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PROPHYLACTIC EFFECTS OF SODIUM AND LITHIUM HYDROXYBUTYRATE DURING STRESS-INDUCED DEPRESSION OF NORMAL KILLER CELL ACTIVITY IN MICE

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Natural or normal killer (NK) cells play an important role in the maintenance of tissue homeostasis [7], in antitumor and anti-infectious protection [10, 11], and in regulation of a number of important biological processes [9, 11]. NK cell activity can be changed as a result of injury to various parts of the brain and, in particular, the hypothalamus [8], and under the influence of hormones, especially glucocorticoids [6]. It was shown previously that NK cell activity is significantly inhibited during emotional-painful [4] and immobilization stress [5], and that this inhibition can be abolished prophylactically by the cyclic derivative of delta-sleep peptide [3]. A marked prophylactic effect also is produced by sodium hydroxybutyrate (Na-OHBA) [3]. The high antistressor efficacy of a new preparation, namely lithium hydroxybutyrate (Li-OHBA), used for the treatment of various nervous and mental disorders, has recently been established [1, 2]. The aim of this investigation was to compare the prophylactic effect of these two substances — Na-OHBA and Li-OHBA — against stress-induced depression of NK cell activity.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 12 weeks. Immobilization stress for 6 h was induced by fixing the animals by their limbs in the supine position. The compounds for testing were given as a single intraperitoneal injection, 30 min before the beginning of exposure to stress, in the following doses: Na-OHBA 50, 100, and 200 mg/kg, Li-OHBA 50 mg/kg, and lithium chloride (LiCl) 10 mg/kg. The animals were divided into five groups: 1) mice receiving an injection of physiological saline (control); 2) stress (immobilization for 6 h); 3) Na-OHBA + stress; 4) Li-OHBA + stress; 5) LiCl + stress.

The animals were decapitated 24 h after the end of exposure to stress. A suspension of splenocytes, obtained by gentle mechanical destruction of the spleen in a glass homogenizer with Teflon pestle, followed by filtration of the cells through a nylon filter and by washing twice in Eagle's medium, was used as the source of NK cells. The cell concentration was adjusted to $20 \cdot 10^6$/ml and the cells were transferred to culture medium (RPMI 1640 medium with 10% embryonic calf serum and 1% glutamine). NK cell activity was determined by the test based on release of ^{51}Cr from labeled YAC-1 target cells (TC; a T-cell mouse lymphoma, maintained by subculture *in vitro*). For this purpose, to $5 \cdot 10^6$ YAC-1 cells in a volume of 1 ml of culture fluid was added 100 μCi of $Na_2{}^{51}CrO_4$ (specific activity 300-500 mCi/mg, from Amersham International, England) and incubated on a water bath at 37°C for 60 min. The TC

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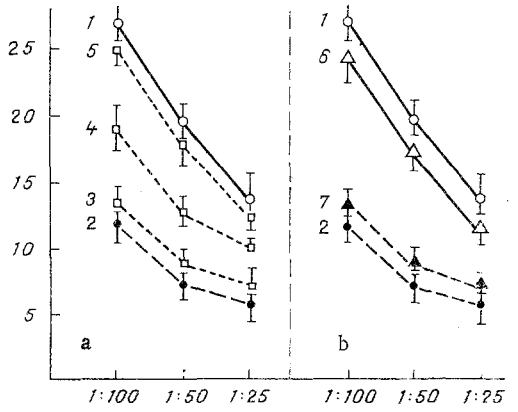

Fig. 1. Prophylactic effect of Na-OHBA (a) and Li-OHBA (b) on stress-induced depression of NK. Abscissa, ratio of EC: TC; ordinate, CI (in %). 1) control; 2) stress; 3, 4, 5) Na-OHBA (in doses of, respectively, 50, 100, and 200 mg/kg) + stress; 6) Li-OHBA (50 mg/kg) + stress; 7) LiCl + stress.

were then carefully washed to remove unbound ^{51}Cr, and their concentration was adjusted to $2 \cdot 10^5$/ml. The TC ($2 \cdot 10^4$) in a volume of 100 µl of culture medium were placed in wells of round-bottomed plates with an equal volume of effector cells (EC), with the ratio of EC to TC equal to 1:100, 1:50, and 1:25. The cells were incubated for 4 h at 37°C in an atmosphere with 5% CO_2. The radioactivity of 100 µl of supernatant was measured with a gamma counter. The cytotoxic index (CTI) was calculated by the equation:

$$CTI = \frac{\text{Number of counts (experiment} - \text{spontaneous release of } ^{51}Cr)}{\text{Number of counts (maximal release} - \text{spontaneous release of } ^{51}Cr)} \times 100\%.$$

The spontoneous release of ^{51}Cr was determined in the supernatant from three wells, to which only TC had been added. To determine the maximal release of ^{51}Cr, instead of EC, 100 µl of the detergent X-100 was added to the TC. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

During immobilization for 6 h a significant decrease in NK cell activity took place (Fig. 1). Injection of Na-OHBA in a dose of 50 mg/kg 30 min before immobilization had virtually no prophylactic effect; increasing the dose to 100 mg/kg protected the natural cytotoxicity system against stress, and a dose of 200 mg/kg had optimal activity in this respect, as regards prevention of stress-induced depression of NK cell activity. Li-OHBA, in a dose of 50 mg/kg, caused significant protection of the natural cytotoxicity system against exposure to stress. To examine the role of lithium ions in this protective effect, mice were injected with LiCl before immobilization; this compound had no significant action: the results were indistinguishable from NK cell activity during exposure to stress alone.

Li-OHBA was thus shown to be highly effective in preventing stress-induced depression of NK cell activity in mice. Its protective effect was exhibited in a dose of only one-quarter of that of Na-OHBA (50 and 200 mg/kg, respectively). The results reveal yet another, hitherto unknown, aspect ot the action of Li-OHBA. It will be noted that Li-OHBA has a many sided physiological normalizing action and possesses properties on the basis of which it can be classed as a nootropic drug [1].

The results of this investigation broaden the range for experimental use and for future clinical use of preparations which are analogs of natural metabolites of stress-limited systems, with a view to prevention of stress-limiting systems, with a view to prevention of stress-induced damage to different target organs and physiological systems of the body.

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