BIOPHYSICS AND BIOCHEMISTRY

Serum from Patients with Multiple Sclerosis Affects Electrical Activity in Neocortical Slices of Guinea Pigs

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The serum from patients with multiple sclerosis changes electrical activity of neocortical slices of guinea pigs. This test can be used for the diagnosis of multiple sclerosis and assessment of treatment efficiency.

Key Words: *multiple sclerosis; diagnostics; neocortical slices; electrical activity*

Multiple sclerosis (MS) is a progressive chronic disease affecting the central nervous system. The peculiarities of its clinical course makes it extremely difficult to assess the efficiency of therapy. Taking into account the medical and social significance of this problem, it seems important to develop new efficient tests for the diagnosis of this disease [1]. For this purpose we tested brain slices from guinea pigs which have been used for many years as a convenient model for the assessment of brain tissue functions.

MATERIALS AND METHODS

In vitro experiments were carried out on neocortical slices from guinea pigs in accordance with the European Communities Council Directive for care and use of animals in experimental procedures. The animals (250-400 g) were decapitated with a guillotine, frontal slices (400-500 μ) of the sensorimotor cortex were prepared with a mechanical slicer and placed in a thermocontrolled experimental chamber perfused with a standard carbogen-saturated solution (35°C, pH 7.2) of the following composition (mmol/l): 131.5 NaCl, 5 KCl, 1.24 KH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 13.5 $NAHCO_3$, 10 glucose. The flow rate of 4 ml/min was provided by a peristaltic pump. The recording of electrical activity was started after 2-h adaptation. The stimulating bipolar tungsten electrodes were placed on the grey/white matter border, the recording electrodes (10-μ tips, filled with 0.1 M NaCl) were placed in the same plane within layers IV-V. Stimulation, recording, and primary analysis of the data were performed with a computer using software developed at the Protvino Branch of the Institute of Nuclear Physics. The evoked population responses (EPR) of cortical neurons with a latency of 2-4 msec, duration of 10-15 msec and the mean amplitude of 0.1-0.5 mV were recorded. Serum samples were obtained by 10-min centrifugation of venous blood and stored in a fridge until use.

The effects of the serum of MS patients during acute attack and remission were compared with those of healthy donors, patients with some other CNS diseases (acute disseminated encephalomyelitis and Parkinson's disease), and sterile serum preparations. The study included 19 patients (n=43) and 5 healthy vo-

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lunteers (n=10). The experiments without serum application (n=20) served as the control.

The data were analyzed statistically at a significance level of 0.95.

RESULTS

In the control series, EPR remained unchanged for at least 5 h. Perfusion with the medium containing 3-4 ml serum obtained from acute MS patients (10-20% of the total circulating volume) induced irreversible decrease in EPR amplitude (Fig. 1, *a*). Despite some variations in the onset and magnitude of this depression with different serum samples, this irreversible decrease in EPR was a typical response to serum from MS patients with acute attack. EPR started to decrease after 10-20-min perfusion and were reduced by 30-100%, *i. e.* could be completely blocked. The serum from healthy donors in the same concentration did not affect EPR, while the serum of MS patients during remission increased their amplitudes almost 1.5-fold. This increase persisted for 1 h and then gradually declined (Fig. 1, *a*). We compared the effects of the serum from acute MS patients (n=18; 9 patients) and patients in remission (n=10). Two portions of a single sample taken from individual patient showed only minor variations in their effects. Despite considerable variations between individual effects, the serum from acute patients always reduced EPR, while that from patients in remission slightly increased them and this enhancement notably exceeded the oscillations observed during perfusion with healthy serum. Thus, these effects were statistically significant.

Four chronic patients were followed up for 1.5 years during which they experienced several acute attacks of the disease followed by remissions. The se-

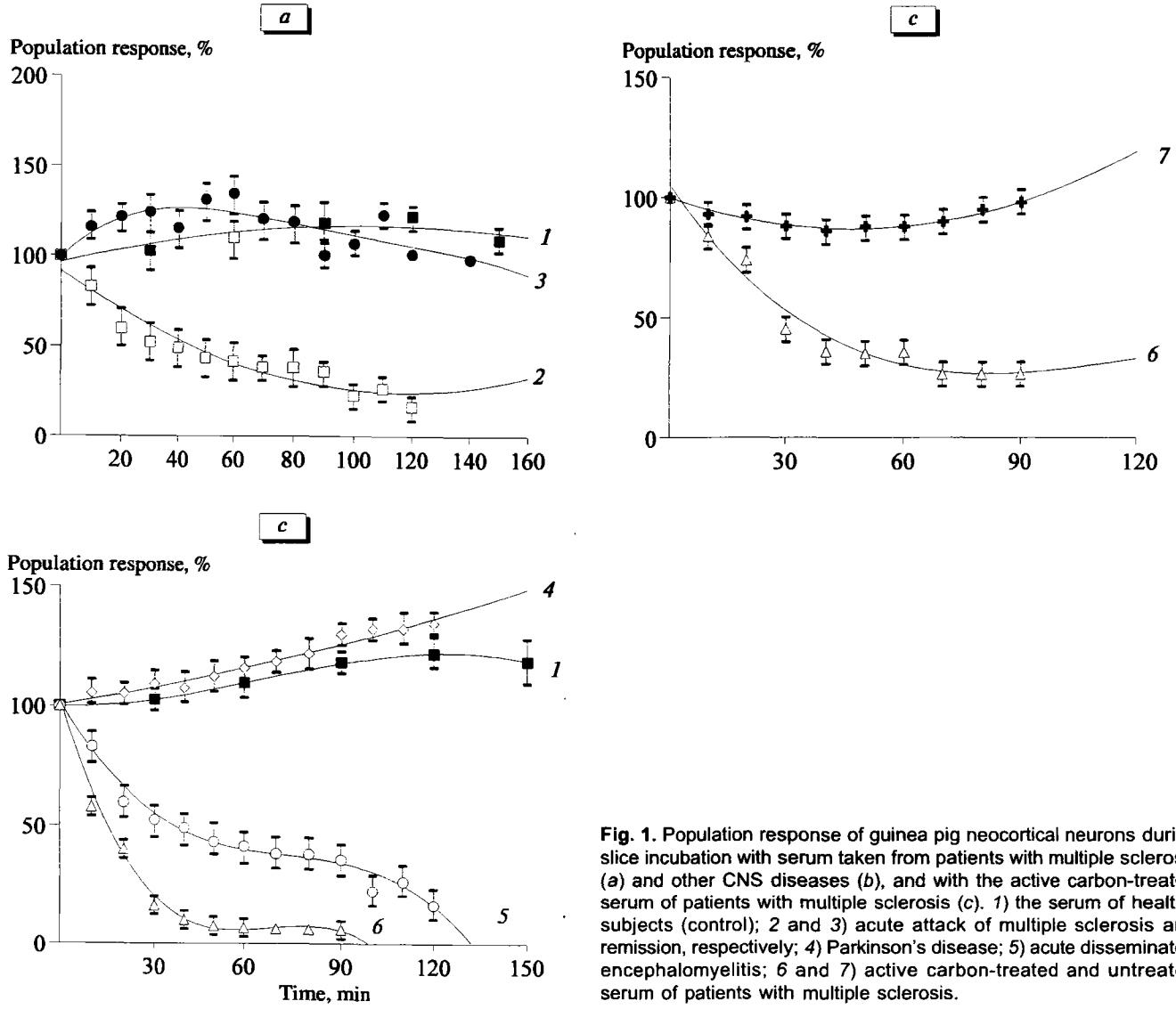

Fig. 1. Population response of guinea pig neocortical neurons during slice incubation with serum taken from patients with multiple sclerosis (*a*) and other CNS diseases (*b*), and with the active carbon-treated serum of patients with multiple sclerosis (*c*). *1*) the serum of healthy subjects (control); *2* and *3*) acute attack of multiple sclerosis and remission, respectively; *4*) Parkinson's disease; *5*) acute disseminated encephalomyelitis; *6* and *7*) active carbon-treated and untreated serum of patients with multiple sclerosis.

rum taken in these different periods always induced distinctive changes in EPR amplitude in neocortical slices.

The serum from patients with Parkinson's diseases (3 patients, n=4) and acute disseminated encephalomyelitis (2 patients, n=5) was tested at the same concentrations to assess the specificity of diagnostic reactions. Clinical syndrome of Parkinson's disease and early manifestations of MS are sometimes very similar despite completely different nature of these diseases. Like MS, acute disseminated encephalomyelitis is accompanied by nerve fiber demyelination [4, 5]. The serum of parkinsonic patients had practically no effect on EPR amplitude, while the serum of encephalomyelitic patients sharply reduced it more potently than the serum of MS patients (Fig. 1, *b*).

Hemosorption is often used to alleviate the state of MS patients [1]. We modeled hemosorption by incubation of serum samples from acute MS patients with an activated charcoal (400 mg/ml). After a 30-min incubation the charcoal was removed by centrifugation. The charcoal-treated serum did not affect EPR, while the same serum before treatment reduced their amplitudes (Fig. 1, *c*).

Histological and electron-microscopic studies on cerebellar tissue culture show that the serum from patients with acute demyelinatind diseases induces structural changes in the nervous tissue [2,3]. These data allowed to apply this test for the diagnosis of MS and experimental allergic encephalomyelitis. Our electrophysiological model offers significant advantages over this test: the results can be obtained within 2-3 h compared to several days or even weeks; thus our model can be used for express-diagnostics; the model allows clear differentiation between the acute attack and remission in MS patients and between remission and healthy condition. Opposite effects of the serum in acute and remission periods are of special theoretical interest. Rapid and pronounced effects attest to the high sensitivity of our model. Since neuronal responses to synaptic stimuli are modified by short (10-20 min) incubation with the serum, it can be suggested that myelin degradation products or corresponding antibodies directly affect the state of synapses and /or postsynaptic membrane receptors.

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