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Activation of ryanodine receptors influences the paired-pulse depression in cultured rat hippocampal neurons

За допомогою методики фіксації потенціалу в конфігурації «ціла клітина» вивчали участь внутрішньоклітинних кальцієвих депо у регуляції короткочасної пластичності викликаних гальмівних постсинаптичних струмів (ГПСС) на синаптичноз'єднаних культивованих нейронах гіпокампа. Струми викликалися зовнішньою стимуляцією пресинаптичного волокна імпульсами напруги тривалістю 500 мкс. При значеннях затримки між стимулами 150 та 500 мс у клітинах спостерігалася депресія (n=18) або фасилітація (n=7) другого ГПСС у парі. Вивільнення кальцію з внутрішньоклітинних депо індукувалося за допомогою локальної аплікації агоністів ріанодинчутливих рецепторів кофеїну та ріанодину. Одна з характеристик пластичності, що викликається парною стимуляцією – відношення амплітуди другого ГПСС у парі до амплітуди першого – змінювалася при аплікації ріанодину (50 нмоль/л) з 0,79±0,02 до 0,71±0,04 (n=10). Подібний ефект спостерігався тільки в тих клітинах, для яких було характерне явище депресії, викликане парною стимуляцією. Також було досліджено вплив кофеїну та ріанодину на спонтанні струми: при аплікації кофеїну (10 ммоль/л) спостерігалася зміна усередненої амплітуди (до 0,71±0,06) та частоти (до 0,42±0,08) спонтанних ГПСС (n=7). При аплікації ріанодину (50 нмоль/л) середня амплітуда струмів не змінювалася, у той час як частота зменшувалась до 0,74±0,09 (n=12). При наявності кофеїну (10 ммоль/л) і ріанодину (50 нмоль/л) амплітуди струмів, які були викликані швидкою локальною аплікацією γ-аміномасляної кислоти (ГАМК, 100 мкмоль/л), пригнічувалися до 0,59±0,03 (n=5) та до 0,56±0,11 (n=7) відповідно. Було зроблено висновок про те, що кальцієві депо ендоплазматичного ретикулума здатні регулювати синаптичну передачу як з пресинаптичного (оскільки вважається, що короткочасна синаптична пластичність і спонтанна активність мають пресинаптичну природу), так і з постсинаптичного боків (тому, що рецептори ГАМК розташовані на постсинаптичній клітині).

INTRODUCTION

Ca^{2+} is known to be involved in regulation of many different processes in cells, especially in the excitable cells. In neurons and neuroendocrine cells calcium is closely linked to such processes as excitability regulation and neurotransmitter release. Katz and Miledi [15] established that action potential-evoked neuromediator release is triggered by Ca^{2+} influx through plasma membrane into the neuronal synaptic terminal. Presence of extracellular calcium is not vital for spontaneous transmitter release since Ca^{2+} ions are available from different intracellular stores [3, 25, 34]. The pool, which is mainly considered to participate in modulation of Ca^{2+}-signals and ionic currents in neurons, is the endoplasmic reticulum (ER). For the present day existence of the ER with ryanodine-sensitive receptors is discovered in dedritic spines as well as in soma, axon and dendrites [9, 10, 14, 16, 29].

It is considered that such close location of calcium stores and calcium channels in synaptic endings may cause complicated interactions between them.

Phenomenon of short-term synaptic plasticity in forms of paired-pulse depression (PPD), facilitation (PPF) or augmentation, post-tetanic potentiation or depression is one of the key points in the mechanisms of memory and learning [18, 31, 32, 36]. According to up-to-date conception PPD is supposed to have dual regulation by depletion of a readily releasable pool of vesicles [12] and by desensitization of postsynaptic receptors [13, 23]. PPF is believed to be related to various effects of residual calcium in terminals [37,38]. However, facilitation and depression presumably coexist in all experimental conditions, so the paired-pulse ratio that is routinely measured in experiments reflects a balance between these two competing processes [5, 6].

It was shown [28] that in rat hippocampal slices intracellular Ca^{2+} stores may participate in neurotransmission. The authors indicate to clear postsynaptic effect of caffeine action. On the other hand, in some publications [7, 19] a controversial site of action of calcium pools' on inhibitory transmission was found. Involvement of intracellular calcium stores in the short-term synaptic plasticity has been already intensively studied [8, 11, 27] but mostly on slices.

In this report we investigate the role of intracellular ryanodine-sensitive calcium stores in modulation of short-term plasticity of inhibitory currents in cultured hippocampal neurons.

METHODS

Preparation of hippocampal culture with low density. Hippocampi samples taken from newborn Wistar rats killed by decapitation were incubated with 0.05% trypsin for 5 min at room temperature and than mechanically dissociated using Pasteur pipettes. Suspension of the cells was plated on poly-L-ornitine- and laminine-covered Petri dishes. The initial density of cells was approximately 30000-35000 cm^{-2}. Neurons were cultured in Eagle's modified medium containing 10% horse serum, 6 μg/ml insulin, 2.2 g/l $NaHCO_3$, with addition of penicillin (25 units/ml) and streptomycin (25 μg/ml) at 37°C in 5% CO_2 humidified incubator. Cytosine-β-D-arabinofuranoside (5μM) was added for 24 hours on the 3rd day after plating to reduce glia proliferation. Experiments were performed after 12 days of cultivation to allow the complete formation of synaptic contacts between cells.

Solutions. Intracellular solution contained (mM): K-gluconate – 100; KCl – 50; $MgCl_2$ – 5; EGTA – 10; HEPES – 20; pH 7.4. Extracellular bath solution contained (mM): NaCl – 140; KCl – 3; $CaCl_2$ – 2; $MgCl_2$ – 2; glucose – 30; HEPES – 20; pH 7.4. To block glutamatergic excitation 20 μM of 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 20 μM of D_L-2-amino-5-phosphonopentanoic acid (D_L-AP5) were routinely added to extracellular solution. Ryanodine (50 nM) and caffeine (10 mM) were used as activators of calcium release from endoplasmic reticular stores [2, 21, 24, 30, 33]. All drugs were applied using a fast local superfusion technique [35] (Fig.1). All drugs and chemicals were purchased from "Sigma" (USA).

Patch-clamp recordings and data processing. Pipettes were pulled out from borosilicate glass ("WPI", USA) on a self-made puller in two steps. Typical values for resistances of the recording pipettes filled with intracellular solution were 5-7MΩ. Currents were recorded using the Axopatch-1D amplifier ("Axon Instruments", USA) in voltage-clamp mode at a holding potential -75 mV and digitized at 10 kHz (with filtering at 2 kHz by Bessel lowpass filter) using LabMaster TL-1 and pClamp 6.0 software ("Axon Instruments", USA). Voltage stimulation was performed by an isolated stimulator DS-2 ("Digitimer", England).

All experiments were performed at room

temperature. Due to large variability observed from cell to cell, all values have been presented as relative ratios of (Value in test conditions)/(Value in Control). Paired Student's *t*-test was performed to identify statistical significance. Data were considered significantly different when $P<0.05$. All data are expressed as mean ± S.E.M with number of cells averaged given in brackets.

RESULTS

To study involvement of calcium stores in neurotransmission we investigated IPSCs in rat hippocampal neurons in culture. In our experiments IPSCs were reversibly (to 0.08±0.01, n=4, data not shown) inhibited by a specific blocker bicuculline methobromide (10 μM) indicating that they were mediated by $GABA_A$-receptors.

We used a paired-pulse ratio (PPR), i.e. a ratio of amplitude of the second IPSC in a pair to the amplitude of the first IPSC, as a quantitative measure of synaptic plasticity. Since it was shown [12] that paired-pulse depression (PPD) is observed up to 4 s intervals between stimuli, we evoked paired currents at a rate of 0.2 Hz. Paired stimuli resulted in paired-pulse depression (n=18) or facilitation (n=7) of the second IPSC at interpulse intervals (IPI) 150 and 500 ms (when the second IPSC appeared during or after complete decay of first IPSC, respectively). A pattern of activity (PPD or PPF) was not changed when switching between IPI=150 ms and IPI=500 ms although PPR was closer to 1 for longer IPI.

Application of ryanodine (50 nM) or caffeine (10 mM) induces Ca^{2+} release from intracellular calcium stores by activation of ryanodine-sensitive receptors. This leads to reduction of relative amplitudes of evoked IPSCs to 0.79±0.05 (n=18) and to 0.23±0.03 (n=7), respectively. However, a significant change of PPR produced by application of ryanodine was seen only in cells in which PPD occurred at IPI=150 ms (from 0.79±0.02 to 0.71±0.04 (n=10), $P<0.05$) (Fig.2). In other cases no statistically significant effect on a paired-pulse ratio was observed.

Spontaneous release of vesicles from synaptic terminals is determined by presynaptic mechanisms [3, 4, 25]. So we also studied the spontaneous activity of hippocampal neurons.

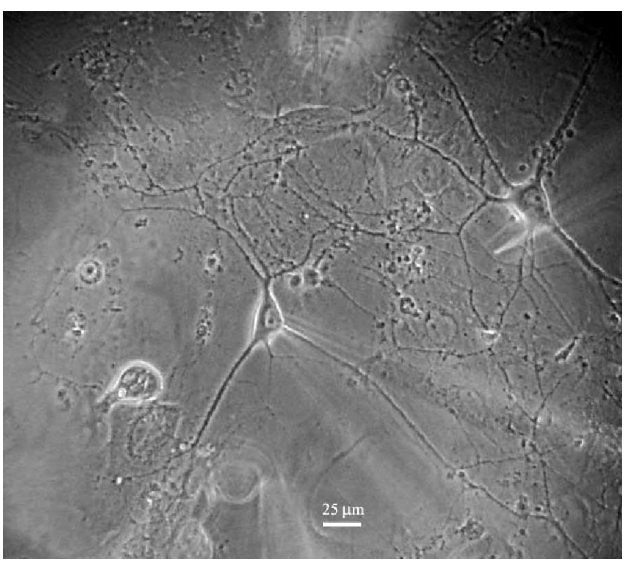

Fig.1. Using of local superfusion technique on cultured hippocampal neurons. Pyramidal neuron lies in the center. Recording pipette is positioned on it. A drop-shape area of drug application is formed between feeding (bottom) and sucking (top) pipettes. Pipette for stimulation is situated at the top right corner

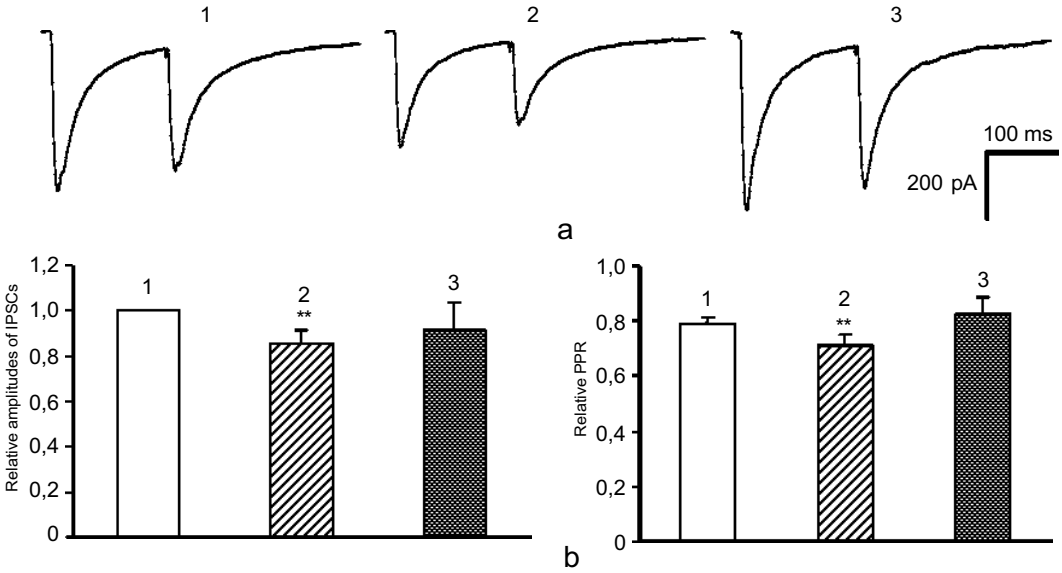

Fig.2. Influence of ryanodine on paired-pulse depression. a – Averages of 30 currents during control (1), application of 50 nM ryanodine (2) and washout (3). b – Changes of relative amplitudes of averaged currents (calculated for 1st IPSC in a pair) and relative PPR during application of ryanodine (50 nM, n=10). 1 – control, 2 – ryanodine 50 nM, 3 – washout

It appeared that ryanodine (50 nM) did not change relative amplitudes of spontaneous IPSCs (0.97±0.07 (n=12)) in contrast to caffeine (10 mM) which caused attenuation to 0.71±0.06 (n=7). Relative average frequency of appearance of spontaneous events

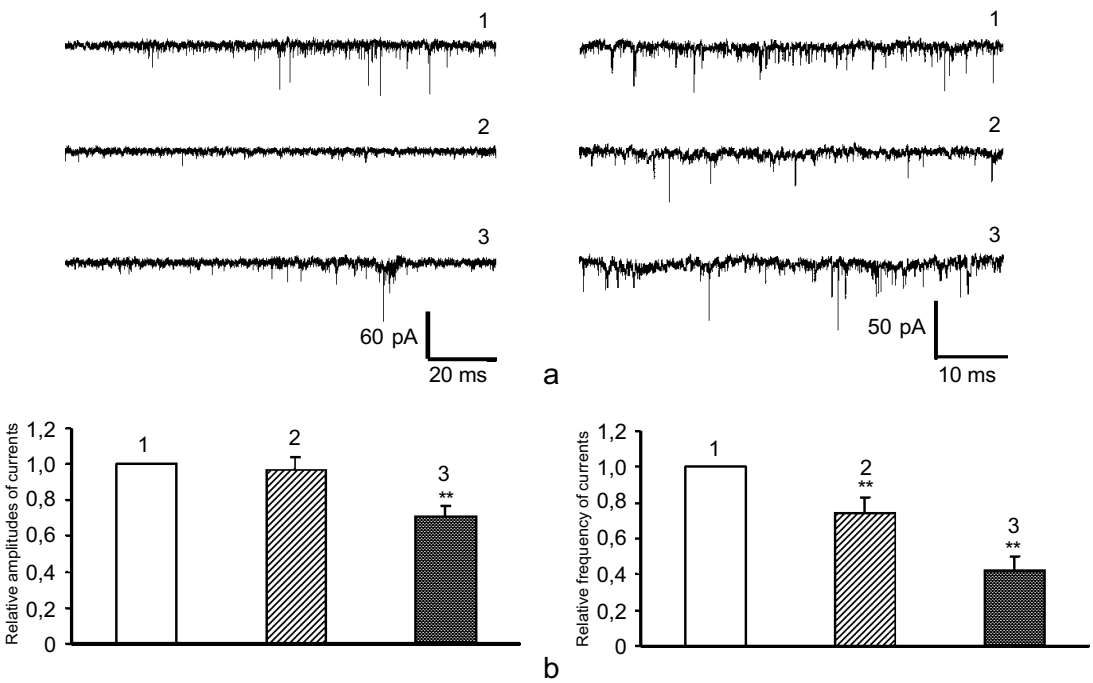

Fig.3. Influence of calcium release activators on spontaneous activity of hippocampal neurons. a – Examples of recordings of spontaneous IPSCs during application of 10 mM of caffeine (left) and 50 nM of ryanodine (right). 1 – control, 2 – test, 3 – washout. b – Averaged effect of action of ryanodine (50 nM, n=12) and caffeine (10 mM, n=7) on amplitudes and frequencies of spontaneous IPSCs. 1 – control, 2 – ryanodine 50 nM, 3 – caffeine 10 mM

during application of these drugs was decreased to 0.74±0.09 and 0.42±0.08, respectively (Fig.3).

Direct action of agonist on $GABA_A$-receptors had been used to clarify whether calcium pools are able to affect inhibitory synaptic transmission postsynaptically. Inhibitory currents were elicited by a direct local application of GABA (100 μM) in short (200 ms long) pulses with period 30 s for receptors to be recovered from previous to application. Fast application of 10 mM caffeine simultaneously with 100 μM of GABA showed inhibition of GABA-induced currents comparing to control to 0.59±0.03 (n=5). When ryanodine (50 nM) was applied in similar way attenuation of GABA-induced currents to 0.56±0.11 (n=7) was observed.

DISCUSSION

Our results suggest that mobilization of calcium from ryanodine-sensitive stores contributes to GABA release in hyppocampal neurons. Activation of ryanodine receptors in culture seems to have a similar effect on IPSCs as blocking of them in slices [8]. By inhibition of spontaneous activity and reduction of PPR (which is determined by probability of transmitter release [1, 22]) the presynaptic action of ryanodine and caffeine was elucidated. On the other hand inhibition of both amplitudes of IPSCs and amplitudes of GABA-evoked currents during ryanodine and caffeine application indicate that ryanodine receptor activation acts at a postsynaptic locus.

We propose that the observation of controversial effects (paired-pulse depression and facilitation) is due to existence of several types of GABAergic inhibitory interneurons in the hippocampus [17, 20, 26] which cannot be distinguished in culture.

Kinetic analysis of IPSC curves using single-exponential fit (coefficient of determination R^2>0.99, data not shown) shows that all values of time-of-decay constants were not statistically significantly different for studied conditions. The absence of clearly detectable effect on this parameter may indicate that ryanodine-receptor activators fail to induce action properly on postsynaptic chlorine channels responsible for observed currents.

Obtained results suggest that activation of ryanodine receptors inhibits release of GABA, showing that both pre- and postsynaptic caffeine-ryanodine sensitive calcium stores play a regulatory role in release of neurotransmitter.

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ACTIVATION OF RYANODINE RECEPTORS INFLUENCES THE PAIRED-PULSE DEPRESSION IN CULTURED RAT HIPPOCAMPAL NEURONS

Role of intraterminal calcium stores in modulation of short-term plasticity of evoked inhibitory postsynaptic currents (IPSCs) was studied in synaptically connected cultured hippocampal neurons using patch-clamp technique in whole-cell configuration. Currents were induced by voltage stimulation which were applied externally to presynaptic fiber. Paired stimuli resulted in paired-pulse depression (n=18) or facilitation (n=7) of the second IPSC at interpulse intervals 150 and 500 ms. Calcium release from intracellular calcium stores was activated by local application of caffeine and ryanodine, ryanodine receptor agonists. One of the characteristics of short-term plasticity, the pair-pulsed ratio (ratio of amplitudes of second IPSC to first IPSC), decreased during addition of ryanodine (50 nM) from 0.79±0.02 to 0.71±0.04 (n=10). This change was observed only for cells that demonstrated pair-pulsed depression. We also studied the influence of caffeine and ryanodine on spontaneous currents. Attenuation of the mean amplitude to 0.71±0,06 and frequency to 0.42±0.08 (n=7) of spontaneous IPSCs was observed during application of caffeine (10 mM). Upon ryanodine application the mean amplitude did not change but frequency of spontaneous events decreased to 0.74±0.09 (n=12). The amplitudes of currents evoked by fast local application of γ-aminobutyric acid (GABA 100 mM) were diminished in the presence of caffeine (10 mM) to 0.59±0.03 (n=5) and in the presence of ryanodine (50 nM) to 0.56±0.11 (n=7). Thus we conclude that endoplasmic reticular calcium stores are able to modulate synaptic transmission from both presynaptic (in assumption that short-

term plasticity and spontaneous activity are believed to have presynaptic nature) and postsynaptic (since GABA-receptors are situated on postsynaptic cell) sides.

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