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Pharmacological blockade of neuraminidase activity does not affect paired-pulse plasticity in hippocampal CA3-to-CA1 network

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Polysialic acids (PSA) of the outer cell membrane are widely distributed in the neuronal tissue. Their linkages to the glycolipids and glycoproteins are essential for synaptic plasticity. Neuraminidase (NEU) is the main enzyme, which controls PSA population by removing sialic acids from sialoglycoconjugates. **Aim.** In the present study, we investigated the role of NEU inhibition in hippocampal short-term memory processing. We previously showed that NEU blockage results in a significant decrease in long-term potentiation (LTP) and an increase in short-term depression of hippocampal CA3-to-CA1 network in the stratum radiatum. **Methods.** Using specific blocker N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid (NADNA), we examined the effect of downregulation of NEU activity on paired-pulse plasticity at Schaffer collateral-CA1 pyramidal cell synapses of the rat hippocampus. **Results.** The present study demonstrates that suppression of endogenous NEU causes an increase in the excitatory post-synaptic potentials without alterations in paired-pulse ratio. **Conclusions.** Inhibition of NEU activity did not affect paired-pulse plasticity, which reflects changes in the release probability of presynaptic site. We hypothesized that effect of NADNA on basal transmission and LTP is due to the involvement of postsynaptic mechanisms.

K e y w o r d s: neuraminidase blocker, synaptic plasticity, hippocampus.

Introduction

Polysialic acids are the negatively charged chains on the outer cell membrane surface. They are widespread in the neuronal tissue. Previously the PSA appearance in the CA1 hippocampus was demonstrated using SNA–I lectin staining [1, 2]. The role of PSA in the excitability was shown on the different models of epileptogenesis, activity-dependent synap-

togenesis and synaptic plasticity of the hippocampal neuronal networks [3–6]. The main regulator of the PSA amount is the endogenous enzyme neuraminidase [7]. High concentrations of NEU significantly decrease the level of PSA on the membrane surface. NEU applied to the hippocampus influences many neural functions including neurotransmitter release, memory, synaptic plasticity, axon outgrowth and neural differentiation [8–10]. NEU blocker has an opposite effect on the PSA cluster quantity and respectively on the neuronal excitability: increases the firing frequency and amplitude of spontaneous synchronous oscillations, and the frequency of multiple unit activity in cultured rat hippocampal slices [1, 11]. Previously it was shown that downregulation of NEU activity decreases synaptic plasticity in both long and short forms in the CA3-to-CA1 apical dendrite synapses [6] and mossy-fibers-to-CA3 pyramidal cell synapses [12]. In this work we examined whether the presynaptic mechanisms associated with neurotransmitter release are affected by neuraminidase inhibition.

Materials and Methods

All experimental procedures were performed in accordance with the requirements of the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes and approved by the Committee on Biomedical Ethics of the Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine (protocol No. 1/23 dated 03/22/2023).

Slice preparation

Rats were deeply anesthetized by diethyl ether, decapitated, then the brain was gently removed and placed into ice-cold oxygenated (95 % O_2–5 % CO_2) artificial cerebrospinal fluid (ACSF) of following composition (in mM): NaCl 119, KCl 2.5, CaCl2 2, MgCl2 1.3, NaHCO3 26, NaH2PO4 1, and glucose 11 (pH 7.35). Hippocampi were isolated, cut into 400 μm slices using vibratome, and incubated in the oxygenated ACSF for 1.5–2 hours.

Electrophysiology

Brain slices were transferred to the incubation chamber and superfused with oxygenated ACSF at a rate of 2 ml/min (22–24 °C). Extracellular recordings were obtained within the CA1 stratum pyramidale (SP) of hippocampus with extracellular glass microelectrodes (3–4 MΩ) filled with ACSF using patch-clamp amplifier (PC 501A, Warner Instruments Corp., Hamden, CT). Stimulating and recording electrodes were placed on the slice surface approximately 400 μm apart from each other. Evoked postsynaptic responses were elicited by stimulation of Schaffer collateral-commissural pathway using a concentric bipolar stimulating electrode (FHC Inc., Bowdoin, ME) connected to a flexible stimulus isolator (ISO-Flex, A.M.P. Instruments, Jerusalem, Israel). Stimulation intensity varied between 150 and 400 μA in all slices. At the beginning of each experiment, the maximal synaptic response was determined by generating input–output curves. For induction of paired-pulse plasticity, two stimuli were delivered to the hippocampal pathway with the interstimulus interval (ISI) ranging from 25 to 500 ms. The paired-pulse ratio was defined as A2/A1, where A1 and A2 are amplitudes of the population spikes evoked by first and second

pulses, respectively. Recordings were digitized at 10 kHz and filtered at 3 kHz using an analogue-to-digital converter (National Instruments, Austin, TX) and stored on a computer using the WinWCP program (Strathclyde Electrophysiology Software, University of Strathclyde, Glasgow, UK).

NEU Blocker Treatment

Brain slices were incubated with NADNA during 2 hr at room temperature, then extensively washed with ACSF before recordings. In all experiments we used NADNA in concentration of 500 μM purchased from Sigma-Aldrich (St. Louis, MO, USA). The specificity of the effect of NADNA as a blocker of the endogenous NEU was shown in histological and electrophysiological studies in our previous reports [1, 5].

Data Analysis

Offline analysis of the recordings was performed using Clampfit (Axon Instruments, USA), Prism 8 (GraphPad, La Jolla, CA), and Origin 8.5 (OriginLab, Northampton, MA) software. Initially, the normality of the distributions was evaluated using the Shapiro-Wilk test. In cases where the data were parametric, an unpaired two-tailed Student's t-test was employed, with Welch's correction applied to accommodate for variances that were different. However, if the data were non-parametric, an unpaired Mann-Whitney test was used instead. A *p*-value less than 0.05 was considered significant. Data are shown as mean ± SEM.

Results and Discussion

To determine whether pharmacological blockade of NEU alters short-term synaptic plasticity in the CA1 pyramidal cell layer of rat hippocampal slices, using a paired-pulse stimulation paradigm, we examined the effect of downregulation of NEU activity on synaptic transmission and neuronal firing. Depending on presynaptic release probability, the paired-pulse plasticity can take the form of facilitation or depression [13–15].

Stimulation of Schaffer collaterals at a 25 ms ISI leads to paired-pulse facilitation of dendritic excitatory postsynaptic potentials (EPSPs), which could be accompanied by paired-pulse depression of somatic EPSPs and pop-spikes (PSs), reflecting diminished propagation of excitatory inputs to the soma and diminished pyramidal cell firing [16]. Incubation with 500 μM NADNA for 2 hr decreased 2nd PSs at ISI 25 ms in 46.2 % of slices (n = 6 out of 13 slices) while in the control slices paired-pulse depression was observed in 33.3 % of slices (n = 3 of 9 slices). We suppose that decrease of 2nd PSs in NADNA-treated slices is observed due to enhanced release probability to the first pulse. Indeed, electrophysiological recordings in the hippocampal CA1 pyramidal cell layer have shown increased basal excitatory transmission following NEU blockage (pop-spike amplitude, 0.26 ± 0.02 mV [n = 19] in control versus 0.55 ± 0.03 mV [n = 27] in NADNA-pretreated group, $P < 0.0001$, unpaired Welch's *t*-test). Analyzing paired-pulse ratio at ISI 25 ms we have not found any significant alterations of short-term plasticity after NEU inhibition (P = 0.85, Mann-Whitney test, Fig. 1).

We also observed paired-pulse depression at ISI 50 ms in 11 % of control slices (n = 1 out of 9 slices) and 20 % of NADNA-treated

slices (n = 3 out of 15 slices). Paired-pulse ratio did not change due to NEU blockage (P = 0.19, Mann-Whitney test, Fig. 1).

At ISI 100 to 500 ms all control and NADNA-pretreated slices demonstrated paired-pulse facilitation, paired-pulse ratio of postsynaptic responses did not change after NEU inhibition (P = 0.81, Mann-Whitney test, Fig. 1).

The main finding of the current study is that regardless of the significant effect of NEU blockage on basal synaptic efficacy and long-term potentiation, it does not affect short-term synaptic plasticity. Our results are in agreement with previous study, when NEU was applied exogenously to decrease concentration of PSA on the outer cell membrane, and had no influence on the short-term plasticity in CA3-to-CA1 networks of organotypic hippocampal culture [17]. In addition, application of another NEU blocker, oseltamivir carboxylate, the active form of anti-influenza drug Tamiflu, did not change the paired-pulse potentiation of population spikes in CA1 region of the hippocampus [18]. However, at mossy fiber — CA3 pyramidal cell synapses NADNA failed to decrease the paired-pulse facilitation (Minami *et al.*, 2016). Thus, the mechanism of synaptic plasticity induction at mossy-fiber-CA3 synapses differs from that in Schaffer collateral-CA1 synapses [19].

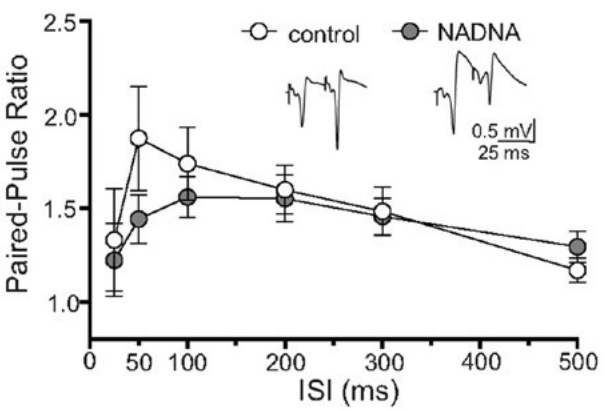

Fig. 1. The effect of neuraminidase inhibition on paired-pulse plasticity in CA1 pyramidal cell layer of hippocampus. The graph summarizes the paired-pulse ratio of pop-spike amplitudes in control (white) and NADNA-pretreated slices (grey). Insets: averaged sample records of pop-spike at CA3-CA1 synapses measured at 25 ms ISI. All data are presented as Mean ± SEM.

Since the paired-pulse plasticity reflects changes in the release probability of presynaptic cell [15], we hypothesized that pharmacological blockade of NEU activity does not affect the mechanisms of release probability on the presynaptic site. The evidences that pretreatment with NADNA influences the LTP [6, 12] are in favor of postsynaptic mechanisms evolved in its manifestation. As NEU blockage promotes desialylation on the cell membrane, it can change cell surface properties. Previous studies indicate that increase in membrane sialylation or downregulation of NEU activity could significantly alter neuronal activity. The possible mechanism of long forms of synaptic plasticity alterations due to desiyalylation is the transient decrease in synaptic strength according to vesicle depletion or desensitization of postsynaptic receptors and activity-dependent receptor internalization [20–22].

Conclusions

Our data indicate that regardless of the ISI the paired-pulse ratio (PPR) of postsynaptic responses did not change after NADNA pretreatment in CA3-to-CA1 network of hippocampal pyramidal cell layer.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Фармакологічне блокування активності нейрамінідази не впливає на пластичність, викликану парною стимуляцією, нейронної мережі CA3-CA1 гіпокампа

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Поліsіалові кислоти (ПСК) зовнішньоклітинної мембрани широко розповсюджені у нервовій тканині. Їх зв'язки із гліколіпідами та глікопротеїнами є вкрай важливими у процесах синаптичної пластичності. Нейрамінідаза (NEU) є основним ферментом, який контролює кількість ПСК шляхом розщеплення сіалових кислот із сіалоглюкокон'югатів. **Мета.** В даній роботі ми досліджували роль блокування NEU у процесах обробки короткотривалої пам'яті. Раніше нами було показано, що блокада NEU призводить до суттєвого зниження довготривалої потенціації та збільшення короткотривалої депресії у нейронній мережі CA3-CA1 радіального шару гіпокампа. **Методи.** Використовуючи специфічний блокатор N-ацетил-2,3-дегідро-2-дезоксинейрамінову кислоту (NADNA), ми досліджували ефект пригнічення активності NEU на пластичність, індуковану парною стимуляцією в синапсах від коллатералей Шаффера до CA1 ділянки пірамідного шару гіпокампа щурів. **Результати.** В даному дослідженні продемонстровано, що блокування NEU викликає збільшення збуджуючих постсинаптичних потенціалів без змін у співвідношенні фасилітації/депресії. **Висновки.** Пригнічення активності NEU не впливає на пластичність, викликану парною стимуляцією, яка відображає зміни вірогідності вивільнення нейромедіаторів на пресинаптичному сайті. Ми припускаємо, що вплив NADNA на базову активність та довготривалу пластичність опосередковується через залучення постсинаптичних механізмів.

Ключові слова: блокатор нейрамінідази, синаптична пластичність, гіпокамп.

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