Generation of Excitatory Postsynaptic Potentials in the Hippocampus after Functional Modification of Glycosaminoglycans

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Experiments on hippocampal slices form 4-week-old rats (n=28) showed that addition of lidase (1.0 and 10.0 U/ml) to the perfusion solution (artificial cerebrospinal fluid) was accompanied by the impaired generation or blockade of excitatory postsynaptic potentials and population spikes in the hippocampal CA1 region during stimulation of Schaffer collaterals. Removal of lidase from this solution normalized the amplitude of evoked responses. Hence, lidase in these concentrations produced a reversible effect on synaptic transmission. Our results indicate that structure and function of glycosaminoglycans in the extracellular matrix determine signal transduction in the nervous tissue.

Key Words: *extracellular matrix; hippocampus; synaptic transmission; lidase*

The volume of the extracellular matrix in different brain regions and in different periods of ontogeny varies from 7-8 to 20-40% [5,9]. Glycosaminoglycans (chondroitin sulfate, heparan, hyaluronan, *etc*.), glycoproteins (laminins, tenascin, fibronectin, *etc*.), and proteoglycans (syndecan, agrin, versican, phosphacan, *etc*.) are the major components of the matrix [5,8,12]. Elements of the extracellular matrix can secrete signal molecules, including bFGF and HB-GAM. Recent studies showed that extracellular matrix has various receptors [3,4,6,12]. It was hypothesized that binding of these receptors with the corresponding ligands or functional transformation of glycosaminoglycans, glycoproteins, and proteoglycans in the matrix is followed by a variety of changes in the nervous tissue [2,4,5,7]. Spatial and functional changes in the extracellular matrix are followed by variations in the function of nerve and glial cells, which impairs signal transduction in the brain. Apart from well-known synaptic transmission, there is a extrasynaptic process (spillover) [10,11]. Taking into account the function of the extracellular matrix, the question arises: what is the role of structural and functional properties of the major components of extracellular matrix in signal transduction in the nervous system and integrative activity of the brain? It remains unclear whether structural and functional modification of the extracellular matrix with lidase or hyaluronidase-containing solutions modulates generation of postsynaptic potentials and to what degree modification of glycosaminoglycans in brain sections affects signal transduction in Schaffer collaterals to neurons of the hippocampal CA1 region? The present study was designed to answer these questions.

MATERIALS AND METHODS

Experiments were performed on transverse sections of the hippocampus from 4-week-old male Wistar

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rats (n=28). The brain was removed and placed in a cold solution (2-3°C) containing 124 mM NaCl, 3 mM KCl, 1.25 mM KH_2PO4, 4 mM $MgCl_2$, 0.5 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose and saturated with carbogen (gas mixture of 95% O_2 and 5% CO_2, pH 7.3-7.4) [6]. Brain tissues were put in a cold solution not later than 50 sec after decapitation. The width of hippocampal sections was 400-450 μ. Sections were bathed in artificial cerebrospinal fluid (124 mM NaCl, 3 mM KCl, 1.25 mM KH_2PO_4, 1.2 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose) at 26°C for 1.5 h to stabilize metabolic processes in the nervous tissue [1]. The section was placed in a temperature-controlled chamber (BSC-ZT, Harward Apparatus) and perfused with carbogen-saturated solution (95% O_2 and 5% CO_2) of artificial cerebrospinal fluid at a flow rate of 2 ml/min. Lidase (Belmedpreparaty) was dissolved in artificial cerebrospinal fluid.

Recording microelectrodes were inserted into the *stratum radiatum* and *stratum pyramidale* (hippocampal CA1 region). Excitatory postsynaptic potentials (EPSP) and population spikes (PS) were monitored between basal dendrites of the hippocampal CA1 region and apical area of pyramidal neurons using a Microelectrode AC Amplifier Model 1800 (A-M Systems Inc.). Electrical stimulation of Schaffer collaterals was delivered through a unipolar platinum-iridium microelectrode. This microelectrode was placed in the *stratum radiatum* at the boundary of CA1 and CA3 regions (Fig. 1). Brain

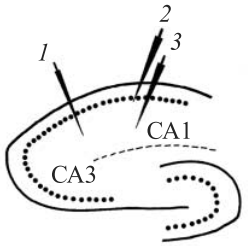

Fig. 1. Location of stimulating (*1*) and recording electrodes (*2*, *3*) in the rat hippocampal section.

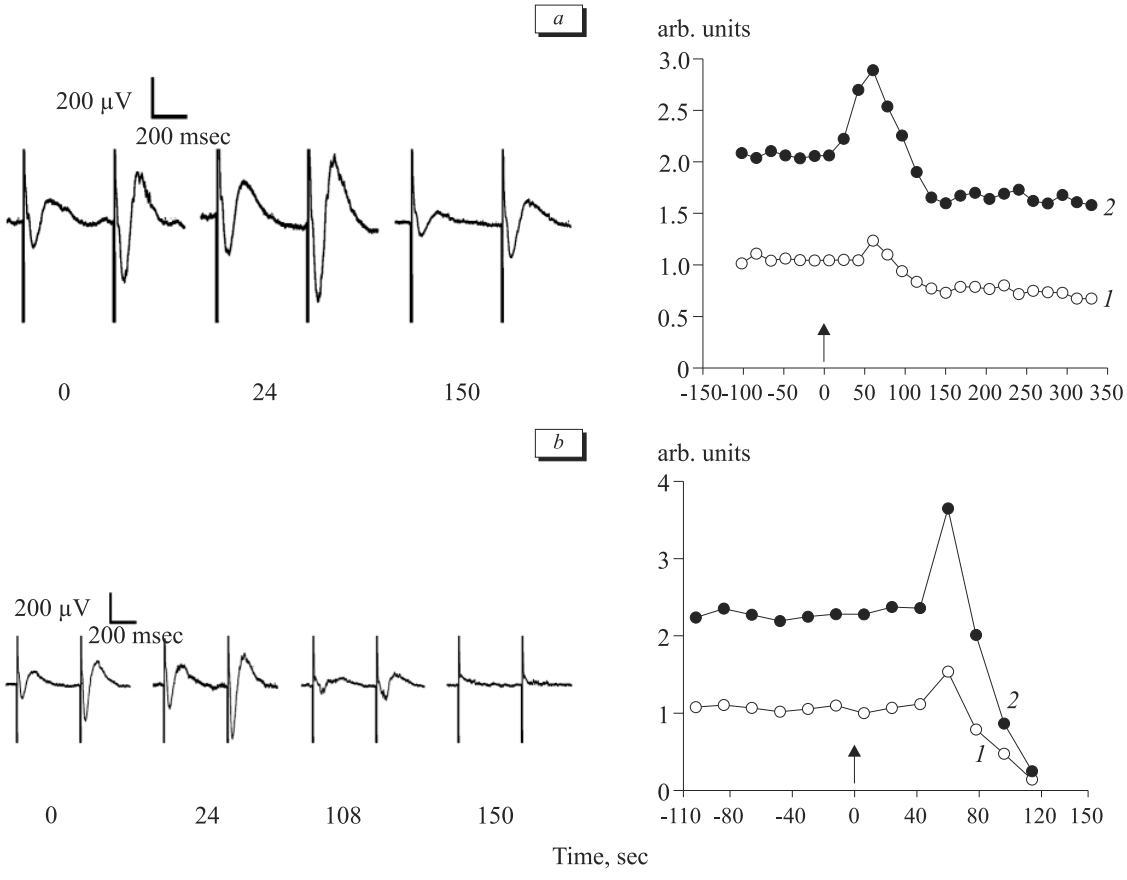

Fig. 2. Amplitude of EPSP (response in the *stratum radiatum*) under the influence of lidase in doses of 1 (*a*) and 10 U/ml (*b*). Arrow: start of perfusion. Average amplitude of the response to the 1st (*1*) and 2nd stimulus (*2*) of paired stimulation. Ordinate: amplitude of evoked responses (averaged in each point by the amplitude of 3 responses), normalization by the average amplitude of responses in the control pretreatment period.

tissue was dissected at the boundary between CA1 and CA3 regions to prevent transmission of spontaneous rhythmic activity from giant pyramidal neurons of the hippocampal CA3 region to hippocampal CA1 cells through Schaffer collaterals.

The stimulation pulse included a negative and positive phase (amplitude 20 μA, duration 200 msec). In the initial state, 60 paired pulses were applied at an interstimulus interval of 50 msec. The interval between paired pulses was 6 sec. During stimulation, the perfusion fluid was replaced by a solution containing lidase in doses of 0.1 (n=8), 1 (n=10), and 10 U/ml (n=10). Hippocampal sections were subsequently perfused with lidase solution (3 min) and artificial cerebrospinal fluid.

The data on electrical activity were transmitted to a computer using an analog-digital converter (ADC-100k/12-8, Spetspribor), recorded, and processed with Origin 6.1 and Statistica 6.0 software.

RESULTS

Perfusion of hippocampal sections with a solution containing 0.1 U/ml lidase had little effect on the amplitude of EPSP and PS in the hippocampal CA1 region during stimulation of Schaffer collaterals. A short-term increase in the amplitude of EPSP and PS in response to paired stimulation was observed after 60-sec perfusion of hippocampal sections with a solution of 0.1 U/ml lidase (Fig. 2). The amplitude of evoked responses progressively decreased over 50-75 sec and corresponded to 75-80% of the basal level after 100-120-sec perfusion. The amplitude of EPSP and PS returned no normal after perfusion of sections with lidase-free solution for 360-400 sec (but not immediately by the end of lidase application). The amplitude of EPSP significantly increased in the early stage of perfusion of sections with the solution containing 10 U/ml lidase (60-90 sec), but progressively decreased and was undetected in the follow-up period (Fig. 2). The removal of lidase from the perfusate normalized the amplitude of EPSP and PS (10-15 min after perfusion of sections with a lidase-free solution).

Our results indicate that the substances not affecting presynaptic and postsynaptic signal transduction in the brain modify EPSP and PS generation in the hippocampal CA1 region [4,5,9]. These findings are of considerable importance. First, the appearance of hyaluronidase in the extracellular space is followed by changes in intercellular communication. It can be hypothesized that the effect of lidase in the central nervous system manifests by changes in the central regulation of systemic functions. And second, application of lidase to the hippocampal section leads to a short-term increase followed by a decrease (or blockade) in the amplitude of evoked responses in the population of nerve cells. These data should be taken into account during central administration of lidase. Neurophysiological study on hippocampal sections showed that structural and functional characteristics of the extracellular matrix in the brain are important for signal transduction in the nervous tissue.

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