

Nonsynaptic epileptiform activity in CA3-CA1 regions of the hippocampus in low-Ca²⁺ and Cd²⁺-containing milieu

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In our work we compare seizure-like-activity in CA1 and CA3 regions of the hippocampus in two common models of nonsynaptic epilepsy in vitro: low-Ca²⁺ and Cd²⁺ models. Under low-Ca²⁺ conditions, the concentration of Ca²⁺ is insufficient to produce synaptic release. Cd²⁺ ions block Ca²⁺ channels, which result in a decrease of intracellular concentration of Ca²⁺ in presynaptic sites. We found that delay time for seizure appearance in CA1 area is longer than in CA3 in both models. The frequency of epileptic-like discharges in low-Ca²⁺ model was higher than in Cd²⁺-model. We did not find a difference in patterns of seizure-like discharges, which suggests a similar mechanism explored in studied models. The difference in distribution and frequency of nonsynaptic seizure-like activity in the hippocampus is discussed in the paper. Key words: nonsynaptic epileptiform activity; low-Ca²⁺ model of seizures; CA3-CA1 regions of the hippocampus; rat brain slices.

INTRODUCTION

Fast glutamatergic and GABAergic synaptic transmission plays a key role in epileptiform synchronization of hippocampal network. However, under certain *in vitro* and *in vivo* experimental conditions the hippocampal formation is prone to dramatic neuronal synchronization that is independent of active chemical synaptic transmission [1, 2]. In the early 1980s, it was shown that perfusion of hippocampal slices with low-Ca²⁺ aCSF (at concentrations sufficient to block synaptic transmission) results in spontaneous long-lasting seizure-like activity (SLA) in area CA1 and under certain conditions in area CA3 and the dentate gyrus of the hippocampus [3, 4]. Later, it was shown that similar discharges could be evoked at normal external Ca²⁺ levels and even with intact synaptic transmission, as long as there was a sufficient increase in neuronal excitability [1, 5]. The observed activity approximated low-Ca²⁺ discharges in frequency,

duration, and propagation speed, and the addition of postsynaptic receptors antagonists to the perfusion aCSF had no effect on this type of bursting, thus proving its nonsynaptic origin [1].

Four mechanisms are considered to account for abnormal neuronal synchronization in the absence of chemical synaptic transmission: electrical field interactions, fluctuations in extracellular ion concentrations, ephaptic transmission, and electrotonic coupling through gap-junctions [6]. Under suitable conditions of tight and laminar hippocampal cellular organization, electrical field effects sustain dramatic neuronal synchronization and provide a means for synaptically-independent propagation of epileptiform activity across the hippocampus [7, 8]. Reduction of extracellular space due to cell swelling promotes the spread of excitation between neuronal membranes through ephaptic interactions and together with field effects accounts for increased neuronal excitability under hypo-osmotic conditions [6, 9]. Excessive fluctuations of extracellular K⁺ during seizures

alter membrane excitability and underlie propagation of nonsynaptic activity across the tissue, although at a rate almost a hundred times slower than electrical field propagation [3, 10, 11]. Increased number of gap junctions occur in the CA1 area during prolonged perfusion with low-Ca²⁺ solutions, affecting the frequency, duration, and amplitude of nonsynaptic discharges [8, 12]. Hence, while nonsynaptic interactions have a subliminal impact on normal network functioning, they become extremely effective in neuronal synchronization under pathological conditions. In this respect, a low-Ca²⁺ milieu provides a suitable conditions for studying the impact of nonsynaptic interactions on hippocampal local synchronization. On the other hand, activity of voltage-dependent Ca²⁺ channels regulates synaptic transmission and blockade of these channels results in appearance of nonsynaptic local synchronization [1]. The aim of our study was to compare effect of removing Ca²⁺ from extracellular milieu and blockade of voltage-gated Ca²⁺ channels on patterns of network activity in hippocampus.

METHODS

All experimental procedures were performed on Wistar rats according to the guidelines set by the National Institutes of Health for the humane treatment of animals and approved by the Animal Care Committee of Bogomoletz Institute of Physiology of National Academy of Science of Ukraine.

Hippocampal slice preparation. Postnatal day 12–14 rats were deeply anesthetized using sevoflurane and decapitated. Transverse brain slices were prepared according to the technique described previously [13]. Briefly, brains were removed and placed in the ice-cold aCSF of the following composition (in mmol/l): NaCl – 125, KCl – 3.5, CaCl₂ – 2, MgCl₂ – 1.3, NaH₂PO₄ – 1.25, NaHCO₃ – 24 and glucose – 11; pH of aCSF was adjusted to 7.3–7.35. The cerebellum and the frontal lobe were removed, and 500 μm thick slices were cut using Vibroslice NVSL

(“World Precision Instruments”, Sarasota, FL). Slices were allowed to equilibrate at the room temperature and under constantly oxygenated aCSF for at least 1.5–2 hours before the experiment.

Extracellular recordings and data acquisition. For extracellular recordings, slices were transferred to a submerged recording chamber and perfused with oxygenated aCSF (23–25°C) at a rate of 2–3 ml/min. Temperature control was performed with the Dual Temperature Controller (TC-144, “Warner Instruments”). Field potentials were obtained from the CA3 and CA1 pyramidal cell layer with extracellular glass microelectrodes (2–3 MΩ) filled with normal aCSF. Signals were amplified using a differential amplifier (“A-M Systems”, Carlsborg, WA), digitized at 10 kHz using an analog-to-digital converter (NI PCI-6221; National Instruments, Austin, TX); online analysis was performed using the WinWCP program (“Strathclyde Electrophysiology Software”, University of Strathclyde, Glasgow, UK). Offline analysis was performed using Clampfit (“Axon Instruments”) and Origin 8.0 (“OriginLab”, Northampton, MA). A two-sample *t*-test, was used for statistical analysis. Data are presented as mean ± SE.

Induction of nonsynaptic epileptiform activity. Nonsynaptic epileptiform discharges were induced by perfusion hippocampal slices with “low-Ca²⁺ aCSF” of the following composition (mmol/l): NaCl – 115, KCl – 5, MgCl₂ – 1, NaH₂PO₄ – 1.25, NaHCO₃ – 24, D-glucose – 11; pH 7.35–7.4. In separate set of experiments epileptiform bursting was induced by perfusing hippocampal slices with cadmium-containing aCSF of the following composition (mmol/l): NaCl – 115, KCl – 5, CaCl₂ – 1, MgCl₂ – 1, NaH₂PO₄ – 1.25, NaHCO₃ – 24, D-glucose – 11 and CdCl₂ – 15 μM; pH 7.35–7.4.

A total of 52 hippocampal slices were used in this study. Student’s *t*-test was used for statistical analysis. Data presented as mean ± SE; the difference between groups was considered significant when *P* ≤ 0.05. All chemicals were purchased from “Sigma” (USA).

RESULTS AND DISCUSSION

Consistent with previous studies, perfusion of hippocampal slices with low- Ca^{2+} or Cd^{2+} aCSF resulted in the appearance of spontaneous epileptiform discharges in both hippocampal zones [5, 10] (Fig.1). In CA1 zone epileptiform discharges were induced in 82% of slices perfused with low- Ca^{2+} aCSF, while in CA3 zone SLA were induced only 41% of slices (29/35 and 15/35 respectively). During perfusion with Cd^{2+} -containing aCSF nonsynaptic bursting was induced in CA1 in 41% of slices and in CA3 – in 82% (7/17 and 14/17 respectively, Fig. 2). Nonsynaptic discharges represented high-frequency population spikes that either fired continuously or were arranged in recurrent bursts (Fig. 2). We refer to the two observed types of recurrent nonsynaptic discharges as intermitted and persistent bursts of population spikes (Fig. 2b, c). Persistent bursts represented a period of high frequency population spikes firing that had a spindle shape with maximum spike amplitude in the middle of the burst (Fig. 2c). Intermitted bursts were never spindle-like, the amplitude of the spikes was greater than in persistent bursts

and normally stayed the same throughout the burst (Fig. 2b). Along with population spikes, we observed slow shifts of baseline potential, which we refer to as slow waves (Fig. 1b).

Nonsynaptic bursting had a longer latency to onset within region CA1 than within CA3 (Fig.3a). The delay time for the SLA at low- Ca^{2+} was 21.08 ± 2.17 min ($n = 31$) in area CA1 and 14.68 ± 1.79 min ($n = 29$, $P = 0.03$) in region CA3 (Fig. 3a). Similarly, at Cd^{2+} spontaneous activity was induced with the latency of 15.66 ± 2.27 min in region CA1 ($n = 15$) and 10.14 ± 1.37 min ($n = 14$, $P = 0.05$) in region CA3 (Fig. 3a).

The frequency of continuous population spikes in region CA1 was 1.48 ± 0.2 s^{-1} ($n = 31$) at low- Ca^{2+} in aCSF and 0.68 ± 0.17 s^{-1} ($n = 13$) in Cd^{2+} aCSF ($P = 0.02$); in area CA3 the frequency of population spikes was 1.79 ± 0.33 s^{-1} ($n = 17$) at low- Ca^{2+} and 0.87 ± 0.2 s^{-1} ($n = 14$, $P = 0.01$) at Cd^{2+} in aCSF (Fig. 3b). Slow waves had duration ≥ 2 sec and appeared irregularly with a mean frequency of 0.02 ± 0.005 Hz at low- Ca^{2+} and 0.05 ± 0.01 Hz at Cd^{2+} ($P = 0.1$). It is worth noting that these discharges do not resemble the negative potential shifts observed originally in low- Ca^{2+} solution and

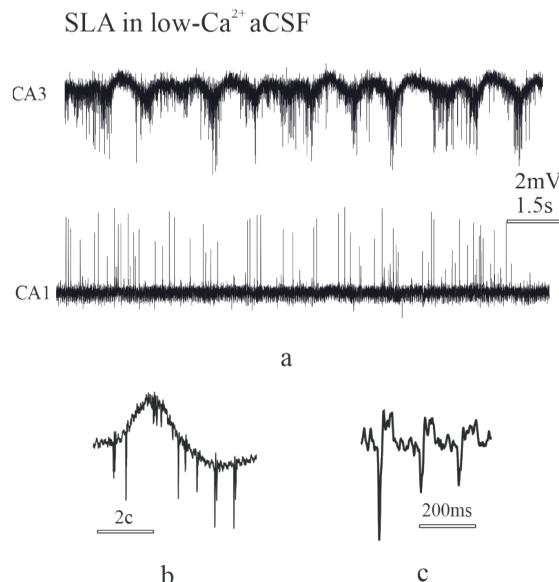


Fig. 1. Electrophysiological recording of nonsynaptic seizure-like activity induced in low- Ca^{2+} aCSF; a – simultaneous recording of spontaneous epileptiform activity in CA3 and CA1 hippocampal zones during perfusion with low- Ca^{2+} aCSF; b – population spikes superimposed on slow potential shift; c – single population spikes

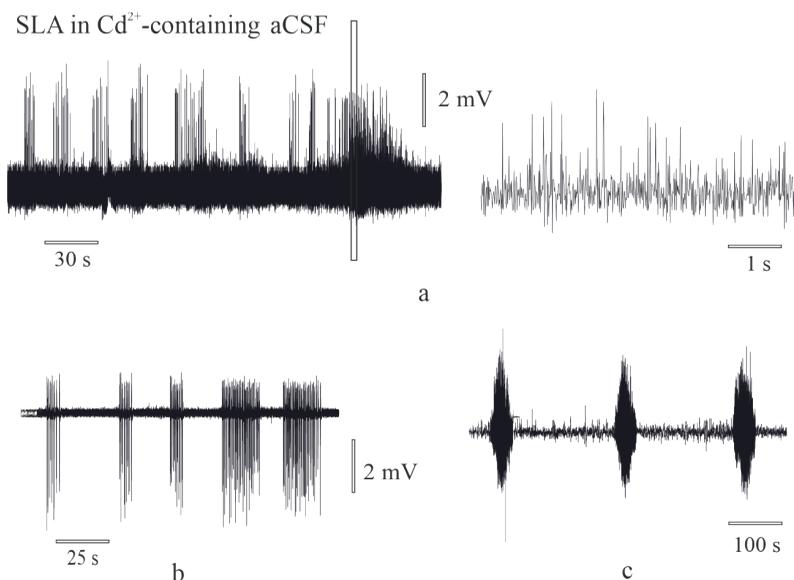


Fig. 2. Types of nonsynaptic seizure-like activity induced by aCSF containing Cd²⁺; a – population spikes recorded in CA1 hippocampal area, the portion of recording outlined in the box is shown enlarged on the left; b – intermitted bursts of SLA; c – persistent bursts of SLA

are not considered the same type of discharges (Haas and Jefferys, 1984).

Perfusion of hippocampal slices with low-Ca²⁺ aCSF is known to induce nonsynaptic bursting [10]. It is important to note that in the present work we did not observe slow negative potential shifts that were previously recorded in low-Ca²⁺ milieu and are known to arise from extracellular accumulation and consequent influx of K⁺ into glia [3, 6]. Slow redistribution of K⁺ transients mediated by glial spatial buffering mechanisms likely accounts for the slow propagation of low-Ca²⁺ negative potential shifts previously seen [6, 14]. However, *in vivo* experiments have shown that elevation of K⁺ transforms low-Ca²⁺ bursts into persistent spike activity [15]. While in the neonatal brain K⁺ buffering mechanisms are not completely developed, it is likely that persistent spike activity, as shown in the present study, represents another form of low-Ca²⁺ nonsynaptic bursting [6].

Previous studies have shown that the CA1 area is more prone to nonsynaptic discharges than CA3 due to compact cellular organization and lower extracellular volumes compared to the remainder of the hippocampus [3, 6]. Our

results are consistent with this observation, as nonsynaptic SLA were more likely induced in the CA1 area than in CA3 at low-Ca²⁺.

In the present study, low-Ca²⁺ and Cd²⁺ aCSF induced a similar profile of epileptiform discharges. In both cases, nonsynaptic SLA represented population spikes that in previous studies were shown to be synchronized action potentials of pyramidal cells. However, the frequency of population spikes was significantly higher in low-Ca²⁺ compared to Cd²⁺ in both the CA3 and CA1 regions respectively. This observation may be explained by the slightly different conditions that are provided by low-Ca²⁺ compared to Cd²⁺ aCSF. Aside from blocking synaptic transmission, under low-Ca²⁺ neuronal excitability is altered mainly via two mechanisms: 1) decreasing surface charge screening and 2) reducing Ca²⁺-activated K⁺ conductance [13, 16]. Both mechanisms bring the cellular membrane closer to the excitation threshold, thus making neurons more sensitive to extracellular potentials generated by the adjacent firing cells, which in term results in increased neuronal synchronization through ephaptic and electrical field interactions. Additionally,

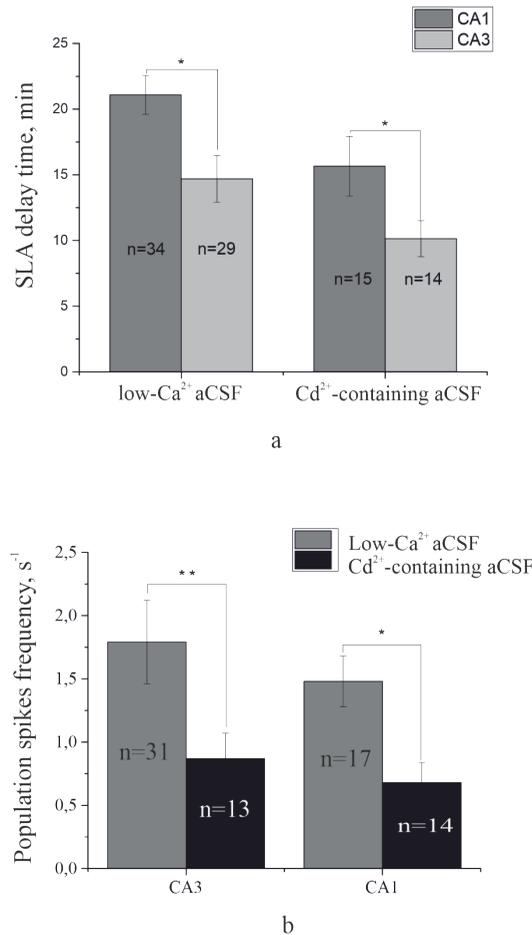


Fig. 3. Comparison of SLA characteristics in low-Ca²⁺ and Cd²⁺-containing aCSF; a – mean values for the delay time for non-synaptic epileptiform activity; b – mean values for the frequency of population spikes

low-Ca²⁺ solution increases dye coupling between hippocampal pyramidal cells, possibly promoting additional synchronization of small neuronal clusters through gap junctions [12]. Taken together, these factors likely account for a higher frequency of population spikes recorded in low-Ca²⁺ compared to Cd²⁺ aCSF.

CONCLUSIONS

1. Application of low-Ca²⁺ aCSF or addition to aCSF of 15 μM Cd²⁺ results in the appearance of recurrent nonsynaptic discharges as bursts of population spikes.

2. Delay time for seizure appearance in

CA1 area is longer than in CA3 in both models of nonsynaptic seizures.

3. The frequency of nonsynaptic seizures in low-Ca²⁺ model of seizures was greater than in Cd²⁺ model.

4. Low-Ca²⁺ model and Cd²⁺ model cause similar patterns of nonsynaptic seizure-like activity.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of coauthors of the article.

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НЕСИНАПТИЧНА ЕПІЛЕПТИФОРМНА АКТИВНІСТЬ У ДІЛЯНКАХ СА3-СА1 ГІПОКАМПА ЩУРІВ У НИЗЬКОКАЛЬЦІЄВОМУ ТА КАДМІЙВІСНОМУ СЕРЕДОВИЩАХ

У нашій роботі порівнювали епілептиформну активність у ділянках СА3-СА1 гіпокампа на двох моделях несинаптичної епілепсії *in vitro*: з низьким вмістом Ca²⁺, а також наявністю Cd²⁺. У разі 1-ї моделі концентрації Ca²⁺ було недостатньо для синаптичного вивільнення медіатора. В іншій серії експериментів іони кадмію блокували Ca²⁺-канали, що призводило до зниження внутрішньоклітинної концентрації кальцію у пресинаптичних ділянках. Ми виявили, що латентний час розвитку епілептиформної активності в зоні СА1 довший, ніж у СА3 в обох випадках. Частота епілептиформних спалахів у дослідах з низьким вмістом Ca²⁺ була більшою, ніж з іонами кадмію. У представлених результатах не виявлено відмінностей у двох моделях епілептиформної активності, що вказує на аналогічний механізм їх розвитку. В роботі також обговорюється різниця в розподілі несинаптичної епілептиформної активності в досліджуваних ділянках гіпокампа.

Ключові слова: несинаптична епілептиформна активність; ділянки СА3-СА1 гіпокампа; кадмій.

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НЕСИНАПТИЧЕСКАЯ ЭПИЛЕПТИФОРМНАЯ АКТИВНОСТЬ В ЗОНАХ СА3-СА1 ГИПОКАМПА КРЫС В НИЗЬКОКАЛЬЦИЕВОЙ И КАДМИЙСОДЕРЖАЩЕЙ СРЕДЕ

В нашей работе мы сравниваем эпилептиформную активность гиппокампа в двух моделях несинаптической эпилепсии *in vitro*: с низким содержанием Ca²⁺, а также в присутствии Cd²⁺. Что касается 1-й модели, концентрации Ca²⁺ было недостаточно для синаптического высвобождения медиатора. В другой серии экспериментов ионы кадмия блокировали Ca²⁺-каналы, что приводило к снижению внутриклеточной концентрации кальция в пресинаптических участках. Мы обнаружили, что латентный период развития эпилептиформной активности в зоне СА1 длиннее, чем в СА3 в обоих моделях. Частота эпилептиформных вспышек в опытах с низким содержанием Ca²⁺ была больше, чем с ионами кадмия. В представленных результатах не выявлено различий в двух моделях эпилептиформной активности, что указывает на аналогичный механизм их развития. В работе также обсуждается разница в распределении несинаптической эпилептиформной активности в исследуемых участках гиппокампа.

Ключевые слова: несинаптическая эпилептиформная активность; СА3-СА1 гиппокамп; кадмий.

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