# CYCLIC AMP CONTENT AND CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVITY IN THE RABBIT BRAIN AFTER MILD HEAD INJURY

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Head injury causes important changes in physiological and biochemical processes in the brain tissue, manifested primarily as disturbance of the mechanisms of regulation of nerve tissue metabolism [4]. The writers have shown, for instance, that during the development of post-traumatic pathology, caused by mild experimental head injury (MHI), disturbances of integrity of the membranes and of the character of their function develop [3]. There is evidence [15] of changes in the kinetic parameters of enzymes of cyclic AMP (cAMP) metabolism, namely phosphodiesterase and adenylate cyclase, in the course of MHI, indicating disturbance of the functioning of the cAMP system. The results of clinical investigations [10] have shown that the cAMP concentration in the CSF of patients after MHI is significantly depressed. We also have found changes in activity of cAMP-dependent protein kinases from the cerebral hemispheres, diencephalon, and brain stem of experimental rabbits.

The aim of this investigation was to study the relationship between the equilibrium concentration of the chemical messenger, cAMP, and activity of cAMP-dependent protein kinases in the course of experimental MHI.

## EXPERIMENTAL METHOD

A model of experimental MHI was created in male rabbits weighing 2-2.5 kg, kept on the standard animal house diet, by means of a dosing device described in [2]. Material for investigation consisted of the diencephalon and brain stem (DBS) and the cerebral hemispheres of rabbits, taken 15 min, 2 h, and 1, 3, 7, and 14 days after trauma. Preparations of cAMP-dependent protein kinases were isolated from the rabbit's brain by a modified method [13]. The cytosol and membrane fractions were obtained from the hemispheres and from DBS of the rabbit's brain by the method in [5]. Protein kinase activity was determined as described in [14]. Adenylate cyclase and cAMP phosphodiesterase activity were measured as in [5] and [7] respectively. The equilibrium concentration of cAMP was calculated as described in [9, 10]. Radioactivity was measured on a Delta-300 liquid scintillation counter (USA). The protein concentration was measured by the method in [12]. The results were subjected to statistical analysis by the method in [1], using Student's t test and linear regression methods.

## EXPERIMENTAL RESULTS

The course of the traumatic process was accompanied by profound disturbances of function of the cAMP-dependent mechanism of regulation of metabolic processes in the rabbit's brain tissue. The equilibrium intracellular cAMP concentration is a factor determining activity of cAMP-dependent protein kinases [9]. The use of the theoretical models described in [8] and of others enabled the equilibrium cAMP concentration to be calculated on the basis of experimentally determined kinetic constants of reactions catalyzed by enzymes of cAMP metabolism, namely adenylate cyclase and cAMP phosphodiesterase. In the course of experimental MHI the value of the equilibrium concentration of cAMP underwent significant changes (Fig. 1). In the cerebral cortex, for instance, there was a 50% increase in the equilibrium concentration of the cyclic nucleotide 15 min after experimental MHI, which was followed by a fall of its concentration by 60% after 24 h. An even greater increase in the

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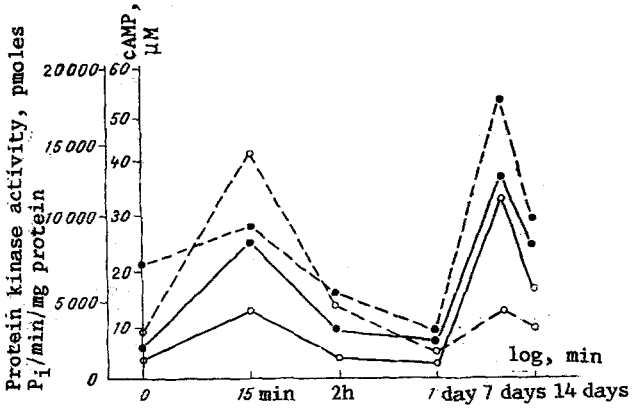


Fig. 1. Changes in calculated cAMP concentration (1, 2) and total activity of cAMP-dependent protein kinases (3, 4) during course of mild head injury. 1, 3) Microsomes of cerebral hemispheres; 2, 4) microsomes from cells of diencephalon and brain stem of rabbits.

equilibrium concentration of cAMP compared with the control took place after 7 and 14 days, by 180 and 60% respectively. A qulitatively similar dynamics of the equilibrium cAMP concentration also is characteristic of the brain stem of rabbits in the post-traumatic period. A more marked increase in the cAMP concentration in DBS of the rabbits' brain than in the cerebral cortex could be observed in the early period (15 min and 2 h) after experimental MHI. In the time course of trauma a biphasic pattern of post traumatic changes in activity of cAMP-dependent protein kinases also was established. For instance, activity of these enzymes in the cytosol and in the microsomal membrane fraction of the regions of the rabbits' brain studied underwent reciprocal changes. In the microsomal fraction both for DBS and for the cerebral hemispheres increased activity of endogenous cAMP-dependent protein kinases was observed in the presence of cAMP, by 5 and 4 times respectively compared with the control, 15 min after trauma. In the later stages (up to 3 days) activity of the enzyme in the brain stem and cerebral hemispheres of the rabbits was reduced, whereas activity of the enzyme in the same fractions on the 7th-14th days after tauma was increased by 7 and 14 times respectively. In the cytosol fraction a decrease in activity of cAMP-dependent protein kinases was found in the presence of cAMP 2 h after MHI, and reduction of its activity in the later stages. Correlation between post-traumatic changes in activity of cAMP-dependent protein kinases in the microsomal fraction and the dynamics of changes in the calculated equilibrium concentration of cAMP will be noted. The cerebral hemispheres were characterized by a linear relationship between cAMP-dependent protein kinase activity and the dynamics of the cAMP concentration, as expressed by the equation:

$$A_{pk} = -1425 + 307.5\ [cAMP]$$

where $A_{pk}$ indicates protein kinase activity (in pmoles/min/g tissue) and [cAMP] denotes the calculated cAMP equilibrium concentration (in μM).

Considering the diversity of molecular mechanisms regulating activity of cAMP-dependent protein kinases, including interaction with cyclic nucleotide and protein inhibitor, autophosphorylation, translocation of subunits and holoenzyme into the membrane fraction of the cells, it can be postulated that molecular mechanisms regulating enzyme activity are injured in the course of the post-traumatic process.

The results point to the possibility of post-traumatic changes in the mechanism of translocation of cAMP-dependent protein kinase from the cytosol into the cell membranes of the rabbit brain. Thus important changes in the state and function of the cAMP system in rabbit brain tissue, manifested at several different levels of the structural and functional organization of the system, were identified in the course of mild head injury. Changes in function of the cAMP system in nerve tissue after mild head injury indicate that post-traumatic changes in the brain are based on disturbances of mechanisms of self-regulation of metabolic processes.

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# EFFECT OF ACUTE INTESTINAL OBSTRUCTION ON THE STATE OF ERYTHROCYTE MEMBRANES

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Despite much progress in the study of all aspects of acute intestinal obstruction (AIO) mortality from this disease remains high [4], due to the irreversibility of the pathological changes in the internal organs, leading to severe disturbances of activity of the internal organs. As the writers showed previously [1, 3], considerable changes in membrane permeability of erythrocytes and lysosomes take place even in the initial stages of AIO.

In order to shed light on the possible causes of these disturbances, it was decided to study the protein component of erythrocyte membranes during the development of experimental AIO.

## EXPERIMENTAL METHOD

A model of AIO was creadted on noninbred albino rats under ether anesthesia. A loop of small intestine 4 cm away from Treitz' ligament was twisted through 360°C and the base of the loop was then fixed with a silk ligature. The rats were decapitated 2, 12, and 24 h after the operation. The results of the investigations were compared with an intact group and with a group of animals undergoing laparotomy only under ether anesthesia. All groups consisted of eight to 10 animals.

Erythrocyte membranes were isolated by the method described in [6]. Membrane proteins were obtained by the method of Wherret and Tower [10]. Amino-acid analysis of the proteins was carried out on a Hitachi KLA-3B automatic amino-acid analyzer after hydrolysis in 6 N HCl. Protein was determined by Lowry's method [8].

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