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EFFECT OF CORTISOL, ALONE AND IMMOBILIZED ON POLYVINYLPYRROLIDONE, AND OF ADENYLATE CYCLASE ACTIVATORS ON CYCLIC AMP LEVELS IN RAT THYMOCYTES

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There is as yet no general agreement regarding the mechanism of the effect of steroid hormones on the adenylate cyclase system of target cells. Some workers have observed a decrease in phosphodiesterase activity of the soluble cell fraction in the presence of steroids [7]. This kind of action is exhibited if the hormones are present in a concentration of about 10^{-6}-10^{-4} M. There is evidence in the literature that steroids may interact with adenylate cyclase at the level of the cytoplasmic membrane of hormone-sensitive cells [2]. By using cortisol immobilized on polyvinylpyrrolidone (PVP-HC), and thus not penetrating into the cell, we have demonstrated in our laboratory that high-affinity glucocorticoid binding sites are present on the cytoplasmic membrane of the thymocytes of adrenalectomized rats [5]. The question of the biological role of the specific binding sites thus revealed needs an answer. The possibility cannot be ruled out that they participate in interaction between glucocorticoids and the adenylate cyclase system of the cells. Thymocytes are a convenient object with which to study the effect of glucocorticoid hormones and adenylate cyclase activators, for on the one hand they are target cells for glucocorticoids, and on the other hand, receptors of biologically active compounds, which exert their action on thymus lymphocytes through activation of adenylate cyclase [9], are located on the plasma membrane of thymocytes.

The aim of this investigation was to study the action of cortisol, PVP-HC, and progesterone on the cyclic AMP (cAMP) concentration in the thymocytes of adrenalectomized rats and to compare it with that of known adenylate cyclase activators (adenosine, isoproterenol, sodium fluoride).

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 120-150 g were used. On the 4th-5th day after bilateral adrenalectomy the animals were killed under superficial anesthesia. Thymocytes were isolated by the method described previously [3]. The viability of the cells was estimated by their ability to stain with trypan blue. In all experiments the viability of the thymocytes after isolation was not less than 95%. After preincubation of the cell suspension for

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TABLE 1. Time Course of cAMP Concentration (in μmoles/10^7 cells) in Thymocytes under the Influence of PVP-HC, Cortisol, and Adenylate Cyclase Activators (M ± m)

Compound	Duration of incubation, min					
	2	5	10	15	20	30
PVP-HC (10^{-6} M)	4,3±0,2 (3)	4.0±0,3 (4)	4,4±0,2 (4)	5,0±0,4 (5)	5,8±0,4* (5)	4,8±0,4 (5)
Cortisol (10^{-6} M)	4,4±0,2 (3)	4.2±0.2 (4)	4,6±0,2 (4)	4,9±0,3 (5)	5,6±0,3* (5)	5,0±0,3 (5)
Adenosine ($0.5 \cdot 10^{-6}$ M)	4,2±0,2 (5)	7,0±0,5** (5)	15,2±1,2** (5)	10,1±0,9** (5)	8,2±0,6** (4)	7,9±0,6** (4)
Adenosine ($0.5 \cdot 10^{-6}$ M) + PVP-HC ($0.4 \cdot 10^{-6}$ M)	4,3±0,3 (5)	6,8±0,4 (5)	20,8±1,1*** (5)	17,3±1,4*** (5)	9,4±0,8 (4)	8,0±0,6 (4)
Isoproterenol (10^{-6} M)	4,2±0,3 (5)	6,5±0,4 (5)	8,4±0,7** (5)	7,3±0,6** (5)	4,8±0,5 (4)	4,9±0,5 (4)
Isoproterenol (10^{-6} M) + PVP-HC ($0.4 \cdot 10^{-6}$ M)	4,3±0,2 (4)	6.6±0,4 (5)	12,6±0,5*4 (5)	8,9±0,4 (5)	5,7±0,6 (4)	4,9±0,6 (4)
NaF (10^{-2} M)	4,6±0,3 (5)	8.3±0,8** (5)	11,5±1,0** (5)	9,5±0,7** (5)	6,0±0,5* (4)	5,2±0,6 (4)
PVP-HC ($0.4 \cdot 10^{-6}$ M) + NaF (10^{-2} M)	4,4±0,3 (5)	8,1±0,6 (5)	11,9±0,9 (5)	9,3±0,7 (5)	5,6±0,4 (4)	5,6±0,4 (4)
PVP ($3 \cdot 10^{-6}$ M)	—	—	4,3±0,2 (4)	4,7±0,4 (4)	4,8±0,4 (4)	
Control (without hormones)	4,1±0,3 (9)					

Legend. *p < 0.05, **p < 0.01 relative to control, ***p < 0.05 relative to action of adenosine, ****p < 0.05 relative to action of isoproterenol. Here and in Table 2, number of experiments given in parentheses.

40 min at 37°C, the test substances were added. The cell concentration in the incubation medium was 10^7 cells/ml medium (Hanks' solution, pH 7.2). At the end of incubation the cells were homogenized in ice-cold Tris-EDTA buffer (pH 7.5), the homogenates were quickly placed in a water bath at 100°C for 3 min, after which the samples were centrifuged (10 min at 8000g) and the cAMP concentration in the supernatants was determined with the aid of standard kits from Amersham International (England). PVP-HC (mol. wt. 23 kilodaltons) containing 4% of cortisol by weight, was synthesized at the Institute of Macromolecular Compounds, Academy of Sciences of the USSR (Leningrad). The significance of the difference between mean values was estimated by Student's t test.

EXPERIMENTAL RESULTS

The results of the study of the effect of free cortisol and of cortisol immobilized on the polymer, on changes in the cAMP concentration in the thymocytes, mediated by adenosine ($5 \cdot 10^{-7}$ M), isoproterenol (10^{-6} M), and NaF (10^{-2} M), are shown in Table 1. Here and subsequently concentrations of PVP-HC are expressed as the content of molecules of PVP-bound hormone in the incubation medium (in M). The adenylate cyclase activators raised the cAMP level, to reach a maximum by the 10th minute of incubation. PVP-HC, like free cortisol in concentrations of 10^{-8}-$0.5 \cdot 10^{-6}$ M, had no effect on the cAMP concentration in the cells. Incidentally, both free cortisol and cortisol immobilized on PVP (in a concentration of 10^{-6} M) caused the same increase in the cAMP concentration, to reach a maximum by the 20th minute of incubation. PVP had no effect on the cAMP level.

The action of PVP-HC and cortisol on activity of adenylate kinase activators with different mechanisms of action was studied. PVP-HC and native cortisol, in a concentration of $4 \cdot 10^{-7}$ M, potentiated the adenosine- and isoproterenol-mediated increase in cAMP concentration at the 10th, 15th, and 20th minutes of incubation. Neither free nor immobilized cortisol had any effect on the rise of the cAMP level induced by NaF (Fig. 1a, b). The type of dependence of the cAMP level on the adenosine and isoproterenol concentrations, in the presence and absence of PVP-HC (competitive activation takes place) suggests the existence of a common stage in the action of cortisol, adenosine, and isoproterenol on the cyclase system. We know that adenosine and isoproterenol, which bind with purine [1] and β-adrenoreceptors of the thymocyte plasma membrane, respectively, modify adenylate cyclase activity through the participation of regulatory N-proteins, whereas NaF directly affects functional activity either of N-proteins or of the catalytic subunits of adenylate cyclase [4].

It can thus be concluded from the results of the study of the effect of free and immobilized cortisol on the increase in the cAMP concentration mediated through adenylate cyclase

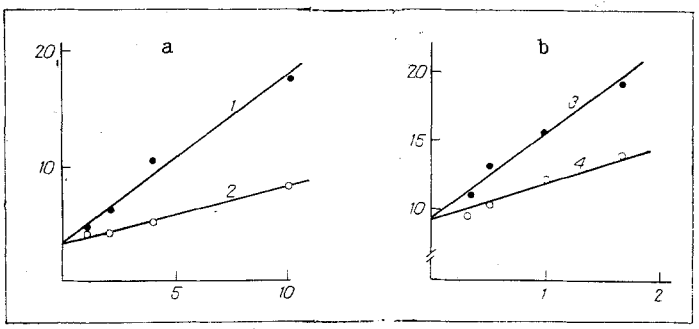

Fig. 1. Dependence of cAMP concentration in thymocytes on adenosine concentration in the absence (1) and presence (2) of PVP-HC (a) and on isoproterenol concentration in the absence (3) and presence (4) of PVP-HC (b), plotted between reciprocal coordinates. Abscissa, 1/C (in μM^{-1}), where C is the concentration of adenosine or isoproterenol in the incubation medium; ordinate, cAMP concentration in thymocytes [in $(pmoles^{-1}/10^7$ cells)$\cdot 10^2$] 10 min after the beginning of incubation. Straight lines drawn by the method of least squares. PVP-HC concentration $4 \cdot 10^{-7}$ M.

TABLE 2. Effect of Adenosine, PVP-HC, and Progesterone on cAMP Concentration in Thymocytes (M ± m)

Substance	cAMP concentration, 10^{-12} moles/10^6 cells
Control	4,1±0,3 (9)
Adenosine (10^{-7} M)	5,6±0,3* (6)
Adenosine (10^{-7} M) + PVP-HC ($0.4 \cdot 10^{-6}$ M)	12,5±1,3**,*** (6)
Adenosine (10^{-7} M) + PVP-HC ($0.4 \cdot 10^{-6}$ M) + progesterone (10^{-5} M)	5,5±0,3* (6)
Progesterone (10^{-5} M)	4,0±0,3 (4)
PVP-HC ($0.4 \cdot 10^{-6}$ M)	4,1±0,2 (3)

Legend. Duration of incubation with hormones was 10 min. *$p < 0.05$, **$p < 0.01$, compared with control; ***$p < 0.05$ compared with action of adenosine.

activators, that the interaction between cortisol, adenosine, and isoproterenol is synergic in character. The potentiating action of cortisol is evidently determined by its effect on coupling of the membrane receptors for adenosine and isoproterenol with the catalytic subunit of adenylate cyclase. In a previous investigation, by radioimmunoassay, of the cortisol concentration in chromatographic fractions obtained by gel-filtration of a sample of PVP-HC on Sephadex G-25, absence of hydrolysis of PVP-HC with the formation of cortisol molecules not bound with the polymer was found during incubation of the polymer preparation with thymocytes for 9 h at 37°C [6]. It was accordingly concluded that PVP-HC exerts its effect on the intracellular cAMP level through cortisol immobilized on PVP.

To study the specificity of action of PVP-HC on the adenylate cyclase system, the action of the glucocorticoid antagonist progesterone on the potentiating effect of PVP-HC was investigated (Table 2). The cAMP level in samples to which progesterone (final concentration 10^{-5} M), PVP-HC ($0.4 \cdot 10^{-6}$ M), and adenosine ($0.1 \cdot 10^{-6}$ M) were added simultaneously, was the

same as in samples containing adenosine. Addition of progesterone or PVP-HC alone to the incubation medium in the above concentrations did not change the cAMP level in the cells. Consequently, progesterone completely abolishes the effect of PVP-HC on the adenosine-mediated increase in the cAMP concentration, or in other words, the effect of PVP-HC was specific for the glucocorticoid hormone. There is as yet no clear idea of the molecular mechanism of action of progesterone as a glucocorticoid antagonist [8]. The data suggest that progesterone can interact directly with thymocyte membrane receptors for glucocorticoids.

It can be concluded from these results that the modulating effect of cortisol on the cAMP concentration in the thymocytes is one way whereby the hormonal activity of glucocorticoids is realized. The results of the study of the effect of progesterone on potentiation of the action of the adenylate cyclase activator, adenosine, by PVP-HC are evidence that the plasma membrane of thymus lymphocytes can be regarded as the first stage in interaction of glucocorticoids and their antagonists with target cells.

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PROTEIN SYNTHESIS AND FREE AMINO ACID LEVELS IN ORGANS OF RATS WITH EXPERIMENTAL PERITONITIS

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Mechanisms of the disturbance of homeostasis which accompanies the development of peritonitis remain partly unexplained, and are largely associated with the character of the metabolic disturbances in this state. The development of peritonitis is characterized, in particular, by a marked catabolic reaction and a negative protein balance, a leading role in the genesis of which is ascribed to irreversible losses of proteins [5]. Meanwhile the disturbance of synthesis and processing of various classes of RNA [1, 2], and also disturbance of the structural integrity of the polysomes of the liver and spleen [4] are evidence that the disturbance of protein metabolism in peritonitis is also linked with damage to the protein-synthesizing apparatus of the cells in the organ studied. Protein synthesis, as we know, includes several consecutive stages, each of which may become the limiting stage in the time course of development of peritonitis.

The aim of this investigation was to study protein synthesis in different organs of rats and to compare it with changes in the reserves of precursors, namely free amino acids, during the development of experimental peritonitis.

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