a ligand of purine receptors, activates lymphocytic adenylate cyclase, thus affecting cortisol reception in the subsequent stages of realization of the hormonal stimulus also [2]. This particular effect of adenosine is mediated by the secondary mediator *c*AMP.

The aim of the present investigation was accordingly to study the action of adenosine and cortisol on the intracellular *c*AMP level and also the effect of adenosine on specific binding of ^{3}H-cortisol by human peripheral blood lymphocytes in normal individuals and patients with bronchial asthma (BA).

Human peripheral blood lymphocytes were chosen as the test object because they contain glucocorticoid and purine receptor systems and are available in the necessary amounts for radioreceptor analysis of clinical material.

EXPERIMENTAL METHOD

The test material consisted of venous blood (30-50 ml) from patients with atopic and infectious-allergic forms of BA. Lymphocytes were isolated by centrifugation in a Ficoll–Hypaque density gradient at room temperature [6]. *c*AMP in the cells was determined by the radioligand method, using kits from "Amersham." To samples containing 0.5 ml of lymphocyte suspension (10^7 cells/ml) the test substances, in a volume of 10 μl, or 10 μl of Hanks' solution (control samples) were added. The samples were then incubated at 37°C for 10 min.

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TABLE 1. Effect of Adenosine and Cortisol on *c*AMP Concentration (in pmoles/10^7 cells) in Peripheral Blood Lymphocytes ($M \pm m$)

Group of subjects	Basal cAMP level	Stimulation of cells by		$cAMP_{stim}/cAMP_{bas}$	
		adenosine 10^{-6} M	adenosine 10^{-6} M + cortisol 3×10^{-6} M	adenosine	adenosine + cortisol
Healthy	6,2±0,3	20,7±0,9	22,3±0,8	3,3	3,6
Patients with BA					
group I	6,1±0,2	16,4±0,6	21,9±1,1	2,7	3,6
p		<0,05			
group II	4,2±0,2	11,0±0,8	18,6±0,8	2,6	4,4
p	<0,05	<0,01			

Legend. *p*) Statistical significance of values in groups of patients relative to group of healthy individuals.

Specific binding of ^{3}H-cortisol (specific activity 91 Ci/mmole, "Amersham") of the cytosol and nuclear fractions was determined by the method described in [9]. Radiometry was carried out by means of a liquid scintillation counter ("Intertechnique," France). The experimental results were subjected to statistical analysis on the "Amstad" PC 1640 computer, using the "Microstat" package of statistical programs.

EXPERIMENTAL RESULTS

The early stages of the action of glucocorticoids and adenosine are associated with an increase in the intracellular *c*AMP concentration, the trigger mechanisms of which are located on the plasma membrane [5]. In the first series of experiments the effect of adenosine and cortisol on the *c*AMP level was compared in lymphocytes from healthy blood donors and patients with BA (Table 1). The basal *c*AMP level in healthy human lymphocytes was 6.2 ± 0.3 pmole/10^7 cells. Incubation of the lymphocytes in the presence of adenosine (1×10^{-6} M) led to a marked increase in the *c*AMP concentration up to a value of 20.7 ± 0.9 pmole/10^7 cells. To investigate the ability of cortisol to intensify the stimulation of *c*AMP formation, the glucocorticoid was added to the incubation medium simultaneously with adenosine. The dose of cortisol used was 3×10^{-6} M or 1 μg/ml, which corresponds to the therapeutic concentrations of glucocorticoids in the blood plasma adopted in the treatment of BA. It was found that on combined action of cortisol and adenosine, the intracellular *c*AMP concentration rose to 22.3 ± 0.8 pmole/10^7 cells. Thus cortisol potentiated the effect of adenosine on the intracellular *c*AMP level, in agreement with data in the literature [8]. Incidentally, under the experimental conditions adopted, cortisol in the absence of adenosine did not change the intracellular *c*AMP level, and for that reason the effect of the hormone was analyzed only on the increase in the *c*AMP concentration, effected through the action of adenosine.

The results of the study of the action of adenosine and cortisol on lymphocytes from patients with BA are given in Table 1. Depending on the character of the response of the lymphocytes to stimulation by the test compounds, the patients with BA were divided into two groups. The basal *c*AMP level in lymphocytes isolated from the patients of group I did not differ significantly from the control values. Addition of adenosine caused an increase in the cyclic nucleotide concentration to 16.4 ± 0.6 pmole/10^7 cells. The combined action of adenosine and cortisol led to an increase in the *c*AMP concentration in the cells (21.9 ± 1.1 pmoles/10^7 cells). Meanwhile, the basal *c*AMP level in lymphocytes obtained from patients with BA in group II was below the control value (4.2 ± 0.2 pmole/10^7 cells). The response of the cells to stimulation by adenosine was depressed (11.0 ± 0.8 pmole/10^7 cells). Addition of cortisol to the incubation medium led to marked potentiation of the effect of adenosine. As a result of the combined action of adenosine and cortisol the *c*AMP concentration in the lymphocytes reached a value of 18.6 ± 0.8 pmole/10^7 cells. For further analysis of the results, the ratio of the *c*AMP content in the cells before and after their stimulation by the chosen preparations was calculated ($cAMP_{stim}/cAMP_{bas}$). A decrease in this ratio was recorded in the patients with BA, evidence of disturbance of the ability of adenosine to stimulate *c*AMP formation in the lymphocytes. Cortisol, as a rule, corrected the observed changes (Table 1). Consequently, differences in the mechanisms of transduction of the hormonal stimulus are already observed at the cellular plasma membrane level in normal subjects of patients with BA.

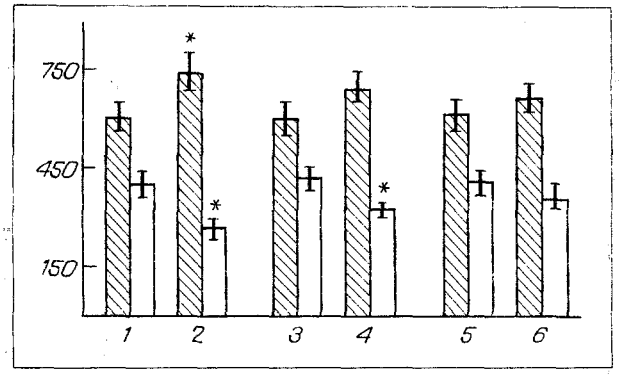

Fig. 1. Distribution of ^{3}H-cortisol-receptor complexes in peripheral blood lymphocytes. Ordinate, specific binding of ^{3}H-cortisol (in cpm/10^7 cells) in cytosol (unshaded columns) and nuclear (shaded columns) fractions of blood lymphocytes from healthy individuals (1) and patients with BA (3, 5), in the absence and in the presence of adenosine (2, 4, 6). *$p < 0.05$ Compared with initial data (samples not containing adenosine).

Elevation of the intracellular *c*AMP level is accompanied by activation of cytosol glucocorticoid-receptor complexes and their subsequent translocation into the nucleus of the target cell [4]. In experiments in vitro, this could be recorded as the distribution of ^{3}H-hormone-receptor complexes (^{3}H-HRC) between the nuclear and cytosol fractions of the cells. Since we had shown that the response of the lymphocytes of patients with BA to stimulation by adenosine was depressed, it was logical to suggest a change in the intracellular distribution of ^{3}H-HRC compared with the normal state. To test this hypothesis, in the next series of experiments we compared the effect of adenosine on specific binding of ^{3}H-cortisol (1×10^{-8} M) by lymphocytes in normal individuals and patients with BA. The results are given in Fig. 1. Clearly, in the absence of adenosine, the ^{3}H-HRC were distributed between the nuclear and cytoplasmic fractions about in the ratio of 3:2. This means that of the total number of HRC formed in the cell, on average 60% were bound with lymphocyte nuclei. This distribution of ^{3}H-HRC was characteristic both of lymphocytes obtained from healthy human blood and blood from patients with BA. The ^{3}H-HRC content in the nuclear fraction varied from 57 to 63%. However, against the background of the action of adenosine (1×10^{-6} M) the distribution of ^{3}H-HRC in the lymphocytes of patients with BA differed appreciably from the normal. For instance, whereas in healthy human lymphocytes adenosine increased the content of ^{3}H-HRC in the nuclear fraction to 75% of their total number in the cell, the effect of adenosine in patients with BA was only slight. This difference was manifested particularly clearly when the effect of adenosine was studied on specific binding of ^{3}H-cortisol by lymphocytes isolated from BA patients of group II (Fig. 1).

The results indicate that in BA a change takes place in the "recognition" of glucocorticoids by target cells at all stages of the receptor mechanism: the membrane stage — HRC formation — binding of the HRC by nuclear acceptors. In patients with BA disturbance of coordination in the realization of the combined action of adenosine and cortisol on peripheral blood lymphocytes also was observed. It must be pointed out that the presence of two groups of patients with BA, differing in the ability of their lymphocytes to respond to the action of cortisol and adenosine, evidently indicates the necessity for a differential approach to the treatment of these two categories of patients.

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CHARACTERISTICS OF INTERSTITIAL CELLS OF THE RENAL MEDULLA DURING LASIX-INDUCED STIMULATION OF PROSTAGLANDIN PRODUCTION

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Loop diuretics, which include Lasix, have not only a direct tubular diuretic action, but also have a stimulating effect on renal prostaglandin production [2, 4, 10]. Renal prostaglandins perform the function of regulators of vascular reactions in the kidney. Under the influence of Lasix prostaglandin production is increased most strongly in the inner medullary layer of the kidneys [13]. The main sources of renal prostaglandins in this zone are the interstitial cells [2, 11, 12]. The mechanism of action of Lasix on prostaglandin synthesis in the interstitial cells is not yet clear. There have been sporadic studies of the effect of a single injection of Lasix on these cells [6]. However, in clinical practice Lasix is often used over a long period of time, and this necessitates the study of the mechanisms of its effect on the interstitial cells under conditions of long-term administration.

The aim of this investigation was to study the structural and functional state of the interstitial cells of the internal medulla of the kidneys during long-term Lasix administration.

EXPERIMENTAL METHOD

Experiments were carried out on 28 noninbred male albino rats weighing 120-150 g. All the animals were divided into three experimental groups: group 1 (n = 8) served as the control, group 2 (n = 10) received a single dose of Lasix, whereas group 3 (n = 10) received Lasix daily for 30 days. Lasix in a dose of 3 mg/kg was injected intraperitoneally. The animals of group 2 were killed 30 min after injection of the preparation. Animals of group 3 were killed 30 min after the last injection. In all the experimental groups the animals were killed by decapitation in the morning before taking food. Pieces of the inner zone of the renal medulla were fixed in 1% glutaraldehyde solution and postfixed in 1% OsO_4 solution. Both fixatives were made up in 0.1 M phosphate buffer, pH 7.4. The material was dehydrated in alcohols and acetone and embedded in Araldite. Ultrathin sections cut on the LKB-4800 ultramicrotome were stained with uranyl acetate and lead citrate and studied under the IEM-100B electron microscope.

EXPERIMENTAL RESULTS

The experiments showed that the structure of the interstitial cells in animals of the control group corresponded to existing views [1, 7-9]. These cells were oriented strictly at right angles to the longitudinal axis of the renal papilla, and were distinguished by their elongated shape and their numerous cytoplasmic processes, which made contact with surrounding structures. Many membranous organelles could be seen in their cytoplasm: the rough and smooth endoplasmic reticulum, Golgi complex, mitochondria, and lysosomes (Fig. 1a).

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