Changes in Electrical Properties of Command Neurons during Protective Effect of Low Doses of Antibodies to S100 Protein on the Development of Long-Term Sensitization in *Helix lucorum*

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We studied the effect of antibodies to Ca^{2+}-binding protein S100 in a dilution of 10^{-12} (LAT-S100) on the development of long-term sensitization in *Helix lucorum,* a neurobiological model of anxious and depressive states. After administration of LAT-S100 preventing the development of long-term sensitization before training, the membrane and threshold potentials in command neurons regulating defense behavior decreased less markedly than during long-term sensitization. It is assumed that the "protective" effect is associated with mechanisms of long-term potential maintenance and changes in intra- and extracellular balance of Ca^{2+}-binding protein S100.

Key Words: *Ca^{2+}-binding protein S100, antibodies to S100, long-term sensitization, neurobiological model of anxiety and depression, membrane and threshold potential*

Ca^{2+} ions participate in the regulation of various neuronal processes, being the most universal intracellular messengers. Due to their specific physical and chemical properties, Ca^{2+} ions provide coupling between electrical events in the cell membrane and reactions in the neuronal cell. High Ca^{2+}-binding capacity of intracellular medium is determined by the presence of efficient buffer system consisting primarily of Ca^{2+}-binding proteins. Ca^{2+} ions are essential for learning and memory processes [13], therefore we tried to modulate the development of some behavioral changes with antibodies to Ca^{2+}-binding protein S100 (AT-S100) for detection of their possible effects on behavior. Long-term sensitization (LTS) in higher invertebrate, *Helix lucorum* snail, can simulate depending on parameters of stimulation (in our case, electrical stimuli) non-species-specific features of a stable pathological phenomenon, homologous to chronic stress, depression, and anxiety [2,9].

We previously demonstrated that administration of antibodies to calcium-binding protein S100 in a dilution of 10^{-12} (LAT-S100) before the start of long-term sensitization training (10 min before the first electric shock) prevented the increase in defense reactions of pneumostome closure and ommatophore withdrawal (eye tentacles). Thus, we demonstrated a protective effect of antibodies to S-100 in low doses on the formation of long-term sensitization as a neurobiological model of anxiety and depression [8]. Since this model allows successful investigation of membrane mecha-

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nisms of the formation of stable excitation foci in the nervous system [11], we studied the membrane mechanisms underlying the protective effect of AT-S100.

MATERIALS AND METHODS

Adult *Helix lucorum* snails of similar weight and size were used in the experiments. The snails were active for at least 2 weeks before the experiment. Long-term sensitization of the defense reflex was trained by a previously developed protocol [2]. Electrical stimuli were applied to the head 4 times daily for 4 days at 1.5-2-h intervals. Each stimulation lasted for 1-2 sec (rectangular pulses, 6-8 mA amplitude, 10 msec duration, and 50 Hz frequency). Significant increase in the time of closed pneumostome status in response to the test stimulation in comparison with the initial reaction served as the criterion of LTS formation. Only complete closure of the pneumostome was regarded as the positive reaction to the stimulus. The tests were carried out daily before presentation of the series of electrical stimulation.

Two experimental series were carried out: one half of snails were injected with 0.1 ml LAB-S100 (Materia Medica), which corresponded to a concentration 6×10^{-11} mg/ml through the sinus node daily before the start of electric stimulation, control snails were injected with the same volume of saline at the same terms. Defense reactions of pneumostome closure, ommatophore withdrawal, and locomotion velocity in response to stimulation were quantitatively estimated during the experiment and a few days after LTS formation. Behavioral responses and locomotion rate were analyzed by recording snail behavior using a video camera. The pneumostome closure reaction was tested during a testing session consisting of several tactile test stimuli of the same strength applied to the mantle roll. The time of the pneumostome closure (duration of closed status of the pneumostome) after application of the tactile stimulus by stimulation of the mantle roll with a hair from the brush was registered. The pneumostome closure reaction was chosen because it is the initial component of the defense reactions [1] and can be objectively recorded. In addition, defense behavior of animals was evaluated by the tentacle withdrawal defense reaction in response to the test stimulus. To this end, the tentacle withdrawal amplitude in response to tactile stimulation of the middle and anterior parts of the foot was measured and tentacle contraction was evaluated visually in percents. The maximum length of tentacles was taken as 100% and the tentacle withdrawal by 0, 25, 50, 75, or 100% was recorded [8]. The velocity of snails movement (per min) on the vertical wall of a rectangular glass terrarium was also measured as described previously [8].

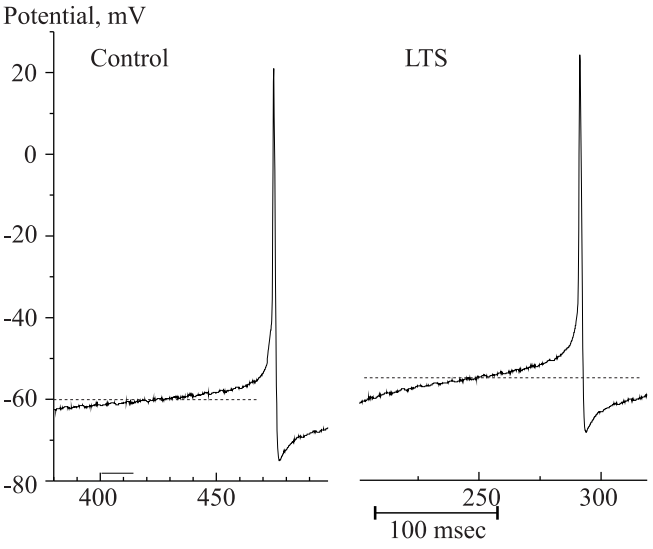

Fig. 1. A record of electrical activity of command neuron of defense behavior LPa3 in control and sensitized (LTS) animals. Dashed line: resting potentials.

After completion of behavioral experiments, electric parameters of command neurons of defense behavior LPa3, RPa3, LPa2 и RPa2 were recorded [1]. To this end, we used preparations of intact, control (saline injection), and experimental (LAT-S100 injection) animals. Electrical parameters were analyzed on isolated preparation of subesophageal ganglion complex from *Helix lucorum* anesthetized by keeping in ice-cold water for 20-30 min before the preparation procedure. Electrophysiological measurements were conducted at room temperature (20-22°C) using intracellular glass microelectrodes (5-40 MΩ resistance) filled with 2.5 M KCl. Physiological saline used for *Helix lucorum* contained (mmol/liter): 80 NaCl, 4 KCl, 10 $CaCl_2$, 5 $MgCl_2$, and 5 $NaHCO_3$ (pH 7.6-7.8). Resting membrane potential (Vm) and the threshold of action potentials generation (Vt) were measured during the experiment.

The data were processed statistically, the mean and standard errors of the mean ($M\pm SEM$) were calculated. Significance of difference between the means for neuronal parameters measured in different experimental sets was evaluated using Student t test and Mann—Whitney U test.

RESULTS

Exposure to electrical stimuli for 4 days increased the duration of closed pneumostome status in snails in response to the test stimulus, which attested to the development of LTS. This result was obtained previously [2]. Administration of saline to snails before a series of electroshocks did not alter the course of LTS development and behavioral indices. However, LTS did not develop after preliminary administration of LAT-S100

judging from both pneumostome and ommatophore defense reactions. At the same time, motor functions (tested by locomotion speed) were not altered. This result was also obtained previously [8].

Electrical properties of command neurons of defense reflex LPa2, RPa2, LPa3 и RPa3 (which are silent at baseline) were analyzed on the next day after LTS training; typical response of these cells to intracellular stimulation is shown on Figure 1. It was found that the probability of generation of action potentials by these cells in sensitized snails is substantially higher than in control animals. Measurement of electric parameters of the neuronal membrane showed a decrease in resting membrane potential and threshold potential by 5-8 mV in sensitized animals compared to controls (Figs. 2 and 3).

Preliminary administration of physiological saline to snails before electroshock did not affect LTS development: similarly to LTS development, depolarization shift of the membrane resting potential and a decrease of threshold potential in command neurons by 5-8 mV compared to intact animals were observed (Figs. 2 and 3). After LAT-S100 administration before LTS training, membrane and threshold potentials in neurons controlling defense behavior decreased to a substantially lesser extent than it was observed after LTS (Figs. 2 and 3). Thus, the effect of AT-S100 on LTS development is followed by partial recovery of the membrane and threshold potentials. It should be noted that after LAT-S100 administration to control group membrane and threshold potentials remained at the baseline control level (data not shown).

LTS was chosen as the test model because it is widely used as the model of anxiety and depression, because command neurons controlling the components of defense behavior were identified, and because of possible of precise measurement of the efferent organs (pneumostome and eye tentacles) response. All these factors make it possible to reconstruct ideal picture of sustained "pathological" disorder of defense behavior and to estimate the involved neural network [2,9].

Our findings clearly indicate that low doses of AT-S100 produce an anxiolytic effect. Published data suggest that low doses of AT-S100 can produce anxiolytic and antidepressant effects in experimentally designed conflict situations in rats [4]. Recent papers confirm these data [6, 10]; antihypoxic (survival under hypoxic conditions) and differentiating (neuritogenic) effects of LAT-S100 were revealed in neuroblastoma C-1300 culture [5]. As an extension of these studies we attempted to modulate some behavioral changes observed during LTS with antibodies to S100B for elucidation of the mechanism of the possible protective effect. Our findings can explain this phenomenon from the viewpoint of the effect of these antibodies on calcium signal transduction system [3]. We are talking about the protective effect of LAT-S100 on Ca^{2+}-dependent K-channels, which is realized not through changes in the action potential generation threshold and its duration, but through ion channels participating in action potential generation and providing calcium entry into the cell [3]. Our results suggest that the S100 protein participates in the functioning of membrane structures via modulation of Ca^{2+}-dependent K channels. This result was obtained using AT-S100, similar results was also described by other authors who used application of the S100 protein [14]. Moreover, we previously demonstrated direct influence of AT-S100 on Ca^{2+}-channels and modulation of changes in Ca^{2+} concentration under the influence of AT-S100. We can hypothesize that a certain role in the manifestation of long-term protective effects of LAT-S100 is played by a signal transduction pathway mediated by Ca^{2+}-dependent MAP-kinase system [7]. Thus, the Ca^{2+}-binding protein S100 imbalance caused by AT-S100 can lead to inhibition or modification of the key

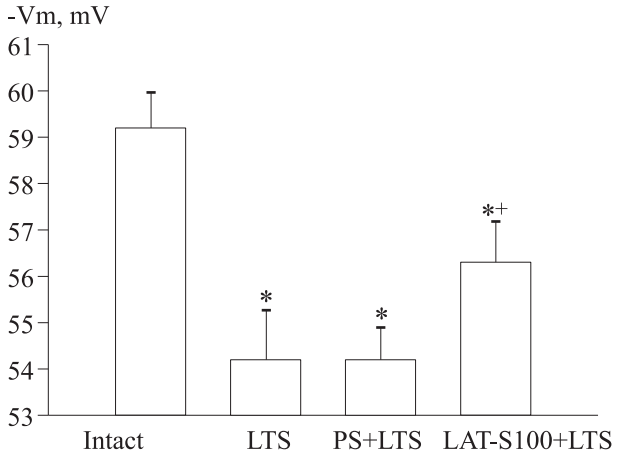

Fig. 2. Resting membrane potential of command neurons (Vm). Here and on Fig. 3: $p<0.01$ compared to: *intact snails, +group "saline+LTS".

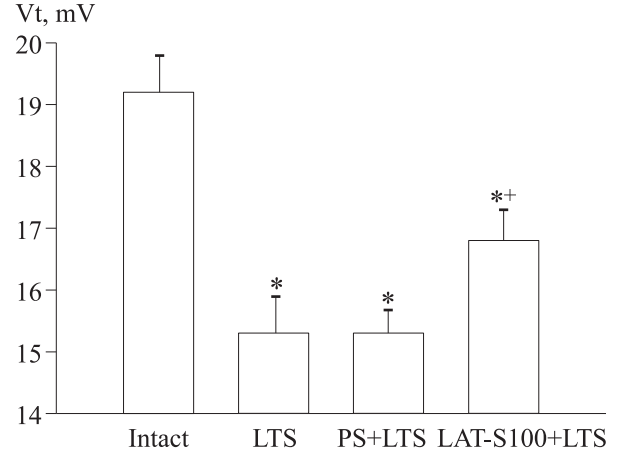

Fig. 3. Threshold potential of command neurons (Vt).

mechanisms accompanying plastic changes in the organism, especially under pathological conditions.

The search for protective effects of various drugs within the framework of preconditioning paradigm is now in progress, this problem is most actively discussed for glutamate and NMDA receptors [15]. We previously studied this phenomenon in experiments with application of antibodies to Ca^{2+}binding protein S100B to hippocampal slices: long-lasting posttetanic potentiation caused by tetanization of dentate gyrus mossy fibers inhibited with anti-S100B antiserum (1.2 mg/ml) was completely restored after preconditioning of slices with the same antibodies in dilution 10^{-12}-10^{-15} [12]. In view of published data, our findings suggest that the protective effect is quite explainable and is aimed at regulation of the physiological level of S100. This sequence of extracellular and intracellular events providing LTS can be used in interpretation of the nature of anxiolytic and antidepressant activity of antibodies to S100B and their antihypoxic effect.

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