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EVALUATION OF THE PROTECTIVE EFFECT OF GAMMA-HYDROXYBUTYRIC ACID IN STRESS

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Stress is known to induce disturbances of the metabolism and function of the heart and also to cause ulcers in the gastrointestinal tract [9, 10]. Meanwhile activation of the inhibitory GABA-ergic system of the brain, protective in character, is observed during stress, for preliminary administration of the GABA metabolite sodium hydroxybutyrate (GHBA) prevents disturbances in the heart and stomach [8-10]. The writers showed previously that stress causes phasic injuries to the structure of the heart muscle which correspond to definite periods with different blood eosinophil counts [2, 5]. It has been found that changes in the blood eosinophil level, reflecting functional activity of the pituitary-adrenal system (PAS) in stress [3, 4], can serve as the criterion for quantitative evaluation of the effectiveness of drugs [6].

Accordingly the aim of the investigation described below was to study the effect of GHBA on the eosinophil, corticosterone, and noradrenal levels and the severity of injuries to the structure of the heart as a result of exposure to stress.

EXPERIMENTAL METHOD

Experiments were carried out on 78 male albino rats weighing 180-200 g with an initial eosinophil count of 220-340/μl peripheral blood at 9 a.m. Stress was produced by Desiderato's method [11]. GHBA in a dose of 100 mg/kg was injected subcutaneously 30 min before exposure to stress and again 2 and 4 h after the beginning of exposure. Control animals received physiological saline at the same time. After the end of exposure to stress at intervals of 3 h the eosinophils in 1 μl peripheral blood were counted in the animals of all groups (in a Goryaev's chamber, using Hinkleman's stain). The plasma corticosterone concentration was determined by chromatography on columns with silica-gel [1] 2 h after the end of stress and the noradrenalin concentration in the heart was determined fluorometrically [7]. To assess the protective effect of GHBA on the animal quantitatively, the method developed previously [6] was used; this involved calculating the ratio between the time required for eosinophilia to appear after the eosinopenia in animals of the control group (without GHBA) and the time taken for eosinophilia to appear in the animals of the experimental group (receiving GHBA). To detect structural injuries to the heart, Perls's reaction was carried out on serial topographic sections, followed by counterstaining with hematoxylin and eosin; the method of polarization microscopy also was used. Animals in all groups were killed for morphological investigation during the period of marked eosinophilia, for it was shown previously that the severest structural changes in the heart are observed at that time [2, 5].

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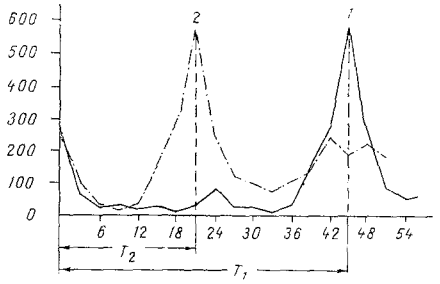

Fig. 1

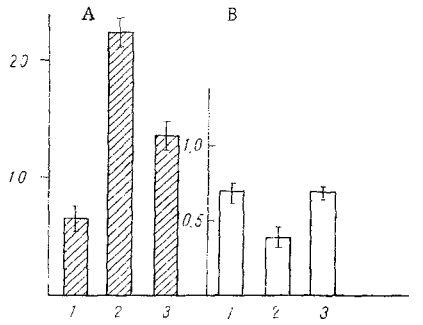

Fig. 2

Fig. 1. Number of eosinophils in 1 μl peripheral blood of rat after end of stress (1) and after stress combined with GHBA (2). Abscissa, time after stress (in h); ordinate, number of eosinophils in 1 μl blood.

Fig. 2. Effect of GHBA on corticosterone concentration in plasma (A) and noradrenalin concentration in heart of rats (B) 2 h after end of stress. 1) Control; 2) stress; 3) stress + GHBA. Ordinate: A) corticosterone concentration (μg%), B) noradrenalin concentration (in μg/g).

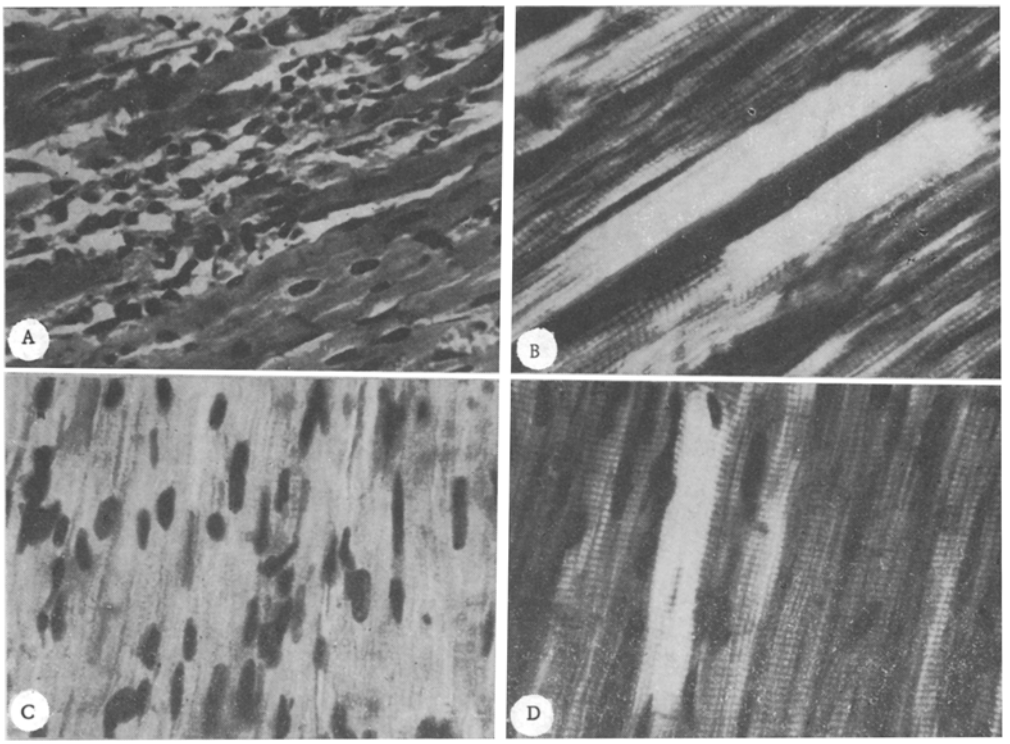

Fig. 3. Effect of GHBA on morphological changes in myocardium or rats during stress. A) Myocardium of rat 45 h after stress. Focal necroses of myocardium with development of inflammatory infiltration. Perls' reaction, 126×; B) myocardium of rat 45 h after stress. Severe contractural changes in myocardial cells of 3rd-4th degree. Polarization microscopy, 197×. C) Myocardium of rat 21 h after stress + GHBA. Positive Perls' reaction in single muscle fibers, 197×; D) myocardium of rat 21 h after stress + GHBA. Single myocardiocytes with contracture of myofibrils of 1st-2nd degree. Polarization microscopy, 197×.

EXPERIMENTAL RESULTS

After the end of exposure to stress marked activation of the PAS was observed during the first 39 h, and was manifested by the development of an initial eosinopenia, followed by a transient eosinophilia (Fig. 1). The

corticosterone concentration in the blood plasma was raised and the noradrenalin concentration in the heart lowered (Fig. 2). Morphological investigation showed that stress causes considerable focal necrotic and contractural injuries to the myocardium (Fig. 3A, B).

Injection of GHBA reduced the severity of the stress syndrome. The plasma corticosterone level showed a much smaller rise and the noradrenalin concentration in the heart did not fall. The effectiveness of GHBA in stress was assessed by the equation [6]:

$$A = \frac{T_c}{T_e},$$

where A is the effectiveness of GHBA and T_c and T_e the times (in h) for eosinophilia to develop in animals of the control (without GHBA) and experimental (with GHBA) groups after the end of stress.

Administration of GHBA to animals exposed to stress reduced by more than half the time required for eosinophilia to develop after eosinopenia. (When intact animals were exposed to stress eosinophilia appeared after 45 h, but when GHBA was given it appeared after 21 h.) This is evidence of the powerful antistress action of GHBA (A = 2.14).

The use of a metabolite of the GABA-ergic inhibitory system in these experiments prevented the development of foci of necrosis and severe contractural injuries to the myofibrillary system of the heart. Only single areas of muscle fibers were observed in which the cross striation had disappeared and anisotropy was rather more pronounced (Fig. 3C, D). Virtually no pathological changes were present in the myocardium incidentally, 45 h after the end of stress in animals receiving GHBA.

The protective effect of GHBA in stress is thus associated with a decrease in the degree and duration of activation of the PAS. Functional activity of the GABA-ergic and pituitary-adrenal systems was found to be interdependent. At the same time, prevention of structural injuries to the heart muscle with GHBA in stress is evidence of the important role of the level of function of the GABA-ergic inhibitory system in the pathogenesis of stress injuries to the heart.

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