Protective Effect of Low-Doses of Antibodies to S-100 Protein on the Formation of Long-Term Sensitization in *Helix Lucorum*

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Injection of antibodies to Ca-binding protein S-100 in a dilution of 10^{-12} before the formation of long-term sensitization in *Helix lucorum* (10 min before the first electric shock) prevented the increase in defense reactions of pneumostome closure and ommatophore withdrawal. Thus, we demonstrated a protective effect of low-dose antibodies to S-100 on the formation of long-term sensitization as a neurobiological model of anxiety and depression.

Key Words: *Ca-binding protein S-10 ; antibodies to S-100; long-term sensitization; neurobiological model of anxiety and depression*

Two neuron types with different reaction to application of antibodies to S-100 protein (AB-S100) in $^1/_5$ dilution were detected in preparations of the isolated nervous system of *Helix lucorum*: the frequency of action potential (AP) generation decreased in neurons B1 and B17, but increased in neurons B4 and B6 [1]. The described effects of AB-S100 on the neuronal spike reactions were not observed, when application of AB-S100 in physiological doses ($^1/_5$ dilution corresponding to 12 mg dry substance/ml) to nervous tissue preparations was preceded by preincubation of these "simple systems" with low doses (10^{-12} dilution) of the same antibodies. The detected phenomenon was called protective. A similar effect of AB-S100 in low doses ($10^{\backslash 12}$ dilution) was observed during the formation of long-term post-tetanic potentiation in rat hippocampal slices [11]. A neuroprotective effect of ultralow doses of glutamate protecting from its toxicity in high doses was recently demonstrated for spinal, cortical, and cerebellar neurons [13] and of low-dose cycloheximide for neuron survival in glutamate [15].

An anxiolytic effect of low-dose AB-S100 was demonstrated under conditions of punished and unpunished behavior in rats [3] and antidepressant effects of low-dose AB-S100 was reported [8]. We studied the mechanisms underlying the effect of low-dose AB-S100 on the model of pathology realized in *Helix lucorum*.

Long-term sensitization (LTS) is a neurobiological model of long-term modifications of behavior attracting now much attention [4,9]. Using this model, it is possible to study the membrane mechanisms of the formation of stable foci of anxiety in animal nervous system [10]. Since LTS is a species-unspecific phenomenon (*i.e.* intrinsic of animals of different levels of organization [6]), reactions of higher animals can be evaluated on relatively simple objects or models convenient for the analysis. Realization of this experimental model in animals with simple nervous system creates actual

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prerequisites for deciphering cellular mechanisms of the formation and development of states accompanying nervous mental disorders, such as stress conditions, anxiety of different kinds, schizophrenia, *etc.* [4,6].

MATERIALS AND METHODS

Adult *Helix lucorum* of similar weight and size were used in the experiments. The snails were kept in glass terrariums at ambient temperature, high humidity, and with food excess. Before the experiment the snails were active for at least 2 weeks. Long-term sensitization of the defense reflex was trained by a previously developed protocol [4]. Electrical stimuli (rectangular pulses of 6-8 mA amplitude, 10 msec duration, and 50 Hz frequency) were applied to the head 4 times daily for 4 days at 1.5-2-h intervals. Each stimulation lasted for $^1/_2$ sec. Actual voltage during stimulation was controlled using an oscillograph monitor. During electrical stimulation the animals were placed on a copper electrode plate covered with wet paper. The other electrode was a metal rod applied to the snail head. Control animals were exposed to the same procedures, but without electric stimulation. A significant prolongation of the 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 a positive reaction to the stimulus. The moment of the pneumostome opening was determined by return of the photoresistor resistance to the stationary level, but no less than 90% of the initial value. The tests were carried out daily before presentation of the series of electrical stimulation.

Two experimental series were carried out: 50% snails (experimental group) were injected with 0.1 ml of AB-S100 solution in low doses (LAB-S100, 10^{-12} dilution, Materia Medica) daily before the start of electric stimulation (10 min before the first electric shock), control snails were injected with the same volume of saline at the same terms. Defense reactions of pneumostome closing, ommatophore withdrawal, and locomotion velocity in response to the stimulus were tested during and after LTS formation.

The pneumostome closure reaction was tested during a testing session including several tactile test stimuli of the same strenght applied to the mantle roll. The testing was carried out in a special device consisting of a water reservoir with a light polyethylene ball and a holder. The snail was tethered by its shell in a manner allowing it to crawl on a ball rotating freely in water [2]. This device enabled objective recording of the movements of the pulmonary cavity and ommatophore (posterior tentacles) muscles and stimulation any site of the snail body. 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 [2] 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 (withdrawal of the middle and fore parts of the foot touched with a Frey hair) 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 [12]. The velocity of 1-min track of snails moving on the vertical wall of a rectangular glass terrarium was measured. The start and finish of straight-line path of each animal over 1 min was marked with a marker and these tracks were measured with a ruler. When measuring the velocity of the wave propagation in the foot, locomotion velocity, and length of the foot, we selected only the tracks with muscle waves (dark narrow strips divided by light intervals and transversely crossing the foot) clearly seen from the caudal part of the foot to the rostrum border [7].

The results were presented as $M \pm SEM$. The significance of differences in the mean values of the neuron parameters in different experimental series was evaluated using Student's t and Mann—Whitney U tests.

RESULTS

At the initial stage of the experiment several parameters were analyzed in order to obtain more complete picture of the effect of LTS training procedure on snail behavior. We found that during the first 2 days of LTS formation the snails were anxious, moved more actively and ate less actively, while during the next 2 days their motor activity decreased. Spontaneous closures of the pneumostome were observed, though it remained open during the greater part of time, and the testing session was carried out during this very period. The time of closed pneumostome status in response to the test stimulus increased significantly during 4 days of LTS formation (Fig. 1). After formation of LTS, tentacle withdrawal in response to tactile stimulus increased significantly in comparison with the initial level and control (from 15 to 30 sec, on aver-

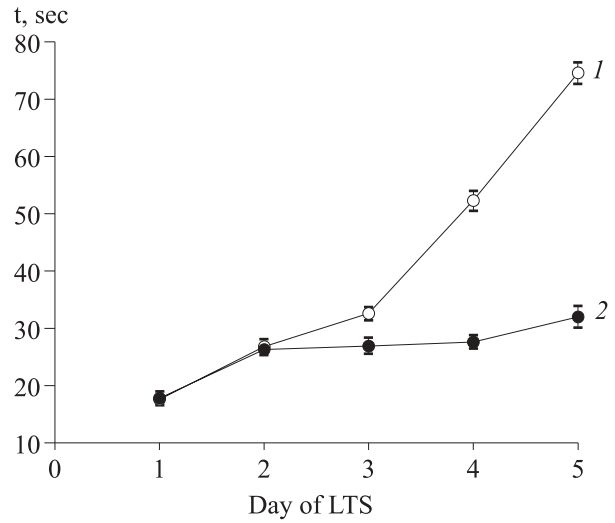

Fig. 1. Modification of the defense reaction of pneumostome (duration of closed status) in response to tactile test stimulation in control (*1*) and experimental (*2*) snails.

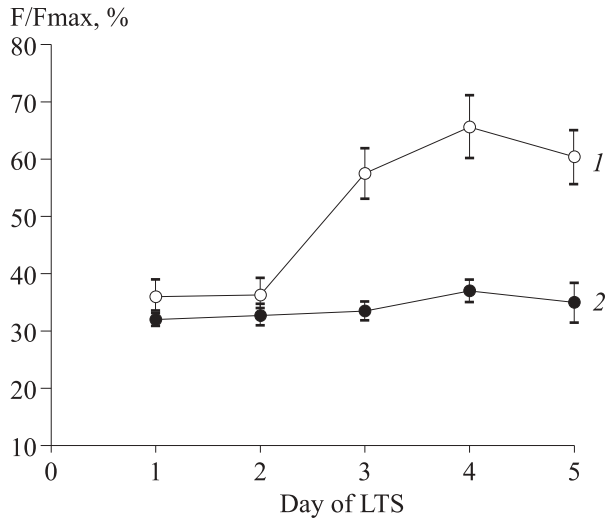

Fig. 2. Modification of the defense reaction of tentacle withdrawal in response to tactile test stimulation in control (*1*) and experimental (*2*) snails.

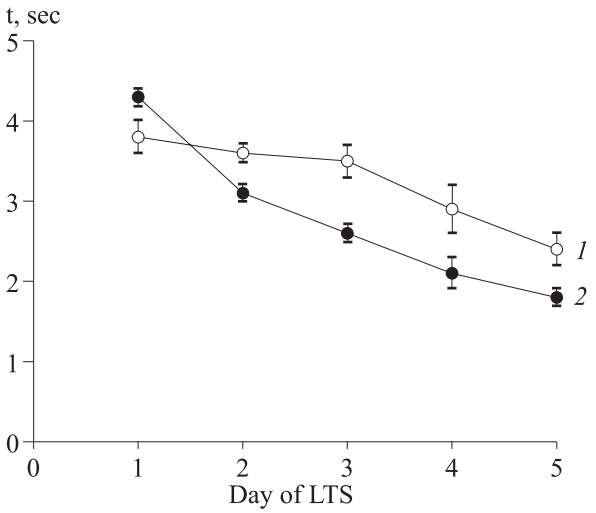

Fig. 3. Changes in locomotion velocity (vertical movement on the terrarium wall) in control (*1*) and experimental (*2*) snails.

age; Fig. 2). The locomotion velocity decreased at the very beginning of LTS formation (1.3 times on average; Fig. 3).

Preinjection of saline before electric shock stimulation did not modify the formation of LTS and parameters of snail behavior. No LTS formed after preinjection of low-dose AB-S100 (10^{-12}) judging from the defense reactions of pneumostome or tentacles (Figs. 1, 2). The motor functions (locomotion velocity) did not change (Fig. 3).

These findings attest to an anxiolytic effect of low-dose AB-S100. This phenomenon can be explained by the effect of antibodies on the cell calcium signal system [5]. We mean the protective effect of LAB-S100 on Ca-dependent K-channels manifesting not through modification of AP generation threshold and duration, but at the level of ionic channels involved in AP generation and providing Ca entry into the cell [5]. The results indicate that S-100 protein participates in the functioning of membrane structures by modulating the functions of Ca-dependent K-channels. These data were obtained with AB-S100; similar results were obtained by other scientists using applications of S-100 protein [14].

Hence, low-dose AB-S100 prevented the formation of LTS, a neurobiological model of anxiety [4,6,9]. The defense reactions in *Helix lucorum* are determined by command neurons, while motor activity is determined by the pedal ganglion motoneurons, whose activity is quite different. Different activity patterns of these neurons presumably indicate that intracellular signal systems act differently during their functioning.

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