EFFECT OF γ-GOLBULINS TO BRAIN-SPECIFIC NONHISTONE CHROMATIN PROTEINS ON CONDITIONED REFLEX PERFORMANCE IN *Helix pomatia*

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There is much evidence of the close interconnection between RNA and protein biosynthesis and the integrative functions of the nervous system [7, 14]. It has been shown that changes in total brain DNA, RNA, or protein metabolism produced by transcription and translation inhibitors lead to marked disturbances of learning and, in some cases, of the recall of an acquired skill [6, 11, 12]. Protein compounds unique for the brain, determining the specificity of its structure and functions [3, 4], are particulary interesting; this is particularly true of brain-specific nonhistone chromatin proteins (NHCP), which are considered to take part in the regulation of activity of genes which function only in nerve cells [2, 5]. Concrete experimental data on the participation and role of particular genes or proteins of brain cells in learning and memory processes can be obtained by deliberate selective intervention in their metabolism. An effective tool for these purposes is provided by antibodies reacting specifically with particular proteins [13, 15].

The aim of this investigation was to use an immunoneurophysiological approach to study the participation of two brain-specific NHCP in learning and memory processes, using the conditioned avoidance reaction in mollusks as the model.

EXPERIMENTAL METHOD

Snails (*Helix pomatia*) weighing 30-40 g, which had been in an active state for not less than 2 weeks, were used. The animals were kept in a special container in which high humidity and alternation of daylight (12 h) and darkness (12 h) were constantly maintained and the food supply (vegetables) was replenished daily. After food deprivation for 2 days a conditioned defensive food (carrot or apple) avoidance reaction was formed in the animals [8]. One of the types of food, which was not the conditioned stimulus (apple in some experiments and carrot in others) was used as the differential stimulus. The reinforcing stimulus was a constant electric current (τ_u = 300 msec, I_m = 2 mA), passed through the food fragment and the snail's body at the time of the first chewing movements. The criterion of successful conditioning was a defensive response as soon as the snail touched the food: closing of the spiracle, contraction of the anterior part of the leg, and refusal to eat for 3 min after presentation of the food.

On the day after preliminary reinforcement of the avoidance reflex (3-5 combinations) test solutions of γ-globulins in a concentration of 0.05 mg and in a volume of 0.5 ml of physiological saline for mollusks were injected into the visceral cavity of the snails (the anterior part of the leg, the region of the mantle fold), after which the presence of the conditioned defensive reaction was tested for 6 h. Food stimuli were presented every 10-20 min.

The effects of γ-globulin fractions obtained from sera of rabbits immunized with HNCP, isolated from bovine brain [9] and identified as Np 3.5 and Np 3.6, were investigated. Nonimmune γ-globulins (γ-H), obtained from sera of intact rabbits, were used as the control.

The time course of movements of the spiracle, contact with food, and chewing movements were recorded on the N-338 high-speed automatic writer by the method in [8].

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Tests were carried out on snails of six groups, with six in each group. A conditioned carrot avoidance reflex was produced in the snails of groups 1, 2, and 3, which received injections of γ-Np 3.5, γ-Np 3.6, and γ-H, respectively. For the animals of group 4, which received γ-Np 3.5, apple was the conditioned stimulus. Animals of groups 5 and 6 (intact) were used as the control to study the time course of extinction of the conditioned food avoidance reflex (apple or carrot) without the use of biologically active substances. The percentage of defensive reactions for each moment of presentation of the conditioned stimulus was calculated for all the groups of snails.

EXPERIMENTAL RESULTS

To produce a defensive food avoidance reflex in the snails from 10 to 15 combinations of food and electrical stimulation were needed. A snail was considered to be trained if it demonstrated a defensive reflex to 10 unreinforced presentations of food. The first signs of extinction of the conditioned reflex during isolated presentation of food every 10-20 min were observed after 6-7 h.

No differences were found in the time course of conditioned reflex formation and extinction in snails taught to reject qualitatively different types of food (apple and carrot).

After injection of γ-globulins into the animals of groups 2, 3, and 4 no changes were found in their behavior or in the time course of extinction of the conditioned defensive reflex: the snails demonstrated defensive behavior in response to the conditioned stimulus — carrot (groups 2 and 3) or apple (group 4).

In all snails of group 1 the behavioral parameters recorded 1.5 h after injection of γ-Np 3.5 were depressed or conditioned defensive reactions to the food stimulus (carrot) disappeared completely: the latent period of food behavior (eating carrot) was shortened, and the components of defensive behavior (withdrawing the anterior part of the leg, closing of the spiracle) disappeared in response to contact with food. Meanwhile the mean amplitude of opening of the spiracle and the snail's motor activity increased. This effect was observed for 60-80 min, after which the conditioned defensive reflex formed previously was restored spontaneously (food behavior was not reinforced by electrical stimulation). Recovery of the conditioned reflex was accompanied by reduction of motor activity and of the mean amplitude of opening of the spiracle, and by cessation of food consumption and the appearance of defensive reactions to its presentation. After injection of γ-Np 3.5, incidentally, the snail's response to presentation of the differential stimulus (apple) was unchanged throughout the period of testing.

The experiments showed that γ-Np 3.5 depressed the defensive behavior formed to presentation of a particular kind of food (carrot), selectively in the course of tens of minutes compared with the other γ-globulins tested, but has no effect on defensive reflexes evoked by the other conditioned stimulus (apple). The reversibility and the comparatively short time of action of γ-Np 3.5 may be due to interaction of antibodies with a rapid NHCP turnover, whose half-life is comparable with the duration of the physiological effects observed [5]. The absence of any effect of γ-Np 3.6 on the conditioned defensive reflex is evidently associated with their action on different molecular and cellular mechanisms from those on which γ-Np 3.5 acts. Differences in the physicochemical properties of the NHCP investigated, and also differences in their effects on protein synthesis in vitro [9] are evidence in support of this conclusion.

Since the reflex formed after brief suppression under the influence of γ-Np 3.5 was restored spontaneously, it can be tentatively suggested that the engram formed under these circumstances was not appreciably affected. Most probably selective action directed toward protein Np 3.5 has an effect on the process of recall of the acquired skill from the memory.

The theory of the functional system [1] suggests that one cause of the effect observed in these experiments may be a disturbance of the mechanisms assessing the quality of food (trigger stimulus). Carrot and apple have both similar and different taste components. It can be tentatively suggested that γ-Np 3.5 disturbs the perception of a particular taste quality that is peculiar to carrot and not present in apple, so that carrot apparently becomes a new kind of food, which the snail does not perceive for a certain time as a conditioned stimulus.

Another explanation of the observed effect may be a change in the level of motivational excitation under the influence of γ-Np 3.5. In particular, it can be submitted that carrot, which is a more valuable food product for snails than apple, induces stronger motivational excitation, and under the influence of γ-Np 3.5, this becomes dominant whereas the initially weak motivation induced by apple remains nondominant. However, this hypothesis is less probable because the time course of formation and extinction of learning, depending on the level of motivation [10], did not differ when carrot and apple were used as the conditioned stimulus. Furthermore, no preference was found for carrot or apple with respect to the rate of their consumption and the quantity consumed by the snails.

The possibility cannot be ruled out that selective action on species-nonspecific, grain-specific NHCP Np 3.5, in the experimental situation used, may affect other mechanisms of behavior of snails also. The facts obtained are evidence that brain-specific NHCP are selectively involved in the processes of recall of information from memory. These processes are evidently closely linked with activity of the genome of the nerve cell, regulating the function of NHCP and playing an operative role in the molecular determination of processes of integrative activity.

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EXPERIMENTAL STUDY OF THE INITIAL STAGES OF PROTEIN ASSIMILATION IN LATE ONTOGENY

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The particular features of protein assimilation in late ontogeny remain inadequately studied. According to data in the literature [9] in old age hydrolysis and absorption of proteins are reduced, and this leads to the reorganization of amino-acid metabolism, with a decrease in the utilization of free amino acids for structural processes, and to changes in synthesis of digestive enzymes. It has also been shown that during aging it is the function of the transport systems that suffers rather than the sorptive properties of the enterocytes and peptidase synthesis [8]. It has been shown [7] that a high level of membrane hydrolysis of proteins, which continues into old age, helps to protect the body against protein deficiency.

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