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Selective Extracellular Stimulation of Pharmacologically Distinct CCK/CB1R Positive Interneuron to Pyramidal Cell Perisomatic Inhibitory Synapses

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Abstract During prolonged whole cell recording, the intracellular contents are progressively dialyzed with the pipette solution; this often leads to significant changes in synaptic efficacy. To overcome this problem, we developed an approach allowing reliable extracellular stimulation of perisomatic synapses formed by CCK+/CB1+ interneurons onto CA1 pyramidal cells. Functional identification of this input was based on the unique features of CCK+/CB1+ terminals: long-lasting asynchronous transmitter release following high-frequency stimulation and exclusive expression of CB1R. Asynchronous release was used as an indication of proper positioning of the theta glass stimulation pipettes. We found that all extracellularly stimulated inputs with characteristic asynchronous release undergo robust DSI in response to 5-s depolarization and could also be almost entirely blocked by application of the CB1R agonist CP55940, which were similar to the data obtained with paired recordings from connected CB1+ and CA1 pyramidal cells. Thus, we have developed an approach allowing the selective and reliable extracellular stimulation of a subtype of hippocampal perisomatic inhibitory synapses.

Keywords Method · Extracellular stimulation · Perisomatic inhibition · CCK

1 Introduction

In long-duration experiments, stability of synaptic transmission is an essential requirement. During prolonged whole cell recording, the intracellular contents are progressively dialyzed with the pipette solution; this often causes significant changes in synaptic efficacy. For instance, it has been shown that prolonged whole cell dialysis of presynaptic GABAergic neurons leads to a significant reduction of postsynaptic responses [1–3]. This rundown is most likely due to washout of compounds essential for maintenance of the vesicular transmitter content and release probability, and, therefore, it can produce substantial problems in experiments designed to study long-term plasticity. Side effects of dialysis in postsynaptic neurons can be successfully avoided by the use of the perforated patch technique. However, this method is not applicable for presynaptic stimulation, since multiple current/voltage injections can lead to eruption of the plasma membrane.

One obvious method to overcome this problem is the usage of focal extracellular stimulation of identified GABAergic inputs. Here we describe an approach allowing selective stimulation of the subtype of perisomatic synapses formed

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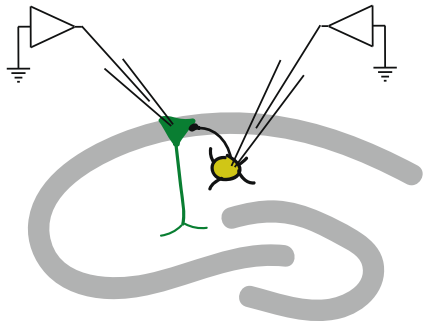

Fig. 1 Schematic drawing of the experimental setup for paired recordings from connected CCK+/CB1R+ interneurons (*yellow*) and CA1 pyramidal cells (*green*)

by cholecystokinin positive (CCK+) and cannabinoid type 1 receptor (CB1R) expressing interneurons onto the cell bodies of CA1 pyramidal cells.

2 Materials and Method

The experimental procedures were performed in accordance with the guidelines for the use of laboratory animals of Kazan Federal University. The experimental protocol met the requirements of the European Communities Council Directive 86/609/EEC and approved by the Ethical Committee of Kazan Medical University.

Transverse hippocampal 300 μm slices were prepared from the brains of 14–21-day-old WT (C57Bl6) mice, killed by cervical dislocation. The slicing chamber contained an oxygenated ice-cold K-based cutting solution (modified from [4]). Slices were incubated for 30 min at 35 °C before being stored at room temperature in artificial CSF (ACSF) containing (in mM) NaCl, 125; $NaHCO_3$, 25; KCl, 2.5; NaH_2PO_4, 1.25; $MgCl_2$, 1; $CaCl_2$, 2; and D-glucose, 25 bubbled with 95 % O_2 and 5 % CO_2. During the experiments, slices were continuously perfused with the same ACSF. Patch electrodes for the postsynaptic pyramidal cells were filled with a solution which

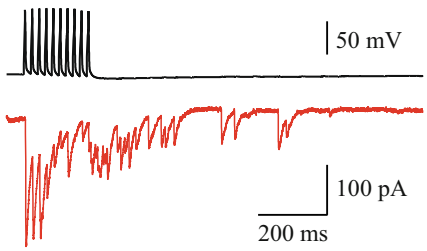

Fig. 2 Asynchronous release evoked in the postsynaptic pyramidal cell (*red trace*) in response to the train of action potentials triggered in the CCK+/CB1R+ interneuron

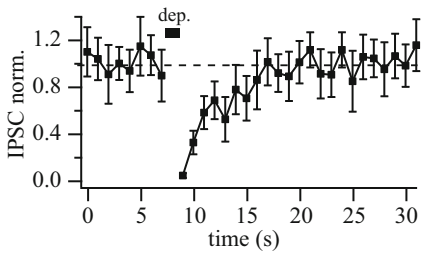

Fig. 3 Typical time course of DSI evoked by 1-s depolarization in the pairs of connected CCK+/CB1R+ interneurons and CA1 pyramidal cells

consisted of (in mM) Cs-gluconate, 100; CsCl, 40; HEPES, 10; NaCl, 8; MgATP, 4; MgGTP, 0.3; and phosphocreatine, 10 (pH 7.3 with CsOH). The intracellular solution for the presynaptic interneurons consisted of (in mM) *K*-gluconate, 100; KCl, 40; HEPES, 10; NaCl, 8; MgATP, 4; MgGTP, 0.3; and phosphocreatine, 10 (pH 7.3 with KOH).

CA1 pyramidal cells were identified visually using IR-video microscopy. In paired recordings, presynaptic CB1+ putative basket cells were identified by location and firing

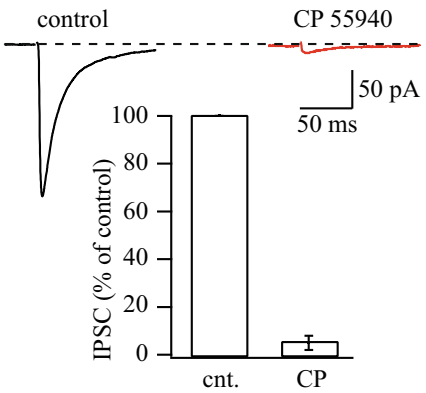

Fig. 4 Effect of CP 55940 application on IPSC amplitudes in the pairs of connected CCK+/CB1R+ interneurons and CA1 pyramidal cells

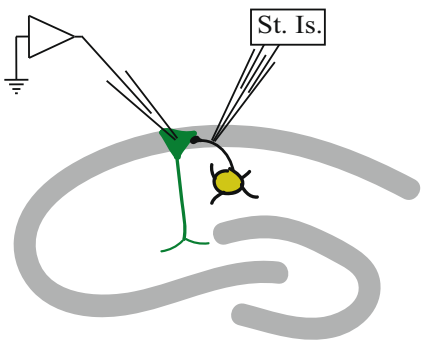

Fig. 5 Schematic drawing of the experimental setup for extracellular stimulation of CCK+/CB1R+ to pyramidal cell synapses

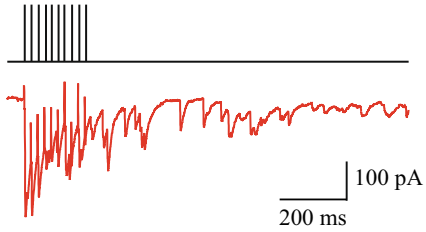

Fig. 6 Asynchronous release evoked in the postsynaptic pyramidal cell (*red trace*) in response to the train of extracellularly delivered stimuli (*black*)

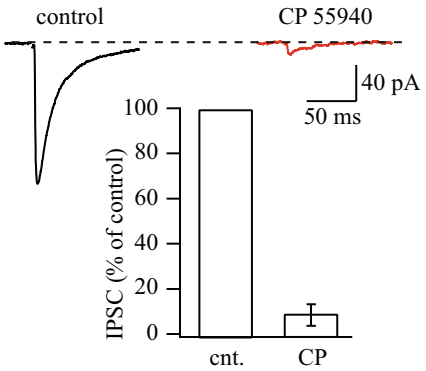

Fig. 8 Effect of CP 55940 application on amplitudes of extracellularly evoked IPSCs in CA1 pyramidal cells

pattern. All experiments were done at room temperature (23–25 °C).

3 Results and Discussion

First we documented the typical physiological and pharmacological features of CCK+/CB1R+ to pyramidal cell connections in identified connected cell pairs. CCK+ interneurons were located in the stratum radiatum close to the border of CA1 region of the stratum pyramidale (20 to 100 μm; Fig. 1). Neurons were identified by multipolar morphology of the cell body and the characteristic firing pattern [5]. After finding the postsynaptic partner (CA1 pyramidal cell), the identity of interneurons was further confirmed by profound asynchronous release evoked by high-frequency stimulation (10 stimuli at 50 Hz), robust depolarization induced suppression of inhibition (DSI) in response to 1-s depolarization to 0 mV, and, finally, by sensitivity of release to the application of the synthetic agonist of CB1R CP 55940 (1 μM; $n = 5$; Figs. 1–4). The combination of all these features is unique for this type of connection and therefore was used as the set of criteria to position the extracellular stimulation pipette and choose the stimulus intensity for selective stimulation of afferents from CCK+/CB1R+ to the CA1 pyramidal cell. To provide the most focal stimulation possible, we used stimulation pipettes pulled from two barrel theta glass capillaries. The best results were achieved with pipettes which had a tip diameter of about 5–7 μm. Chlorinated silver wires inserted inside of each compartment of the theta glass pipette were connected to a stimulus isolator. Stimulation electrodes were placed in the stratum pyramidale ~20–100 μm of the soma of the recorded neurons (Fig. 5). The stimulus intensity was reduced to the lowest amplitude that would trigger a response. Excitatory synaptic transmission was blocked during recordings by the addition of 10 μM NBQX to the perfusion ACSF (Fig. 2).

In all cases where high-frequency minimal stimulation triggered long-lasting asynchronous release (Fig. 6), the evoked synaptic transmission could be significantly attenuated by either 1-s depolarization of the postsynaptic neuron or by bath application of CP 55940 (Figs. 7 and 8; $n = 5$; $p < 0.01$). Thus the properties of extracellular stimulated putative CCK+/CB1R+ to CA1 pyramidal cell inputs were identical to those measured in identified cell pairs.

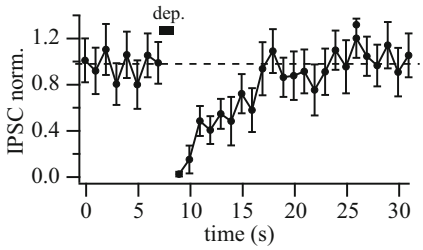

Fig. 7 Time course of DSI in extracellularly evoked IPSCs in CA1 pyramidal cells

4 Conclusion

The technique described above allows the “noninvasive” presynaptic stimulation of this subpopulation of perisomatic hippocampal inhibitory synapses. This can be crucial for long-lasting experiments where prolonged whole cell dialysis often affects the fidelity of synaptic transmission. We also defined a number of relatively simple tests that can be used to ensure input specificity at the beginning of the experiments: asynchronous release and DSI. At the end of the assay, sensitivity to CB1R agonists can also be used as a test for input

specificity. A similar approach could be developed for extracellular stimulation of perisomatic synapses formed by parvalbumin positive interneurons; in this case, the absence of asynchronous release and DSI in combination with sensitivity of release to mu opioid receptor activation would be used as criteria.

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Compliance with Ethical Standards The experimental procedures were performed in accordance with the guidelines for the use of laboratory animals of Kazan Federal University. The experimental protocol met the requirements of the European Communities Council Directive 86/609/EEC and approved by the Ethical Committee of Kazan Medical University.

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