ELECTROPHYSIOLOGICAL AND NEUROCHEMICAL INVESTIGATION OF LONG-TERM SENSITIZATION IN SNAILS

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The study of long-term sensitization (LTS) is of both theoretical and practical interest. As one form of nonassociative learning, LTS may have mechanisms in common with associative learning, and its study is one way of analyzing the cellular and molecular bases of behavior, without the need to resort to complicated methods [3]. Meanwhile, the extreme form of LTS is a model of the nonspecific hypertrophied alarm reaction [3, 9], on which the character of development of chronic pathological states of the CNS can be studied.

The writers previously described a model of long-term sensitization of the defensive reflex of pneumostome closure in *Helix pomatia* and demonstrated a quantitative redistribution of individual protein fractions in the subesophageal ganglion complex of the CNS at different stages of LTS [5].

The aim of this investigation was to study the distribution of water-soluble proteins in functionally different groups of nerve cells and changes in the electrical characteristics of command neurons of defensive behavior during LTS formation.

EXPERIMENTAL METHOD

Snails whose pneumostome closure time in response to the testing stimulus did not exceed 10 sec were chosen for the experiment. LTS of the defensive reflex of *Helix pomatia* was formed in the course of 4 days by daily application of four electrical stimuli to the head with an interval of 1.5-3 h (alternating current, 10 mA, 50 Hz, duration 1 sec, from the ÉS-50-1 electrical stimulator). The animals were tested 24 h after the end of the procedure of LTS formation. The criterion that LTS was present was a significant increase in the pneumostome closure time in response to testing tactile stimulation in the region of the mantle fold compared with this parameter in control animals kept under the same conditions. This same criterion also was used in other studies involving LTS formation [5, 8, 9]. Before dissection the snail was anesthetized by placing it for 15-30 min in a mixture of ice and water. To analyze the electrical characteristics a semi-intact snail preparation with maximally preserved connections between the CNS, the mantle region, and the caudal part of the foot, was used. The mantle region was stimulated mechanically with a silver electrode (diameter of tip about 1 mm), which was set in motion by means of an electromagnet, triggered in accordance with the stimulation program by a computer. Electrical activity of the neurons was recorded through a microelectrode, filled with 2.5 M KCl solution, and with resistance of between 10 and 50 MΩ after sharpening, inserted into the neuron. Values of membrane potential were transformed into digital form by an analog-to-digital converter and analyzed by computer [1]. Command neurons of the defensive reflex of pneumostome closure of the snail — LPa3, RPa3, LPa2, RPa2 [2, 6], were chosen as the test objects. The response parameters of these neurons were studied in control and sensitized animals to tactile stimulation of the foot (resting potential, amplitude of action potentials and threshold of their generation, amplitude of the excitatory postsynaptic potential — EPSP).

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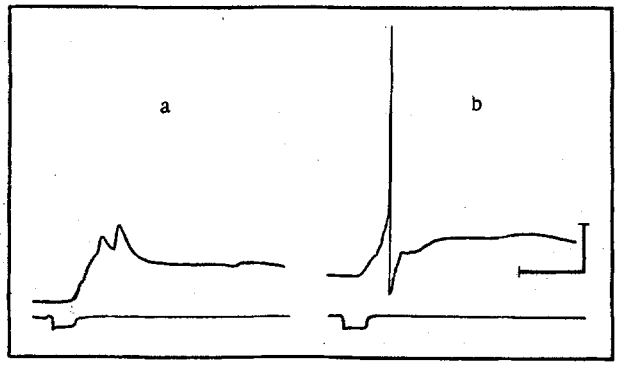

Fig. 1. Responses of command neurons LPa3 of defensive behavior of *H. pomatia* to tactile stimulation of mantle (the same point was stimulated) of control (a) and sensitized (b) animals. Marker of tactile stimulus shown on lower curve (current passing through electrode while it touched the mantle). Calibration: 10 mV, 400 msec.

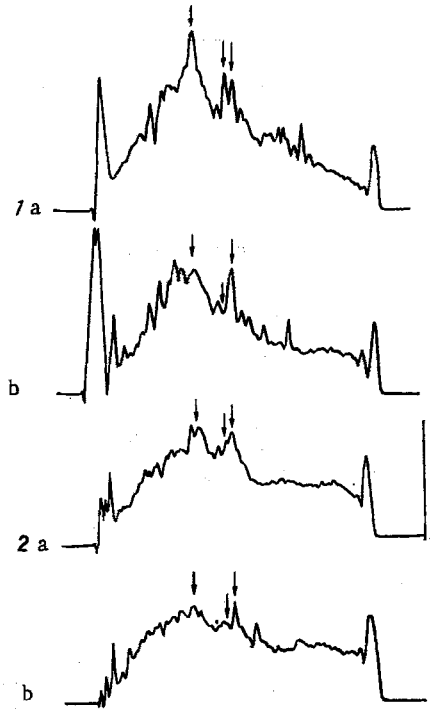

Fig. 2. Densitograms of water-soluble proteins of groups of identified neurons in control (a) and sensitized (b) snails. 1) Command neurons of defensive behavior LPa2, 3 and RPa2, 3; 2) neurons with bursting activity RPa1 and RPa5-8. Double arrows — peak of proteins with mobility of 0.42 and 0.40; single arrows — proteins with mobility of 0.54 and 0.56.

After LTS formation in the snail a comparative analysis also was made of the protein spectra of the identified cells in two groups of animals: control and experimental (after LTS formation). Command neurons LPa3 and LPa2, RPa3 and RPa2, were isolated (with the aid of tungsten needles and pipeting) without preliminary enzyme treatment. Neurons RPa1 and RPa5-RPa8 (classification of Sakharov [6] and Maksimova and Balaban [2]), and cells of the D pool were isolated in one group. The neurons were placed in glass test tubes 1.5 mm in diameter and the proteins were extracted at 0°C by homogenization of the contents of the tubes with a resolving stainless steel wire (diameter 0.15 mm) in buffer 0.075 M Tris-HCl, 10% sucrose, 0.02% NaN_3, 0.5% Triton, and 7 M urea. The homogenates were centrifuged at 12,000g (type K24 centrifuge) for 30 min at 4°C. The supernatants were subjected to PAG

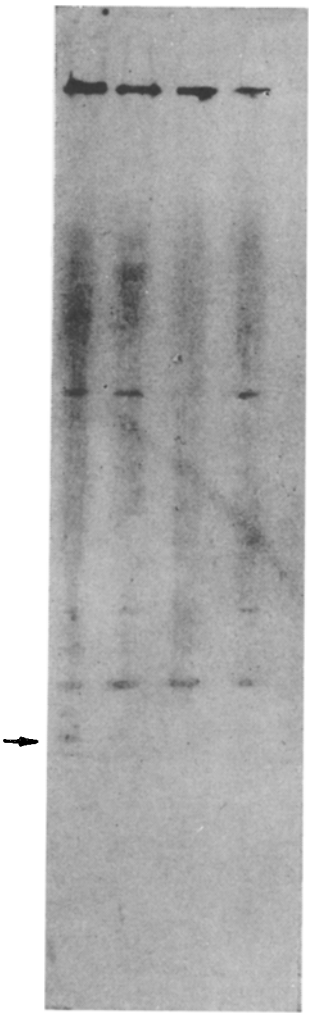

Fig. 3. Electrophoresis of water-soluble proteins of neurons of the D pool in control (c, d) and sensitized (a, b) snails. Arrow indicates protein fraction with mobility of 0.75.

gradient electrophoresis (C-5, T-10-30) in vertical plates (300 × 450 × 0.7 mm) at 4°C. Proteins in the gel were stained with Coomassie bright blue R-250 and then impregnated with $AgNO_3$ [10]. The gels were scanned on an "Ultroscan 2202" densitometer (LKB, Sweden).

EXPERIMENTAL RESULTS

Data characterizing the response of neuron LPa3 to tactile stimulation of the mantle fold of the sensitized and control snails are given in Fig. 1 in the form of an EPSP, which could change into an action potential. During tactile stimulation of equal strength the probability of appearance of action potentials in the response of command neurons LPa3 and RPa3 in sensitized snails was higher than in the controls; no changes were observed in the shape of the action potentials. These neurons also differed in the value of their initial resting potential: -66.6 ± 1.5 mV for cells LPa3 and RPa3 in the control snails ($n = 20$) and -57.5 ± 1.0 mV in the sensitized snails ($n = 17$). Differences in the mean values of the resting potential in the control and sensitized animals were statistically significant at the $p < 0.01$ level (Student's t test [4]). These results demonstrate the increased excitability of pneumostome closure command neurons. A considerable change of membrane potential must be noted in these experiments, which was not obtained in [8]. These differences in the results are evidently due to the fact that electrical activity was recorded in different elements of the reflex arc.

Analysis of water-soluble proteins of the CNS showed that functionally different identified neurons had individual protein spectra (Figs. 2 and 3), in agreement with data obtained

previously in our laboratory by L. N. Grinkevich et al. [7] by capillary microdisk electrophoresis. However, the method of microelectrophoresis in gel gradient plates, which was used in the present investigation, has higher resolving power, and impregnation of the proteins with silver nitrate can reveal a much richer spectrum of proteins, especially those of low molecular weight. The quantitative dynamics of the proteins in fractions with mobility of 0.56, 0.54, 0.42, and 0.40, described previously by Safronova in the subesophageal complex and cerebral ganglion [5], is similar (a significant decrease in the protein content in the fractions with mobility of 0.54, 0.42, and 0.40 24 h after the end of the procedure) in all neurons examined, evidence of the involvement of many cells of the nervous system in the response to LTS. It was shown in the D pool (neurosecretory and serotoninergic cells [6]) that a protein with mobility of 0.75, which cannot be detected in control animals or at the early stages of sensitization, was present in a high proportion of the sensitized cells (Fig. 3).

Thus the formation of LTS of the defensive reflex is accompanied by an increase in excitability of the command neurons of this reflex. One of the mechanisms of the increase in excitability of the nerve cells is their depolarization. A quantitative redistribution of the individual protein fractions of the identified neurons, involving both neurons lying in the arc of this reflex (command neurons) and those not included in it (bursting neurons and cells of the D pool) also takes place.

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LITERATURE CITED

1. S. V. Astaf'ev, V. P. Tret'yakov, M. B. Shtark, and G. Ya. Yanovskii, Avtometriya, No. 4, 44 (1984).
2. P. M. Balaban, I. S. Zakharov, and O. A. Maksimova, Problems in Cybernetics [in Russian], No. 109, Moscow (1985), pp. 29-45.
3. E. R. Kandel, Cellular Bases of Behavior [Russian translation], Moscow (1980).
4. G. F. Lakin, Biometrics [in Russian], Moscow (1973).
5. O. G. Safronova, Byull. Éksp. Biol. Med., 103, No. 3, 266 (1987).
6. D. A. Sakharov, The Genealogy of the Neuron [in Russian], Moscow (1974).
7. M. B. Shtark, L. N. Grinkevich, and Kh. L. Gainutdinov, Dokl. Akad. Nauk SSSR, 230, No. 5, 1254 (1976).
8. W. N. Frost, V. F. Castellucci, R. D. Hawkins, and E. R. Kandel, Proc. Natl. Acad. Sci. USA, 82, 8266 (1985).
9. H. M. Pinsker, W. A. Hening, T. J. Carew, and E. R. Kandel, Science, 182, 1039 (1973).
10. E. C. Rochette and G. C. Styssi, Electrophoresis, 5, 285 (1984).