

Anti-ganglioside Antibodies Present in Serum of Patients with Multiple Sclerosis and of Immunized with Gangliosides Rabbits Alter Neuronal Electrical Characteristics

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Abstract. Multiple sclerosis (MS) is considered to be the prototype of primary demyelination. However, imaging and morphological studies of recent years have challenged this view. There is new evidence that the neurons themselves are target in the disease process. On the other hand, a significant increase of some neuronal gangliosides and of anti-ganglioside antibodies (AgA) was detected in the serum of MS patients during the first attacks of the disease. In order to obtain more information concerning the effect of AgA on the neuronal electrogenesis we used sera of MS patients and of immunized with gangliosides rabbits, containing AgA. The studies were performed on the Retzius neuron of the leech. The frequency of the spontaneous activity, the amplitude and the duration of the spontaneous action potential, the threshold, the latency period and the reaction to the synaptic stimulation were determined. There was an increase of the threshold and the latency period after incubation in human and rabbit sera. The neuron generated double spontaneous impulses. The disturbance of the processes of habituation and sensitization was quite similar. The control sera had no effect on the neuronal electrogenesis. These findings give us ground to assume that AgA play a role in the alteration of neuronal electric characteristics. They further support the new concept of MS as a neuronal disease.

Keywords

Neuronal electrogenesis, anti-ganglioside antibodies, serum, multiple sclerosis.

1 Introduction

Gangliosides are major cell surface determinants and the predominant sialoglycoconjugates in the nervous system [26]. They occur most prominently in the neurons [13]. The major neuronal gangliosides in human brain are GM1 and GD1a, which are principally synthesized in neuronal perikarya [1]. There is new evidence that the neurons are target of the disease process in human demyelinating neurological disease multiple sclerosis (MS) [25, 27, 30, 31]. A significant increase of GM1 and GD1a was detected in the serum of MS patients during their first attacks of the disease suggesting neuron injury in the early pathogenesis [30, 31]. A considerable increase of GM1 and GD1a was also revealed in the brain and spinal cord of Lewis rats with chronic relapsing experimental allergic encephalomyelitis, an animal model of MS, just before the onset of clinical signs and during the first clinical episode of the disease [28, 29, 32]. On the other hand, antibodies to GM1 and GD1a have been detected in the sera of MS patients [21, 34]. The data on the influence of anti-ganglioside antibodies (AgA) upon the electrical characteristics of the excited neuronal membrane are very scanty [20]. In order to obtain more information concerning the effect of AgA on the neuronal electrogenesis in the present paper we used sera of MS patients and of immunized with gangliosides rabbits, containing AgA to elucidate their influence on the Retzius neuron of the leech. The leech neurons are very suitable experimental models for such experiments because of their superficial location in the second ganglion of the abdominal nervous chain, the presence of gangliosides [19] and the absence of myelin sheath surrounding the axons [12].

2.1 Material and Methods

The studies were performed on the Retzius neuron of the leech (*Hirudo medicinalis*). The second ganglion of the abdominal nervous chain was fastened on the bottom of a chamber (first chamber) filled with a Ringer solution (Fig.1).

Then this solution was substituted with a Ringer solution containing 20 % anti-ganglioside serum (AG-serum). The ganglion was released from the connective tissue membrane and stained with a 0.01 % solution of neutral red. After this procedure two large (80-100 μm in diameter) Retzius neurons were seen on the ganglion surface. A metallic (gold) electrode for extracellular recording of the impulse activity (IA) was applied under visual control to the surface of one of these neurons. The rest part of the abdominal nervous chain (6-8 ganglia) was placed in a second chamber. The irritating electrodes were put on the nerve connective between the 4-th and 5-th ganglia. The synapses of the investigated neuron are located in the 3-th and 4-th abdominal ganglia, which were in the second chamber without AG-serum [12]. This experimental procedure permits the application of different agents only on the postsynaptic somatodendritic membrane of Retzius neuron. The stimulation strength was equal to two thresholds, the duration of the electric current – 0,2 ms and the stimulation frequency was variable.

The experiments were carried out in the following manner. At first, the frequency of spontaneous IA, the amplitude and the duration of the spontaneous action potential (AP), as well as the threshold of the synaptic irritation stimulation of the Retzius neuron in a Ringer solution were recorded. After that the Ringer solution was changed with a 20 % serum solution. The ganglion was incubated during 20 minutes in this solution. The frequency of the spontaneous IA, the amplitude of AP, its duration and the threshold of the neuron were determined. Then the synaptic stimulation of the neuron was carried out with current strength more then two thresholds during 10 min with frequency of 0.5, 3 and 10 Hz. The provoked IA was registered every 3 minutes during 10 minutes of stimulation.

In our experiments human and rabbit sera were used.

Human sera were obtained from patients with clinically definite MS (according to Poser's criteria [15]) during the first attacks of the disease and from healthy individuals. The sera were evaluated by the enzyme-linked immunosorbent assay (ELISA) for the presence of antibodies to GM1 according to a modification [34] of the method of Mizutamari et al [14].

Rabbit sera were obtained from animals (Chinchilla rabbits) immunized with total brain gangliosides after the procedure of Rapport et al [16]. The total ganglioside fraction from bovine brain homogenates was isolated following the method of Ilinov et al [10]. Antibodies to gangliosides were determined by ELISA. The test antigen for antibody titration was a homogeneous ganglioside fraction – GM1 (Sigma).

2.2 Results

Four series of experiments were performed, as follows.

In the first experimental series (the first control) ($n=35$), the Retzius neuron was incubated during 40 minutes in a 20 % solution of serum from healthy individuals. The frequency of the spontaneous IA was 0.25 ± 0.04 imp/s, the amplitude of the action potential (AP) – 51.1 ± 8.6 mV, its duration – 6.1 ± 0.4 ms, (Fig.2, a) the latency period – 24.5 ± 3.2 ms. During the synaptic stimulation for 10 min at frequency of 0.5 Hz. the neuron generated in response to each shock of current 6-7 AP with amplitude of 38-56 mV and duration of 6.8 ± 0.4 ms (Fig.3, curve a). Such type of the neurophysiological reaction was described as sensitization of the neuron [17]. During synaptic stimulation at frequency of 3 Hz in response to each shock of current the neuron generated one AP, with amplitude of 59.4 ± 2.1 mV and duration of 6.1 ± 1.1 ms (Fig.3, curve b). This reaction was reported as repetition of the stimulation rhythm [17]. During synaptic stimulation at frequency of 10 Hz the neuron developed habituation process. The neuron generated some AP at the first stimuli. Than the cell reaction decreased. Retzius neuron has begun to transform IA frequency. There was no generation of AP in response to each shock of current (Fig.3, curve c). The amplitude of AP was 28.2 ± 0.8 mV and its duration – 12.1 ± 0.1 ms.

In the second experimental series (the second control) ($n=68$), the Retzius neuron was incubated during 40 minutes in 20 % serum from non sensitized with gangliosides rabbits. After the incubation the neuron generated spontaneous impulses at frequency of 0.2 ± 0.04 imp/s, the amplitude of AP was 50.4 ± 10.2 mV and its duration – 6.2 ± 0.8 ms (Fig.2, b). The latency period was 25.7 ± 1.2 ms. During synaptic stimulation for 10 min at frequency of 0.5 Hz neuron generated in response to each shock of current 6-7 AP with amplitude of 40-58 mV and duration of 6.2 ± 0.2 ms (Fig.4, curve a). During synaptic stimulation at frequency of 3 Hz in response to each shock of current the neuron generated one AP, with amplitude of 56 ± 3.3 mV and duration of 6.1 ± 1.4 ms (Fig.4, curve b). During synaptic stimulation at frequency of 10 Hz the neuron developed habituation process. Retzius neuron has begun to transform the IA frequency. There was no generation of AP in response to each shock of current (Fig.4, curve c). The amplitude of AP was 30.3 ± 0.6 mV and its duration – 12.1 ± 0.1 ms. Neuron reaction to a synaptic activation does not differ from the neuron reaction to serum from healthy individuals.

In the third experimental series ($n=34$), the Retzius neuron was incubated during 40 minutes in 20 % serum from MS patients containing AgA. After the incubation the neuron generated double spontaneous impulses. The amplitude of the first AP was 45.2 ± 12.1 mV and its duration – 6.8 ± 0.8 ms, the amplitude of the second AP was 28.9 ± 9.3 mV and its duration – 8.4 ± 1.7 ms, the threshold has increased to 20.2 ± 8.3 % (Fig.2, c), the latency period was 30.7 ± 14.6 ms, the frequency IA has decreased to 28.4 ± 4.6 %. During synaptic stimulation for 10 min at frequency of 0.5 Hz the neuron in response to each shock of current generated only one double AP with amplitude of 48.4 ± 18.2 mV and 30.1 ± 14.1 mV. Their durations were 6.4 ± 0.2 ms and 8.1 ± 0.9 ms (Fig.5, curve a). There was no reaction of sensitisation. During

synaptic stimulation at frequency of 3 Hz in response to each shock of current the neuron generated one AP, with amplitude of 56.3 ± 6.2 mV and duration of 6.6 ± 0.8 ms (Fig.5, curve b). During synaptic stimulation at frequency of 10 Hz the neuron generated one AP in response to each shock of current (Fig.5, curve c). The amplitude of AP was 42.8 ± 8.0 mV and its duration – 7.0 ± 0.4 ms. There was no reaction of habituation.

In the fourth experimental series (n=68), the Retzius neuron was incubated during 40 minutes in 20 % serum from immunized with gangliosides rabbits, containing AgA. After the incubation the neuron generated double spontaneous impulses. The amplitude of the first AP was 40.4 ± 0.2 mV and its duration – 7.2 ± 0.4 ms, the amplitude of the second AP was 30.0 ± 0.6 mV and its duration – 10.1 ± 0.8 ms (Fig.2, d), the threshold has increased to 18.4 ± 3.2 %, the latency period was 32.2 ± 4.2 ms, the frequency of IA has decreased to 22.4 ± 4.1 %. During synaptic stimulation for 10 min at frequency of 0.5 Hz the neuron generated in response to each shock of current a double AP with amplitude of 48.4 ± 11.2 mV and 28.4 ± 11.6 mV and duration of 6.4 ± 0.1 ms and 8.7 ± 0.4 ms (Fig.6, curve a). There was no reaction of sensitisation. During synaptic stimulation at frequency of 3 Hz in response to each shock of current the neuron generated one AP, with amplitude of 31.4 ± 0.4 mV and duration of 11.2 ± 0.2 ms (Fig.6, curve b). During synaptic stimulation at frequency of 10 Hz the neuron generated one AP in response to each shock of current (Fig.6, curve c). There was no reaction of habituation The amplitude of AP was 44.4 ± 8.1 mV and its duration – 6.1 ± 0.4 ms. The neuron reaction to a synaptic activation does not differ from the neuron reaction to a serum from MS patients.

In the third and the fourth experimental series only sera with elevated antibody titres to GM1 were used.

2.3 Figure

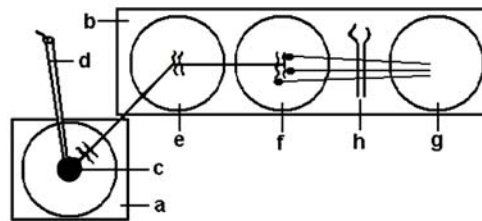


Fig. 1. Experimental scheme.

- a. First experimental chamber. b. Second experimental chamber. c. Retzius neuron of second ganglion. d. Metallic electrode for extracellular recording of the impulse activity (IA). e-g. Second, third and fourth ganglia of the leech abdominal nervous chain. h. Irritation electrodes between the 3-rd and 4-th ganglia.

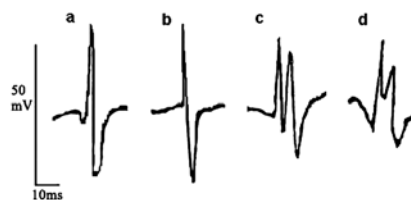


Fig. 2. Action potential (AP) of Retzius neuron.

- a. Normal AP after incubation in serum of healthy individuals. b. Normal AP after incubation in serum of non sensitized with gangliosides rabbits. c. Double AP after 40 minutes incubation in serum of MS patients containing anti-gangliosides antibodies. d. Double AP after 40 minutes incubation in serum of immunized with gangliosides rabbits containing anti-gangliosides antibodies.

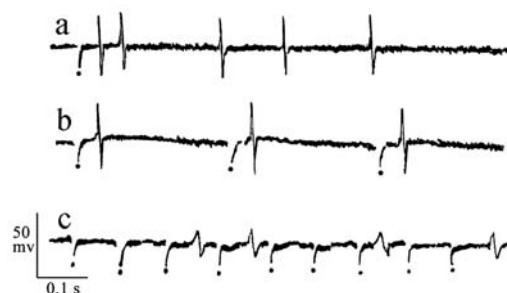


Fig. 3. The changes of the IA of the Retzius neuron incubated in serum of healthy individuals.

a. Reaction of sensitisation during synaptic stimulation at frequency of 0.5 Hz. b. Reaction of repetition to synaptic stimulation rhythm during a stimulation at frequency of 3 Hz. c. Reaction of habituation during synaptic stimulation at frequency of 10 Hz.

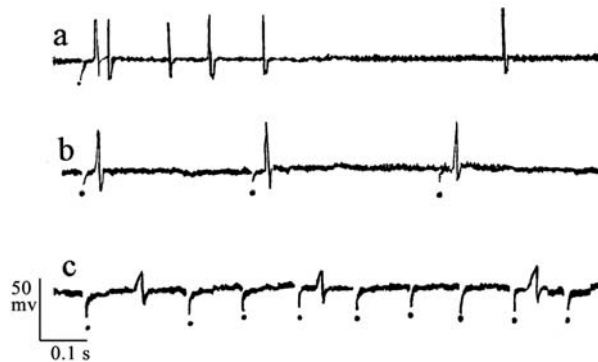


Fig. 4. Changes of the IA of Retzius neuron incubated in serum of non sensitized with gangliosides rabbits. a. Reaction of sensitisation during synaptic stimulation at frequency of 0.5 Hz. b. Reaction of repetition to synaptic stimulation rhythm during stimulation at frequency of 3 Hz. c. Reaction of habituation during synaptic stimulation at frequency of 10 Hz.

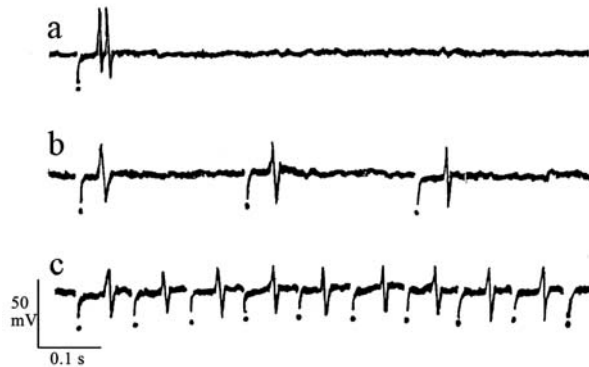


Fig. 5. The changes of the IA of the Retzius neuron incubated in serum of MS patients containing anti-gangliosides antibodies.

a. Double AP during synaptic stimulation at frequency of 0.5 Hz. There is no reaction of sensitisation. b. Normal reaction of repetition of stimulation rhythm during synaptic stimulation at frequency of 3 Hz. c. Reaction of repetition of the stimulation rhythm during synaptic stimulation at frequency of 10 Hz. There is no reaction of habituation.

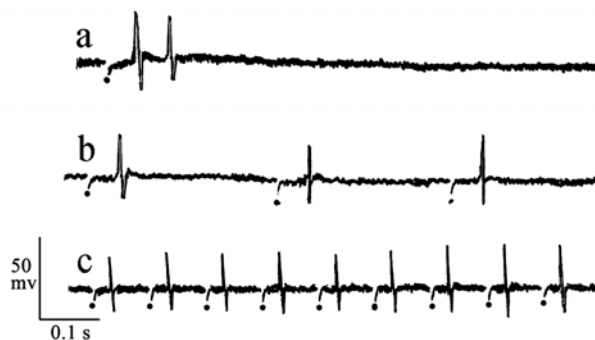


Fig. 6. The changes of the IA of Retzius neuron incubated in serum of immunized rabbits with gangliosides containing anti-gangliosides antibodies.

a. Double AP during synaptic stimulation at frequency of 0.5 Hz. There is no reaction of sensitisation. b. Normal reaction of repetition of stimulation rhythm during synaptic stimulation at frequency of 3 Hz. c. Reaction of repetition of the stimulation rhythm during synaptic stimulation at frequency of 10 Hz. There is no reaction of habituation.

3.1 Discussion

The present study demonstrate that the serum of MS patients and of immunized with gangliosides rabbits containing anti-ganglioside antibodies altered electrical characteristics of leech Retzius neuron. The effect of both sera on neuronal electrogenesis was quite similar. There was an increase of the threshold and the latency period, the neurons generated double spontaneous impulses. The processes of sensitization and habituation were disturbed at the same way.

The MS serum tested in our experiments had high titres of antibodies to GM1. The serum of immunized rabbits was tested for antibodies to GM1 and also contained elevated antibodies titres to GM1. The more important advantage of this serum is that it does not contain antibodies to serum albumin because of the absence of albumin in the immunizing mixture. On the other hand, the control sera from healthy individuals and non sensitized rabbits had no effect on the neuronal electrogenesis. These findings give us ground to assume that the anti-ganglioside antibodies play a role in the neuronal membrane changes in our experiments.

Our experiments demonstrate that the antigen-antibody reaction blockades those functions of gangliosides which are connected with the electrical capacities of the neuron. There is increasing body of evidence that gangliosides play an important role in a variety of cellular events, as well as in a number of neurological functions. They exhibit receptor or coreceptor functions for many bioactive agents and are considered to be involved in differentiation and morphogenesis, cell-cell interactions, cellular recognition and growth regulation. GM1 ganglioside has neuritogenic and neuronotrophic activity and facilitates repair of neuronal tissue after mechanical, biochemical or toxic injuries [8, 22]. Several studies have focused on the immunological properties of gangliosides and this field has undergone a remarkable development. Rabbits immunized with gangliosides developed a chronic partially remitting disease with clinical and pathological features reminiscent of MS [2, 3, 4,]. As it was mentioned above, an increase of GM1 and GD1a, the major neuronal gangliosides, as well as of antibodies to GM1 and GD1a in serum of MS patients during the first attacks of the disease has been reported.

The etiology and pathogenesis of multiple sclerosis have been debated for decades. In the majority of the text books of neurology MS is considered to be the prototype of acquired primary demyelinating disease of the central nervous system (CNS) with relative preservation of neurons. It was indicated that autoimmune mechanisms play an important role in the pathogenesis of CNS demyelination [9, 24]. Imaging and morphological studies of recent years have challenged the historical view of preserved neuronal and axonal integrity in MS. Early axonal damage in patients with MS has been demonstrated in vivo by magnetic resonance spectroscopy (MRS), which shows decreased levels of neuron-specific marker N-acetylaspartate (NAA) in the early stages of MS [5, 7]. Direct evidence of axonal damage in MS has been provided by morphological investigations [6, 11, 23]. Early neuronal and axonal damage has been demonstrated also in chronic relapsing experimental allergic encephalomyelitis (CREAE), an animal model of MS. Our electronmicroscopic studies on the brain and spinal cord of Lewis rats with CREAE at the preclinical stage and the first clinical episode of the disease revealed axonal degeneration, which preceded demyelination very early in the disease [33]. All these data further support the new concept of MS as a neuronal disease [25].

3.2 Conclusions

The findings in this study provide for the first time evidence that the serum of patients with MS during the first attacks of the disease and of immunized with gangliosides rabbits, containing anti-ganglioside antibodies provokes changes of neuronal electrogenesis. They argue for the early neuronal damage in multiple sclerosis and neuroprotective therapy in the first stages of the disease.

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