NAD, deficient during hypoxia, is formed and the excess of free ammonia is removed through the synthesis of glutamic acid and glutamine.

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EFFECT OF EXTREMAL STIMULATION ON BRAIN LEVEL OF CYCLIC AMP

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The content of cyclic adenosine-3',5'-monophosphate (cAMP) in the brain was investigated after electrical stimulation of immobilized rats, leading to the development of degenerative lesions in the internal organs. A marked decrease was found in the cAMP content in the rats' brain, which appeared 15 min after the beginning of extremal stimulation and remained at the same level during stimulation for 3 h. The lowering of the cAMP level was evidently connected with a deficiency of noradrenalin and, perhaps, of other biologically active amines also in the brain during electrical stimulation.

KEY WORDS: rat brain; cyclic AMP; electrical stimulation; degeneration of organs.

The appearance of neurogenic degenerative changes in the internal organs has been shown to be associated with disturbance of regulatory influences of the CNS and, in particular, of the hypothalamic region [1]. By means of biochemical analysis in addition to pharmacological, the processes taking place in the brain on the arrival of extremal impulses, leading to a disturbance of central trophic influences, were investigated more fully. In particular, during extremal stimulation a decrease in the level of biogenic amines and, in particular, of noradrenalin (NA) has been established [3]. Cyclic adenosine-3',5'-monophosphate (cAMP) is known to be an intermediary in the action of most biologically active substances and in the mechanism of their metabolic effects.

The object of this investigation was to study the concentration of cAMP in the brain during the development of neurogenic degenerative lesions arising as a result of extremal stimulation.

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TABLE 1. Concentrations of cAMP, ATP, and Glucose and Hexokinase Activity in Rat Brain during Electrical Stimulation (M ± m)

Conditions	cAMP, $\cdot 10^{-9}$ moles/g wet weight of tissue	ATP, μmoles/g wet weight of tissue	Glucose, μmoles/g wet weight of tissue	Hexokinase, μmoles NADP/min/mg protein
Control	2,00±0,14 (8)	2,30±0,01 (9)	1,60±0,01 (6)	8,4±0,5 (6)
Electrical stimulation:				
5 min	—	—	2,80±0,10 (4)	10,9±0,7 (5)
15 min	1,00±0,08 (5)	3,10±0,05 (5)	3,30±0,10 (4)	12,4±1,0 (5)
3 h	1,00±0,14 (5)	2,30±0,05 (4)	1,80±0,03 (4)	10,3±0,4 (4)

end. Number of experiments shown in parentheses.

EXPERIMENTAL METHOD

Immobilized rats were stimulated by an electric current (5-10 V, 50 Hz, 10 msec) through electrodes implanted into the forelimbs [2]. As a result of this stimulation the animals developed marked degenerative lesions of their internal organs.

Cyclic AMP was determined by an enzymic method [4, 5] with certain modifications . Brain tissue frozen in liquid oxygen was homogenized in 5 volumes of 10% TCA and the cAMP was isolated from the other nucleotides by adsorption on Zn–Ba followed by high-voltage electrophoresis on paper. The localization of the cAMP was established with an ultrachromatograph and it was eluted with 50% ethanol. After removal of the ethanol by means of a vacuum evaporator the cAMP was dissolved in 0.2 ml 0.5 M Tris-HCl, pH 7.7, and 1 mM $MgSO_4$. To 0.08 ml of the solution of cAMP 0.02 ml (40 μg) of 3',5'-AMP-phosphodiesterase (purified lyophilized bovine heart preparation with an activity of 0.45 unit/mg; Sigma, USA) was added. To the control sample (0.08 ml) 0.02 ml of bidistilled water was added. After incubation (30 min, 37°C) the reaction was stopped by boiling. The samples were cooled and an ATP-generating system was added: 0.15 ml of reagent containing 0.2 M Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 0.1 M KCl, 15 mM dithiotreitol, 7.5 mM phosphoinolpyruvate, 0.5 mM EDTA, $1 \cdot 10^{-8}$ M ATP, myokinase 30 μg/ml (preparation from hog muscle with an activity of 940 units/mg; Sigma, USA), and pyruvate kinase 80 μg/ml (from rabbit muscle, activity 100 units/mg; Reanal, Hungary). The samples were incubated for 3 h at 30°C, after which an ATP-utilizing system was added: 0.02 ml of reagent containing 10 mg/ml hexokinase (Koch-Light, England) and 60 mM glucose. After incubation for 1 h at 37°C the samples were placed in boiling water for 2 min. After cooling and centrifugation, 0.03 ml of the supernatant was added to 4 ml of a solution containing 0.1 M Tris-HCl, pH 7.5, 0.1 mM NADP, and 1 μg/ml of glucose-6-phosphate dehydrogenase (Fluka, Switzerland). The quantity of reduced NADP was measured fluorometrically on a modified BIAN-130 fluorometer. The final concentration of cAMP in standard samples was $0.4 \cdot 10^{-7}$ M.

Adenylate cyclase activity was determined by a radioactive method from the rate of conversion of ATP-C^{14} into cAMP-C^{14} [7]; cAMP was separated from the other nucleotides by high-voltage electrophoresis on paper. The concentration of ATP [6] and glucose [8] was investigated by the hexokinase method. Hexokinase activity was determined in the fraction including soluble and microsomal hexokinase.

EXPERIMENTAL RESULTS AND DISCUSSION

The cAMP level in the brain was studied at the beginning of electrical stimulation, and after stimulation for 15 min and 3 h, when marked degenerative lesions in the tissues began to appear. The experiments showed that the cAMP concentration 15 min after the beginning of electrical stimulation was reduced by 50% and it remained at that level until the end of stimulation (Table 1). Since activation of the cAMP system might have been expected during the first few minutes after the beginning of stimulation, adenylate cyclase activity and the cAMP level were investigated 1 min after the beginning of electrical stimulation. However, adenylate cyclase activity after stimulation for 1 min was indistinguishable from normal (967 ± 67 and 986 ± 73 counts/min/mg protein respectively), and the cAMP concentration did not exceed the control values.

To analyze the changes in cAMP during electrical stimulation some indices of energy metabolism were investigated in the brain: hexokinase activity and the levels of glucose and ATP, the substrate for adenylate cyclase during cAMP formation. An increase in the intensity of brain energy metabolism was observed 5 min and, to a greater degree, 15 min after the beginning of electrical stimulation (Table 1). The hexokinase activity and the concentrations of ATP and glucose in the brain tissues were significantly increased 15 min after the beginning of stimulation, whereas after 3 h these indices did not differ significantly from the control values.

It can be concluded that the decrease in the cAMP level in the brain after extremal stimulation was not associated with a disturbance of energy metabolism or with inadequacy of ATP synthesis. The main cause of the decrease in the cAMP concentration in the brain during extremal stimulation was the decrease in the level of NA and, perhaps, of other biologically active amines. In the writers' laboratory a sharp decrease in the NA level in the brain tissue was observed after electrical stimulation of the immobilized rats for 3 h, when a marked decrease in the NA concentration is observed in the hypothalamic region [3].

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EFFECT OF CARBON TETRACHLORIDE ON RNA METABOLISM IN THE RAT LIVER

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The effect of systematic administration of carbon tetrachloride (CCl_4) to rats on the RNA content in the liver and the intensity of incorporation of the labeled precursor (uridine-H^3) into it was investigated. Comparison of the results of morphological and biochemical studies revealed two consecutive stages of the toxic process, terminating in the formation of septal fibrosis. The sharpest changes in rapid RNA turnover in the rat liver were observed during the first 3 months of action of the toxic agent. The disturbance of metabolism also was reflected in a lowered RNA level and changes in the nucleo-cytoplasmic ratio in the tissue of the affected liver.

KEY WORDS: carbon tetrachloride; liver; total, nuclear, and cytoplasmic RNA; RNA turnover.

In experimental models of nonspecific liver damage administration of carbon tetrachloride (CCl_4) to animals is widely used [3, 11]. Resting on a firmly established morphological basis [9], these models have nevertheless been inadequately studied as regards the metabolism of nucleic acids, the most important class of biological compounds.

The object of this investigation was to study the effect of prolonged systematic administration of CCl_4 on the content and intensity of incorporation of labeled precursor in RNA and its various fractions in the tissues of rat liver, with an accompanying morphological control of the stages of the toxic changes.

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