PHYSIOLOGY

Modulatory Action of Combined Treatment with Antibodies against S100 Protein and Low Doses of this Protein on Membrane Effects of Quinine

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Antibodies against calcium-binding protein S100 (AB-S100) 1.5-fold increased, while quinine 2-fold decreased the frequency of action potential generation in B4 and B6 neurons. Application of quinine against the background of AB-S100 treatment returned this parameter to the initial level. Pretreatment with AB-S100 in low doses prevents changes the frequency of action potential generation induced by application of AB-S100 in the initial dilution. The duration of action potential increased by 1.6 times after application of AB-S100 and quinine, while after application of quinine increased this parameter by more than 6 times. AB-S100 decreased maximum inward current by 20%. Our experiments demonstrated a modulating effect of combined administration of AB-S100 and its low doses on the membrane effects of quinine.

Key Words: *calcium-binding protein S100; anti-S100 antibodies; quinine; neuronal excitability; ion influx*

S100 proteins belong to a group of low-molecular-weight Ca^{2+}-binding proteins carrying Ca^{2+}-binding domains. Nerve-specific S100 proteins are characterized by brain specificity and evolutionary stability and are capable of binding calcium ions [9,12]. These proteins regulate cell-cell communications, cell growth and motility, energy metabolism, and transcription [6].

Monospecific antibodies against S100 (AB-S100) modify electrical characteristics of the neuronal membrane and decrease the amplitude of action potential (AP). These changes are accompanied by membrane depolarization and dysfunction of Ca^{2+} channels [2,4,5,10,15]. Previous studies showed that AB-S100 impair synaptic transmission in hippocampal slices and abolish the induction of long-tern postsynaptic potentiation [7,13,14].

Experiments on isolated nervous system of *Helix lucorum* identified two types of neurons demonstrating different reaction to AB-S100 application: the frequency of AP generation decreased in B1 and B17 neurons, but increased in B4 and B6 neurons [1,7]. The main properties of the neuronal membrane and synaptic structures do not decrease when nervous tissue preparations are preincubated with low doses of AB-S100 (10^{-12} M, LAB-S100) prior

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to AB-S100 treatment (so-called protective phenomenon). The bipathic or neuroprotective effect is observed during combined administration of the compound and its potentiated form in high dilutions [7,11]. The mechanisms of this phenomenon require further investigations.

Recent studies showed that S100 modulate function of K^+ channels in *Helix pomatia*, including Ca^{2+}-dependent K^+ channels [12]. Here we studied the effect of LAB-S100 ("Materia Medica Holding" Research-and-Production Company) on ion channels in a relatively simple neuronal system of *Helix lucorum*.

MATERIALS AND METHODS

Experiments were performed on mature *Helix lucorum* of similar weight and size. The mollusks were maintained in glass terrariums at room temperature, high humidity, and excess of food. The snails were in the active state for at least 2 weeks before the experiment. The study was conducted on identified spontaneously active B4 and B6 neurons of the subesophageal ganglion [3]. The preparation of CNS included the complex of subesophageal ganglia. Before the study all animals were cooled in ice-cold water for 20-30 min [1]. The measurements were performed at 20-22°C using intracellular glass microelectrodes (resistance 5-40 MΩ) filled with 2.5 M KCl. Monospecific AB-S100 were diluted 1:5 with a saline solution (PS) for snails [15].

Monospecific immune serum to Ca^{2+}-binding protein S100 was kindly provided by S. M. Sviridov (Institute of Cytology and Genetics). The immune serum was obtained by immunization of rabbits with S100 protein from bovine brain (*i.e.* S-100b fraction). Methylated bovine serum albumin served as the carrier during immunization. The immune serum was dialyzed in distilled water, lyophilized, and stored until the study. The concentration of the basic solution was 12 mg lyophilized product per 1 ml solution. Nonimmune rabbit serum served as the control.

Quinine (Sigma) in a concentration of 0.2 mM was used for blockade of Ca^{2+}-dependent K^+ channels [8].

Membrane characteristics of identified neurons were determined in PS and then recorded in AB-S100 or LAB-S100 solutions. The modulation of the effects of quinine after preincubation with AB-S100, LAB-S100, and LAB-S100+AB-S100 was studied. Since quinine significantly increases AP duration, electrical characteristics of neurons do not return to normal even after washout. Hence, the study was performed in 4 variants:

- 20 min PS→20 min quinine→washout;
- 20 min PS→30 min AB-S100→20 min quinine+AB-S100→washout;
- 20 min PS→20 min LAB-S100→20 min quinine+LAB-S100→washout;
- 20 min PS→20 min LAB-S100→20 min LAB-S100+AB-S100→20 min LAB-S100+AB-S100+quinine→washout.

In series I the frequency of AP generation (number of impulses per minute), resting membrane potential, AP generation threshold, and AP duration were recorded using intracellular microelectrodes. We evaluated changes in the frequency after application of the test solutions (% of the basal level). In series II we recorded inward and outward ion currents in B4 and B6 neurons of the visceral ganglion using the patch-clamp technique. The experiments were performed in 3 variants: AB-S100 (n=13), LAB-S100 (n=17), and LAB-S100+AB-S100 (n=9).

The results were analyzed by Student's t test and Mann—Whitney U test.

RESULTS

Replacement of PS for LAB-S100 practically did not affect the frequency and electrical characteristics of neurons. Application of quinine after pretreatment with LAB-S100 decreased the frequency of AP generation to 28% (p<0.01, Fig. 1, *a*) and 5-fold increased AP duration (p<0.01, Fig. 1, *b*). The observed changes did not differ from those in PS. AB-S100 1.5-fold increased the frequency of AP generation (p<0.01, Fig. 1, *a*), but had no effect on resting membrane potential, AP generation threshold, and AP duration.

Combined treatment with AB-S100 and quinine decreased the frequency of AP generation to the basal level (Fig. 1, *b*). AP duration increased by 1.6 times, less markedly than after treatment with quinine (more than by 6 times, Fig. 1, *b*). Therefore, AB-S100 prevent lengthening of AP after blockade of Ca^{2+}-dependent K^+ channels. LAB-S100 produced a protective effect on the frequency of AP generation during application of AB-S100 (Fig. 1, *a*).

After treatment of the preparation with LAB-S100 and then with LAB-S100+AB-S100, quinine did not decrease the frequency of AP generation: it 1.5-fold surpassed the baseline. AP duration in the solution of LAB-S100+AB-S100+quinine increased by 2.2 times (p<0.01). Similar changes were observed under the influence of AB-S100 without preexposure to LAB-S100 (Fig. 1, *b*).

The maximum inward current decreased by 20% after application of AB-S100 (Fig. 2). Application

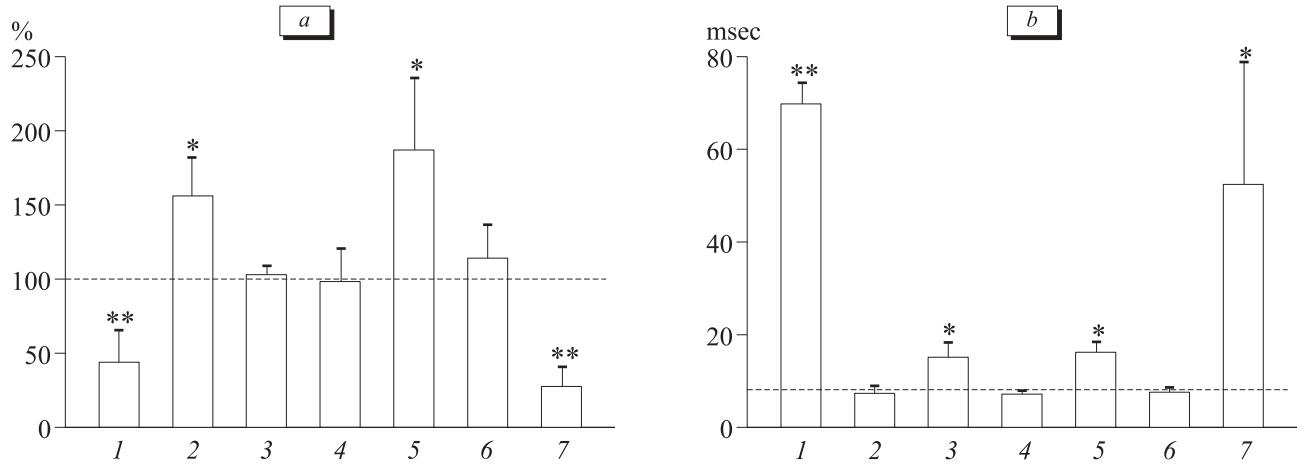

Fig. 1. Ratio between the frequency of AP generation in B4 and B6 neurons before and after application of solutions (*a*); AP duration in B4 and B6 neurons (*b*). Quinine (*1*); AB-S100 (*2*); AB-S100 and quinine (*3*); AB-S100 in LAB-S100 solution (*4*); LAB-S100, AB-S100, and quinine (*5*); LAB-S100 (*6*); LAB-S100 and quinine (*7*). Dotted line: frequency in PS. *$p<0.05$ and **$p<0.01$ compared to PS.

of LAB-S100 increased the maximum inward current by 7%.

Our results suggest that the protective effect of LAB-S100 on Ca^{2+}-dependent K^+ channels is not mediated by changes in AP generation threshold and AP duration. Therefore, LAB-S100 have a protective effect on ion channels involved in AP generation and providing Ca^{2+} entry into cells. These data show that S100 protein plays a role in the function of membrane structures by modulating Ca^{2+}-dependent K^+ channels. Our results are consistent with published data on the effect of S100 protein [12]. AB-S100 had opposite effects on cells of various types, which depended on the function of the antibody-affected membrane element. It is generally accepted that the major biological function of S100 proteins is binding of Ca^{2+} [9]. Calcium ions regulate physiological activity and membrane processes in cells [6]. These data are consistent with the notion that S100 proteins play a role in extracellular signal transduction to the intracellular space of neurons [6,9]. The cascade chain of signal amplification in intracellular regulatory systems is an important element for the analysis of the effects of AB-S100 in low doses. For example, water-soluble cAMP analogue 8-Br-cAMP abolishes the inhibitory effect of AB-S100 on long-term potentiation [14]. The cascade amplification system probably mediates the influence of autoantibodies in low doses on metabolic and transformational processes in nervous structures. We showed that AB-S100 in low doses invert the effects of quinine and basic solution of AB-S100. This is probably a general mechanism for the effect of compounds in low doses on the cascade chain of signal amplification in intracellular regulatory systems.

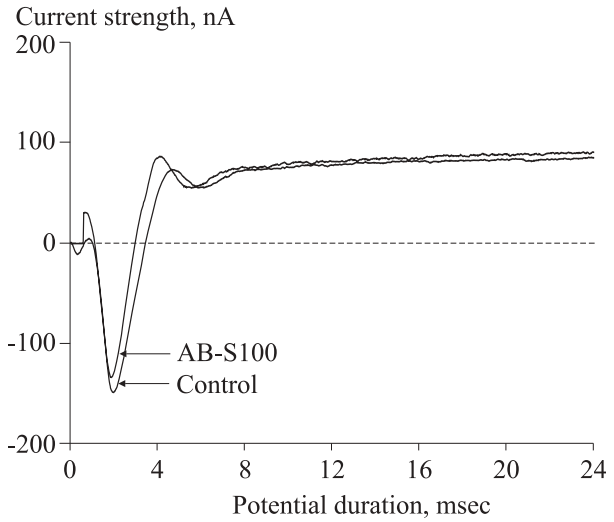

Fig. 2. Effect of AB-S100 on inward and outward currents in the B4 neuron. This cell exhibits the increase in spike activity in response to AB-S100 treatment. Maintained and test potential, 50 mV.

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