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The effect of neuraminidase blocker on gabazine-induced seizures in rat hippocampus

Concentration of neuraminidase (NEU), an enzyme which cleaves negatively charged sialic acids from carbohydrate moieties of the cellular memabrane, could vary depending on physiological conditions. Multiple evidences suggest that fluctuations of NEU extracellular concentrations can influence neuronal activity. In the present study we examined the effect of down regulation of endogenous NEU activity on seizure-like activity (SLA) induced by gabazine (specific blocker of inhibitory synaptic transmission) in the hippocampal CA1 pyramidal region of cultured slices. We show that in slices pretreated with the blocker of endogenous NEU, N-acetyl-2,3-dehydro-2-deoxyneuraminic acid (NADNA), duration of synchronous oscillations induced by gabazine was considerably increased comparatively to control untreated slices. This study adds further information that changes in the level of NEU activity is an important factor, which can affect neuronal network excitability.

Keywords: polysialic acid, neuraminidase blocker, seizure, gabazine, hippocampus.

INTRODUCTION

Polysialic acid, a large cell-surface negatively charged carbohydrate, regulates manifold physiological functions including cell migration, axon outgrowth, neurogenesis, synaptogenesis and neuronal excitability [7,10,16]. A key enzyme, which regulates the level of sialic acid in the cell outer membrane, is endogenous NEU [14, 15]. Concentration of NEU in the brain could vary in dependence on physiological conditions [2] and many studies suggest that increasing in the level of the extracellular concentrations of NEU can affect cell-to-cell interactions, synaptogenesis and influence neuronal activity [7,13,17]. However, there is a lack of studies devoted to investigation of the effect of endogenous NEU deficiency on the cellular and neuronal network activity. In our recent study we showed that seizures induced by infusion of the high-potassium/low magnesium (High-K^+/low Mg^{2+}) artificial cerebrospinal fluid (ACSF) into hippocampus were significantly longer and seizure threshold was decreased in rats pretreated by the NEU blocker [7]. A major goal of the present study was to determine how oversialylation following blockade of endogenous NEU affected the hippocampal seizures evoked by gabazine, a specific $GABA_A$ receptor inhibitor. The mechanism of seizure induction in gabazine model of seizures is based on the blockage of synaptic inhibition. The etiology of temporal lobe epilepsy is closely associated with hippocampal changes in $GABA_A$ receptor expression and function [3,11,18,20]. In the present study we showed that in hippocampal slices pretreated with specific NEU blocker epileptiform activity induced by blockade GABAergic transmission was considerably exacerbated.

METHODS

All procedures used in this study were approved by the Animal Care Committee of Bogomoletz Institute of Physiology.

Slice cultures were prepared using the method of Stoppini et al. [12,19]. Briefly, Wistar rat pups were anesthetized and decapi-

tated at postnatal day 7. The brains were removed and hippocampi were cut into 350 μm transverse sections using a McIlwain tissue chopper. Slices were then transferred to sterile porous membrane inserts (Millicell, Bedford, MA, USA), which were placed in a 6-well plate containing 1 ml culture medium/well (50% of MEM, 25% horse serum (HS), 25% HBSS, 5 mM Tris, 2 mM $NaHCO_3$, 12,5 mM HEPES, 15mM glucose, 100 U/ml penicillin and 100 μg/ml streptomycin, pH 7.2) and cultivated at +35°C at air atmosphere with 5% CO_2. The culture medium was changed the next day after preparation of the slices and then twice a week. All experiments with organotypic hippocampal slice cultures were performed at 14-21 days in vitro. For recordings, slices were transferred to a submersion-type chamber mounted to the microscope (Olympus BX50WI, Japan) and superfused with the oxygenated ACSF of the following composition in (mM): NaCl 126, KCl 3.5, $CaCl_2$ 2.0, $MgCl_2$ 1.3, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11. Extracellular field potentials were recorded from hippocampal CA1 pyramidal layer using borosilicate glass pippetes filled with ACSF. Pipette resistance was 1-3 Mï. Recordings were performed using AC differential amplifier (A-M Systems, Carlsborg, WA, USA) (bandpass 0.1 Hz1 kHz; 100) and digitized at 10 kHz online with an analogue-to-digital converter (NI PCI-6221, National Instruments, Austin, TX, USA) and stored on a computer using WinWCP program (Strathclyde Electrophysiology Software, University of Strathclyde, Glasgow, UK). Off-line analysis of the recordings was performed using Clampfit (Axon Instruments, Sunnyvale, CA, USA) and Origin 7.0 (Microcal Software, Northampton, MA, USA).

RESULTS AND DISSCUSION

Field potential recordings were performed from hippocampal CA1 pyramidal layer in organotypic slice culture. Bath application of 10 μM of gabazine led to the increase of neuronal activity following spontaneous interictal-like discharges in all control slices (Fig 1). To examine the effect of downregulation of the endogenous NEU activity cultured hippocampal slices were incubated with NADNA (2mM) during 2 hours. SLA was induced in all 10 slices pretreated with NADNA (Fig 1). This activity persisted as long as gabazine was kept in extracellular solution in control as well as in NADNA pretreated slices. The fre-

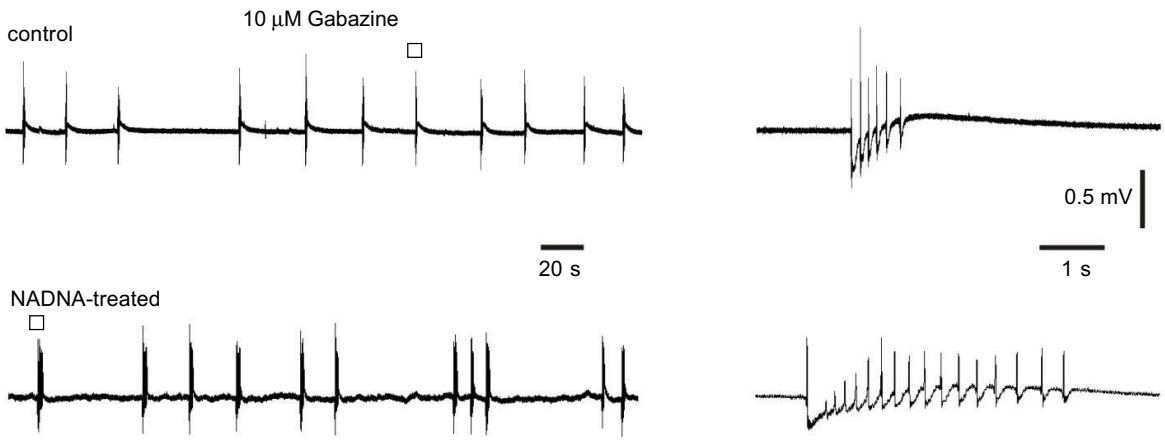

Fig.1. Effect of NEU activity suppression on gabazine-induced SLA in cultured hippocampal slices. Extracellular field potential recordings from CA1 pyramidal cell layer in the presence of 10μM gabazine in control (upper panel) and NADNA pretreated (lower panel) slices. Spontaneous discharges marked with squares shown in expanded scales in the right panel

quency of synchronous discharges during SLA for both control and NADNA-pretreated slices was 0,03± 0,01 Hz (Fig 2a). However the duration of synchronous oscillations was significantly increased in NADNA pretreated slices comparatively to controls (2,1 ± 0,3 s in control vs 3,8 ± 0,7 s in NADNA pretreated slices; $p< 0.05$, Fig 2b). These data are in agreement with our previous report when blockade of endogenous NEU significantly reduced seizure threshold and aggravated hippocampal seizures induced by infusion of High-K^+/low Mg^{2+} solution in vivo [7]. Previous studies have shown that pretreatment of slice with NEU significantly altered kinetic properties of the voltage-gated sodium channels [1,7,15]. The authors connected this phenomenon with a presence of the negatively charged sialic acid residues on the extracellular surface region of the channel [6,9]. The charge created by these carbohydrates constantly influences the gating apparatus of the channel. Pretreatment with NEU removes sialic acid residues from the extracellular membrane and as a result shifts channel activation curve to the depolarized direction [7,15]. Further support of the idea that sialic acids contribute to the voltage dependence of sodium channel gating was obtained using recombinant deletion of likely glycosylation sites from the sodium channel sequence [1]. The deletion of the channels glycosylation sites resulted in mutant channels that gated at voltages up to 10 mV more positive than wild-type channels. In our previous study pretreament with NEU led to increase in the action potential threshold following decreasing of neuronal activity [7]. The blockade of the endogenous NEU in our present study has an opposite effect on the neuronal network activity. It was demonstrated that seizure intensity in a kindling model of epilepsy were not altered when NADNA was administered concurrently with NEU. So there is no direct proconvulsant effect of NADNA on SLA [7]. We proposed that NEU deficiency lead to accumulation of sialic acid in extracellular region and as a result increases open probability of the sodium channels which leads to enhancement of the neuronal excitability. Recent studies strongly support this assumption. It was shown that NEU inhibitors induced paired-pulse facilitation in population spikes without changes in excitatory postsynaptic potentials in the CA1 region of hippocampal slices and enhanced synchronization in rat hippocampal CA3 networks [8,21]. Also the fact that inherited diseases (sialidosis, galactosialidosis, Salla disease etc.) concerned with defective or deficient metabolism of endogenous NEU

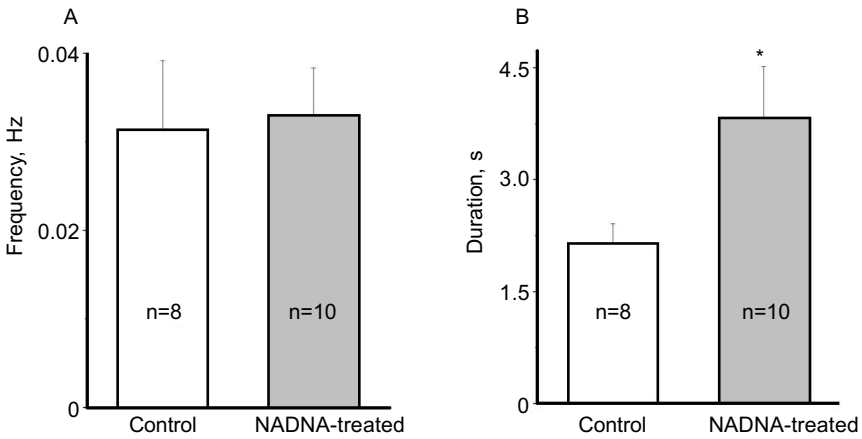

Fig.2. Effects of NADNA pretreatment on different characteristics of gabazine-induced SLA in CA1 pyramidal layer. Summary plots show the PS frequency (A) and duration (B) during the epileptiform discharges in control (white) and NADNA pretreated (grey) slices. All values are mean ± SEM, *P < 0.05 versus control. N designates the total number of slices in each experimental group

and sialic acid are often accompanied with epilepsy, exemplifies a substantial role of the level of sialylation in regulation of neuronal activity [4,5,22]. Present study adds further evidence that modulation of NEU activity renders a substantial influence on neuronal network excitability.

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ЕФЕКТ БЛОКАТОРА НЕЙРАМІНІДАЗИ НА ВИКЛИКАНІ ГАБАЗИНОМ НАПАДИ В ГІПОКАМПІ ЩУРІВ

Концентрація нейрамінідази – ферменту, який відокремлює негативно заряджені сіалові кислоти від вуглеводів клітинної мембрани, може змінюватися залежно від фізіологічних умов. Численні відомості свідчать про те, що флуктуації позаклітинної концентрації нейрамінідази можуть впливати на нервову активність. Ми досліджували ефект зменшення активності ендогенної нейрамінідази на епілептичну активність, викликану габазином (специфічним блокатором синаптичного гальмування) у пірамідальному шарі зони CA1 гіпокампа культивованих зрізів. Ми показали, що у зрізах, оброблених блокатором ендогенної нейрамінідази N-ацетил-2,3-дегідро-2-деоксинейраміновою кислотою, тривалість синхронних осциляцій, викликаних габазином, була значно збільшена порівняно з контрольними необробленими зрізами. Наше дослідження доповнює та розширює дані про те, що зміна активності нейрамінідази є важливим фактором, який може впливати на нервову збудливість.

Ключові слова: полісіалова кислота, блокатор нейрамінідази, габазін, гіпокамп.

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ЭФФЕКТ БЛОКАТОРА НЕЙРАМИНИДАЗЫ НА ВЫЗВАННЫЕ ГАБАЗИНОМ ПРИПАДКИ В ГИПОКАМПЕ КРЫС

Концентрация нейраминидазы – фермента, который отделяет негативно заряженные сиаловые кислоты от углеводов клеточной мембраны, может изменяться в зависимости от физиологических условий. Многочисленные данные свидетельствуют о том, что флуктуации внеклеточной концентрации нейраминидазы могут влиять на нервную активность. В этой работе мы исследовали эффект уменьшения активности эндогенной нейраминидазы на эпилептическую активность, вызванную габазином (специфическим блокатором синаптического торможения) в пирамидном слое зоны CA1 гиппокампа культивированных срезов. Мы показали, что в срезах, обработанных блокатором эндогенной нейраминидазы N-Ацетил-2,3-дегидро-2-деоксинейраминовой кислотой, длительность синхронных осцилляций, вызванных габазином, значительно увеличивалась по сравнению с контрольными необработанными срезами. Настоящая работа дополняет и расширяет сведения о том, что изменение активности нейраминидазы является важным фактором, который может влиять на нервную возбудимость.

Ключевые слова: полисиаловая кислота, блокатор нейраминидазы, габазин, гиппокамп.

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