PHYSIOLOGY

Effects of Antibodies against Protein S100b on Synaptic Transmission and Long-Term Potentiation in CA-1 Hippocampal Neurons in Rats

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Effects of idiotypic and antiidiotypic antibodies against protein S100b on synaptic transmission and long-term potentiation of CA1 pyramidal neurons in rat hippocampus were studied. Idiotypic antibodies against protein S100b inhibited synaptic transmission from Schaffer collateral axons to pyramidal neurons, but had no effect on neuronal responses to antidromic stimulation. Antiidiotypic antibodies against protein S100b facilitated neuronal responses to orthodromic stimulation. Idiotypic antibodies against protein S100b suppressed changes in neuronal responses to orthodromic stimulation during long-term potentiation, while antiidiotypic antibodies facilitated these effects. Our findings suggest that the effects of idiotypic and antiidiotypic antibodies to protein S100b are associated with their regulatory influences on synaptic transmission. These mechanisms differ from mechanisms of synaptic plasticity involved in long-term potentiation.

Key Words: *hippocampus*; *neuron*; *long-term potentiation*; *protein S100b*

Similar molecular basis of learning and development stimulates more intensive investigation of the role of neurotrophic and growth factors in learning and memory [5,6,8]. Special attention is paid to protein S100b belonging to a family of S100 calcium-binding protein. This protein exhibits growth-stimulating, trophic, and mitogenic activities, participates in the integrative function of the brain, and plays an important role in the pathogenesis of various diseases, including multiple sclerosis, Down's syndrome, Alzheimer's and Parkinson's diseases, *etc.* [9,14]. Antiserum to S100b protein inhibits long-term memory formation in chicks, disrupts learning and consolidation of defense behavior in adult rats, changes characteristics of neuronal membranes [6, 12]. Overexpression of S100b gene in transgenic mice impairs spatial learning and suppresses induction of long-term potentiation in hippocampal neurons [10]. In mollusks, protein S100b affects neuronal activity by modulation of potassium currents [11]. At the same time, neurophysiological and neurochemical mechanisms underlying the involvement of S100 protein in learning remain unclear.

Long-term posttetanic potentiation (LTP) is widely used in studies of cellular and molecular mechanisms of neuronal plasticity underlying learning and memory processes [3,13].

In this work, we studied the effect of antibodies against protein S100b on synaptic transmission and LTP in hippocampal CA1 neurons of adult rats for evaluation of the role of protein S100b in synaptic and neuronal plasticity.

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MATERIALS AND METHODS

Experiments were carried out on mature male Wistar rats weighing 150-200 g. The rats were anesthetized with ether and decapitated. The hippocampus was extracted and 350-400-μ slices were prepared. The slices were placed in a non-immersion recording chamber [7] and perfused with physiological saline at 35.0± 0.5°C and flow rate of 2 ml/min. The saline containing (in mM) 126 NaCl, 1.25 NaH_2PO_4, 1.2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, 3 KCl pH 7.4 was continuously bubbled with carbogen (95% O_2+5% CO_2) [10]. Orthodromic and antidromic electrical stimulation was applied via bipolar platinum wire electrodes to Schaffer collateral or alveus, respectively. Test stimulation was performed with 3-8-V pulses of 0.1 msec duration delivered at 100 Hz. LTP was produced by tetanic stimulation of Schaffer collaterals with three stimulation series (300 pulses each, 100 Hz repetition rate, 1 sec interval between pulses). Parameters of pulses were similar to those in test stimulation. Population spikes in CA1 hippocampal field were recorded via monopolar glass microelectrodes filled with 0.15 M NaCl (resistance 1-3 MΩ). Effects of polyclonal idiotypic antibodies (IAB) and antiidiotypic antibodies (AAB) to protein S100b were studied. In control series preimmune antibodies from nonimmune rabbits were used. Anti-S100b-protein were isolated from rabbits immunized with S100b. AAB were obtained from rabbits immunized with immunoglobulins containing Anti-S100b [2]. The stock solution containing antibodies (1 mg/ml) was diluted 1:5 or 1:10 before application to the slices.

The results were averaged and expressed in percent of initial values, all values are presented as mean±SEM. The differences between the means were statistically analyzed by Student's *t* test.

RESULTS

In series I, we studied the effect of anti-S100b antibodies on responses of non-potentiated CA1 pyramidal neurons to antidromic and orthodromic stimulation of the alveus and Schaffer collaterals, respectively. The antibodies were applied for 40 min. In the presence of preimmune antibodies neuronal responses (n=5) remained relatively stable throughout the observation period (1-1.5 h, Fig. 1). Five-ten minutes after the start of incubation with anti-S100b IAB (diluted 1:5; n=5) the amplitude of orthodromic population spikes decreased by 28-35% compared to the initial value (p<0.001, Fig. 1). After termination of IAB application (washout), neuronal responses to orthodromic stimulation recovered within 30-50 min. Anti-S100b produced no effect on neuronal responses to antidromic stimulation (n=5, Fig. 1). AAB to S100b (1:5, n=5) increased population response to orthodromic stimulation by 40-45% after 10-15-min exposure (p<0.001, compared to the initial values; Fig. 1). This effect persisted after termination of AAB application until the end of recording.

In series II, the effect of antibodies on LTP-related synaptic plasticity of CA1 pyramidal neurons was studied. In this experimental series, antibodies were applied 30 min before tetanization and removed 30 min after it.

Application of preimmune antibodies increased post-tetanization responses of hippocampal neurons (1:5, n=5) to orthodromic stimulation by 97-103% (p<0.001) and this effect persisted until the end of recording (Fig. 2).

IAB against S100b diluted 1:5 (n=5) and 1:10 (n=5) applied 30-min before tetanization reduced the amplitude of the response to orthodromic stimuli by 53 and 24%, respectively (Fig. 2). After tetanization, the amplitudes of these responses increased by 15 and 42% (for IAB application in doses of 1:5 and 1:10, respectively), compared to the preapplication values. However, compared to the value of population spikes measured after 30-min application of IAB just before tetanization (taken as 100%), the increase in the responses observed in the presence of IAB in doses of 1:10 and 1:5 constituted 91% and 140% (p<0.001), respectively.

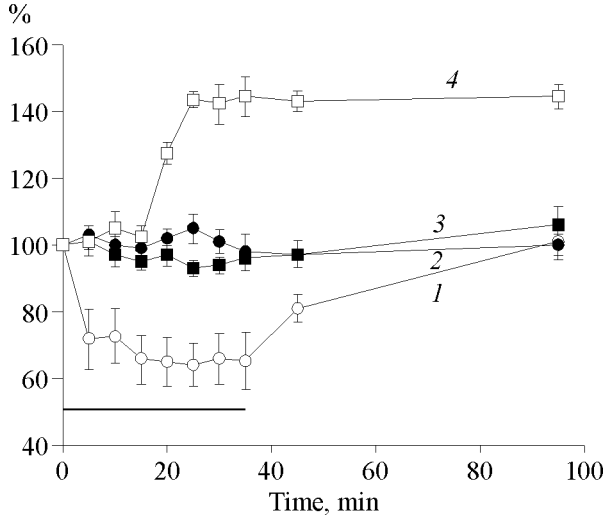

Fig. 1. Effects of idiotypic and antiidiotypic antibodies against protein S100b on non-potentiated responses of CA1 pyramidal neurons in rat hippocampal slices. Effects of idiotypic antibodies against S100b (dilution 1:5) on neuronal responses to orthodromic stimulation of Schaffer collaterals (*1*) and antidromic stimulation of the alveus (*2*); orthodromic responses in the presence of preimmune (*3*) and antiidiotypic antibodies (*4*). Here and in Fig. 2: ordinate: amplitude of population spike; 100% corresponds to the amplitude of initial neuronal response before application of antibodies. Horizontal solid line indicates period of application.

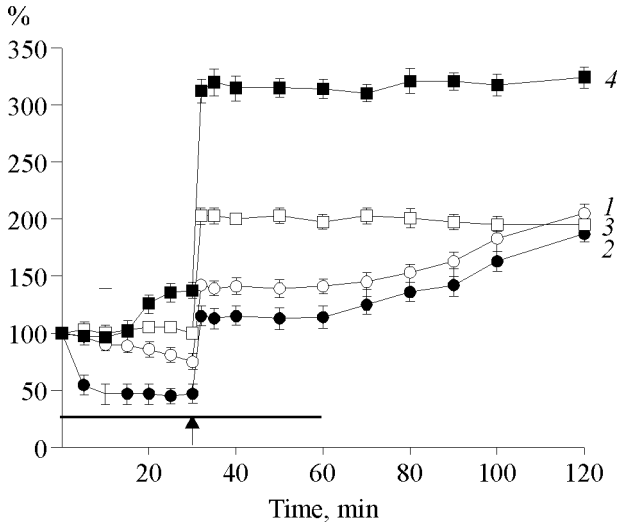

Fig. 2. Effects of idiotypic and antiidiotypic antibodies against protein S100b on potentiated responses of CA1 pyramidal neurons. Application of idiotypic antibodies against S100b in doses of 1:10 (*1*) and 1:5 (*2*); application of preimmune (*3*) and antiidiotypic antibodies (*4*) against S100b. Arrow indicates the moment of tetanic stimulation of Schaffer collaterals.

These amplitudes continued to increase for 40-50 min after completion of IAB application and approximated the amplitude of orthodromic responses of tetanized neurons in control slices ($p>0.05$, Fig. 2).

Anti-S100 AAB (1:5, $n=5$) increased the amplitude of population response by 28-34% compared to the initial value (Fig. 2). After tetanization on min 30 of AAB exposure, the response amplitude to orthodromic stimuli increased by 200-220% ($p<0.001$) compared to the initial values and by 110-120% ($p<0.001$) compared to the response amplitude before tetanization. The observed facilitation persisted until the end of observation.

Thus, anti-S100b IAB reversibly inhibited synaptic transmission from Schaffer collaterals to CA1 pyramidal neurons of the hippocampus. At the same time, IAB did not affect neuronal membrane excitability, judging from the responses to antidromic stimulation. Anti-S100b AAB facilitated respon-ses to orthodromic stimulation and this effect was irreversible throughout the observation period (1.0-1.5 h). IAB and AAB to S100b produced opposite changes (inhibition and facilitation, respectively) on CA1 neuronal responses to orthodromic stimulation during LTP.

The opposite effects of IAB and AAB observed in our experiments are due to the fact that AAB act like a model of the initial antigen, *i. e.* S100b protein, and, therefore, they can play similar role [1]. The observed effects of anti-S100b antibodies are determined by different mechanisms. For example, both protein S100b and anti-S100b antibodies can modulate the synthesis, release, and reception of neurotransmitters such as acetylcholine, GABA, glutamate, and serotonin [6,9]. Moreover, it was found that S100b modulates potassium currents of various types in *Helix pomatia* neurons [11], while application of IAB to S100b during nociceptive sensitization substantially reduced its effects on neural transmission and membrane excitability in snail neurons [4]. Changes in membrane excitability, in its turn, can affect the efficacy of synaptic transmission.

Our findings suggest that the effects IAB and AAB to S100b are determined by their influence on synaptic transmission, which differ from the synaptic plasticity associated with LTP induction. This assumption is based on the following facts. First, both in control slices and in neurons exposed to IAB and AAB, we revealed similar facilitation of responses during LTP (by 90-140%) compared to those before tetanization. Second, similar amplitudes of population spikes were observed during washout in neurons potentiated in the presence of IAB against S100b and in control neurons during LTP. After induction of LTP in the presence of AAB to S100b, no recovery was observed during washout. This implies that the effect of AAB is highly resistant.

Thus, our results indicate that IAB and AAB against protein S100b produce plastic modification of synaptic inputs to non-potentiated CA1 area neusrons in rat hippocampal slices. It seems unlikely that changes in LTP parameters produced by these antibodies are related to the involvement of S100b protein in the mechanisms responsible for LTP induction and its early phase. Recent evidence suggests that the neurotrophic protein S100b plays a role in LTP maintenance characterized by pronounced morphological transformations. However, further studies are required to validate this hypothesis.

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