EFFECT OF FUNCTIONAL STATE ON DEVELOPMENT OF LONG-TERM POTENTIATION AND CHANGES IN S-100 PROTEIN CONTENT IN HIPPOCAMPAL SLICES OF RATS

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Genetic strains of animals with different levels of excitability of their nervous system are being used on an ever-widening scale in studies of normal and pathological behavioral reactions. Differences between these strains have been demonstrated with respect to a whole range of normal and pathological features, including conditioned-reflex activity and differential sensitivity to the action of neurosis-inducing agents [5]. It has also been shown that neuro-specific antigens and, in particular, protein S-100, participate in conditioned reflex formation [7], and that at the neuronal level they are involved in the formation of long-term trace phenomena such as prolonged post-tetanic potentiation (PPTP) which is nowadays regarded as the cellular analog of memory [14].

The aim of this investigation was to compare two strains of rats differing in the threshold of excitability of their peripheral nervous system, with respect to the content of protein S-100 and the development of PPTP in hippocampal slices.

EXPERIMENTAL METHOD

Experiments were carried out on two strains of male rats bred at the I. P. Pavlov Institute of Physiology (St. Petersburg) with respect to the threshold of excitability of their neuromuscular apparatus [1, 2]: strains with a high threshold of excitability (TE) and with a low threshold (LT). Altogether 32 rats took part in the experiments. Half of the animals of each strain underwent induction of neurosis over a period of 15 days, using Hecht's stochastic scheme [9]. Experiments were carried out 2 months after the end of induction of neurosis. Transverse slices of the hippocampus 300-350 μ thick were placed in a thermostated chamber, 30 min before the experiment began, through which physiological saline, saturated with carbogen (95% O_2 and 5% CO_2) was pumped continuously. The composition of the incubation medium was (in mM): NaCl 124, KCl 5, KH_2PO_4 1.24, $MgCl_2$ 1.3, $CaCl_2$ 2.6, $NaHCO_3$ 20, D-glucose 10 [15]. Electrical activity of pyramidal neurons in hippocampal area CA3 was recorded extracellularly by means of a glass microelectrode with broken off tip filled with physiological saline. To stimulate mossy fibers, bipolar tungsten electrodes were used. During tetanization the fibers were stimulated by bursts of polar pulses for 1.5 min. The frequency of pulses in the burst was 200 Hz, the duration of the burst 75 msec, the duration of each pulse 0.1-0.5 msec, and the burst frequency 0.2 Hz. The strength of the stimulating pulse was chosen so that the amplitude of the population spike was 50% of its peak value. Evoked potentials were recorded before and 30 min after the end of tetanization. The experiment was controlled and the data processed by "Iskra-226" microcomputer. Ten hippocampal slices from each animal were used in the electrophysiological experiments. Five of them were subjected to tetaniza

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TABLE 1. Changes in Content of Protein S-100 during Development of PPTP in Hippocampal Slices of Rats differing in Level of Excitability of the Nervous System (n = 7)

Strain of animals	PPTP		Control	
	WS	MB	WS	MB
Wistar	1,809±0,08*	0,634±0,011**	2,09±0,11	0,439±0,05
HT	1,787±0,13*	0,687±0,04**	2,26±0,12	0,446±0,09
LT	1,91±0,12	0,490±0,16	2,02±0,17	0,456±0,12

Legend. Concentration of protein S-100 expressed in mg/mg total protein of water-soluble (WS) and membrane-bound (MB) fraction. *$p < 0.05$, **$p < 0.01$.

tion, the rest were controls. Slices for biochemical analysis were taken from the medium 1 h after stimulation of the last slice. Part of the sensomotor cortex, the hippocampus, and cerebellum also was removed from the animal's brain for biochemical tests. Tissue samples were homogenized on a microblender in plastic test tubes in 400 μl of 10 mM potassium-phosphate buffer, containing 1 mM EGTA-Na_2, and centrifuged at 20,000*g* for 1.5 h. The supernatant was withdrawn for analysis as the water-soluble protein fraction. The residue was resuspended in the same buffer with 1 mM EDTA-Na_2 and 0.5% Triton X-100, and kept for 3 h in a cold room (4°C). The sample was then recentrifuged at 20,000*g* for 2 h and the supernatant withdrawn for analysis of the membrane-bound fraction. Total protein content was determined by binding with Coomassie Brilliant Blue G-250 [13]. The content of protein S-100 was analyzed by ELISA, using pure protein and monospecific antibodies to it [6].

EXPERIMENTAL RESULTS

On statistical analysis of the parameters of the evoked potentials no significant differences were found for all four groups of animals studied. However, the increase of the population spike after tetanization was significant for slices prepared from the hippocampus of rats with a high threshold, but without an induced neurosis, compared with the remaining groups of animals. Interlinear differences in the efficacy of formation of PPTP in animals with low and high thresholds may be due to several causes. First, the great mobility of the synapses in high-threshold animals. Second, the increased calcium ion concentration in the intercellular fluid of the brain of low-threshold rats [3]. This latter hypothesis is supported by data obtained in several investigations, indicating that PPTP can be induced on hippocampal slices by increasing the calcium ion concentration in the physiological saline [8]. Probably as a result of the increased calcium ion concentration, many of the synapse population are in a state corresponding to peak efficiency. Clearly in this case tetanization of the mossy fibers led only to a very small increase in amplitude of the population spike.

It can also be concluded from the results of this investigation that development of neurosis of the HT animals led to inhibition of processes connected with PPTP formation. It is hardly possible at the present time to discuss the mechanisms lying at the basis of this phenomenon. However, attention is drawn to the positive correlation existing between the degree of development of PPTP and ability to learn the conditioned active avoidance reflex. It has been shown that the HT strain have better capacity for forming a conditioned active avoidance reflex [4]. Meanwhile, neurosis development leads to a disturbance of this process only in the HT strain, in agreement with the hypothesis that the PPTP phenomenon at the cellular level can be regarded as a model of learning [14]. Neurosis development, however, did not change the level of the protein S-100 fraction in the cerebral cortex, hippocampus, and cerebellum.

The development of PPTP in hippocampal slices was accompanied, as shown previously [12], by a decrease in the concentration of protein S-100 in the water-soluble fraction and an increase in its concentration in the membrane-bound fraction; the quantitative ratios, moreover, suggest switching of the protein from one fraction into the other. A similar pattern also was observed during PPTP formation in the strain of animals with a high threshold of excitability (Table 1), whereas in the case of the LT strain no such redistribution of the protein was observed. Since the development of PPTP in rats with LT was not significant, it can be postulated that redistribution of protein S-100 may be one of the genetically determined factors that determine the molecular mechanisms of PPTP. This hypothesis is in agreement with the known facts, that injection of antibodies to-protein S-100 into the brain of animals disturbs their ability to learn [10], whereas application of the same antibodies to hippocampal slices inhibits the development of PPTP [11].

LITERATURE CITED

1. N. P. Aleksandrova, N. V. Shiryaeva, N. G. Lopatina, et al., Dokl. Akad. Nauk SSSR, **259**, No. 5, 1233 (1981).
2. A. I. Vaido, Yu. S. Dmitriev, D. A. Kulagin, et al., Genetika, **19**, No. 9, 1446 (1983).
3. A. I. Vaido, N. V. Shiryaeva, M. M. Sokolova, et al., Content of Cations in Tissues of the Nervous System in Strains of Rats Bred for Excitability of the Neuromuscular Preparation [in Russian], Moscow (1986). Lodged with the All-Union Institute of Scientific and Technical Information, 1986, No 3626, V86.
4. A. I. Vaido, I. V. Zhdanova, and N. V. Shiryaeva, Zh. Vyssh. Nerv. Deyat., **37**, No. 3, 575 (1987).
5. A. I. Vaido, V. V. Vlivtseva, V. G. Lukashin, et al., Zh. Vyssh. Nerv. Deyat., **40**, No. 3, 518 (1990).
6. M. V. Starostina and S. M. Sviridov, Biokhimiya, **46**, No. 11, 2030 (1991).
7. M. B. Shtark, Brain-Specific Proteins (Antigens) and Function of the Neuron [in Russian], Moscow (1985).
8. T. V. Dunwiddie and G. S. Lynch, Brain Res., **169**, No. 1, 102 (1979).
9. K. Hecht, K. Treptov, S. Choinowski, and M. Peschel, Die räum-zeitliche Organization der Reiz–Reactions: Beziehungen bedingtreflectorischer Prozesse, Jena (1972), p. 103.
10. S. E. Karpiak, M. Serokosz, and M. M. Rapport, Brain Res., **102**, No. 2, 313 (1976).
11. D. Lewis and T. J. Teyler, Brain Res., **383**, 159 (1986).
12. N. Popov, S. Schulzeck, T. M. Pankoua, et al., Biomed. Biochim. Acta, **47**, No. 2, 185 (1988).
13. S. M. Read and D. H.Northcon, Analyt. Biochem., **116**, No. 1, 54 (1981).
14. T. J. Teyler and P. Di Scenna, Ann. Rev. Neurosci., No. 10, 131 (1987)
15. C. Yamamoto and S. Sawada, Exp. Neurol., **74**, No. 1, 122 (1981).