CHANGES IN ANTIGENIC PROPERTIES OF SYNAPTIC MEMBRANE PROTEINS IN THE CEREBRAL CORTEX OF TRAINED RATS

L. G. Voronin, G. T. Zhangel'dina, and R. A. Danilova

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Rabbit antisera against the synaptic membrane fraction (SMF) of the cerebral cortex, which reacted with corresponding antigens in the complement fixation test, were obtained from control rats and from rats with a defensive conditioned reflex. In the "trained" serum, after removal of antibodies against heterologous antigens and against antigens of the control SMF, antibodies specifically reacting with SMF of the brain of the trained animals were found.

KEY WORDS: synaptic membranes of the brain; learning; specific antigens.

The study of the biochemical mechanisms leading to an increase in the efficiency of the synapses during learning envisages chiefly the investigation of proteins of the synaptic membranes. The immunologic approach to this problem is determined by recent attempts to explain the mechanisms of conditioning from the immunologic standpoint [1, 11] and the high specificity and sensitivity of immunochemical methods. Considering data showing the role of proteins of synaptic structures in the conditioning process, and also the fact that the synaptic membrane fraction of the rat brain contains brain-specific antigens [5], it was decided to carry out an investigation with the aim of obtaining antisera against the synaptic membrane fraction (SMF) of the cerebral cortex of control rats and of conditioned rats and to determine their specificity during interaction with antigens of the "control" and "trained" brain in the complement fixation test (CFT).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 120-150 g. To obtain immune sera, SMF of the cerebral cortex of control rats and of rats after defensive conditioning in a specially equipped chamber with a floor consisting of a metal grating were used as the antigen. The action of the conditioned stimulus, namely light (for 10 sec), concluded with an electric shock. In response to presentation of the light the rat had to jump on to a shelf in order to avoid the "punishment." The reflex was considered to be formed if the animal gave five correct responses to six presentations. Immediately after conditioning the rats were decapitated and SMF isolated by the method of De Robertis [10]. Six rabbits (three rabbits were immunized with control SMF and three with SMF isolated from the brain of the trained animals) were immunized by weekly subcutaneous injections of suspensions of SMF (4 mg protein per rabbit) in physiological saline together with Freund's stimulator (from Difco) (1:1). Blood was taken from the marginal vein of the ear on the eighth day after the third immunization. The resulting antiserum was tested for reactivity by the immunodiffusion method in agar and by the CFT [3]. If it was necessary to increase the antibody titer the rabbits were reimmunized two months after the end of the first immunization cycle. The method of back titration of complement was determined against a standard hemolysis scale. During the titration of complement its activity in 50% hemolytic units was calculated from a table compiled by means of Krogh's formula [7]. The results, shown in Tables 1 and 2, were expressed as percentages of hemolysis after the addition of five hemolytic units of complement, and the range from 5 to 70% hemolytis was taken to be the working range. To remove antibodies against heterologous antigens the antiserum was exhausted with corresponding fractions as follows: Fractions of microsomes, mitochondria, and vesicles were sedimented by centrifugation (50,000g, 30 min), 1 ml serum was added to the residue of the fractions, the residue

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TABLE 1. CFT of Non-Exhausted Rabbit Antisera with SMF of Cerebral Cortex of Trained and Control Rats

Antigen	Types of antisera and their dilution													
	against control SMF							against "trained" SMF						
	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Control SMF	5	10	15	20	30	40	50	30	40	40	50	60	—	—
Trained SMF	20	30	40	50	60	—	—	10	15	20	30	40	50	60
	$P<0,01$							$P<0,05$						

TABLE 2. CFT of Exhausted Rabbit Antiserum against SMF of Cerebral Cortex of Trained Animals

Antigen	Dilution of antiserum					
	1/2	1/4	1/8	1/16	1/32	1/64
Control SMF	—	—	—	—	—	—
Trained SMF	30	40	45	50	60	—

was suspended and allowed to stand for 18 h at 4°C, after which it was sedimented at 10,000g for 40 min and the supernatant was tested for reactivity in the CFT. In the absence of a reaction with the antigens of the above-mentioned fractions the serum was regarded as exhausted. Sera from three control and three experimental rabbits were tested separately in the CFT. In all series of experiments 10 independently obtained SMF from control and trained animals were used as the antigen. The results are summarized in Tables 1 and 2.

EXPERIMENTAL RESULTS

Antibodies in the immune sera were detected and tested for reactivity in experiments with homologous and heterologous tissues in the CFT. The antisera to SMF were found not to react positively with homogenates of liver, spleen, heart, and muscle, i.e., they possessed organ-specific differentiation. Antisera against "control" SMF and SMF of the "trained" brain reacted with fractions of mitochondria, microsomes, and synaptic vesicles, evidence of the existence of common antigens in these subcellular fractions of the rat brain, and in agreement with data in the literature [8, 9]. The titer of the control antiserum in the reaction with these antigens was 1/128 (mitochondria), 1/256 (microsomes), and 1/64 (vesicles). Antiserum against SMF of the "trained" brain reacted with titers of 1/256, 1/128, and 1/128, respectively. A comparative investigation with antigens of SMF of the trained and control animals in crossed tests showed that antiserum against the control SMF reacted with the control SMF in a titer of 1/512-1/1024, whereas in the reaction with "trained" SMF the titer was lower, namely 1/256 (Table 1). On the other hand the "trained" antiserum reacted with SMF of the "trained" brain with a titer of 1/512-1/1024, and with the control SMF with a titer of 1/256. These results indicate changes in the synaptic membranes of the trained rats used as antigen, and also that the antisera obtained were not identical. After removal of antibodies against heterologous antigens the exhausted control antiserum did not react with the fractions of microsomes, mitochondria, and vesicles, but gave a positive reaction with the control SMF with a titer of 1/64, confirming the presence of specific antigens in the synaptic membranes [5].

The reaction of SMF from the "trained" brain with exhausted monospecific immune serum was very interesting. Antisera against SMF of the trained animals were exhausted with antigens of the corresponding subcellular fraction and with antigens of the control SMF. Complete exhaustion was obtained by the use of 7.5 mg protein of the microsomes fraction, 4.5 mg of vesicle protein, 21 mg of mitochondrial protein, and 25 mg protein of the control SMF. The exhausted "trained" serum did not react with any of the subcellular fractions of the rats, including the control SMF and, as a rule, it was still able to react only with antigens of the "trained" SMF, but the activity of its antibodies in the CFT was reduced (titer of the reaction 1/16-1/32; Table 2). Hence, in antiserum against SMF from the brain of trained ani-

mals, after removal of antibodies against heterologous antigens and antigens of the control SMF, antibodies reacting specifically with SMF from the brain of trained animals were found. This fact reflects changes taking place in the proteins of the synaptic membranes during learning. It can tentatively be suggested that the differences observed in these experiments were due to changes in the so-called "conformational" determinants. In fact, the presence of antibodies and their possible conversion to conformationally dependent antigenic determinants have been demonstrated [6]. On the other hand, the writers' previous results indicate that conditioning is accompanied by conformational changes in the structure of the synaptic membranes of the cerebral cortex of rats during learning [2, 4], and as a result of this, changes may appear in the conformational determinants, leading to changes in the antigenic properties of the synaptic membrane proteins.

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