EFFECT OF HETEROLOGOUS ANTIBODIES ON ACTION POTENTIAL GENERATION IN THE NODE OF RANVIER OF THE ISOLATED NERVE FIBER

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A single node of Ranvier of the isolated nerve fiber in frogs becomes unable to generate action potentials as a result of treatment with immune γ-globulin isolated from the serum of a rabbit immunized with frog skeletal muscle tissue. After exhaustion of the serum with antigen the γ-globulin caused virtually no change in the amplitude of the action potential or in the rate of its development. Hyperpolarization of the node membrane by a direct current and an excess of Ca^{++} ions in Ringer's solution counteract the development of the effects of immune γ-globulin.

In the study of tissue incompatibility, it is important to determine the mechanism of action of organ-nonspecific antibodies, i.e., antibodies produced against antigens common to different organs and tissues of animals of the same species (class), on cell membranes.

This paper describes the results of a study of the effect of antibodies against frog skeletal muscle antigen on the electrical activity of the node of Ranvier. The investigation is part of a series [1] aimed at studying changes in the properties of excitable membranes during cytotoxic reactions.

EXPERIMENTAL METHOD

Isolated nerve fibers of the frog (Rana temporaria) sciatic nerve were the test objects. The fiber was placed on two insulating air bridges. The theoretical circuit of the apparatus for polarization, stimulation, and recording the potentials from the single node of Ranvier was described earlier [2]. The γ-globulin used in the experiments was obtained by precipitation with 50% alcohol from rabbit serum at a temperature of 5°C. The antiserum was produced in the usual way: rabbits were immunized with frog muscle tissue extract, after preliminary removal of traces of blood (the sartorius muscles were chosen for immunization). The presence of antibodies in the rabbits' serum was determined by the complement fixation test. Frog muscle tissue extract served as the antigen. The resulting sera reacted in the CFT in dilutions of 1:160 to 1:320, and in the ring-precipitation test with homologous antigen in dilutions of up to 30 μg protein. The original solution of immune γ-globulin contained 48 mg protein per ml. In the experiments the γ-globulin solution was diluted 1:10 with Ringer's solution. In the control tests, exhausted γ-globulin was used. Exhaustion was carried out with serum of the donor animal and frog muscle extract. The exhausting dose of antigen was chosen to be excessive: 1.5 volumes antigen to 1 volume γ-golobulin. The mixture was incubated for 2 h at 37°C and the precipitate was discarded. Completeness of exhaustion was verified by the agar diffusion test. No complement was added to the γ-globulin solution.

EXPERIMENTAL RESULTS AND DISCUSSION

In these experiments immune γ-globulin induced total inhibition of action potential generation by the node of Ranvier while the resting potential was virtually unchanged. The effects developed gradually over a

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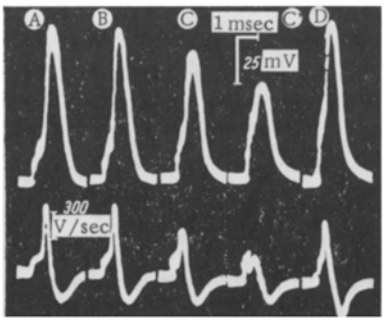

Fig. 1

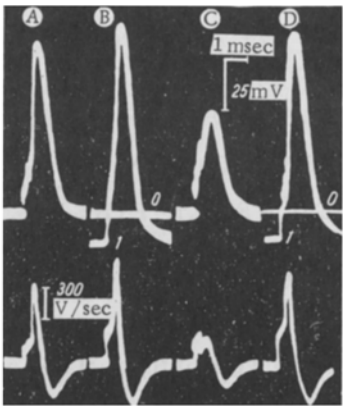

Fig. 2

Fig. 1. Action of exhausted and immune γ-globulin on electrical activity of a single node of Ranvier. A) Fiber in Ringer's solution of ordinary composition; B) exhausted γ-globulin; C and C') immune γ-globulin; D) immune γ-globulin, with 20-fold increase in Ca^{++} concentration in the solution. In all frames, bottom curve represents rate of change of action potential in time.

Fig. 2. Effect of hyperpolarization of node membrane on effects of immune γ-globulin. A) action potential in Ringer's solution; B) the same during hyperpolarization of the membrane; C) response of fiber in solution of immune γ-globulin; D) recovery of action potential generation during hyperpolarization of the membrane.

period of 6-10 min. The resistance of the membrane measured in 2 experiments by pulses of direct current fell during the action of immune γ-globulin to 81 and 64% respectively of the initial value, measured in Ringer's solution of normal composition. When the fiber was washed with Ringer's solution the node gradually regained its ability to generate action potentials. As Fig. 1 shows, exhausted γ-globulin (Fig. 1B) caused virtually no change in the amplitude of the action potential or in the rate of its development, whereas immune γ-globulin (Fig. 1C and C') changed these parameters considerably. The blocking action of immune γ-globulin on the node of Ranvier was considerably weakened by increasing the Ca^{++} ion concentration in the solution (Fig. 1D).

The degree of recovery of amplitude of the action potential under the influence of Ca^{++} ions varied from specimen to specimen within wide limits. By contrast, hyperpolarization of the node membrane (Fig. 2), when altered by the immune γ-globulin, caused the almost complete recovery of the action potential – to 97.2 ± 3.3% (five experiments) of the maximal spike amplitude during hyperpolarization of the membrane by 15 mV. Recovery of the maximal rate of development of the ascending portion of the action potential was less complete (85.8 ± 12.9%).

The effects of immune γ-globulin are outwardly very similar to those of procaine on the node of Ranvier [2, 3]. Like procaine (10^{-4} g/ml), immune γ-globulin inhibits the ability of the node membrane to generate action potentials. Hyperpolarization of the membrane and an excess of Ca^{++} ions in the solution in either case weaken or almost completely abolish the alteration. The steepness of rise and, in particular, the amplitude of the action potential return close to their initial values. Since both factors used (excess of Ca^{++} and hyperpolarization) weaken sodium inactivation of the membrane [4] it is natural to conclude that the inhibitory action of immune γ-globulin on nerve fibers is due chiefly to inactivation of sodium permeability. However, it must be emphasized that, unlike the effect of procaine, the action of immune γ-globulin develops after a considerable latent period.

In previous experiments on cells of the guinea pig myocardium the writers showed that the cytotoxic effect of serum antibodies may develop in the absence of complement [1]. The results of the present investigation give further confirmation of this conclusion.

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