Role of Neuronal Cell Adhesion Molecules and N-Cadherin in Passive Avoidance Training of Rats

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A comparative study of the role of specific adhesion proteins, NCAM (neuronal cell adhesion molecules) and N-cadherin, was carried out on rats subjected to passive avoidance training procedure. It was shown that antibodies against the Ca^{2+}-dependent adhesion protein N-cadherin injected into the rat somatosensory cortical zone 6 h after passive avoidance training had been completed did not evoke a loss of the habit by experimental animals. At the same time, an absolute amnestic effect with respect to this reflex developed after injection of antibodies against NCAM. After injection of antibodies against the above-mentioned proteins into the dorsal part of the hippocampus, the avoidance habit also disappeared in the case of treatment with antibodies against NCAM and was kept under the influence of antibodies against N-cadherin. The data obtained testify that NCAM and N-cadherin play dissimilar roles in the formation of a memory trace in the course of training.

Keywords: NCAM, N-cadherin, neocortex, hippocampus, processes of memory formation, amnesia.

INTRODUCTION

One of the most important problems in modern neurobiology is elucidation of the molecular mechanisms underlying learning processes and formation of memory. Investigations of this problem are not only of theoretical significance; the respective data can be useful in the treatment of many diseases characterized by memory disturbances. It was shown that fixation of an acquired habit in mammals is related to expression of a number of specific proteins within the synaptic loci of the neocortex and hippocampus [1, 2]. For example, it was noted [3] that 5-6 h after a training session has been successfully performed, expression of the neuronal cell adhesion molecules (NCAM) within the synaptic membranes of the forebrain structures is nearly doubled. It is reasonable to believe that other adhesive neurospecific proteins can be involved in the mnestic processes. One of the above proteins is N-cadherin, which is known to be a member of the family of Ca^{2+}-dependent adhesion proteins. As was shown earlier [4], N-cadherin is localized mostly within the postsynaptic membrane regions. It should also be noted that homophilic bonds formed with cadherins (by the principle of protein–protein bonds) are the strongest among all the known types of adhesive interactions. The cytoplasmic domain of N-cadherin is capable of binding with cytoskeletal structures of the actin filaments; this fact suggests that the above process can represent an especially important component in the circuit of events leading to the formation of acquired habits [5]. We undertook an attempt to compare the roles performed by adhesive proteins, which belong to various protein families, in the course of training.

METHODS

Experiments were carried out on 24 mature Wistar rats weighing 150-200 g. As a model of the learning process, we tested the passive avoidance reaction (PAR), or, as it is usually called, the passive avoidance conditioning reflex (PACR), described earlier [6]. To evoke this behavioral reaction, we placed the rat in the center of the illuminated compartment of an experimental chamber so that the animal's tail was oriented toward

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the passage to the dark compartment. After careful investigation of the illuminated compartment, the animal found the passage to the dark compartment and entered the latter. We measured the latent period of such a reaction as the time from the moment we put the animal in the illuminated compartment to the complete entry of the rat into the dark compartment. Fifteen seconds after the appearance of the animal in the dark space, 50-Hz alternating current was applied to a grid floor in the dark compartment; the current intensity provided a nociceptive reaction (a range from 35 to 45 V). The passage between the above compartments remained open. The animal's behavior was monitored for 3 min. If the animal did not try to return to the dark compartment, the PAR was considered successfully trained in one presentation. Rats that repeatedly entered the dark compartment over 3 min were excluded from the experiment.

All the rats under study were divided into four groups; each group consisted of four experimental and two control animals. Six hours after the training procedure had been completed, the animals were anesthetized with kalipsol (5 mg/kg, i.p.). Four experimental animals of each of the first two groups were injected with antibodies against NCAM (first-group rats) and N-cadherin (second-group rats) into the somatosensory cortex zone through an opening in the skull. The same antibodies were injected into the dorsal region of the hippocampus of the experimental rats of two other groups (third-group rats received antibodies against NCAM, whereas fourth-group animals received antibodies against N-cadherin). Continuous single injections of antibodies with the use of a Hamilton syringe into the brain structures were performed according to the stereotaxic coordinates (p = 2 mm, D = 1 mm, H = 3.5 mm [7]). The volume of injected antibody solutions was 10 μl per each animal. Control rats were injected with physiological solution (10 μl per each animal), and these animals were subjected to a similar training procedure. Antibodies against NCAM and N-cadherin were from Sigma, USA (titer, 1:1000). Twenty-four hours after the PAR had been successfully learned, we tested the acquired habit.

RESULTS AND DISCUSSION

Our study showed that injection of antibodies against NCAM into both the somatosensory cortex zone and the dorsal hippocampal region 6 h after the PAR had been trained caused complete loss of the avoidance habit in all the experimental animals of the first and third groups. With testing of the PAR 24 h and even 48 h after the habit training, all the experimental animals of the above groups entered the dark compartment 15-25 sec after they have been placed in the illuminated compartment, i.e., the amnestic effect was fully preserved. In all control rats of these groups, the avoidance habit was completely kept, and the animals made no attempts to enter the dark compartment.

Our data are in good agreement with results of studies aimed at determining the role of neuronal cell adhesion molecules (NCAM) during formation of an acquired habit. According to the published reports [1, 2, 4], the process of formation of long-term memory in mammals is accompanied by formation of novel synaptic contacts and increased expression of neurospecific membrane glycoproteins, first of all in cellular structures within the neocortex and hippocampus. Among the agents having a special relation to the processes of transformation of synaptic apparatus, different neuronal cell adhesion molecules should be considered especially important.

In this respect, the finding that the PAR was preserved in all experimental animals injected with antibodies against N-cadherin into the neocortex and hippocampus (the second and fourth groups) was to a great extent unexpected. Testing of rats 24 h or 48 h after injection of these antibodies showed that all the experimental animals of the above groups made no attempts to enter the dark compartment for 5 min from the moment of their appearance in the experimental chamber; the behavior of these rats sharply distinguished them from the behavior of animals injected with antibodies against NCAM.

The absence of the amnestic effect after injection of antibodies against N-cadherin can be interpreted considering two suggestions. First, it is quite probable that greater doses of this kind of injected antibodies are necessary for attainment of the effect of amnesia. Yet, such an interpretation is not favored. It is reasonable to believe that under conditions of our experiments at least the phenomena of partial amnesia should be observed. The second suggestion is based on the fact that proteins of the cadherin family can play only a mediating role in training events; in this case, blocking of the extracellular domains of the above proteins with the help of antibodies will not exert a crucial effect on the processes of formation and transformation of synapses within the neuronal systems of the neocortex and hippocampus.

According to the published data, a sharp decrease in the level of a protein, drebrin, in the hippocampus is observed in patients with Alzheimer's disease [8]. The cytoplasmic domain of drebrin, similar to that of N-cad-