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A slow excitatory postsynaptic current mediated by a novel metabotropic glutamate receptor in CA1 pyramidal neurons

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ABSTRACT

Slow excitatory postsynaptic currents (EPSCs) mediated by metabotropic glutamate receptors (mGlu receptors) have been reported in several neuronal subtypes, but their presence in hippocampal pyramidal neurons remains elusive. Here we find that in CA1 pyramidal neurons a slow EPSC is induced by repetitive stimulation while ionotropic glutamate receptors and glutamate-uptake are blocked whereas it is absent in the *VGLUT1* knockout mouse in which presynaptic glutamate is lost, suggesting the slow EPSC is mediated by glutamate activating mGlu receptors. However, it is not inhibited by known mGlu receptor antagonists. These findings suggest that this slow EPSC is mediated by a novel mGlu receptor, and that it may be involved in neurological diseases associated with abnormal high-concentration of extracellular glutamate.

This article is part of the Special Issue entitled 'Metabotropic Glutamate Receptors, 5 years on'.

1. Introduction

The vast majority of excitatory synapses in the brain use glutamate as their neurotransmitter. The excitation is mediated by three subtypes of ionotropic glutamate receptors, which act on the time scale of milliseconds. In addition to the ionotropic receptors, glutamate also activates metabotropic glutamate receptors (mGlu receptors), which engage downstream signaling pathways acting on the time scale of hundreds of milliseconds. mGlu receptors modulate synaptic transmission throughout the brain and have been implicated in a variety of neuropsychiatric diseases, such as depression, anxiety disorders, schizophrenia, pain syndromes, and epilepsy (Niswender and Conn, 2010; Rondard and Pin, 2015). mGlu receptors have been found to mediate slow excitatory postsynaptic currents (EPSCs) in several different neuronal subtypes such as cerebellar Purkinje cells (Hirono et al., 1998; Tempia et al., 1998) and hippocampal interneurons (Eguchi et al., 2016; Huang et al., 2004). However, their presence in hippocampal pyramidal neurons remains elusive.

In CA1 hippocampal pyramidal cells group I mGlu receptors (mGlu1 receptor and mGlu5 receptor) are predominately expressed postsynaptically and their selective activation by DHPG causes a reversible inward current, a reversible inhibition of the slow after hyperpolarization, and a long term depression (LTD) of excitatory synaptic transmission (Fitzjohn et al., 1998; Mannaioni et al., 2001; Volk et al., 2006). However, in the presence of ionotropic glutamate receptor antagonists, stimulation of excitatory synapses evokes no response in CA1 pyramidal cells (Castillo et al., 1997; Granger et al., 2013). There are two possible explanations for the absence of a synaptic response. First, the mGlu receptors, which are expressed in CA1 pyramidal cells, are excluded from the synaptic region and presynaptically release glutamate cannot access them. Alternatively, synaptically released glutamate does activate these receptors, but they are not coupled to the signaling pathway that generates an inward current. It would appear that the latter scenario maybe the case. It has been reported that low frequency synaptic stimulation in neonatal slices induces an mGlu receptor-dependent LTD (Bolshakov and Siegelbaum, 1994; Luscher and Huber, 2010; Oliet et al., 1997), thus indicating that synaptically released glutamate can activate mGlu receptors. However, in older animals the linkage of mGlu receptors to NMDAR-independent LTD appears to be more variable (Kemp and Bashir, 1999; Luscher and Huber, 2010; Volk et al., 2006).

In the present study we sought to uncover a synaptic role for

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mGlu receptors at CA1 synapses. We confirmed that in the presence of AMPAR and NMDAR antagonists, neither single stimulus nor high frequency repetitive stimuli elicited any postsynaptic response. However, to our surprise in the presence of the glutamate transporter blocker TBOA, high frequency repetitive stimulation reliably evoked a slow EPSC. This response was lost in the *VGLUT1* KO mouse, in which presynaptic glutamate release is lost, further suggesting that it is mediated by glutamate. Remarkably, this response is not blocked by a cocktail of mGlu receptor antagonists, which are known to block the action of all cloned mGlu receptors. Thus we conclude that the responses we observe are mediated by a novel mGlu receptor.

2. Materials & methods

2.1. Animal care and husbandry

Wild type and *VGLUT1* knockout (KO) littermate mice, as well as Sprague Dawley rats, were housed with 12 h light/dark cycle and unlimited food and water. From postnatal day 15 (P15) and after weaning, *VGLUT1* KO mice are fed daily with Nutra-Gel Diet (Bio-Serv) food supplement to promote their survival. Both male and female mice were used for the experiments.

2.2. Slice preparation

Organotypic hippocampal slice cultures were made as previously described (Schnell et al., 2002) from P6-P8 rats. Acute transverse hippocampal slices (300 μm) were prepared with a Microslicer DTK-Zero1 (Ted Pella) from wild type (P16-30 and P70-90) or *VGLUT1* KO (P70-90) mice. Animals were anesthetized with isoflurane and killed by decapitation in accordance with American Veterinary Medical Association and the UCSF Institutional Animal Care and Use Committee. The chilled high sucrose cutting solution contained the following (in mM): 2.5 KCl, 0.5 $CaCl_2$, 7 $MgSO_4$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 7 glucose, 210 sucrose, 1.3 ascorbic acid and 3 sodium pyruvate. The slices were then incubated for 30 min at 34 °C in artificial CSF (ACSF) contained the following (in mM): 119 NaCl, 2.5 KCl, 26.2 $NaHCO_3$, 1 NaH_2PO_4, 11 glucose, 1.3 $MgSO_4$and 2.5 $CaCl_2$. The ACSF was bubbled with 95% O_2 and 5% CO_2 to maintain pH and the slices were allowed to recover at room temperature for 30 min to 1 h before recording at room temperature.

2.3. Electrophysiological recording

Acute slices were transferred to a perfusion stage on an Olympus BX50WI upright microscope and perfused at 2.5 ml/min with ACSF containing the GABA receptors inhibitors picrotoxin (100 μM), bicuculline (20 μM) and CGP55845 (2.5 μM). Simultaneous whole-cell voltage-clamp and field recordings were performed by placing a field electrode in stratum radiatum of CA1 between the stimulating electrode and the whole-cell voltage-clamped CA1 pyramidal neuron. The field electrode was filled with ACSF. For whole-cell voltage-clamp recordings, the K-gluconate internal recording solution contained the following (in mM): 140 K-gluconate, 10 HEPES, 7 NaCl, 4 Mg-ATP and 0.3 Na-GTP. The $CsMeSO_4$ internal solution contained the following (in mM): 135 $CsMeSO_4$, 8 NaCl, 10 HEPES, 0.3 EGTA, 5 QX314-Cl, 4 Mg-ATP, 0.3 Na_3-GTP and 0.1 spermine. Osmolarity was adjusted to 290–295 mOsm and pH buffered at 7.3–7.4. Synaptic responses were evoked by a bipolar stimulation electrode placed in stratum radiatum, and responses were evoked at 0.1 Hz. To ensure stable voltage-clamp recording, membrane holding current, input resistance, and pipette series resistance were monitored throughout the recordings. Cells were discarded if series resistance varied by 25% or more during a recording session, or series resistance exceeded 40 MΩ. Data were gathered through a MultiClamp 700 A amplifier (Molecular Devices), filtered at 2 kHz, and digitized at 10 kHz and all these data were analyzed off-line with custom software (IGOR Pro).

3. Results

We first examined the effect of the selective group I mGlu receptors agonist DHPG on the holding current of CA1 pyramidal cells. As reported previously (Mannaioni et al., 2001) DHPG caused a reproducible inward current when recordings were made with a potassium-based internal pipette solution (Fig. 1A). The DHPG-induced current was blocked by a cocktail of mGlu receptor antagonists (Fig. 1B). This cocktail contained LY367385 which blocks mGlu1 receptor (Fitzjohn et al., 1999), MPEP which blocks mGlu5 receptor (Gasparini et al., 1999) and LY341495 which blocks all cloned mGlu receptors (Fitzjohn et al., 1998). The application of DHPG to neurons recorded with a cesium-based pipette solution (Fig. 1C) failed to generate any current, consistent with this current

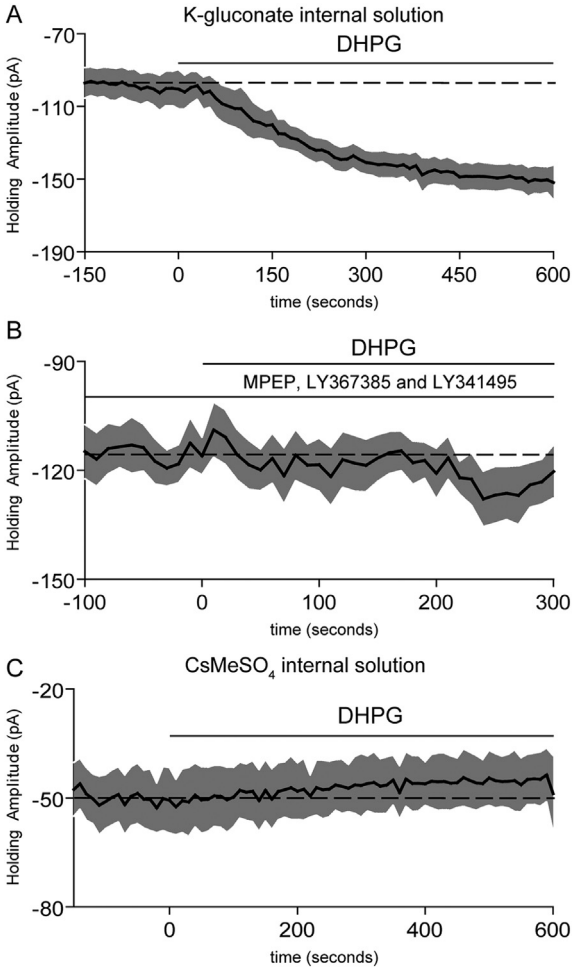

Fig. 1. DHPG-mediated activation of group I mGlu receptors induces inward currents in CA1 hippocampal neurons. Time course of the effect of DHPG (100 μM) on holding current of CA1 pyramidal neurons from organotypic hippocampal cultured slices patched with K-gluconate internal solution (A, n = 3) or in the presence of mGlu1 receptor antagonist LY367385 (100 μM), mGlu5 receptor antagonist MPEP (10 μM) and group II/III mGlu receptor antagonist LY341495 (100 μM) (B, n = 6). (C) The same experiments as in (A) except that $CsMeSO_4$ internal solution was used (n = 3). The traces represent the mean of holding amplitude and the shaded regions represent the corresponding ±SEM. The horizontal bars indicate the time of agonist applications. TTX (0.5 μM) was applied throughout for all recordings.