DOI 10.1007/s10517-016-3234-4

Corticosterone Induces Rapid Increase in the Amplitude of Inhibitory Response in Hippocampal Synapses with Asynchronous GABA Release

E. P. Volkova[1], A. V. Rozov[2], G. G. Nadareishvili[1], and A. P. Bol'shakov[1,3]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 160, No. 11, pp. 572-576, November, 2015
Original article submitted February 9, 2015

Experiments were performed on cultured slices of rat ventral hippocampus. Using extracellular stimulation and patch clamp recording from pyramidal neurons in the hippocampal CA1 area, we studied characteristics of GABAergic synapse formed on these neurons by cholecystokinin-expressing interneurons. This synapse was characterized by asynchronous release of GABA and depolarization-induced suppression of inhibitory response. It was observed that administration of corticosterone increased the amplitude of evoked inhibitory postsynaptic currents in 5 minutes, but the paired ratio did not significantly change. Obtained data reflect that corticosterone can induce rapid genome-independent effects on inhibitory neurotransmission in one of hippocampal synapses.

Key Words: *asynchronous release; corticosterone, GABA; hippocampus; interneuron*

Chronic or severe stress serves can be the cause of mental disorders. It was shown that these types of stress modulate plastic properties of neurons and neuronal networks [2]. Stress triggers a variety of signal cascades associated with elevated blood concentrations of steroid hormones. Corticosterone (CS) is one of the main steroids released from the cortex of adrenal glands of rodents during stress. CS binds to high-affinity mineralocorticoid and low-affinity glucocorticoid receptors and triggers cascade mechanism regulating expression of various genes. However, recent studies showed that CS and specific agonists of glucocorticoid and mineralocorticoid receptors could have not only slow, but also rapid effects developing within few minutes after exposure to the agents [5].

CS easily crosses the blood–brain barrier and penetrates into the brain. The hippocampus, the key structure involved in stress response, is characterized by high density of glucocorticoid and mineralocorticoid receptors [5]. Increased level of CS modifies plastic properties of hippocampal synapses [8]. For example, CS can modulate activity of inhibitory synapses in the hippocampal CA1 region, the physiological effect depends on area of the hippocampus. In the dorsal hippocampus, activation of glucocorticoid receptors by CS or specific agonist dexamethasone is followed by an increase in the amplitude of spontaneous inhibitory postsynaptic currents (sIPSC) in CA1 pyramidal neurons. In the ventral hippocampus, CS does not affect sIPSC in CA1 region, but reduces the frequency of generation of spontaneous responses. It was shown that this reduction was determined by activation of mineralocorticoid receptors. It should be emphasized that the described effects of CS appeared in 15 min after hormone application and were most likely mediated by activation of signal cascades not related to the genome [8].

Methodical approach based on registration of sIPSC has a disadvantage: sIPSC registered on the soma of the neuron are generated by all synapses localized on the soma and proximal dendrites of the postsynaptic neuron. This method does not allow estimating

[1]N. I. Pirogov Russian National Research Medical University, Ministry of the Health of the Russian Federation, Moscow; [2]Laboratory of Neurobiology, Kazan Federal University, Kazan, Russia; [3]Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia. ***Address for correspondence:*** ocracher@yahoo.com. A. P. Bol'shakov

0007-4888/16/1605-0628 © 2016 Springer Science+Business Media New York

synapse specificity of measurements and CS effects. It was hypothesized that activity of various types of perisomatic interneurons can play a pivotal role in generation of rhythmic neuronal activity in the brain cortex and especially in the hippocampus [4]. In view of this hypothesis, not only the effects of various biochemical agents on summary activity of inhibitory synapses (that can be measured during sIPSC registration), but their selective effects on various synapses are of importance. Interneurons expressing parvalbumine and cholecystokinin (CCK) are the main types of perisomatic interneurons involved in generation of various types of rhythmic activity in the hippocampus. CCK-positive interneurons also express cannabinoid CB1-receptors [4]. It is known that CS can modulate properties of synapses formed by CB1-positive interneurons [3,7,9].

Here we studied the effects of CS on IPSC amplitude in perisomatic synapses formed on CA1 pyramidal neurons with typical characteristics of CB1-positive synapses.

MATERIALS AND METHODS

Wistar rats were kept in a vivarium with natural illumination and free access to water and food. Dams got pregnant, gave birth, and fed the progeny under vivarium conditions. Rats aging 22-33 days were used in the experiments.

The rats were immediately decapitated, the brains were removed and placed in ice-cold solution containing (in mM): 140 potassium gluconate, 5 sodium gluconate, 10 HEPES, 4 NaCl, and 0.2 EGTA (pH 7.2). Horizontal 400-μ slices were prepared on EMS 5000 vibratome and placed in incubation chamber with artificial cerebrospinal fluid (35-37°C) containing (in mM): 125 NaCl, 25 $NaHCO_3$, 25 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, and 1 $MgCl_2$ and constantly bubbled with carbogen (95% O_2+5% CO_2). This method allows preparing slices of the ventral hippocampus.

The slices were incubated for 30 min in the incubator; then, one slice was placed into an experimental chamber on a Slicescope microscope platform where it was constantly perfused with artificial cerebrospinal fluid at room temperature. Single pin CA1 pyramidal neurons were found using differential interference contrast. Neuronal responses were recorded using patch-pipettes filled with (in mM): 145 calcium gluconate, 30 KCl, 10 HEPES, 4 MgATP, 10 Na-phosphocreatine, and 0.3 NaGTP (pH 7.2). Resistance of patch-pipettes was 3-6 MΩ. Signals were recorded using Multiclamp 700 B amplifier and digitized (Digidata 1440; Axon Instruments). Series resistance was controlled in the experiments and data from cells with changes in series resistance >15% were excluded from the analysis.

The section was perfused with artificial cerebrospinal fluid with 10 μM CNQX and evoked IPSC (eIPSC) were recorded at -70 mV. Extracellular stimulation was performed using a patch-pipette filled with artificial cerebrospinal fluid and introduced into in the pyramidal layer of hippocampal CA1 region. The interval between impulses during paired stimulation was 100 msec; paired stimulation was repeated every 10 sec.

Electrophysiological data were analyzed using Clampfit software. The curves averaged through 30-40 records (sweep) were used for analysis. Paired Studentэs *t* test was used for comparisons. The data were presented as mean±standard deviation.

RESULTS

Pyramidal neurons for patch-clamp recording were chosen based on their morphology and location in the pyramidal layer of hippocampal CA1 region with apical dendrite directed towards the radial layer (stratum radiatum). Before the experiment, the electrophysiological parameters of neurons were recorded in current clamp regimen. Typical responses of CA1 pyramidal neurons to hyperpolarization and depolarization currents of various intensities are presented in Figure 1, *a*.

For detection and selective stimulation of inhibitory synapses formed by axons of CCK/CB1-expressing interneurons, we used well-known properties of these synapses. First, high-frequency rhythmic stimulation of synaptic activity in these synapses with a series of 10-15 action potentials is followed by asynchronous release of the neurotransmitter [1,4,6]. Second, these synapses demonstrate depolarization-induced suppression of inhibition (DISI): eIPSC amplitude decreases in a few seconds after severe depolarization of the postsynaptic cell [1,6,10]. Typical response of the synapse to a series of 10 stimuli with the frequency of 50 Hz (Fig. 1, *b*, curve *1*) shows that neurotransmitter release lasts dozens of milliseconds after termination of stimulation. This release is hereinafter referred to as asynchronous release of the neurotransmitter. In other synapse, the same stimulation (Fig. 1, *b*, curve *2*) induced weak asynchronous release. We also checked the presence of DISI in the studied synapse. To this end, we evaluated the effects of depolarization of the pyramidal neuron from -70 to 0 mV over 2.5 sec on eIPSC amplitude. Depolarization of postsynaptic neuron resulted in significant suppression of postsynaptic responses (depolarization induced suppression of eIPSC amplitude to 15.4±9.6% from the control level; Fig. 1, *c*). The presence of IPSC and asynchronous release in the studied synapses with high probability suggest that we selectively stimulated synapses formed

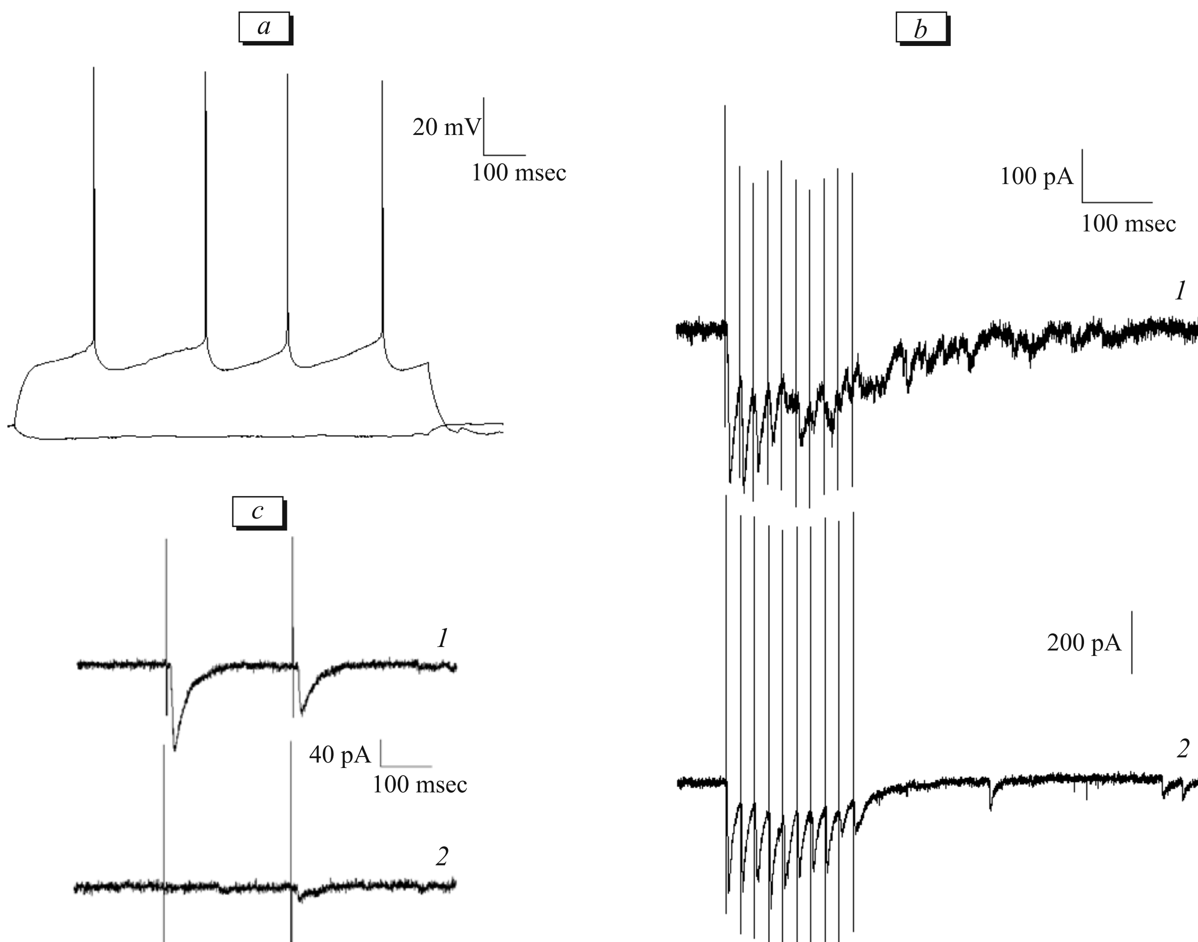


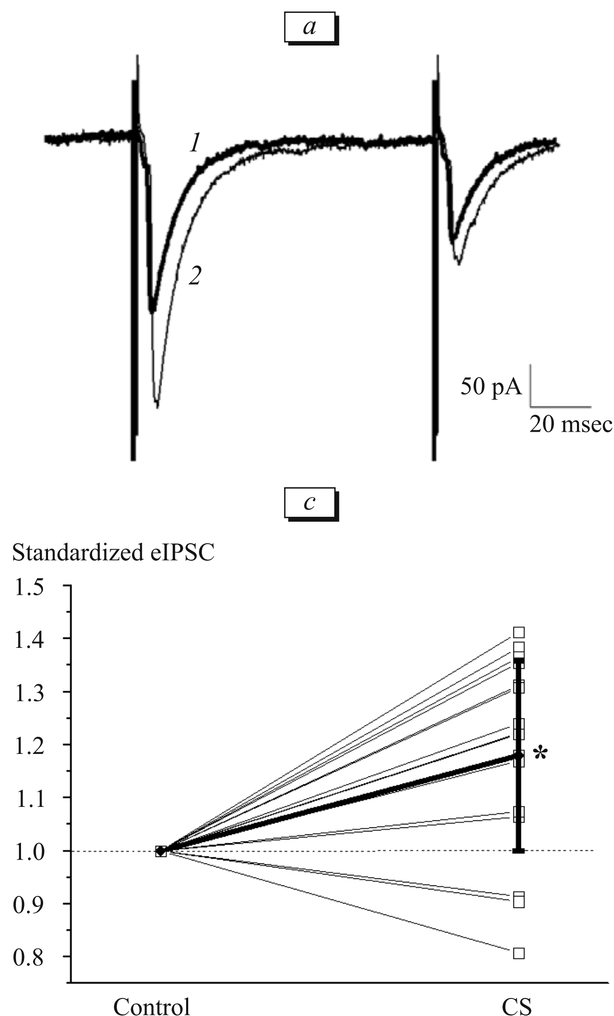
Fig. 1. Parameters of a pyramidal neuron and GABAergic synapse formed by CCK-expressing interneuron on the pyramidal CA1 cells of the hippocampus. *a*) Typical response of CA1 pyramidal neurons of rat hippocampus to hyperpolarization (-60 pA) and depolarization (300 pA) for 1 sec. *b*) Asynchronous GABA release after rhythmic stimulation with high-frequency series of stimuli (curve *1*); response to similar stimulation of the synapses without asynchronous release are presented for comparison (curve *2*). *c*) DISI in synapses with asynchronous release of the neurotransmitter. Responses recorded before (*1*) and after (*2*) depolarization of the pyramidal neuron are shown.

by interneurons expressing CCK and CB1. Synapses without one of these characteristics were excluded from the experiment.

In our experiments extracellular stimulation resulted in the appearance of two-phase responses: the second eIPSC developed 2.5 msec after the first eIPSC. DISI induction was followed by suppression of both responses, and we suggest that in these cases extracellular stimulation can induce generation of two action potentials instead of one. For estimation of CS effects, we measured the total amplitude of eIPSC (from basic level to the level of second peak).

Addition of CS (100 nM) to the section was followed by a significant increase in eIPSC amplitude (Fig. 2) within 5 min. In our experiment, we also estimated paired pulse ratio, *i.e.* response amplitude ratio to two consequential stimulations with an interval of 100 msec. CS did not significantly affect paired ratio in the studied synapse (0.70±0.11 *vs.* 0.75±0.18 in the control). The absence of changes in paired pulse ratio suggests that the effects of CS are most likely mediated by the influence on the postsynaptic cell.

As it was mentioned before, CS induced an increase in sIPSC amplitude in the ventral hippocampus in 15 min after addition [8]. Spontaneous inhibitory activity had irregular pattern, and it was impossible to specify the contribution of a particular synapse into spontaneous activity in certain time interval. CS probably produced rapid effects similar to the effect observed in our investigation, but it was not found in previous experiments [8] due to simultaneous reduction in spontaneous activity of the studied synapse. Increased sIPSC amplitude registered in 15 min can also result from CS-induced increase in response amplitude in synapses of various types. We solved the problem of specificity of sIPSC recording by using selective stimulation of CCK-expressing fibers and this allowed us to reveal quick effects of CS. Further investigations



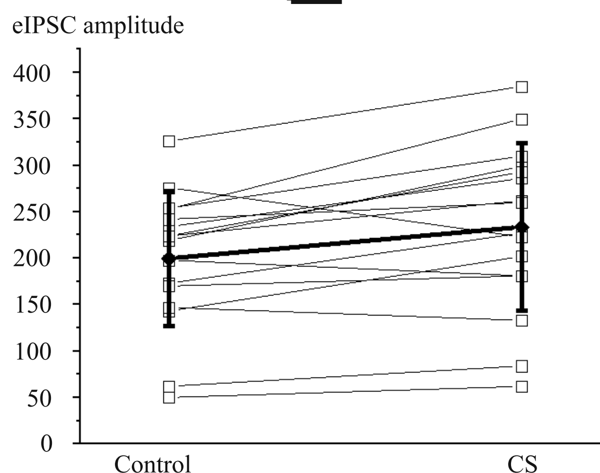


Fig. 2. CS-dependent increase in eIPSC amplitude in the synapse formed by CCK/CB1-expressing interneuron on CA1 pyramidal neuron of the hippocampus. *a*) eIPSC amplitude in control (*1*) and 5 min after CS administration (*2*). *b*) Effects of CS on eIPSC amplitude in individual experiments (squares linked with lines show the data from the same experiment). Mean changes are shown with black line. *c*) Data presented in fragment *b* after standardization for eIPSC amplitude under control conditions. *significant difference from the control (p=0.00382, n=16).

of signal mechanisms mediating CS-induced increase in eIPSC amplitude in CCK-positive synapses in the hippocampus are required.

The study was supported by the Russian Foundation for Basic Research (grant No. 15-04-06115) and State Program of Competitive Growth of Kazan Federal University.

REFERENCES

1. A. B. Ali and M. Todorova, *Eur. J. Neurosci.*, **31**, No. 7, 1196-1207 (2010).
2. E. R. de Kloet, *Endocr. Regul.*, **37**, No. 2, 51-68 (2003).
3. S. Di, M. M. Maxson, A. Franco, and J. G. Tasker, *J. Neurosci.*, **29**, No. 2, 393-401 (2009).
4. T. F. Freund and I. Katona, *Neuron*, **56**, No. 1, 33-42 (2007).
5. A. Galeeva, M. Pelto-Huikko, S. Pivina, and N. Ordyan, *Vitam. Horm.*, **82**, 367-389 (2010).
6. S. Hefft and P. Jonas , *Nat. Neurosci.*, **8**, No. 10, 1319-1328 (2005).
7. W. Hu, M. Zhang, B. Czeh, *et al.*, *Neuropsychopharmacology*, **35**, No. 8, 1693-1707 (2010).
8. N. Maggio and M. Segal, *ScientificWorldJournal*, **10**, 462-469 (2010).
9. M. Wang, M. N. Hill, L. Zhang, *et al.*, *J. Psychopharmacol.*, **26**, No. 1, 56-70 (2012).
10. R. I. Wilson and R. A. Nicoll, *Nature*, **410**, 588-592 (2001).