Cholinergic modulation of distinct types of perisomatic region targeting interneurons and their involvement in carbachol induced fast network oscillation in the CA3 region of the hippocampus

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I. INTRODUCTION

Information processing of the brain requires the control of various types of information processing, including the hippocampus. GABAergic inhibitory cells targeting the perisomatic region of pyramidal cells can effectively modulate the fast network and inhibit potentially the sodium-dependent action potential activity of large assemblies of pyramidal cells. The group of perisomatic inhibitory cells consists of basket cells (BCs) with a spiking (RS BCs) phenotype, as well as agranular interneurons (AACs). BCs innervate the apical dendritic segments (AIS) of pyramidal cells, whereas AACs surround the soma of pyramidal cells.

Cholinergic neuromodulation effects on different cognitive functions. GABAergic interneurons are modulated by cholinergic modulation in many brain areas and transmit an overall influence on large assemblies. Although several investigations regarding the cholinergic receptors and their sensitivity to cholinergic modulation have been performed, cholinergic receptor activation has been tested specifically on distinct types of perisomatic targeting interneurons.

A typical example of cholinergic modulation is observed in the switching of cortical networks behavior and its response to the changes of acetylcholine tone. When the cholinergic tone is high, gamma oscillation can be observed in the potential recording, reflecting the state of the hippocampus. A similar oscillation of a similar oscillation range (30-100 Hz) can be induced in hippocampal slices by using the cholinergic receptor agonist carbachol.
Studying the network mechanisms underlying CCh induced oscillations and the participation of the distinct types of perisomatic region targeting interneurons may help in understanding the processes of gamma oscillations recorded in vivo.

II. AIMS

The main goal of this thesis was to investigate the involvement of perisomatic region targeting interneurons in the generation of cholinergically induced fast network oscillation. Therefore, two objectives were outlined:

The first objective was to determine the output properties of perisomatic region targeting interneurons in the hippocampal CA3 region and to clarify their sensitivity to cholinergic receptor activation. To this end, it was necessary to develop a method by which the distinct types of these interneurons can be distinguished from each other. Furthermore, we aimed to reveal the mechanisms by which the cholinergic receptor agonist exerts its action on the synaptic inhibition originated from these cell types.

The second objective of the thesis was to investigate the contribution of these interneurons to the CCh induced fast network oscillation. Therefore, the firing of interneurons was monitored during CCh induced oscillation in acute hippocampal slices, then the involvement of all types of perisomatic region targeting interneurons to the maintenance of oscillation was also tested by using pharmacological tools.

III. METHODS

All experiments were carried out in accordance with Hungarian Act of Animal Care (XXVIII, section 243/1998), an institutional ethical code, which regulates animal experiments by the European Union. C57Bl/6 mice or transgenic mice expressing fluorescent protein (eGFP) decarboxylase-65 (GAD65) promoter were used in the pair studies. Mice were anaesthetized with isoflurane and quickly removed and placed into a holding chamber at room temperature. The brain was bubbled with carbogen gas (200–350 µl/min) and placed into a recording chamber in standard artificial cerebrospinal fluid (ACSF). In the first study, we performed potassium- or cesium-glucuronate in the pre- or postsynaptic cells of CA3. Electrically evoked inhibitory postsynaptic currents (IPSCs) were isolated by using pharmacological tools including picrotoxin (600–650 µM) and 50 mM kynurenic acid to block glutamatergic receptor-mediated currents. To record extracellularly. The field pipette was filled with ACSF. Electrically evoked inhibitory postsynaptic currents (IPSCs) were pharmacologically isolated by using pharmacological tools including picrotoxin (600–650 µM) and 50 mM kynurenic acid to block glutamatergic receptor-mediated currents.
All recordings were performed at room temperature, except the oscillation experiments demonstrating the similarity of CCh induced oscillation to the in vivo gamma, recorded at 33°C.

In both studies the intrapipette solution contained also 0.3-0.5 % biocytin, and the different cell types were identified post-hoc based on their morphological characteristics. An additional double immunofluorescent labelling process was developed to distinguish AACs from FSBCs by using an antibody against ankyrin-G protein, which labels the AISs of neurons.

IV. RESULTS

Part I.: Comparison of CA3 perisomatic region targeting interneurons regarding their synaptic properties and their sensitivity to cholinergic receptor activation

Using transgenic mice with GFP expression controlled by the PV or GAD65 promoters allowed us to selectively target the FSBCs and AACs as well as RSBCs. We performed paired recordings between these interneurons and their postsynaptic counterparts and determined their synaptic properties. In the slices prepared from PV-eGFP transgenic mice, AACs were unequivocally identified and distinguished from FSBCs, if the biocytin labeled axon terminals of the recorded cells formed close appositions with the ankyrin G immunoreactive AIS in a climbing fiber like manner. RSBCs sampled in the GAD65-eGFP slices were identified based on the regular spiking phenotype and the morphology of reconstructed cells.

The AACs proved to produce IPSCs with the highest peak amplitude and significantly slower decay values compared to FSBCs. This latter property could be due to synaptic cross-talk between adjacent boutons at AAC-pyramidal cell synapses at room temperature.

RSBCs were capable of releasing transmitter in an asynchronous manner, compared to the PV expressing interneurons that only released GABA synchronously. Analyzing IPSCs we found RSBCs to have different from the PV expressing produced IPSCs with higher amplitudes, furthermore they had much lower decay time constants.

In the next set of experiments for the sensitivity to cholinergic receptor activation we administered CCh into the recording chamber and measured the changes in IPSC properties. We observed that IPSCs in all cases but to a different extent in the cell group CCh exerted a robust effect on IPSC amplitude, measured in FSBC- and AAC-matured slices. CCh almost completely blocked IPSCs in all cases but to a different extent in the cell group CCh. Using pharmacological approaches expressing cell-pyramidal cell pairs muscarinic acetyl-choline receptor presynaptically. In contrast, at PV expressing cell-pyramidal cell pairs affected M1 or M3 muscarinic receptors, the complete muting of the presynaptic receptors. The complete muting of the postsynaptic membranes of endocannabinoid release, which is linked to activation of the presynaptic CB1 receptors. The complete muting of the pyramidal cell pairs suggests that CB1 receptors have a significant role in the generation of oscillations.

Part II.: Participation of interneurons in fast network oscillation in the CA3 region

The second part of the experiments aimed to reveal the behavior of CCh induced fast network oscillations genesis and maintenance of oscillations and their participation in the generation of oscillations. We found that all three cell types participate in the oscillation, although with different patterns.
During these oscillations, FSBCs fired the most with the highest accuracy compared to the discharge of AACs and RSBCs. The weak phase coupling of RSBCs further strengthens our hypothesis that these cells do not have a key role in the rhythm generation of CCh induced fast network oscillations.

To reveal the contribution of the other two types of perisomatic region targeting neurons to the perisomatic inhibition in CCh induced fast network oscillations, we investigated the consequence of the µ-opioid receptor (MOR) activation to the synchronous activities. Previous studies showed that MORs were present at the axon terminals of PV expressing interneurons. Bath application of a MOR agonist DAMGO ([D-Ala2,N-Me-Phe4,Gly5-ol]enkephalin acetate) substantially disrupted the oscillation. We demonstrated that application of DAMGO significantly decreased the amplitude of IPSCs recorded in pyramidal cells without any effects on excitatory synaptic transmission or the excitability of neurons. These results suggest that the GABA released from the terminals of PV expressing interneurons may play a role in the oscillogenesis. To further reveal the contribution of AACs and FSBC we tested the effect of DAMGO on FSBC and AAC-pyramidal cell pairs in the presence of CCh. We found that DAMGO caused a further decrement in the amplitude of unitary IPSCs at FSBC-pyramidal cell pairs, whereas similar effect could not be observed at AAC-pyramidal cell pairs. Taken together these results strongly suggests that FSBCs play the main role in the generation of CCh induced fast network oscillations in hippocampal slices.

V. DISCUSSION

The main goal of this thesis was to investigate the properties of the distinct types of inhibitory neurons and to reveal their contribution to the perisomatic inhibition in CCh induced fast network oscillations. The first part of the thesis revealed that AACs and FSBCs contribute to the perisomatic inhibition in CCh induced fast network oscillations. The different properties regarding the intrinsic excitability and the excitatory synaptic transmission of these inhibitory neurons might fulfill their specific role in the organization of hippocampal network. The results of the second part of the thesis strongly suggest that AACs and FSBCs contribute to the perisomatic inhibition in CCh induced fast network oscillations, whereas RSBCs might play a minor role in oscillogenesis.

In the second part of the thesis, we investigated the properties of the distinct types of inhibitory neurons during CCh induced fast network oscillations. This hypothesis, since the spiking activity of FSBCs is strongly synchronized to the oscillation, is extended to the gamma oscillation model of fast network oscillation. The results of the second part of the thesis strongly suggest that FSBCs play the main role in the generation of CCh induced fast network oscillations in hippocampal slices.
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VII. LIST OF PUBLICATIONS

Publications related to the dissertation

Szabo GG, Holderith N, Gulyas A, Szabo GG
Distinct synaptic properties of parvalbumin-containing neurons and their different modulation by TF in the CA3 region of the mouse hippocampus.
EUROPEAN JOURNAL OF NEUROLOGY 2234-2246. (2010)

Gulyas AI, Szabo GG, Ulbert I, F, Szabo G, Freund TF, Hajos N
Parvalbumin-containing fast-spike field potential oscillations induced by theta-activation in the hippocampus.
JOURNAL OF NEUROSCIENCE (2010)

Other publication

Hajos N, Holderith N, Nemeth E, Zemankovics R, Freund TF, Hall E
The Effects of an Echinacea-Transmission and the Firing Propagation in the Hippocampus.
PHYTOTHERAPY RESEARCH