SOME INDICES OF BRAIN METABOLISM IN DYSTROPHIC LESIONS OF THE STOMACH

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KEY WORDS: dystrophic injuries of the stomach; brain metabolism; trophic processes.

Research over a period of several years, conducted in the Department of Pharmacology and aimed at studying the mechanism of development of neurogenic dystrophic lesions of the internal organs resulting from exposure to extremal factors, has demonstrated the role of the hypothalamus and sympathetic nervous system in this pathology. Pharmacological analysis of the pathways of formation of dystrophic changes has shown that they can be prevented by agents blocking transmission of nervous impulses in different components of the reflex arc. Besides peripheral adrenolytics and sympatholytics and ganglion-blockers, these substances also include central cholinolytics and substances blocking excitation in the cerebral cortex [2]. It has been shown by electrophysiological methods that electrical activity in the cerebral cortex is enhanced and desynchronized in the presence of reflex dystrophic injury to the stomach [1].

The object of this investigation was to study the role of central nervous regulatory factors in the mechanism of development of dystrophic injuries of the internal organs. The state of brain metabolism was studied during the formation of a dystrophic lesion of the stomach by application of a stimulus to its reflexogenic zone, the duodenum.

EXPERIMENTAL METHOD

Experiments were carried out on rats in which a dystrophic injury to the gastric mucosa was produced by application of Pean's forceps to the region of the duodenum. The rats were killed 3 h after application of the forceps for 10-15 min, and tissue was taken from the cerebral hemispheres for investigation. This procedure led to a well-marked lesion of the gastric mucosa, consisting of ulceration, hyperemia, and edema.

The following indices of metabolism in brain tissue were studied: the levels of nucleic acids – DNA in the nuclear fraction of the cells, RNA in the microsomal fraction. The concentration of histone proteins in the nuclei and their ratio to DNA, characterizing template activity of the nuclear chromatin, was investigated. To investigate the energy balance of the brain, concentrations of creatine phosphate and glycogen also were determined. Concentrations of oxidized and reduced forms of nicotinamide nucleotides, acting as coenzymes of dehydrogenases in reactions for the conversion of carbohydrates and characterizing the state of oxidation–reduction processes in the tissues, were investigated.

The cell nuclei were isolated by the method described in [4]. The purity of the nuclear fractions was verified microscopically after staining with toluidine blue. The DNA content in the nuclei was determined by the method in [6], and RNA in the microsomes by the spectrophotometric method in [9]. Histones were isolated by the usual method of double extraction of the nuclei with 0.2 N HCl. The content of histone and nuclear proteins was determined by Lowry's method. Creatine phosphate was determined quantitatively by a spectrophotometric method [7]. Glycogen was extracted from brain tissue by the method in [8], hydrolyzed, and determined as glucose by an enzymic method [10]. Nicotinamide nucleotides were extracted separately for oxidized and reduced forms [5]. NAD was determined by an enzymic method based

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TABLE 1. Content of Creatine Phosphate, Glycogen, Nicotinamide Nucleotides, and Nucleic Acids and Histones/DNA Ratio in Rat Brain 3 h after Traumatic Injury to Duodenum (M ± m; n = 5)

Index	Control	Experiment
Creatine phosphate, μmoles/g	1,94±0,10	1,40±0,13*
Glycogen, μg/g	86,7±3,0	63,3±3,5*
Nicotinamite nucleotides, μg/g:		
NAD	270,2±14,2	309,5±22,0
$NAD \cdot H_2$	138,6±9,3	170,5±11,9
NADP	27,7±1,8	32,9±1,8
$NADP \cdot H_2$	36,5±2,6	51,0±2,6*
ratio of oxidized forms to reduced forms	1,70	1,54
Nuclear DNA, μg/mg protein	320,0±13,5	248,6±8,1*
Histones/DNA	1,13	0,80
RNA of microsomes, μg/mg protein	4,22±0,20	3,59±0,41

*P < 0.05.

on reduction by alcohol dehydrogenase; NADP was determined by the method in [11].

EXPERIMENTAL RESULTS

As the results showed, a disturbance of metabolic processes arises in the brain during the development of dystrophic lesions of the gastric mucosa caused by injury to the duodenum (Table 1). The quantity of energy resources in the brain tissue, namely creatine phosphate and glycogen, was considerably reduced. The ratio between oxidized and reduced forms of nicotinamide nucleotides was changed: The content of reduced forms was increased, indicating a disturbance of oxidation–reduction processes in the brain tissue.

A fall in the DNA level was observed in the cell nuclei and in the RNA level in the microsomes. The histone content and the histones–DNA ratio in the nuclei fell considerably. The decrease in the DNA and RNA content evidently points to a decrease in synthetic activity as a whole in the cells. Changes in the histone content in the nuclei and the fall in the histones/DNA ratio, considering the role of these nuclear proteins in genetic induction and repression, indicate intensification of the template activity of the nuclear chromatin. It can be tentatively suggested that activity of the genetic apparatus of the cells underwent reorganization aimed at maintaining synthesis of the so-called adaptive enzyme systems, supplying the necessary energy under abnormal conditions [3].

The changes observed in brain metabolism during the development of reflex dystrophic injury to the stomach demonstrate considerable inhibition of trophic processes in the brain tissue.

According to the views of classical physiologists, all functions taking place in the body are determined in the CNS and, in particular, in the cerebral cortex, and it is there where the function of control and integration of processes determining the course of the metabolic reaction in every cell and organ is effected. This function, in turn, depends on the state of metabolic trophic processes in the nerve tissue itself. Disturbance of trophic processes in brain tissue arising during stimulation of the interoceptive zone of the internal organs is evidence of the part played by central nervous factors in the mechanism of development of visceral dystrophic injuries.

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EFFECT OF ACETYLCHOLINE ON THE CYCLIC GMP LEVEL IN THE RAT HEART AT DIFFERENT AGES

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The cholinergic regulation of the heart undergoes changes during aging. Frol'kis and co-workers showed [3, 6] in a series of investigations that the thresholds of the negative chronotropic influences of the vagus nerve on the heart are lower in old animals and that smaller doses of acetylcholine (ACh) cause changes in myocardial contractility. Effects of ACh on function and metabolism of the heart may be realized through activation of guanylate cyclase and elevation of the intracellular level of cyclic guanosine monophosphate (cyclic GMP) [7, 8, 10-12].

The object of this investigation was to study changes in the cyclic GMP level *in vitro* in the myocardium of adult and old rats under the influence of different doses of ACh.

EXPERIMENTAL METHOD

Experiments were carried out on sections through the heart muscle of adult (6-8 months) and old (24-26 months) Wistar rats. The cyclic GMP content was determined in TCA extracts of sections, previously frozen in liquid nitrogen, neutralized with water-saturated ether, by means of the cyclic GMP kit from the Radiochemical Centre, Amersham (England). ACh was used in concentrations of 0.5, 1, and 2 μM; the sections were incubated with ACh for 1 min.

In the experiments with acetylcholinesterase blockade, neostigmine was used in a concentration of 10 μg/ml incubation medium.

EXPERIMENTAL RESULTS

No data could be found in the literature on age changes in the cyclic GMP content in heart muscle. The only information available was of a decrease in the intensity of synthesis and breakdown of cyclic GMP with age in the tissues [13] and a decrease in its excretion [9].

Our own observations showed that the cyclic GMP level in the rat myocardium does not change with age: 31.5 ± 2.9 pmoles/g tissue in adults and 18.5 ± 1.5 pmoles/g tissue in old animals. In late ontogeny the basal cyclic GMP level is evidently maintained, so that under resting conditions definite stability of this component of intracellular regulation is ensured.

Different relationships are observed in response to activation of guanylate cyclase by ACh. As Fig. 1 shows, both in adult and in old animals ACh caused a considerable and significant increase in the cyclic GMP concentration. With an increase in the ACh concentration,

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