BIOCHEMICAL CHANGES IN THE BRAIN DURING REFLEXES CAUSING DEGENERATION OF THE GASTRIC MUCOSA

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Investigations conducted earlier in the Department of Pharmacology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, have shown that degenerative lesions arising in the gastric mucosa following excessive stimulation of animals are reflex in character [2-4]. Pharmacological analysis showed that blocking the central link of the reflex arc prevents the development of degenerative lesions in the stomach wall. It was found that the maximal protective effect was exhibited by central neurotropic substances which interrupt the flow of impulses mainly in the region of the subcortical structures – the central cholinolytics amizil (benactyzine hydrochloride) and metamizil, blocking the cholinergic synapses in the ascending part of the reticular formation, and the brain-stem sedatives – the barbiturates. Meanwhile, drugs belonging to the group of the cortical sedatives – chloral hydrate, paraldehyde, and urethane – do not prevent reflex degeneration of the stomach wall [4, 5, 6, 7]. On the basis of these results the hypothesis was put forward that the switching of afferent impulses from the reflexogenic zones to the efferent part of the reflexes, disturbing the nutrition of the stomach, takes place at the level of the subcortical structures [1].

This undoubted participation of the cholinergic structures of the central nervous system in the development of the reflex degenerative lesions in the stomach wall was the reason for the present investigation of the changes in the acetylcholine balance, in both the region of the subcortical structures and the cerebral hemispheres, in the presence of reflex degenerative lesions of this type.

EXPERIMENTAL METHOD

Degenerative lesions in the wall of the stomach were produced by O. N. Zabrodin's method: rats were exposed for 3 h to the action of an electric current while immobilized. The stimuli consisted of rectangular pulses of alternating current, generated by an electronic stimulator, with a frequency of 50 cps and an output voltage of 8-10 V. The animals took part in the experiments after fasting for 24 h. Electrodes were inserted into the muscles of the forelimbs. Intact fasting animals were used as controls.

Immediately after the cessation of stimulation, the rats were decapitated and their heads were immersed in a cooling mixture consisting of acetone and dry carbon dioxide. After a few minutes the brain was removed and homogenized in cooled mortars. Pieces of the cerebral hemispheres and the region including the subcortical formations were taken for investigation. The free and bound acetylcholine were determined on a biological test object – the rectus abdominis muscle of a frog, treated with neostigmine – by the method recommended by N. A. Verzhbinskaya. The isolated muscle was kept in a refrigerator in Ringer's solution for 24 h. During testing the bath with the muscle and all the solutions were placed on ice. The acetylcholine was extracted with Ringer's solution containing neostigmine ($2 \cdot 10^{-5}$). For the determination of free acetylcholine the extraction was carried out on ice; for the determination of bound acetylcholine the brain tissue was treated by repeated homogenization at pH 2.0, after which the homogenate was placed on a boiling water bath for 1 min. Immediately before testing, all the extracts were alkalified to pH 7.0, after which a known volume of extract was added to the bath containing the muscle. The amplitude of the muscular contraction produced by the test extract was compared with the amplitude of the contraction caused by a known concentration of acetylcholine.

The stomach of the rats stimulated as described above was opened and examined macroscopically. In 85% of the experimental rats marked macroscopic changes were found: the stomach was filled with blood-stained contents, the epithelium of the mucous membrane had desquamated, and multiple hemorrhagic lesions of the mucous

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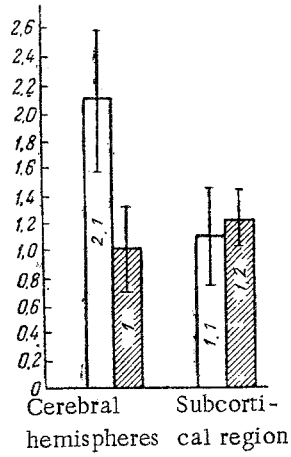

Fig. 1. Acetylcholine content of the brain of normal rats (in μg/g fresh tissue). Here and in Fig. 2: unshaded columns) free acetylcholine. shaded) bound. Each column reflects the results of 12 experiments.

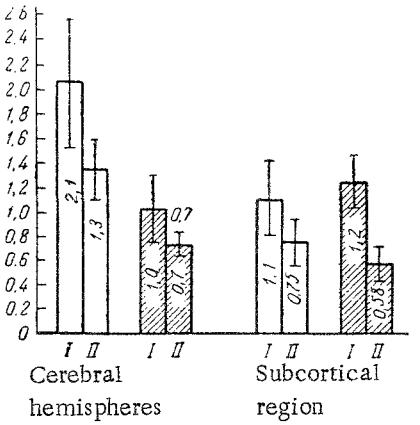

Fig. 2. Effect of prolonged stimulation on the acetylcholine content of the brain in rats exposed to an electric current (in μg/g fresh tissue). I) Control; II) experiment.

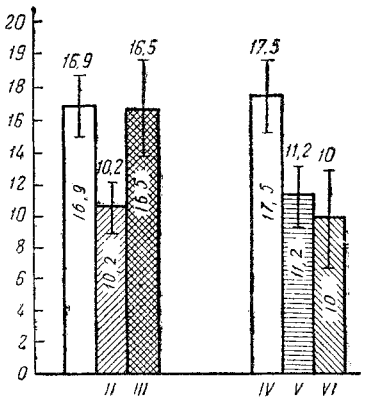

Fig. 3. Content of GABA in the rats' brain (in mg%). I and IV) control (25 animals in each); II) immediately after stimulation for 3 h with an electric current (16 animals); III) 15 min after stimulation ceased (13 animals); V) stimulation for 3 h against the background of amizil (3-5 mg/kg, 15 animals); VI) stimulation for 3 h against the background of phenobarbital (100 mg/kg, 10 animals).

membrane were present. The content of γ-aminobutyric acid (GABA) in the cerebral hemispheres of the rats was determined by the method of the descending paper chromatography followed by spectrophotometry of the eluate [8].

EXPERIMENTAL RESULTS

In the normal rats the content of free acetylcholine in the cerebral hemispheres was slightly higher than in the region of the subcortical formations, whereas the content of bound acetylcholine in the two regions investigated was the same (Fig. 1).

Stimulation of the immobilized fasting rats for 3 h with an electric current was accompanied by a considerable fall in the level of the bound acetylcholine, mainly in the subcortical region where its content was more than halved – from 1.2 to 0.58 μg/g fresh tissue; in the cerebral hemispheres the level of the bound acetylcholine showed a less marked change, falling from 1.0 to 0.7 μg/g (Fig. 2). The content of free acetylcholine, on the other hand, fell by a greater degree in the cerebral hemispheres – from 2.1 to 1.3 μg/g fresh tissue; in the region of the subcortical formations the difference in the content of free acetylcholine was small ($P > 0.05$).

Hence, stimulation of the immobilized rats for 3 h with an electric current, causing the development of degenerative lesions of the stomach wall, was accompanied by a fall in the acetylcholine content in the brain. It was noteworthy that the decrease in the content of bound acetylcholine was more marked in the subcortical region. This demonstrates that in the conditions of these experiments the cholinergic processes in the hypothalamus had undergone more profound changes than those in the cerebral hemispheres.

Since the excitability of the neurons of the central nervous system is influenced by GABA, which disturbs the transmission of impulses in some of the central synapses, it was interesting to study the changes in the GABA level in the brain in conditions causing the development of reflex degenerative lesions of the stomach wall.

Immediately after prolonged stimulation of the rats with an electric current, the GABA content of the brain tissue was appreciably lowered – from 16.9 to 10.2 mg% (Fig. 3). These changes were readily reversible, for 15 min after cessation of stimulation (the earlier periods were not investigated) the GABA level returned to normal.

It may be concluded from these results that a factor possibly having a bearing on the protective effect of certain central neurotropic agents which prevent the development of reflex degenerative changes in the stomach is a

change in the GABA content of the brain tissue. For this reason the effect of drugs of this type on the GABA level was investigated in rats subjected to prolonged stimulation with an electric current. According to findings obtained by I. S. Zavodskaya and O. N. Zabrodin (1963), the strongest protective action was shown by amizil (3 mg/kg) and phenobarbital (100 mg/kg), administered 15-30 min before the beginning of stimulation. Neither amizil nor phenobarbital was found to prevent the fall in the GABA level in the cerebral hemispheres of the experimental animals.

Finally, an attempt was made to discover whether the GABA content was changed by the action of substances stimulating and depressing the cholinergic processes in the central nervous system. For this purpose central muscarinelike and nicotinelike cholinomimetic drugs (arecoline, nicotine) were used in doses causing hyperkinesias, and central muscarinelike and nicotinelike cholinolytics [amizil, diphacil (adiphenine hydrochloride)] in doses preventing them.

Not one of the substances mentioned had any significant effect on the GABA content in the brain tissue of the experimental animals (see Fig. 3).

A combination of electrical stimulation with immobilization of fasting rats for 3 h, causing degenerative lesions of the stomach wall, was accompanied by obvious disturbances of the acetylcholine balance in the brain. These disturbances were expressed most clearly by a decrease in the content of bound acetylcholine in the region of the subcortical formations and by a decrease in the content of free acetylcholine in the cerebral hemispheres.

SUMMARY

Degenerative lesions of the gastric mucosa were induced in starving rats by exposing them for 3 h to an electric current while being inmobilized. It was shown that the development of reflex degeneration of the gastric wall was accompanied by disturbances of the acetylcholine balance in the brain. These disorders were manifested by a reduction in the level of bound acetylcholine mainly in the hypothalamic region and by a decrease in the amount of free acetylcholine mainly in the cerebral hemisphere.

Experiments made under analogous conditions revealed also a reduction in the level of γ-aminobutiric acid in the brain of experimental rats.

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