Effect of Proteins from the Spirochete *Borrelia burgdorferi sensu lato* on Myelinated Nerve Excitability

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We studied the effect of spirochete *Borrelia burgdorferi sensu lato* cell membrane proteins on excitability of myelinated nerve fiber. It was found that cell surface proteins of spirochetes *B. burgdorferi s. s.* bind to Ranvier nodes of the axon and to Schwann cells. Binding of *B. burgdorferi s. s.* and *B. garinii* to the nerve fiber modulates the amplitude and conduction velocity of the action potential, while *B. afzelii* had no effect on these parameters. The decrease in the spike amplitude and conduction velocity during sorption of *B. burgdorferi s. s.* or cell wall proteins was accompanied by desorption of membrane-bound calcium.

Key Words: *Lyme disease; spirochete Borrelia burgdorferi sensu lato; myelin; action potential; membrane-bound calcium*

Structural changes and decrease in myelin content during demyelination (DM) result in the loss of nerve excitability and decelerate conduction of action potential (AP). DM can be caused by various factors, including gene mutations, poisoning, radiation exposure, avitaminosis, traumas, and inflammation. Tick-borne borreliosis (Lyme disease) is a disorders associated with DM.

Lyme disease is a systemic disorder of humans and animals caused by *Borrelia burgdorferi sensu lato* (*B. burgdorferi s. l.*) transmitted by ticks from the population of small animals to the population of large animals and humans. Only 3 of 11 specimens of this species, *Borrelia burgdorferi sensu stricto* (*B. burgdorferi s. s.*), *Borrelia garinii* (*B. garinii*), and *Borrelia afzelii* (*B. afzelii*), are pathogenic for humans [7,12,13]. *B. garinii* genospecies causes pathology of the nervous system, including radiculoneuritis, encephalopathy, and meningitis. *B. afzelii* genospecies causes skin diseases, including erythema migrans, borrelial lymphocytoma, and acrodermatitis chronica atrophicans. Various spirochete *B. burgdorferi s. l.* species are selectively adsorbed on different types of cells, which is determined by binding of surface proteins to proteoglycans [11], integrins [9], galactocerebroside, lactosylceramide, or some gangliosides [5,10].

Here we studied the effect of proteins from various spirochete species on nerve fiber excitability.

MATERIALS AND METHODS

Experiments were performed on myelinated nerves and single myelinated nerve fibers of the sciatic nerve from grass frog *Rana temporaria*. Three bacterial strains of spirochete *B. burgdorferi s. l.* species isolated in Moscow region in 2002 [13] were used: strain Mr9 (*B. burgdorferi s. s.*), strain Nr198 (*B. garinii*), and strain Mr2 (*B. afzelii*). Spirochetes were grown in BSK-II medium [6] and isolated by differential centrifugation. For isolation of surface proteins, spirochetes were incubated with 1% octylglucoside (Sigma) for 15 min. Solubilized surface proteins from the bacteria were dialyzed in phos-

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phate buffered saline with 0.001% NaN_3 for 3 days to remove the detergent. Changes in myelinated nerve excitability (spike amplitude and conduction velocity) were recorded extracellularly [4]. Nerves were incubated in a medium containing 5.85 mg/ml NaCl, 14.9×10^{-2} mg/ml KCl, 11.9×10^{-2} mg/ml $CaCl_2$, and 64.4×10^{-2} mg/ml Hepes (pH 7.4) in the presence of bacteria or isolated proteins and the kinetics of changes in AP amplitude and conduction velocity was recorded.

The nerve was stimulated with rectangular pulses (1 V, 0.3 msec, 100 Hz). The AP amplitude and conduction velocity were estimated on a S1-18 oscillograph. The data were processed using original software for calculation of AP amplitude and conduction velocity. The content of membrane-bound calcium (MBC) and localization of isolated bacterial proteins on the nerve fiber were assessed fluorometrically. MBC content was measured using a fluorescent probe chlortetracycline. Localization of *B. burgdorferi s. s.* proteins was estimated with FITC [1]. Fluorescence of the probe was recorded intracellularly under a Lyumam I-3 luminescence microscope at 520 nm (excitation wavelength 400 nm) [2,8].

RESULTS

The fraction of supernatant proteins contained surface bacterial proteins that were identified with a marker of outer membrane protein (OspA), while intracellular bacterial proteins were present in the precipitate (identified by the presence of flagellin, a protein localized in spirochete periplasmic space, Fig. 1, *a*). During incubation with the nerve fiber, surface proteins from the wall of *B. burgdorferi s. s.* strain Mr9 bind to the axolemma of the nerve fiber in the Ranvier node and to Schwann cell plasma membrane (Fig. 1, *b*, *c*). It can be hypothesized that bacteria of the genus *Borrelia* can bind to these segments of the nerve fiber. We found no primary accumulation of proteins in a certain compartment of the nerve fiber. The bacteria binding over all length of the myelinated nerve fiber either produce a passive effect on the nerve or trigger DM process, both should affect excitability of the nerve fiber.

The AP amplitude and conduction velocity decreased after treatment with *B. burgdorferi s. s.* strain Mr9 (Fig. 2). The decrease in AP amplitude was probably associated with an increase in the re-

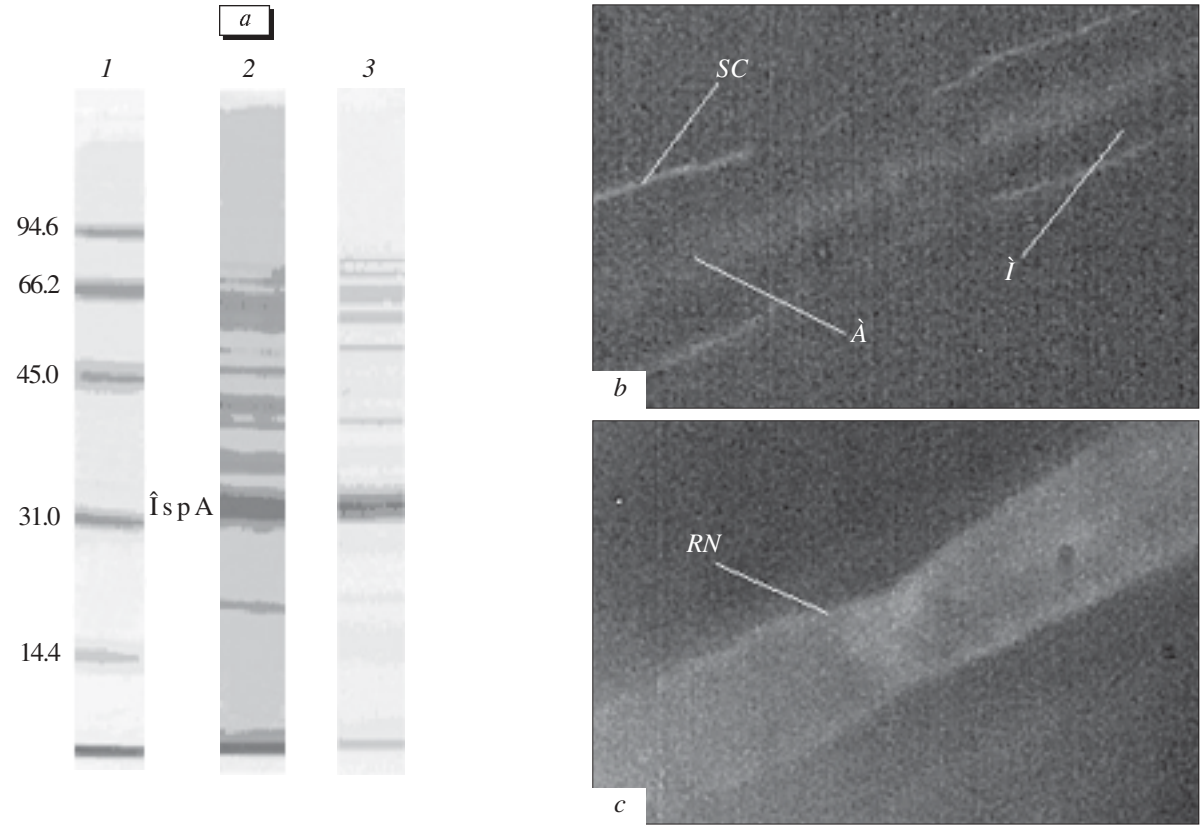

Fig. 1. Localization of surface proteins from *B. burgdorferi s. l.* in the myelinated nerve. (*a*) Protein electrophoresis: (*1*) protein molecular weight markers; (*2*, *3*) proteins from bacteria and bacterial supernatant (*B. garinii* strain Nr198). OspA, outer membrane protein of A. (*b*) Fluorescence of the nerve fiber without FITC-labeled proteins. (*c*) Distribution of FITC-labeled surface proteins from *B. burgdorferi s. s.* in the nerve fiber. SC, Schwann cell; A, axon; M, myelin; RN, Ranvier node. (*b*, *c*) ×60.

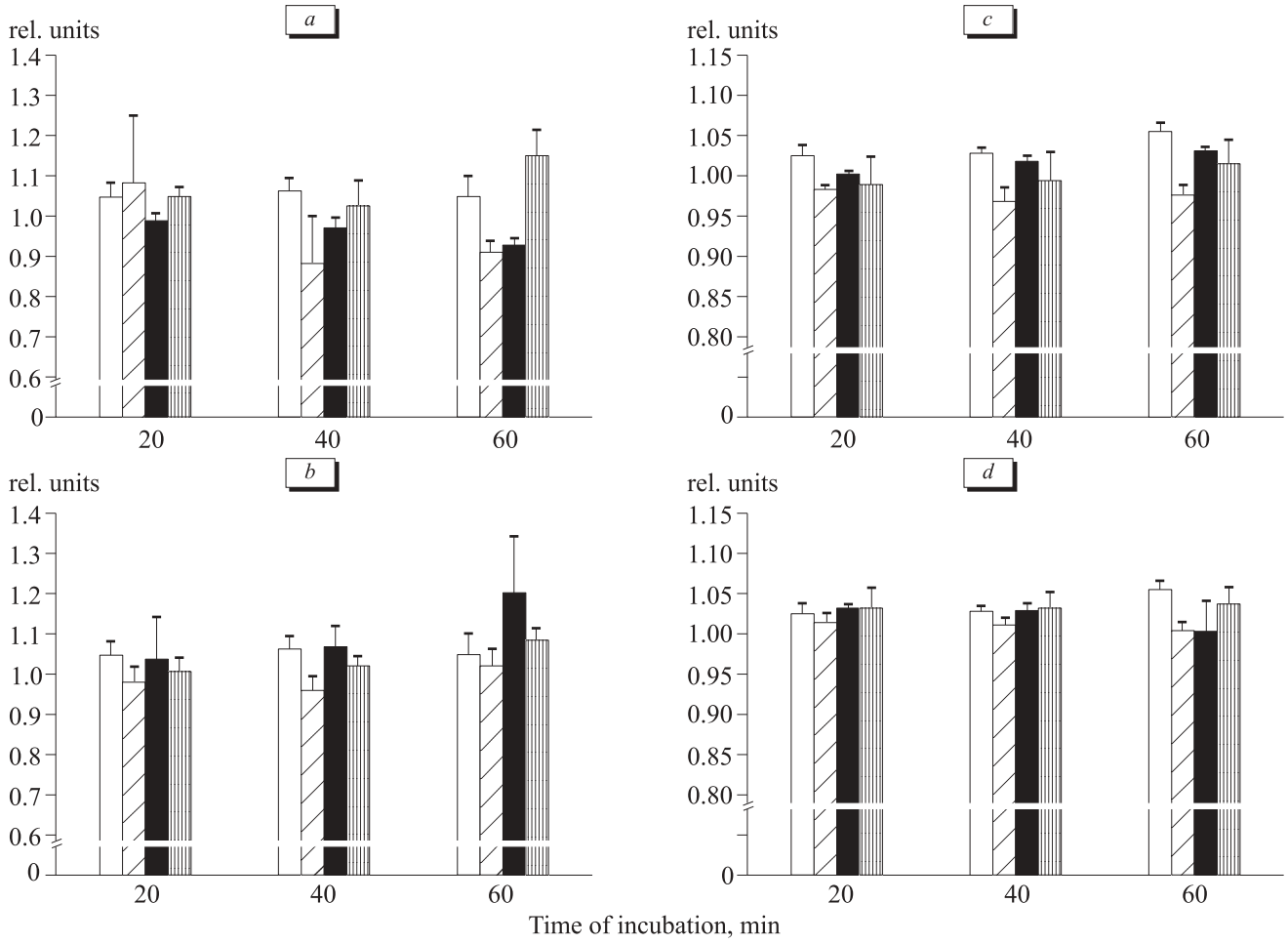

Fig. 2. Amplitude (*a*, *b*) and conduction velocity of AP (*c*, *d*) in the myelinated nerve under the influence of bacteria (*a*, *c*) and cell wall proteins (*b*, *d*). Light bars, control; slant shading, Mr9 (*a*, *c*) or Mr9 proteins (*b*, *d*), 0.05 mg/ml; dark bars, Nr198 (*a*, *c*) or Nr198 proteins (*b*, *d*), 0.05 mg/ml; vertical shading, Mr2 (*a*, *c*) or Mr2 proteins (*b*, *d*), 0.05 mg/ml.

sistance of the medium between single fibers and/or DM. In the latter case, changes in myelin structure and content in the paranodal region contribute to changes in nerve permeability. The paranodal regions of the axolemma contain Ca^{2+}- and ATP-dependent K^+ channels. Function of these channels determines rhythmic excitation during DM. Adsorption of the bacteria to nerve fiber surface probably impairs the function of Na^+ channels in the Ranvier node, which results in blockade of AP. This reversible process is most pronounced by the 40th minute of incubation. It should be emphasized that the decrease in AP conduction velocity occurred earlier than AP block. Hence, binding of bacteria to the surface of the myelinated nerve or structural change in myelin were accompanied by activation of additional K^+ channels, membrane hyperpolarization, and decrease in AP amplitude.

Incubation of the nerve with proteins from strain Mr9 was also followed by a decrease in AP amplitude and conduction velocity by the 40th and 60th minutes, respectively (Fig. 2). It can be hypothesized that the effects of proteins on the nerve are associated with a decrease in Na^+ channel conductance, rather than activation of additional K^+ channels.

The observed changes in excitability of the myelinated nerve fiber under the influence of bacteria and isolated proteins can be determined by changes in the concentration of ionized MBC. Myelin can accumulate extracellular Ca^{2+} and, therefore, can serve as a source of additional Ca^{2+} [3]. In light of this, we measured MBC concentration on the axon surface (Fig. 3). Incubation of the nerve in the medium with proteins from strain Mr9 (0.05 mg/ml) for 15 min was followed by reversible desorption of Ca^{2+}. Ca^{2+} is probably desorbed from myelin, but its sorption proceeds in the Ranvier node. The redistribution of MBC was accompanied by changes in AP amplitude and conduction velocity (Fig. 2, *b*, *d*). Ion sorption in the Ranvier node probably blocks AP, while desorption of MBC from myelin initiates DM. Published data show that the optimal ratio of

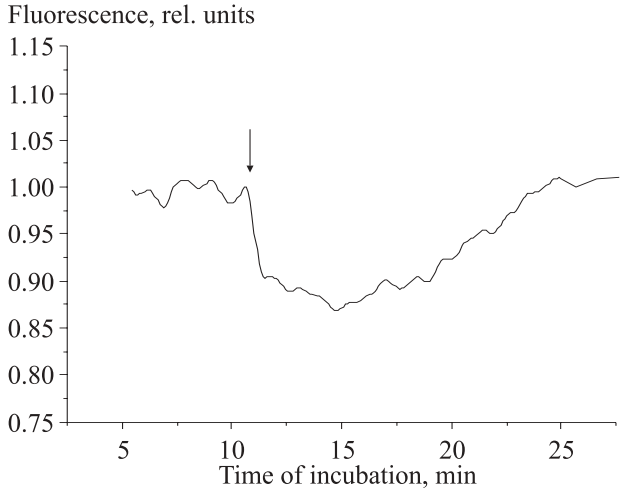

Fig. 3. MBC concentration during incubation of the myelinated nerve with proteins from strain Mr9 (0.05 mg/ml). Arrow: administration of substance to the preparation.

MBC in myelin maintains its compact structure, while ion desorption leds to myelin loosening, decrease in its viscosity, and deceleration of AP conduction [3].

The influence of *B. garinii* strain Nr198 on the nerve was accompanied by a decrease in AP amplitude and conduction velocity (Fig. 2, *c*). Binding of *B. garinii* cells to the nerve fiber also induces DM and decreases conductance of Na^+ channels. However, the amplitude of AP did not return to normal under these conditions. Incubation of the nerve with *B. garinii* proteins had no effect on nerve excitability (Fig. 2) and MBC concentration. The study of nerve excitability after incubation with *B. afzelii* strain Mr2 or isolated proteins revealed no changes in AP amplitude (Fig. 2, *a*), conduction velocity (Fig. 2, *b*, *d*), and MBC concentration.

Our results show that various spirochete species bind to the surface of the myelinated nerve fiber and modulate (*B. burgdorferi s. s.* strain Mr9 and *B. garinii* strain Nr198) or do not modulate (*B. afzelii* strain Mr2) the amplitude and conduction velocity of AP. The decrease in AP amplitude and conduction velocity is induced by sorption of *B. burgdorferi s. s.*, *B. garinii*, and proteins from *B. burgdorferi s. s.* and is accompanied by MBC desorption (*B. burgdorferi s. s.*).

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