Modulation of the hippocampal propensity to non-synaptic epileptiform synchronization in low-calcium model of epilepsy

O.S. Zapukhliak, O.V. Netsyk, O.S. Rasulova, D.S. Isaev

O.O. Bogomoletz Institute of Physiology of National Academy of Science of Ukraine, Kyiv; e-mail: dmytro.isaev@gmail.com

The CA3 and CA1 regions are the main stages of the “three-synaptic pathway”, which plays a role in the generation of hyper-synchronous events in the hippocampus. Under certain experimental conditions, this brain structure might support pathological epileptiform synchronization that is independent of active chemical synaptic transmission. In present work, we estimated the conditions that would facilitate non-synaptic synchronization of the hippocampus. Non-synaptic epileptiform activity was induced in hippocampal slices by the omission calcium ions from the extracellular milieu. The propensity of hippocampal regions to nonsynaptic interactions was estimated by measuring the delay time needed for the development of low-Ca\(^{2+}\) discharges in the CA3 and CA1. Next, an increase of neuronal excitability was induced by the pre-incubation of hippocampal slices in 4-aminopyridine (4-AP) and by the reduction of extracellular osmolarity. Pre-incubation of hippocampal slices with 4-AP under normal osmotic conditions resulted in decreased latency for non-synaptic discharges in the CA3, but not in the CA1. However, hypo-osmotic conditions caused increased excitability of the CA3 region, which resulted in decreased delay time for nonsynaptic discharges and this level of cellular excitability was not further enhanced by the pre-incubation with 4-AP.

Key words: low-Ca\(^{2+}\) seizure-like activity; 4-aminopyridine; hypo-osmolarity; hippocampus.

INTRODUCTION

Temporal lobe epilepsy (TLE) is the most common type of partial epilepsy that is characterized by the recurrent seizures generated in temporal lobe structures, such as hippocampus, amygdala, and parahippocampal gyrus [1]. Furthermore, the hippocampus is considered one of the primer seats of seizure generation in the temporal lobe and hippocampal sclerosis is among the most common causes of TLE [2]. Since the mid of the last century, unilateral excision of the hippocampal formation is known to terminate seizures in humans [3]. However, the dramatic effect of hippocampal removal on memory functioning makes temporal lobectomy the least-choice procedure for seizure termination, and the lack of therapeutic methods for epilepsy treatment remains to be one of the problems in modern neuroscience.

The question of how the process of seizure generation can be interrupted or prevented is one of the intensively studied. During the last several decades a multitude of in vitro and in vivo experimental models of hippocampal seizures has been developed, but still, the precise identification of functional deficits that underlie seizure generation and spread, remains to be illusive [4]. One of the modern experimental approaches in epilepsy research is to study the molecular conditions that arise in the nerve tissue during seizures and to model these conditions in vitro in order to manipulate them with various pharmacological agents [5].

In the 1980s it was demonstrated that lowering extracellular calcium (to the levels that block Ca\(^{2+}\)-dependent neurotransmitter release) induces seizure-like activity in the hippocampus in vitro [6]. Similar discharges were later evoked...
with Ca$^{2+}$-chelating agents in vivo [7]. Additionally, the observation that during seizures local calcium levels may drop to as low as 0.2 mmol/l, which is sufficient to block chemical synaptic transmission, indicates that low-Ca$^{2+}$ (zero-Ca$^{2+}$) model of epileptic discharges represents a relevant tool for studying molecular and electric processes that occur in the brain during seizures [8].

The CA3 and CA1 regions are the main stages of the “three-synaptic pathway” of the hippocampus, which might play a role in the generation of hyper-synchronous events in this brain structure [9]. Hippocampal region CA1, due to its tight and laminar cellular organization and decreased extracellular volume fraction, can support local neuronal synchronization and give a way to synthetically free propagation of epileptiform events under low-Ca$^{2+}$ conditions [8]. The region CA3 of the hippocampus has more recurrent synaptic connections than CA1 and is known to facilitate synchronized “inter-ictal” discharges due to CA3 pacemaker activity [10]. However, under non-synaptic conditions of low-Ca$^{2+}$ milieu CA3 zone is reported to be less prone to seizure-like discharges than CA1 [6]. Thus, in present work, we aimed to estimate conditions that would facilitate synchronization of the CA3 neuronal net through non-synaptic interactions.

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 [11]. Briefly, brains were removed and placed in the ice-cold aCSF of the following composition, (in mmol/l): NaCl - 125, KCl - 3.5, CaCl$_2$ - 2, MgCl$_2$ - 1.3, NaH$_2$PO$_4$ - 1.25, NaHCO$_3$ - 24, and glucose - 11; pH of the 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 (25-27°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 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). Two-sample t-test, was used for statistical analysis. Data is presented as mean ± SE.
O.S. Zapukhliak, O.V. Netsyk, O.S. Rasulova, D.S. Isaev

**Induction of epileptiform discharges by low-Ca\(^{2+}\) aCSF and pre-incubation in 4-aminopyridine containing aCSF.** In the first set of experiments, non-synaptic epileptiform discharges were induced by perfusion hippocampal slices (n = 9) with “normal osmolarity” low-Ca\(^{2+}\) aCSF of the following composition, (in mmol/l): NaCl - 125, KCl - 5, MgCl\(_2\) - 1, NaH\(_2\)PO\(_4\) – 1.25, NaHCO\(_3\) - 24, D-glucose - 11; pH = 7.35-7.4.

In the experiments with pre-incubation of slices with 4-aminopyridine (4-AP), prior to switching to low-Ca\(^{2+}\) aCSF, hippocampal slices (n = 9) were perfused with “4-AP aCSF” of the following composition, (in mmol/l): 125 NaCl - 125, KCl - 5, CaCl\(_2\) - 2, MgCl\(_2\) - 1, NaH\(_2\)PO\(_4\) – 1.25, NaHCO\(_3\) - 24, D-glucose - 11, 4-AP - 100µM.

Following 20 min of 4-AP pre-incubation, perfusion solution was switched to the aCSF with omitted Ca\(^{2+}\) ions (“low-Ca\(^{2+}\) + 4-AP aCSF”) to induce non-synaptic epileptiform activity.

**Induction of epileptiform discharges in hypo-osmotic aCSF.** In next set of experiments (n = 30), non-synaptic epileptiform discharges were induced under hypo-osmotic conditions using “hypo-osmotic low-Ca\(^{2+}\) aCSF” of the following composition (in mmol/l): NaCl - 115, KCl - 5, MgCl\(_2\) - 1, NaH\(_2\)PO\(_4\) – 1.25, NaHCO\(_3\) - 24, D-glucose - 11. Similarly, pre-incubation with 4-AP was performed in “hypo-osmotic 4-AP aCSF” (n = 10), of the following composition, (in mmol/l): NaCl - 115, KCl - 5, CaCl\(_2\) - 2, MgCl\(_2\) - 1, NaH\(_2\)PO\(_4\) – 1.25, NaHCO\(_3\) - 24, D-glucose - 11, 4-AP - 100µM. Following 20 min of 4-AP pre-incubation, perfusion was switched to hypo-osmotic aCSF with omitted Ca\(^{2+}\) ions (“hypo-osmotic low-Ca\(^{2+}\) + 4-AP aCSF”) to induce non-synaptic epileptiform activity. A total of 58 hippocampal slices were used in this study. All chemicals were purchased from «Sigma» (USA).

**RESULTS AND DISCUSSION**

Perfusion hippocampal slices with low-Ca\(^{2+}\) aCSF is known to block active chemical synaptic transmission and induce non-synaptic epileptiform discharges in rat hippocampal slices [12]. Low-Ca\(^{2+}\)-induced epileptiform discharges represent synchronized action potentials of thousands of pyramidal cells, which can be recorded extracellularly in the CA3 and CA1 regions as continuously firing population spikes (Figure, a), [13,14]. Unlike the most in vitro models of epilepsy (low-Mg\(^{2+}\), bicuculline, and 4-AP), in low-Ca\(^{2+}\) aCSF epileptiform discharges are not synchronized between the CA3 and CA1 zones due to the absence of active chemical synaptic transmission [6,15,16,17]. Therefore, low-Ca\(^{2+}\) milieu induces “local” hippocampal synchronization, which originates mostly in the CA1 region. The hippocampal propensity to non-synaptic synchronization depends on several factors among which are: i) laminar organization of neuronal structure; ii) resistivity and composition of extracellular medium; iii) excitability of neuronal membranes and number of electrical contacts between them [6,8].

In the first part of present work, we compared the delay time for low-Ca\(^{2+}\) epileptiform activity in “control” hippocampal slices with the delay time in hippocampal slices exposed to 4-AP pre-incubation. These experiments were performed under normal osmotic conditions (NaCl - 125 mmol/l). The delay time was measured as latent period in minutes from the start of perfusion slices with low-Ca\(^{2+}\) aCSF until the occurrence of non-synaptic epileptiform activity in the CA3 and CA1. Perfusion hippocampal slices with low-Ca\(^{2+}\) aCSF induced non-synaptic bursting with the latency of 22.9±2.54 min in CA3 and 13.54±1.82 min in CA1 (n\(_{CA3}\) = 5, n\(_{CA1}\) = 8, P = 0.01, Figure, a-c). This observation is consistent with previous results that CA1 region is more prone to low-Ca\(^{2+}\) epileptiform activity than CA3, possibly due to more tight cellular organization in CA1 compared to CA3 [8]. Pre-incubation with 4-AP resulted in decreased latency time of low-Ca\(^{2+}\) discharges in CA3 but not in CA1: the delay time for non-synaptic discharges in 4-AP pre-incubated slices was
13.12±2.34 min in CA3 and 13.89±3.70 min in CA1 (n_{CA3} = 8, n_{CA1} = 8, P = 0.86, Figure, c). Thus, under normal osmotic conditions (NaCl - 125 mmol/l), compared to the control, pre-incubation with 4-AP resulted in decreased latency of low-Ca^{2+} discharges in the CA3, but not in the CA1 zone.

Decrease in extracellular osmolarity is known to promote non-synaptic interactions between the cells [8]. Shrinkage of extracellular space due the cell swelling under hypo-osmotic conditions results in the increased proximity of cellular membranes. The latter enables currents produced on the one membrane to affect the

Non-synaptic epileptiform discharges induced by perfusion hippocampal slices with low-Ca^{2+} aCSF. a – extracellular recording obtained from CA3 region in low-Ca^{2+} aCSF of normal osmolarity (Na 125 mmol/l). b – extracellular recording of epileptiform activity obtained from CA3 region, slice was pre-incubated with 4-AP, 100 µM, perfusion solution was then switched to the low-Ca^{2+} aCSF, which resulted in appearance of non-synaptic epileptiform discharges. c – delay time (min) for low-Ca^{2+} non-synaptic epileptiform discharges under normal osmotic conditions (NaCl – 125 mmol/l): pre-incubation of slices with 4-AP significantly decreased the delay time for low-Ca^{2+} epileptiform activity in CA3 (P = 0.019), but not in CA1 (P = 0.93). d – under hypo-osmotic conditions (NaCl - 115 mmol/l), pre-incubation of hippocampal slices with 4-AP had no significant effect on delay time of low-Ca^{2+} epileptiform discharges both in CA3 (P = 0.84) and CA1 (P = 0.17)
membrane potential of the opposing membrane and to reduce action potential threshold of it. Therefore, we also studied the impact of 4-AP pre-incubation on the latency of low-Ca\(^{2+}\) epileptiform discharges under hypo-osmotic conditions, induced by reducing extracellular sodium concentration from 125 mmol/l to 115 mmol/l. Under hypo-osmotic conditions, (NaCl - 115 mmol/l), CA3 region became more prone to non-synaptic bursting than CA1. The delay time for low-Ca\(^{2+}\) discharges was 13.65±3.74 min in CA3 and 22.84±2.97 min in CA1 (n = 8, n = 20, P = 0.09, Figure, d). Pre-incubation with 4-AP in hypo-osmotic conditions had no effect on the latency of low-Ca\(^{2+}\) discharges both in CA3 and CA1: the delay time in CA3 was 14.5±1.76 min and in CA1 30.59±3.70 min (n = 8, n = 7, P = 0.001, Figure, d).

Our results indicate that “epileptic experience” induced by 4-AP pre-incubation can increase CA3 propensity to non-synaptic discharges under normal osmotic conditions. However, under hypo-osmotic conditions increased excitability of the CA3 region results in decreased delay time for nonsynaptic discharges and this level of cellular excitability cannot be further enhanced by the pre-incubation with 4-AP.

While low-Ca\(^{2+}\) aCSF is a model of artificial epileptiform discharges, conditions that promote non-synaptic hippocampal activity occur in vivo during seizures and include: drop in extracellular calcium, increase in extracellular potassium, and decrease in extracellular volume fraction (due to activity-dependent cell swelling) [8, 18, 19]. Thus, during seizure episodes in the brain, mechanisms of non-synaptic neuronal interactions might affect cellular excitability and contribute to pathological hippocampal synchronization.

Decreased osmotic pressure of extracellular medium in the brain may occur during various pathological states that may include over-hydration, hypothalamic or renal dysfunction, and seizures [8]. Reduction of extracellular osmolality results in neuronal swelling and promotes increased cellular excitability, which is crucial for seizure generation. Thus, understanding the mechanisms that affect hippocampal susceptibility to non-synaptic epileptiform discharges may contribute to the better understanding and control of the pathological synchronization in the brain.

**CONCLUSIONS**

1. Under normal osmotic conditions, CA1 region of hippocampus is more prone to non-synaptic epileptiform discharges than CA3.
2. Pre-incubation of hippocampal slices with 4-AP under normal osmotic conditions results in decreased latency for non-synaptic discharges in CA3 but not in CA1.
3. Under hypo-osmotic conditions, CA3 area of hippocampus becomes more prone to non-synaptic discharges than CA1.
4. Pre-incubation of hippocampal slices with 4-AP under hypo-osmotic conditions has no effect on the hippocampal propensity to non-synaptic epileptiform discharges.
Несинаптическая эпилептиформная активность индуцировалась в срезах гиппокампа методом инкубации срезов в безкальциевом внеклеточном растворе. Склонность участков гиппокампа к несинаптическому взаимодействию оценивалась измерением времени задержки, необходимого для развития низкокальциевых разрядов в зонах CA1 и CA3. Увеличение нейронной возбудимости было вызвано преинкубацией гиппокампальных срезов с 4-аминопиридиноном (4-АР) и снижением внеклеточного осмотического давления. Преинкубация срезов в 4-АР при условиях нормального осмотического давления вызывала уменьшение латентного периода несинаптической разрядки в зоне CA3, но не в CA1. Полученные результаты показали, что гипоосмолярные условия и преинкубация с 4-АР моделируют способность гиппокампальной зоны CA3 к генерации несинаптических эпилептиформных явлений.

Ключевые слова: низкокальциевые эпилептиформные явления; 4-аминопиридин; гипоосмолярность; гиппокамп.

REFERENCES


Received 03.08.2015