EFFECT OF ANTIBODIES AGAINST TISSUES OF THE CRUSTACEAN CNS ON ELECTRICAL CHARACTERISTICS OF THE ISOLATED STRETCH-RECEPTOR SENSORY NEURON

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The action of antibodies against the tissues of the crayfish central nervous system on the slowly adapting neuron of the stretch receptor was investigated. Immune γ globulins, unlike nonimmune, were shown to increase the firing rate of the neuron and then to inactivate its membrane. The absence of a physiological effect of nonimmune γ globulins is regarded as evidence of the specificity of the phenomenon. The possibility that immune sera may contain antibodies against proteins directly connected with the mechanism of action potential generation is discussed.

A new and promising section in modern neurobiology is that of immunoneurophysiology, the aim of which is to study the antigenic protein structure of the brain and the physiological role of nerve-specific proteins [2-5, 9, 10]. At the present time the effect of homologous and heterologous antibodies on the memory and behavior of animals [1, 10], integral bioelectrical effects [3, 11], passive and active characteristics of the membranes of neurons and muscle cells [6, 7, 12, 14], and the ultrastructure of neurons and of glial cell elements [13] is being intensively studied.

The object of the present investigation was to study the effect of homologous antibodies against tissues of the crayfish CNS on the electrical characteristics of the slowly adapting sensory neuron (SAN) of the stretch receptor.

EXPERIMENTAL METHOD

The dissected muscle receptor organ of the crayfish was placed in a cell containing Harreveld's solution and stretched by means of a micrometer screw. The emerging segment of nerve, 5-8 mm long, containing axons of receptor neurons, was fixed to a platinum electrode. Action potentials (APs) were led through the UBPl-02 amplifier to a Cl-19A oscilloscope, an ISS-3 count rate meter, and an N-373-1 automatic writer.

The tissue of the crustacean nerve chain was homogenized in physiological saline and resuspended with adjuvant. Rabbits were immunized by the scheme described previously [3]. Blood was taken 7-9 days after the third immunization. The immune serum (IS) was dialized against Harreveld's solution, freeze-dried, vacuum-packed, and kept in a refrigerator. Nonimmune serum (NS) proteins were obtained from the blood of intact rabbits in the same way. Before the experiment the proteins were dissolved in distilled water (60 mg/ml) or Harreveld's solution (0.6%).

The background electrical characteristics of the SAN were investigated for 20-40 min, after which the effect of immune globulins, nonimmune globulins, 0.1-0.05% trypsin solutions used to destroy the capsule

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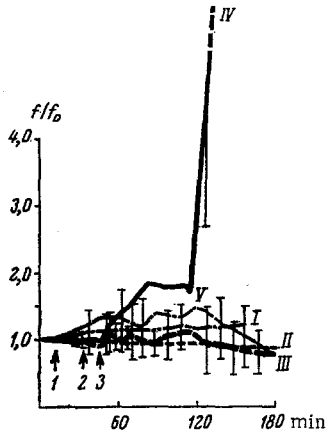

Fig. 1

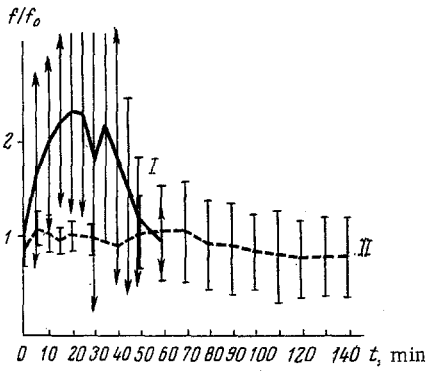

Fig. 2

Fig. 1. Mean relative spike activity of slowly adapting neuron of the stretch receptor under various conditions: I) background; II) exposure for 20 min to 0.05% trypsin solution; III) trypsin – non-immune γ globulin; IV) trypsin – immune γ globulin; V) immune γ globulin without preliminary treatment of NS by trypsin. 1) Beginning of application of 0.05% trypsin solution; 2) beginning of rinsing to remove trypsin; 3) beginning of application of γ globulins. Values shown here and in Fig. 2 are $M \pm 2.5m$.

Fig. 2. Changes in mean relative firing rate of neurons induced by application of 0.6% IS (I) and NS (II). Time counted from moment of application.

covering the sensory neuron, and also the effect of immune globulins without preliminary trypsinization of the preparation were studied. In addition, the relationship between the effect of the antibodies and their concentration in the serum was examined. In the course of the experiments the tension of the receptor organ remained unchanged. Altogether 126 cells were investigated.

EXPERIMENTAL RESULTS AND DISCUSSION

The SAN exhibited repetitive activity which maintained its initial frequency for 200-240 min (Fig. 1, I). The effect of the antibodies developed after 1-5 min and was manifested as a rapid increase in AP frequency (Fig. 1, IV). The first phase of the effect was characterized by a smooth (for 30-35 min) increase in firing rate up to a level on the average twice as high as initially, at which it remained for 20-30 min. In the second phase the spike activity of the cell became unstable: for 1-5 min its frequency increased rapidly, after which AP generation ceased. The inactivation of the neuron membrane was irreversible. The time of development and the intensity of the effect varied considerably for different neurons.

Under the influence of NS the spike activity of the neuron was unchanged for 2 h (Fig. 1, III). The results of preliminary investigations indicated that the ordinary responses of the SAN to acetylcholine and GABA remained intact. This suggests that neither the cholinergic membrane nor the inhibitory postsynapse was in any way damaged by NS.

Trypsin solutions likewise caused no change in the electrical properties of the SAN (Fig. 1, II), in agreement with data in the literature [8]. On immersion of the SAN in IS solution without preliminary trypsinization of the capsule some increase in the firing rate was observed (Fig. 1, V) but the considerable dispersion of the mean relative frequency does not permit the statistical significance of the effect to be estimated. In this series of experiments no inactivation of the SAN was observed in the course of the experiment, and the cells retained their spike activity for 80-120 min.

During investigation of the effects of 0.6% solution of immune globulins a unimodal increase in frequency was observed. The rising phase lasted from 10 to 40 min in the different experiments, after which the firing rate gradually fell to its initial level (Fig. 2).

The results are in agreement with data in the literature on the harmful action of heterologous anti-brain antibodies on nerve cell membranes [6, 11, 12]. The presence of complement in the IS used suggests that they had a lytic action on the excitable membrane of the neuron.

In some investigations [12-14] IS without complement caused no changes in the generation and conduction of APs. However, according to Khodorov et al. [7], absence of complement in IS does not abolish the disturbances of the electrical responses of the muscle cell. Possibly antibodies against proteins directly connected with the specific activity of the neuron are absent in heterologous IS. The character of development of the effects of the heterologous IS does not conflict with such a suggestion: the action of heterologous IS is exhibited after 20-60 min [12, 14], while the effect of homologous antibodies is recorded much sooner. The time required for the irreversible disturbance of AP generation under the influence of 6% IS (40-60 min) in the present experiments agrees with that obtained by other workers [6, 14].

The mechanism of AP generation in the SAN is analogous to that in the spontaneously contracting myocardial cell, so that the effects of IS can be compared in such widely different objects. The similarity between the initial phases of the changes brought about by the action of IS on the heart [7] and on SAN suggests that antibodies against membrane antigens directly connected with electrogenesis may be present in the IS which were used. In that case, the first phase of the increase in frequency could be determined by interaction between antibodies and antigens directly connected with the electrical processes on the membrane. Phenomena reflecting lysis of membrane structures indirectly connected with electrogenesis could then be superposed upon this process. Nevertheless, the fine physiological mechanism and the morphochemical nature of the effects described still remain uncertain.

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