duced aggregation: the cGMP concentration was increased by 5 and 3 times, and the cAMP concentration by 4.8 and 2.6 times in concentrations of $5 \cdot 10^{-4}$ and 10^{-4} M, respectively.

It is generally considered that the process of platelet aggregation is determined by the ratio between the Ca^{++} and cAMP levels in the cell; these substances are secondary messengers in the process of signal transmission from the cell surface to the intracellular structures concerned in the processes of aggregation and disaggregation [4]. Meanwhile the question of the role of cGMP in platelet aggregation has not been settled. It is suggested that cGMP in platelets, just as in cell of other types, can behave as a cAMP antagonist [6].

The results of this investigation are evidence that the ability of 3-HP derivatives to raise the cGMP level, but not significantly, has probably no relation to their ability to inhibit platelet aggregation.

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EFFECT OF MINERALOCORTICOIDS ON RAT BRAIN Na,K-ATPASE ACTIVITY

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UDC 612.822.1.015.1:577.152.361].014.46:615.357.453

KEY WORDS: Na,K-ATPase; brain; microsomes; deoxycorticosterone.

Much progress has been made in the study of the cellular and molecular action of mineralocorticoids, but the mechanism of this action has not been finally explained. The ability of aldosterone and deoxycorticosterone to stimulate Na,K-ATPase activity in the kidneys [1], urinary bladder [11], and skin of the frog [4], and heart [12] is well known. However, this effect develops not sooner than 1-3 h after the action of the hormone. It has been suggested [9, 10] that the effect of mineralocorticoids on Na,K-ATPase is mediated through primary acceleration of nuclear metabolism and synthesis of specific proteins, which may perhaps be Na^+ carriers. According to this view, integrity of the cell is essential. More recently the attention of research workers has been drawn to the contribution of the plasma membranes to the mechanism of hormonal action. In this connection there has been some interesting work to study the effect of aldosterone on Na^+ transport through the anuclear erythrocyte membrane [3]. Aldosterone in vivo depresses the Na,K-ATPase activity of human erythrocytes. The action of the hormone on the erythrocyte membrane thus revealed is not connected with the process of genetic induction. Investigations on erythrocyte membranes have shown that the addition of hormone to them induces conformational changes in them [5].

There has been very little experimental research on plasma membranes, but such investigations could make an important contribution to the study of the mechanism of action of mineralocorticoids, for they enable the possible participation of the cytosol receptor in

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 10, pp. 434-436, October, 1986. Original article submitted April 18, 1986.

0007-4888/86/0010-1380$12.50

TABLE 1. Na,K-ATPase Activity (in μg P_i/mg protein/h) of Rat Brain Microsomes under the Influence of DOCA

Statistical parameter	In vivo		In vitro		
	control	DOCA	control	DOCA, μg/ml	
				5	15
M	16,08	24,93	22,7	9,8	10,6
n	12	15	23	12	12
P	—	<0,05	—	<0,001	<0,001

the process of binding and transfer of the hormone through the cell plasma membrane to be eliminated.

In this investigation activity of rat brain Na,K-ATPase was studied during the action of deoxycorticosterone both in vivo and in vitro.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats in vivo and in vitro. In the experiments in vivo deoxycorticosterone acetate (DOCA) was injected intramuscularly in a dose of 5 mg/100 g body weight, and the rats were decapitated 1 h later. In control experiments, mineral oil was injected intramuscularly in corresponding volumes (0.2 ml/100 g). Na,K-ATPase activity was determined in rat brain microsomes. Na,K-ATPase activity, inhibited by strophanthin K (1 mM) was determined by measuring the rate of accumulation of inorganic phosphate (P_i) in the incubation medium [15] and protein was determined by Lowry's method [6]. An alcoholic solution of DOCA (Koch-Light, England) in the experiments in vitro was added to the incubation medium in doses of 5 and 15 μg/ml (in the control experiments corresponding volumes of ethanol in a concentration of 20 mM were added). The duration of preincubation of the enzyme with DOCA, with the full composition of the substrate solution (6 mM $MgCl_2$, 300 mM NaCl, 60 mM KCl, 160 mM Tris-HCl, pH 7.4, and 6 mM ATP) was 10 min, and the duration of combined incubation with the hormone and strophanthin was a further 10 min. The experimental results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney test [2].

EXPERIMENTAL RESULTS

A marked increase in Na,K-ATPase activity in the rat brain was observed 1 h after intramuscular injection of DOCA (Table 1). To study the mechanism of action of mineralcorticoids on Na,K-ATPase activity in detail, the effect of the hormone was studied in vitro. Preincubaiton of microsomes with DOCA in doses of 5 and 15 μg/ml caused a fall in Na,K-ATPase activity; this effect, moreover, was found as early as 3-5 min after addition of the hormone to the incubation medium.

Two hypotheses have been put forward to explain the action of aldosterone [8]. The "sodium pump" hypothesis postulates that aldosterone directly stimulates activity of the Na pump on the outer side of the cell membrane. According to the "permeability theory," aldosterone increases Na^+ permeability through gaps in the mucous membrane. Supporters of the "metabolic theory" claim that aldosterone regulates the ATP reserves. Most workers [12] until recently considered that the metabolic pathway may be a key mechanism of action of mineralocorticoids. However, investigations of the action of the hormone on whole erythrocytes or on their membrane have demonstrated that it can directly affect the membrane [3, 4].

Our own experimental results suggest that mineralocorticoids can exert their action on rat brain Na,K-ATPase in several ways. One way begins with interaction of the hormone with the plasma membranes, detected by experiments in vitro. The response to the hormone develops essentially without any latent period, which in the case of mineralocorticoids might be expected to be 3-5 min. These facts suggest that the effect of DOCA on Na,K-ATPase is unconnected in this case with the process of genetic induction. Most probably the mineralocorticoid has a direct action on Na,K-ATPase, leading to conformational changes in the enzyme molecule.

Several explanations can be put forward regarding the possibility of the direct action of the hormone on the enzyme. First, there are hypothetical models in the literature to explain the mechanism of action of hormones [13, 14]. According to the "bivalent ligand" model [14], the hormone on the cell surface can bind simultaneously with two independent macromolecules. One of these is a receptor, whereas the other, transmitting the action of the hormone to the cell, is an effector. The other model [13] is based on the hypothesis that receptors for steroid hormones are multicomponent complexes, which in addition to a steroid-recognizing subunit, also contain a protein kinase, activated when the hormones bind with receptors. These models satisfactorily explain rapid responses to steroid hormones, similar to responses to neurotransmitters. Second, we know that in the repsence of aldosterone the inhibitory action of strophanthin on erythrocytic Na,K-ATPase is reduced, possible evidence of competition between these substances for cell membrane receptors. This is connected with the similarity of structure of the mineralocorticoids and cardiac glycosides [1, 3]. These facts completely explain the inhibition of Na,K-ATPase activity observed in our experiments in vitro. The other pathway for the action of DOCA on the brain (in experiments in vivo) is evidently connected with stimulation of biosynthetic processes in brain cells, as a result of which synthesis of new enzyme molecules and, in particular, of the Na pump, which is concerned with active ion transport, is increased. This is shown by the considerable latent period (1 h) required before the effects of mineralocorticoids are manifested.

These data are in agreement with the results of experiments to study the action of steroids on cells of the CNS, which showed that the response is often biphasic [7, 13], and the first phase is formed very quickly. Attempts to link this fast repsonse with changes at the transcription level have met with certain difficulties. The authors cited accordingly have accepted the possibility of direct participation of the cell membrane in the formation of fast repsonses to steroids. The second phase is linked with acceleration of nuclear metabolism and with synthesis of specific proteins, such as Na,K-ATPase, under the influence of hormones.

The diversity of ways in which the action of deoxycorticosterone can be realized can thus be attributed to its effect both directly on the Na,K-ATPase of the plasma membranes of the brain cells and on biosynthetic processes.

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