Effects of Homeopathic Doses of Antibodies to S100 Antigen on Electric Characteristics of Neuronal Membranes

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Various dilutions of antibodies to brain-specific protein S100, including those prepared by multiple consecutive dilutions up to 10^{-12} and 10^{-400} of total weight, produced similar effects on the membranes of *Helix pomatia* giant neurons, which varied only quantitatively. They induced membrane depolarization, reduced the amplitude or completely blocked the action potential, accelerated the maximal rise of the action potential, reduced maximal conductance, and facilitated the steady-state inactivation of ionic channels.

Key Words: *brain-specific S100 antigen; antibodies to S100; passive and active properties of neuronal membrane; homeopathic dilutions of antibodies to S100.*

Previously, when analyzing the physiological role of brain-specific S100 protein on the subesophageal complex of *Helix pomatia* ganglia, we found that anti-S100 antibodies (AS100) reversibly changed both the passive and active properties of the neuronal membranes [1]. The study of antibody-antigen interaction on the excitable membrane was continued in the present work by investigating the effects of physiologically active concentrations and homeopathic dilutions (10^{-12} and 10^{-400} fractions of total weight) of AS100 on the electric properties of neuronal membranes.

MATERIALS AND METHODS

Experiments were carried out on *Helix pomatia* which had been active for at least 2 weeks before the experiment. Electric activity of spontaneously active (V2-V6, PPa1 and PPa2) neurons of the subesophageal complex of ganglia [2] was recorded with a Hitachi apparatus. The following parameters were analyzed: the resting membrane potential, the amplitude of action potential, slope and maximal increment of the action potential (V_{max}), frequency of spike discharges, current-voltage and inactivation curves for inward and outward currents. Some measurements of Ca^{2+} currents were performed in isolated neurons by the voltage clamp technique.

Electric activity was recorded by glass microelectrodes with 7-20 mΩ resistance. The same electrodes were used for intracellular stimulation by de- and hyperpolarizing pulses up to 30 V voltage.

AS100 were applied in physiological concentrations of 1.2%, 2%, 6%, and 12%, and in potentiated forms (homeopathic dulitions 10^{-12} and 10^{-400}).

Nonimmune serum and antiserum to sheep erythrocytes [5] served as the controls. The data were analyzed statistically using Student's *t*-test.

RESULTS

Both nonimmune serum and antiserum to sheep erythrocytes caused no significant changes in the electric properties of neuronal membranes (Fig. 1).

AS100 produced a dose-dependent decrease in V_{max}. The effect measured 20 min after application was 22-28% for 0.2% AS100 and 37-45% for 2% concentration. When applied in higher concentrations (6% and 12%), AS100 reduced V_{max} by 60-80% within the

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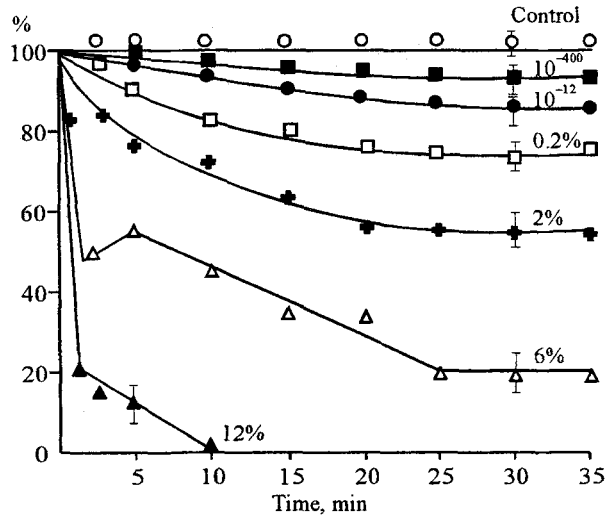

Fig. 1. Changes in the maximum rate of action potentials in giant neurons of the *Helix pomatia* subesophageal ganglion caused by various dilutions of antibodies against S100.

first 10-15 min (Fig.1). Potentiated forms of AS100 caused similar although less pronounced depression, reducing V_{max} by 8-14% after 30-35-min application (Fig. 1).

Irrespective of the concentration, AS100 diminished the amplitude of inward current and increased the steady-state inactivation at zero conditioning pulse: the voltage-current plots and inactivation curves were shifted towards negative membrane potentials (Fig. 2). It is likely that the decrease in the amplitude of inward current was related to decreased conductivity of inward current channels, rather than increased steady-state inactivation. These two factors probably explain the decrease in the amplitude of action potential observed in this and previous work [3, 7]. AS100 did not affect the characteristics of outward current.

Thus, various dilutions of AS100 including so-called homeopathic dilutions applied to the subesophageal ganglia of the *Helix pomatia* caused similar changes in the passive and active properties of neuronal membranes.

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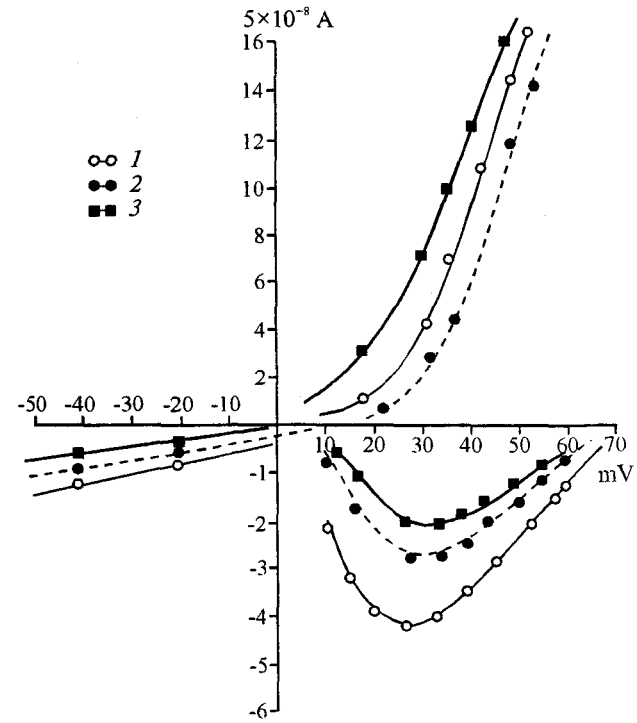

Fig. 2. Current-voltage (*I/V*) characteristics of inward current channels in giant neurons of the *Helix pomatia* subesophageal ganglion measured in saline (*2*) and after application of AS100 in concentration of 12% (*1*) and at a 10^{-12} homeopathic dilution (*3*). The initial membrane potential was 43 mV.

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