Serotonin Precursor 5-Hydroxytryptophan Disturbs the Protective Effect of Low Doses of Antibodies to S100B Protein during the Formation of Long-Term Sensitization

R. R. Tagirova, A. Kh. Timoshenko, Kh. L. Gainutdinov, M. B. Shtark*, and O. I. Epshtein**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, Suppl. 1, pp. 202-204, September, 2009
Original article submitted August 1, 2008

We studied the effect of ultralow doses of antibodies to calcium-binding protein S-100B and 5-hydroxytryptophan, a metabolic precursor of serotonin, on the formation of long-term sensitization as a neurobiological model of anxiety and depression. Daily administration of antibodies to S-100B to edible snails before the formation of long-term sensitization prevents its development. 5-Hydroxytryptophan administered before the formation of long-term sensitization abolished the protective effect of antibodies to S-100B protein.

Key Words: *calcium-binding S-100B protein; antibodies to S-100B protein; long-term sensitization; serotonin; 5-hydroxytriptophan*

Long-term sensitization (LTS) now attracts great attention as a model for studies of membrane mechanisms of the formation of stable excitation focuses in the nervous system of experimental animals [10]. Realization of this experimental model on the simple nervous system creates real prerequisites for elucidation of cell mechanisms of the formation of this state developing in various mental disorders (stress, depression, anxiety) [3,4,10].

In edible snail, defense reactions are modulated by command neurons, while motor activity is controlled by pedal ganglion motoneurons characterized by other type of activity. Different type of activity of these neurons probably suggests that intracellular systems operate in different modes during their functioning.

The formation of LTS involves pathways mediated by serotonin (5-HT) as the neurotransmitter and neuromodulator. Administration of neurotoxin 5,7-dihydroxytryptamine inducing degeneration of 5-HT-ergic terminals and considerably reducing the concentration of 5-HT in CNS [12] prevents the formation of LTS [1,2,11]. Preliminary injection of antibodies to S-100B protein in low doses (LAB-S100B) before the series of electroshocks interrupts the formation of LTS [9].

Here we studied the effect of metabolic precursor of 5-HT, 5-hydroxytryptophan (5-HTP) on the protective effect of LAB-S100B during the formation of LTS.

MATERIALS AND METHODS

Experiments were performed on mature edible snails (*Helix lucorum*) of similar weight and size. The snails were maintained in glass terrariums at room temperature, high humidity, and with food excess. They were in the active state for at least 2 weeks before the experiment. LTS of the defense reflex was trained according to a routine protocol [2]. Electric stimuli were delivered to the snail head 4 times a day with 1.5-2.0-h intervals for 4 days. The duration of each stimulation was 0.5 sec; rectangular pulses with amplitude of 6-8 mA, duration 10 msec, and frequency 50 Hz were used. During stimulation the animals were on a cop-

Kazan' Physicotechnical Institute, Russian Academy of Sciences; *Institute of Molecular Biology and Biophysics, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk; **Materia Medica Holding Research-and-Production Company, Moscow. ***Address for correspondence:*** gainutdinov@maU.knc.ru. Kh. L. Gainutdinov

0007-4888/09/1483-0560

per electrode plate coated with water-soaked paper. Another electrode was a metal rod applied to the snail head. Considerable lengthening of the closed state of the pneumostome in response to presentation of the testing stimulus compared to the initial reaction served as the criterion of LTS training.

Group 1 snails received daily injections of LAB-100B (Materia Medical Holding) before LTS training (30 min before the first electroshock). The control animals received physiological saline (PS) in the same volume and at the same terms. Group 2 snails received 5-HTP in a dose of 10 mg/kg dissolved in 0.1 ml PS 7 min before the start of LTS training. Group 3 snails received daily injection of LAB-100B and 5-HTP 30 and 7 min before LTS training, respectively.

Quantitative evaluation of the defense reaction of pneumostome closure in response to the testing stimulus was performed before the start of the experiment, daily before injections and a series of electroshocks, and one day after attaining LTS training criterion. The animals were tested in a special setup consisting of a reservoir with water, a polyethylene ball floating in water, and a stand with a holder; the snail fixed to the holder by its shell freely moved on the ball, thus rotating it. The setup allowed objective registration of pneumostome muscle movement and reaction of ommatophores and stimulation of any point on the snail body. The testing included several testing tactile stimuli of the same strength applied to the area of mantle cushion. The time of pneumostome closure (*i.e.* the duration of its open state) after application of the tactile stimulus to the area of mantle cushion was recorded.

The significance of differences between the mean parameters of neurons was evaluated by Student's *t* test and nonparametric Mann—Whitney *U* test.

RESULTS

During the first 2 days of LTS training the snails became restless, moved more actively, and ate less food, while during the next 2 days their motor activity sharply decreased. During LTS training, the time of closed state of pneumostome in response to testing stimulus considerably increased over 4 days (Fig. 1).

Preliminary injection of PS before the series of electroshocks did not affect the development of LTS and parameters of snail behavior. Daily injections of LAB-S100B before training prevented the development of LTS, the time of pneumostome closing did not increase. Combined treatment with 5-HTP and LAB-S100B abolished this protective effect. In group 2 snails, LTS developed earlier than in group 3 animals (Fig. 1).

The function of S-100B proteins are related to regulation of metabolism of various neurotransmitter

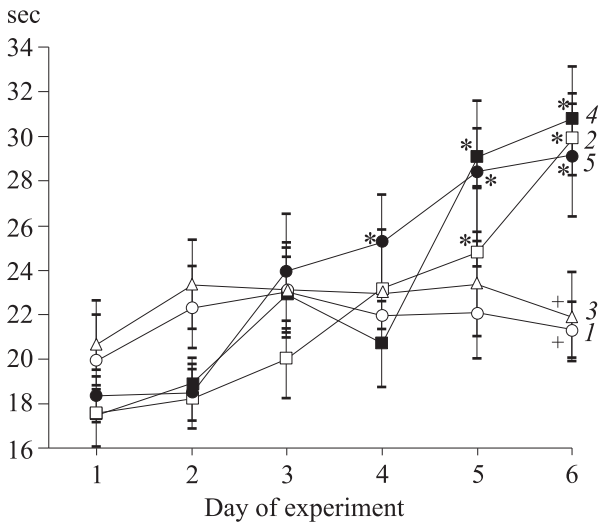

Fig. 1. Time of pneumostome cosing reaction in response to testing tactile stimulation after LTS formation. *1*) snails receiving PS; *2*) PS+LTS training; *3*) snails receiving LAB-S100B; *4*) 5-HTP; *5*) LAB-S100B+5-HTP. **p*<0.05 compared to initial values in the group; ^{+}p<0.05 compared to *2*.

systems and second messenger systems [8]. The interaction of S-100B proteins is most clearly seen in the serotoninergic system: interrelation of the metabolism of S-100B proteins and 5-HT and their regulatory effects on the growth, development, and plasticity of the nervous tissue were demonstrated [13]. The dynamics and the degree of the effects of 5-HT on membranes of command neurons in edible snail are similar to those produced by LAB-S100, in particular, by LAB-S100B [1,7]. LAB-S100B exhibit anxiolytic and antianxious effects [9].

The participation of the 5-HT-ergic system in the realization of anxiolytic and antidepressant activity of LAB-S100B was demonstrated: combined treatment with LAB-S100B and 5-HT-ergic agents reduced the anxiolytic and antidepressant effects of the preparation in rats [6]. Since the effect of LAB-S100B is abolished by increased 5-HT level, two assumptions on the nature of the protective effect can be made:

- LAB-S100B produce a protective effect during LTS training by directly modulating 5-HT-ergic system;
- LAB-S100B create a protective effect via common loci [5], *e.g.* 5-HT-1 receptors.

REFERENCES

1. P. M. Balaban and I. S. Zakharov, *Learning and Development, the Basis of Two Phenomena* [in Russian], Moscow (1992).
2. Kh. L. Gainutdinov, V. V. Adrianov, and T. Kh. Gainutdinov, *Zn. Vyssh. Nervn. Deyat*., **49**, No. 1, 48-58 (1999).
3. Kh. L. Gainutdinov, V. V. Adrianov, R. R. Nazarova, and T. Kh. Gainutdinov, *Ibid*., No. 6, pp. 1063-1065.

4. E. Candel, *Cellular Bases of Behavior* [Russian translation], Moscow (1980).
5. A. S. Pivovarov and V. L. Nistratova, *Byull. Eksp. Biol. Med.*, **136**, No. 8, 132-134 (2003).
6. I. A. Kheyfets, Yu. L. Dugina, T. A. Voronina, *et al., Byull. Eksp. Biol. Med.*, **143**, No. 5, 535-537 (2007).
7. A. V. Shevelkin, V. P. Nikitin, and S. A. Kozyrev, *Zn. Vyssh. Nervn. Deyat*., **47**, No. 3, 532-542 (1997).
8. V. V. Sherstnev, *Brain-Specific Proteins in Mechanisms of Effective Behavior* [in Russian], Moscow (1990).
9. O. I. Epshtein, M. B. Shtark, A. Kh. Timoshenko, *et al., Byull. Eksp. Biol. Med.*, **143**, No. 5, 490-493 (2007).
10. E. G. Antzoulatos, M. L. Wainwright, L. J. Cleary, and J. H. Byrne, *Learn. Mem*., **13**, No. 4, 422-425 (2006).
11. D. L. Glanzman, S. L. Mackey, R. D. Hawkins, *et al., J. Neurosci.*, **9**, No. 12, 4200-4213 (1989).
12. L. Hernadi, L. Hiripi, A. Vehovszky, *et al. Brain Res*., **578**, No. 1-2, 221-234 (1992).
13. D. B. Zimmer, E. H. Cornwall, A. Landar, and W. Song, *Brain Res*. *Bull.*, **37**, No. 4, 417-429 (1995).