EFFECT OF LEARNING ON DYNAMICS OF THE RAT BRAIN ANTIGEN SPECTRUM

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The extremely important role of protein metabolism in brain tissues in the formation of temporary connections can be taken as proven. During learning opposite changes (an increase or decrease) in the synthesis of total neuronal proteins [2] have been found in various brain structures (auditory and visual cortex, hippocampus, basal amygdaloid nucleus, and so on). The antigenic spectra of these structures, performing different physiological functions, differ in the composition and quantity of specific and nonspecific brain proteins [7, 9, 11]. It has been suggested that so-called neurospecific proteins play the most important role in the processes of formation and fixation of the memory trace, and that the investigation of the dynamics of their content could help to shed light on the nature of these processes [4, 7, 8]. The problem of whether preservation of a temporary connection, once it has arisen, is linked with preservation of the metabolic changes which accompanied its formation, merits special attention.

The object of this investigation was to study the dynamics of the content of certain brain antigen proteins in different structures of the CNS immediately and 1 and 7 days after conditioned reflex formation.

EXPERIMENTAL METHOD

Male Wistar rats weighing 130-150 g, aged 3-4 months, were used in the experiments. The animals were divided into three groups: trained (T group), active control group (AC), and passive control group (PC). Training, i.e., conditioning, was carried out in a Y-shaped maze [6]. The animals of group AC received the same number of uncombined photic stimuli and electric shocks as the rats of group T: The animals of groups T and AC, in turn, were divided into three subgroups depending on the time elapsing after the beginning of the conditioned-reflex procedure and of pseudoconditioning: The brain of the animals of subgroup 1 was studied immediately, that of subgroup 2 was studied 1 day, and that of subgroup 3, 7 days after the beginning of the experiment. Each subgroup consisted of 6 rats. The PC group comrised 12 intact animals. The rats were decapitated, the brain was removed, and the hippocampus, caudate nucleus, brain stem (region of the gigantocellular nucleus of the reticular formation), and the motor, visual, and pyriform areas of the cortex were isolated in the cold. Each structure was investigated from two rats and paired formations were isolated on both sides. The

TABLE 1. Changes in Length of Arc of Brain Antigens P_1, P_2, and P_3 Depending on Their Concentrations (M ± m)

Dilution of homogenate	Length of arc of brain antigen ($l \pm \sigma t$)		
	P_1	P_2	P_3
Undiluted	23,3±4	13,8±0,9	14,1±1,3
1:2	14,4±1	9,1±0,3	9,8±0,7
1:4	8,7±1,4	5,9±0,4	6,0±0,3

Legend. Length of arc of brain antigen given in conventional units; σt) confidence interval at $P \leq 0.05$.

KEY WORDS: antigen proteins; brain structures; conditioned reflex.

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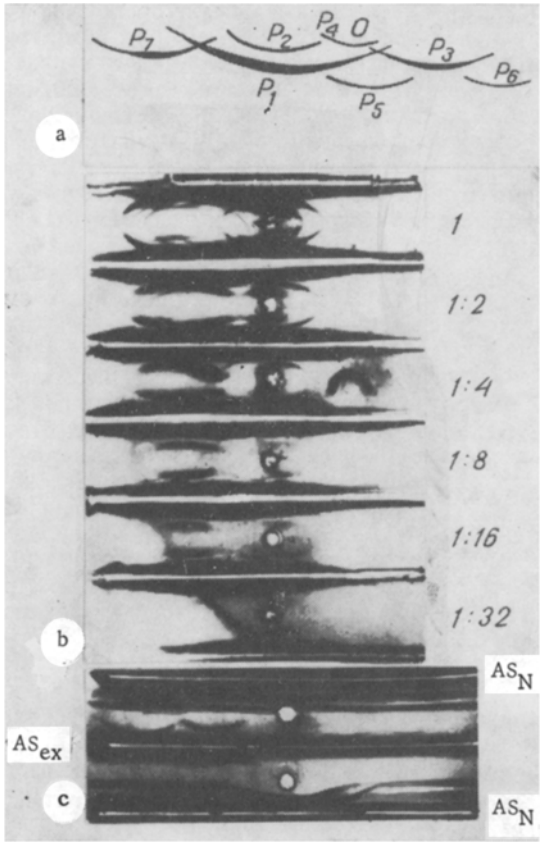

Fig. 1. Antigenic spectrum of brain structure and its dependence on concentration of antigen proteins: a) scheme of antigenic spectrum of rat brain; b) changes in lengths of arcs depending on concentration of antigen (example of motor cortex): Wells contain unexhausted rabbit serum against rat brain; c) neurospecific and nonspecific rat brain proteins: Upper and lower gutters contain unexhausted rabbit serum against nerve tissues antigen (AS_N), middle gutter contains the same AS, exhausted with extract of homologous organs (AS_{ex}), top well contains homogenate of hippocampal tissue, bottom well mixture of extract of homologous organs.

tissue was homogenized in 1.5 volumes of cold 0.005 M sodium-potassium-phosphate buffer, pH 7.4. Homogenates of structures and their dilution (by 2, 4, 8, and 16 times with the above-mentioned buffer) in all experiments were analyzed by immunoelectrophoresis in accordance with the scheme in [7]. A standard mixture of 20 individual unexhausted rabbit antibrain sera, obtained after several reimmunizations, was used. Antigens forming precipitation bands of immunoelectrophoresis were tested for the presence of carbohydrates, polysaccharides, and lipid impurities. Their relative electrophoretic mobility also was determined [4]. Differences in the concentration of antigens were assessed by student's criterion and by the method of dispersion analysis, using Fisher's criterion [6]. Differences were considered to be significant at the $P < 0.05$ level.

EXPERIMENTAL RESULTS

The antigenic spectra of the various brain structures were similar; they consisted of 5-7 precipitation bands and differed mainly in the concentrations of their component antigens. The length of the precipitation arc obtained during immunoelectrophoresis is known to be a linear function of protein concentration provided that the qualitative composition of the antigen forming it (molecules with different electrophoretic mobility, but immunologically identical) is equal [3]. This situation is valid only if the technical conditions are absolutely identical, especially the quantity and titer of antiserum.

The results showed that of the 5-7 antigens revealed by antibrain serum, three were stable in all structures tested. These were conventionally described as P_1, P_2, and P_3 (Fig. 1a). Data on dependence of the length of the corresponding precipitation arcs on the concentration of these proteins are given in Fig. 1c and in Table 1. To analyze and eliminate individual differences between the antigenic spectra of the animals, protein dilutions of five homogenates of each structure were tested.

The data in Table 1 reveal differences between the length of the corresponding arcs during two- and fourfold changes in protein concentration, despite certain variations in the lengths of the arcs depending on individual differences between the animals. With 1:8 and 1:6 dilutions of antigens the corresponding precipitation lines disappeared in most of the immunophoretic analyses, and this concentration of the test antigens indicated the limit of sensitivity of the method.

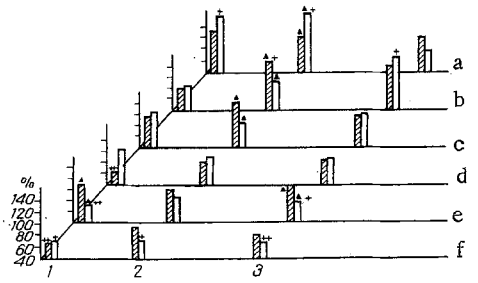

Fig. 2

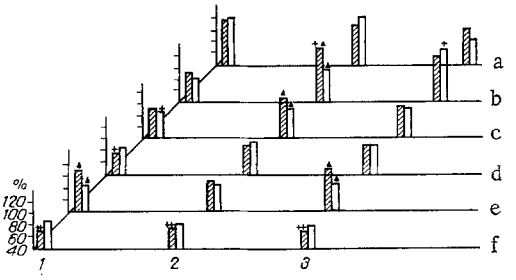

Fig. 3

Fig. 2. Dynamics of concentration of nonspecific protein P_1 in various rat brain structures in the course of training. Abscissa: 1) investigation immediately after experiment; 2) 1 day; 3) 7 days after experiment; ordinate, length of arc P_1 (in percent of that for rats of group PC); shaded columns — group T; unshaded columns — group AC. a) Brain stem; b) pyriform cortex; c) visual cortex; d) motor cortex; e) caudate nucleus; f) hippocampus. +) Difference significant when compared with group PC, $P \leqslant 0.05$; ++) the same, $P \leqslant 0.01$; triangles— differences between groups T and AC significant, $P \leqslant 0.05$.

Fig. 3. Dynamics of concentration of brain-specific protein P_3 depending on training, in different rat brain structures. Legend as to Fig. 2.

Proteins P_2 and P_3 are brain-specific, protein P_1 is nonspecific, for it was not revealed by antibrain serum, exhausted with homologous organs (Fig. 1b). With respect to mobility in an electric field, proteins P_1 and P_2 belong to the β_1-globulins and P_3 to the α_1-globulins. Histochemical staining of precipitates P_1, P_2, and P_3 showed the absence of carbohydrates, polysaccharides, and lipid impurities in their composition.

Data on the content of proteins P_1, P_2, and P_3 in the brain structures are given in Figs. 2 and 3.

During conditioning the most interesting results were obtained in the study of the visual cortex. Significant differences were found in this structure between animals of groups T and AC, characteristic of the learning process only and visible in the dynamics of all three brain antigens studied 1 day after the beginning of the experiment. The content of antigens P_1, P_2, and P_3 was higher in the rats of group T than in those of the animals of group AC. The differences between groups T and AC in the caudate nucleus were discovered in the concentrations of proteins P_1 and P_3 immediately after conditioning; this can be explained by assuming that this part of the brain participates in the organization of motor programs [12] and also in the integration of visual functions [8]. Changes also were noted in the levels of these proteins in the animals of group T 1 week after the experiment also, evidence in support of the direct participation of the caudate nucleus in memory trace fixation processes [1].

In the hippocampus conditioned reflex formation was accompanied by a sharp decrease in the P_3 level, which was not restored 1 week after the beginning of the experiment, and in the animals of group AC the decrease in the content of this protein was significant only after 1 week. Protein P_1 in the hippocampus, on the other hand, was quickly used up in the rats of group AC, and this process still continued 7 days later. The concentration of P_1 changed during learning immediately after conditioned reflex formation, but its original level in the rats of group PC was not restored after 1 day. However, the significant changes in the content of P_1 and P_3 compared with their initial level, discovered in these experiments, were not specific for learning, for no significant differences were found between the trained and active control group.

Specific changes in protein concentration likewise did not occur in the motor cortex, although deviations were observed in the content of P_1 and P_3 from their levels characteristic of PC. Differences between the P_1 and P_3 contents in the rats of groups AC and T were observed 1 day after the experiment in the pyriform cortex also) the protein concentration in the animals of group T, moreover, was higher than in the rats of group AC and in the intact animals. The P_1 level in the brain stem 1 day after conditioned reflex formation was lower than that in rats of the AC group.

Both neurospecific and nonspecific brain proteins were thus shown to participate in the processes accompanying formation of the memory trace. The results are evidence that the structure most concerned in conditioned reflex formation in a Y-shaped maze is the visual cortex. Even the level of the neurospecific protein P_2, which is quite "inert" in the other structures tested, changes in this part of the brain. The reason may perhaps be that conditioned stimulus in this model of learning was light.

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IS THE PRESYNAPTIC ACTION OF CARBACHOL LINKED WITH ACTIVATION OF THE POSTSYNAPTIC MEMBRANE?

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Besides depolarization of the postsynaptic membrane (PSM) of the muscle fiber, carbachol also causes a decrease in the quantum composition of end-plate potentials (mEPP) [2, 3]. This last fact is in harmony with data in the literature showing that other cholinomimetic drugs possess a presynaptic action [2, 6-8, 13]. Solution of the problem of whether the presynaptic action of cholinomimetics is due to their direct effect on motor nerve endings or whether it is brought about indirectly through ionic shifts is an essential preliminary to the understanding of the mechanism of this effect. In particular, the view is sufficiently widely held that the presynaptic action of cholinomimetics is linked with depolarization of motor nerve endings by potassium ions leaving the muscle fiber during activation of its PSM [9]. However, there is no factual evidence in support of the role of K^+ in the mechanisms of the effect of carbachol or of other cholinomimetic drugs on evoked liberation of mediator. Moreover, there are serious grounds for considering that a decrease in mEPP, taking place in the presence of carbachol, is due to the direct action of the mimetic on nerve endings [3].

To shed light on the role of end-plate depolarization in the mechanism of the presynaptic effect of carbachol, the investigation described below was undertaken with the following aim:

KEY WORDS: nerve-muscle preparation; carbachol; presynaptic action; end-plate depolarization.

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