WATER-SOLUBLE AND MEMBRANE PROTEINS OF THE SNAIL CENTRAL NERVOUS SYSTEM AT DIFFERENT STAGES OF LONG-TERM SENSITIZATION

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Long-term sensitization (LTS), in its extreme form, was first described as a model of the hypertrophied alarm reaction by Pinsker and co-workers on the marine mollusk *Aplysia* [4]. The time course of LTS in *Aplysia* and some aspects of the behavior and vegetative changes of these animals during its formation, have now been well studied [3]. A start has been made on the study of possible changes in the electrophysiological characteristics of CNS neurons during the formation of this response.

It was shown previously that CNS proteins play an important role in different versions of modification of behavior (in particular, during learning) [1, 2]. It was considered important to study changes in the protein spectra of the CNS during the development and formation of LTS.

In the investigation described below a model of LTS was developed and used, in the form of the defensive reflex of closing of the pneumostome of the snail *Helix pomatia*. The aim of the research was to study the effect of LTS on water-soluble and membrane-bound CNS proteins.

EXPERIMENTAL METHOD

LTS was formed in the snail: during 4 days electrical stimulation of the snail's head was carried out 4 times a day at intervals of 100-150 min. Control snails were kept in a separate room under the same conditions as the experimental snails, but were not subjected to any experimental procedure. Throughout the period of the experiment the behavior of each snail was kept under observation. A testing session was carried out, including 10 tactile stimulations of the peripneumostomal region, 7 days before the beginning of formation of sensitization and 24 h after its end.

At different stages of formation of LTS (after two electrical stimulations, 2 h, and 1 and 3 days after the end of stimulation) the subesophageal system of ganglia and the cerebral ganglion were removed from the snails, and water-soluble proteins were extracted in buffer A (0.075 M Tris-HCl, pH 6.7, 10% sucrose, 0.1 mM EDTA, 0.02% NaN_3), in the presence of 0.5% Triton X-100. Membrane proteins were extracted from the residue in buffer A in the presence of 1% sodium dodecylsulfate for 2 h at 55°C.

Proteins were fractionated by disk electrophorsis in a PAAG porosity gradient (T = 10-30, C = 5) in vertical slabs (60 × 90 mm), with a thickness of gel of 0.8 mm, at 4°C. The proteins in the gel were stained with 0.15% Coomassie R-250 in 10% acetic acid and 50% methanol for 1 h, followed by staining of the lower part of the gel, which contained low-molecular-weight proteins, with silver nitrate [5].

The gels after electrophoresis were scanned on a Skan-400 densitometer (Joyce-Loebl, England). The densitograms were analyzed statistically by Student's test.

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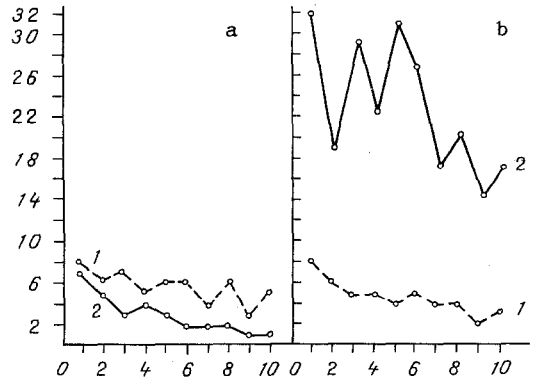

Fig. 1. Average time during which pneumostome remained closed after each of 10 testing stimuli. a) Group of 15 control snails; b) group of 15 experimental snails. 1) First testing (7 days before beginning of sensitization); 2) second testing (24 h after end of procedure). Abscissa, number of testing stimuli; ordinate, time (in sec).

EXPERIMENTAL RESULTS

Observation of the animals' behavior during the formation of LTS showed that on the first two days of sensitization the snails became restless, moved about more, and ate less. On the next 2 days their motor activity was sharply reduced, and the time spent by the snails hidden in their shell after routine electrical stimulation lengthened from 5-10 min on the first day to 60-150 min on the last day. Some snails preserved a sufficiently high level of activity.

It follows from the results of testing that 24 h after the end of stimulation the time during which the pneumostome remained closed after the preceding tactile stimulus became 3.5 times longer than before sensitization in the same animals, and 6 times longer than in the control (Fig. 1).

The study of changes in water-soluble CNS proteins showed that 2 and 24 h after the end of LTS formation the protein content in the fractions with mobility of 0.54, 0.42, and 0.40 relative to the front was reduced by 3.5, 3, and 3 times, respectively, and differed significantly from the control (Fig. 2). The level of these proteins 3 days later was 1.5 times less and differed significantly from the control.

The content of protein with mobility of 0.56, described previously [2], in the fraction increased by 1.7 times after the first two electrical stimulations and differed significantly from that in the control. At the remaining times of LTS the mean quantity of this protein did not differ from that in the control, although its level in the different animals showed wide scatter, probably due to the different degree of activity and level of arousal of the animals after LTS. Quantitative changes in the proteins took place both in the subesophageal system of ganglia and in the cerebral ganglion.

The study of changes in the membrane proteins showed that 2 and 24 h after the formation of LTS the content of the protein in the fraction with mol. wt. of 24 kilodaltons was lower by 1.5 times.

In the initial stage of sensitization (after the first two electrical simulations) no change was found in the levels of the proteins tested (except the 0.56 fraction). This suggests that the changes observed were either linked with late stages of LTS and persisted for several days after the end of stimulation, or accumulated gradually in the course of prolonged stimulation.

The experiments thus showed that LTS affects the turnover of individual CNS proteins in the snail, leading to their substantial quantitative redistribution. The changes de-

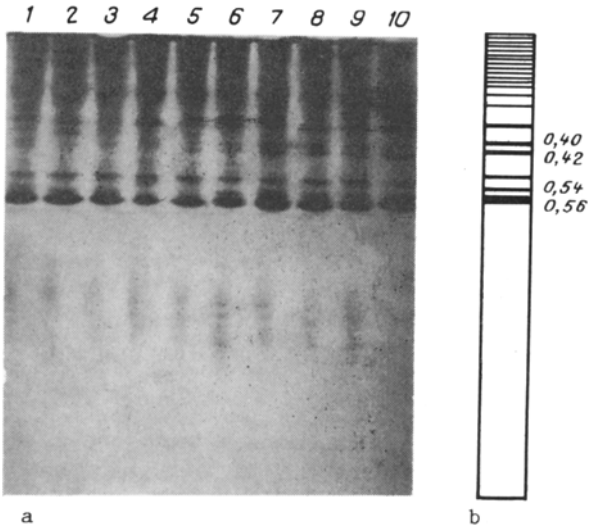

Fig. 2. Electrophoresis of water-soluble proteins from cerebral ganglion (3, 9) and subesophageal system of ganglia of *Helix pomatia* (1, 2, 4-8, 10). a) Electrophoregram. 1-3) Experimental animals 24 h after LTS; 7-10) control animals. b) Scheme of gel: Numbers indicate mobility of corresponding protein fraction in polyacrylamide gel.

scribed above may reflect stages in the development of LTS and may be connected with changes in the functional characteristics of particular cells of the defensive behavior network. Analysis of the protein spectra of identified neurons could provide proof of this hypothesis, and research in that direction is in hand.

LITERATURE CITED

1. L. N. Grinkevich, Dokl. Akad. Nauk SSSR, 252, 248 (1980).
2. L. N. Grinkevich, O. G. Safronova, and M. B. Shtark, Byull. Éksp. Biol. Med., No. 10, 429 (1984).
3. E. R. Kandel, The Cellular Basis of Behavior [Russian translation], Moscow (1980).
4. H. M. Pinsker, W. A. Hening, and E. R. Kandel, Science, 182, 1039 (1973).
5. E. C. Rochette and G. C. Styssi, Electrophoresis, 5, 285 (1984).