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SURFACE CHARGE IMPACT IN NONSYNAPTIC MODEL OF EPYLEPSY IN RAT HIPPOCAMPUS

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Abstract

In the present study, we evaluated the contribution of the effect of altering Mg^{2+} concentration in the extracellular solution on the cellular surface charge in the initial stage of the low-Ca^{2+} model of epilepsy. Our results suggest that the change in the surface charge dramatically affects the probability of induction of low-Ca^{2+} seizure like events (SLA), providing evidence that Mg^{2+} can reduce cerebral excitability by screening surface charge and support the usefulness of Mg^{2+} as an anti-seizure and anti-epileptic agent.

Keywords: epilepsy, surface charge

Introduction

The epilepsies are characterized by the abrupt and highly synchronous discharge of brain neurons. Most complex focal seizures either originate or are elaborated in the hippocampus. During a hippocampal seizure (ictal episode) large neuronal aggregates are recruited into excessive highly synchronized discharges that last several seconds or minutes. In the interseizure (interictal) period the epiloptogenic focus remains active generating brief (lasting tens or hundreds of milliseconds) bursts of synchronized neuronal discharge. Both spontaneous interictal- and ictal-like epileptiform discharges can be readily induced in vitro by exposing hippocampal slices to convulsant agents or by modulation of the extracellular ionic milieu. It is well established that excitatory synaptic connections mediate the initiation and propagation of interictal epileptiform discharges. However in vitro experiments have shown that ictal epileptiform activity can be nonsynaptic in nature.[1]. In 1982 two reports were published, each described a dramatic form of neuronal synchronization in hippocampal slices exposed to media containing low concentration of Ca^{2+}. Both papers showed that that CA1 , and under certain conditions CA3 zones of the hippocampal slices generated large and highly synchronized discharges that could last for many seconds [3]. These experiments revealed that epileptiform activity can be nonsynaptic in nature, however it remained unclear which of the effects of reduction of extracellular Ca^{2+} can account for neuronal synchronization. Four types of nonsynaptic mechanisms are generally considered to account for neuronal synchronization: a) ephaptic transmission (between clusters of neurons with closely opposed membranes); b) field effects (during synchronous neuronal activity of large groups of neurons with a suitable, usually parallel, arrangement; c) electrotonic coupling through gap junctions; d) extracellular ions fluctuations (such as increase in K^+ or decrease in Ca^{2+} or Mg^{2+} during sustained neuronal activity). However there is no doubt that these nonsynaptic mechanisms together dramatically alter neuronal excitability and exert influence on seizure threshold; the issue of how each particular mechanism contributes to neuronal synchronization remains unclear.

Surface charge on the cellular membrane produced by sialic acid, phosphates, charged lipids, charged amino acids, and other hydrophilic residues of channel proteins creates local electrical fields near the channel voltage sensor. The effect of surface charge on the membrane channel can be assessed by changing extracellular concentrations of cations, which produce a screening effect on surface charge. Decreasing extracellular Ca^{2+} or increasing the amount of extracellular negatively charged polysialic acids results in a significant hyperpolarizing shift of activation of voltage-gated Na^+-channels [2]. In the present study, we evaluated the contribution of altered Mg^{2+} concentration in the extracellular solution on surface charge in the low-Ca^{2+} model of epilepsy.

1. Methods

Animals. Wistar rat pups of postnatal day 12 (P12) were used throughout the study and treated in accordance with the guidelines set by the Animal Care Committee of Bogomoletz Institute of Physiology.

Slice preparation. Rats were deeply anesthetized with sevoflurane and decapitated. Brains were rapidly removed and placed in ice-cold artificial cerebro-spinal fluid (aCSF). Cerebellum and frontal lobe were removed and transverse brain slices ($500\mu m$) were cut using vibroslicer. The resulting slices were then transferred to the incubation chamber and left to recover for at least one hour at room temperature. All manipulations were performed in constantly oxygenated (95% O_2 – 5% CO_2) aCSF of the following composition (in mM): 125 NaCl, 3.5 KCl, 1.3 $MgCl_2$ ·

$6H_2O$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 24$NaHCO_3$, 11 glucose, $pH = 7.35$. All drugs were obtained from Sigma.

Extracellular recordings. For extracellular recordings slices were transferred to the submersion-type chamber and perfused with the low-Ca^{2+} solution containing in mM: 115 NaCl, 5 KCl, 0 $CaCl_2$, 0 $MgCl_2 \cdot 6H_2O$, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 10 glucose, pH = 7.4.

A total of 57 hippocampal slices were used throughout the study. In first group of slices ($n = 10$) $MgCl_2$ was omitted from the perfusion solution. In subsequent recordings $MgCl_2$ was added to the low-Ca^{2+} solution in concentration of 1mM ($n = 24$), 2 mM ($n = 12$) and 3 mM ($n = 11$). Extracellular recordings were performed in CA1 and CA3 pyramidal cell layers of rat hippocampus. Spontaneous field potentials were obtained using glass micropipettes (2-5MΩ) filled with artificial CSF. Recordings were amplified with a differential amplifier, digitized at 10 kHz using analog-to-digital converter and stored using `WinWCP` program. Off-line analysis was performed using Clampfit 10.2 (Axon Istruments), `Origin 8.5` (OriginLab Northampton, MA). All data represented as means±Se.

2. Results

Following the application of low-Ca^{2+} aCSF synaptic transmission was blocked and spontaneous epileptiform discharges occurred both in CA1 and CA3 areas of hippocampus with different delay time.

Two distinct types of spontaneous field potentials were observed: slow waves that represented a potential shift usually with spikes superimposed. We recorded two types of slow waves: single peak slow wave and regular slow waves that appeared in clock-like fashion. The second type of nonsynaptic activity was separated spikes, it was the most common pattern of SLA and appeared in every slice that was capable of generating nonsynaptic bursting. The frequency of spikes and the duration of consistent firing varied between the groups, but differences were not significant.

Next we compared the effect of changing Mg^{2+} concentration on the induction of low-Ca^{2+} SLA. Removing Mg^{2+} from extracellular solution caused nonsynaptic bursting in 50% of slices (5 from 10). In the next 3 groups of slices we increased Mg^{2+} consentration up to 1 mM, 2 mM, and 3 mM respectively. We observed that increased Mg^{2+} concentration resulted in significant decrease in probability to induce low-Ca^{2+}SLA: In 1mM Mg^{2+} aCSF nonsynaptic SLA was recorded in 25% of slices (6 from 24). Low-Ca^{2+} aCSF containing 2 mM Mg^{2+} indused epileptiform dicharges in 16% of slices (2 from 12). We did not observed spontaneous nonsynaptic SLA in slices perfused with 3 mM Mg^{2+}.

Discussion

Our data suggest that a decrease in the surface charge screening is required condition to induce seizures in the low-Ca^{2+} model of epilepsy. Both cellular depolarization and hyperpolarizing shift of the voltage-gated

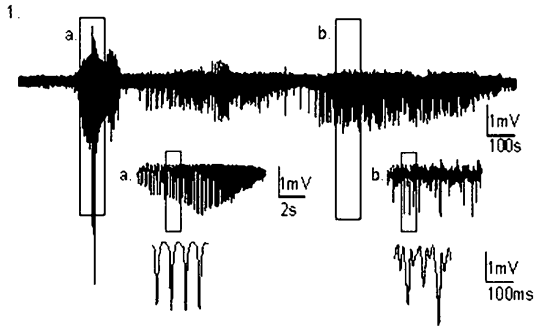

Fig. 1. Extracellular recordings of spontaneous field potentials in low-Ca^{2+} solution, CA1 area of hippocampus. Two types of nonsynaptic discharges outlined with boxes: a) burst of waves with clock-like fashion; b) burst of separated spikies.

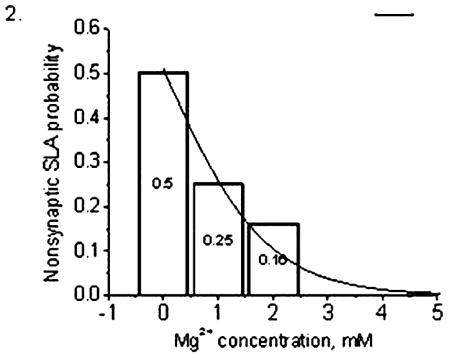

Fig. 2. Cumulative histogram shows probability of induction of low-Ca^{2+} SLA in different Mg^{2+} concentrations, the curve represent theoretical probability statistically assessed with logistic regression.

channel activation cause similar effects on neuronal network activity due to reduced voltage difference between the resting membrane potential and potentials where Na+-channels are activated. Our results suggest that the change in in the surface charge dramatically affects the probability of induction of low-Ca^{2+} SLA, providing evidence that Mg^{2+} can reduce cerebral excitability by screening surface charge and support the usefulness of Mg^{2+} as an anti-seizure and anti-epileptic agent. This study further suggests that surface charge may represent a novel therapeutic target in the treatment of epilepsy.

References

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