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The interaction between mGluR1 and the calcium channel $Ca_{V2.1}$ preserves coupling in the presence of long Homer proteins

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ABSTRACT

Group I metabotropic glutamate receptors (mGluR1 and 5) are G protein coupled receptors that regulate neuronal activity in a number of ways. Some of the most well studied functions of group I mGluRs, such as initiation of multiple forms of mGluR-dependent long-term depression, require receptor localization near the post-synaptic density (PSD). This localization is in turn dependent on the Homer family of scaffolding proteins which bind to a small motif on the distal C-termini of mGluR1 and 5, localize the receptors near the PSD, strengthen coupling to post-synaptic effectors and simultaneously uncouple the mGluRs from extra-synaptic effectors such as voltage dependent ion channels. Here the selectivity of this uncoupling process was examined by testing the ability of Homer-2b to uncouple mGluR1 from multiple voltage dependent calcium channels including $Ca_{V2.2}$ (N-type), $Ca_{V3.2}$ (T-type), and $Ca_{V2.1}$ (P/Q-type) expressed in rat sympathetic neurons from the superior cervical ganglion (SCG). Of these, only the mGluR1–$Ca_{V2.1}$ modulatory pathway was insensitive to Homer-2b expression. Uncoupling from this channel was achieved by co-expression of an mGluR1 C-terminal protein designed to disrupt a previously described direct interaction between these two proteins, suggesting that this interaction allows incorporation of $Ca_{V2.1}$ into the mGluR1/Homer signaling complex, thereby preserving modulation in the presence of scaffolding Homer proteins.

This article is part of a Special Issue entitled 'Metabotropic Glutamate Receptors'.

1. Introduction

Metabotropic glutamate receptors (mGluRs) are a family of G protein coupled receptors activated by the excitatory neurotransmitter glutamate. There are 8 mammalian mGluR genes with widespread expression in the central nervous system, and more limited expression peripherally (Schoepp, 2001). Two of the mGluRs (mGluR1 and 5) comprise the group I mGluR subfamily, which couple to the $G_{q/11}$ and $G_{i/o}$ proteins and commonly exhibit post-synaptic expression at glutamatergic synapses (Hay and Kunze, 1994; Choi and Lovinger, 1996; Kammermeier and Ikeda, 1999; Schoepp, 2001).

Group I mGluRs localize near the post-synaptic density (PSD) through their interaction with the Homer family of scaffolding proteins, which anchor not only mGluR1/5 to the PSD but also several other proteins including IP_3 receptors (IP_3Rs) (Brakeman et al., 1997; Ehrengruber et al., 2004; Duncan et al., 2005), some transient receptor potential (TRP) cation channels (Yuan et al., 2003), dynamin (Gray et al., 2003), and others (Ehrengruber et al., 2004; Shiraishi-Yamaguchi et al., 2009). Interestingly, a natural dominant negative Homer subtype has been described which interacts with Homer binding partners, but does not act as a scaffold (Brakeman et al., 1997; Xiao et al., 1998; Tu et al., 1999). These so called "short" Homers (Homer-1a and Ania-3), exhibit regulated expression, and as such are upregulated following periods of strong neuronal activity (Brakeman et al., 1997; Kato et al., 1997). Interaction with the scaffolding, or "long," Homers induces strong mGluR coupling to PSD localized effectors such as AMPA receptors (Kammermeier and Worley, 2007), and to synaptic responses such as mGluR-dependent forms of long-term plasticity (Kirschstein et al., 2007; Ronesi and Huber, 2008; Ueta et al., 2008). At the same time, the scaffolding Homers uncouple group I mGluRs from other effectors such as the plasma membrane ion channels $Ca_{V2.2}$ and KCNQ potassium channels (Kammermeier et al., 2000).

To date, long Homer protein mediated uncoupling from mGluR signaling has been demonstrated only for $Ca_{V2.2}$ channels and KCNQ

Abbreviations: mGluR, Metabotropic glutamate receptor; SCG, Superior cervical ganglion; Glu, L-Glutamate.

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channels (Kammermeier et al., 2000), but that uncoupling should occur from any effector localized outside the mGluR–Homer–PSD signaling complex. As such, any effector localized within this complex should remain coupled to mGluR1/5 in the presence of long Homers, even if these effectors are closely related to $Ca_{V2.2}$. Recently, a direct interaction between mGluR1 and $Ca_{V2.1}$ has been reported by Kitano et al. (2003), who showed that these proteins directly interact in expression systems and in cerebellar neurons. This interaction allowed us the opportunity to test the dogmatic model explaining the regulation of mGluR signaling by Homer proteins. Specifically, we hypothesized that the mGluR1–$Ca_{V2.1}$ interaction should allow incorporation of this channel into the mGluR–Homer signaling complex and thus preserve its modulation by mGluR1 when Homer proteins are expressed, in contrast to the mGluR1–$Ca_{V2.2}$ pathway (Kammermeier et al., 2000; Kammermeier, 2008). If this were the case, it would represent the first demonstration of group I mGluR mediated modulation of a voltage dependent channel that remains robust when scaffolding Homers are abundantly expressed. Thus, modulation of $Ca_{V2.1}$ currents by mGluR1, and its sensitivity to Homer protein over-expression, was examined in rat sympathetic neurons from the superior cervical ganglion (SCG), a system in which Homer-dependent regulation of mGluR signaling has been demonstrated (Kammermeier et al., 2000; Kammermeier, 2008; Won et al., 2009).

2. Materials and methods

2.1. Electrophysiology and data analysis

Pipettes for patch-clamp experiments were generated using a Sutter (Novato, CA) P-97 horizontal puller with 8250 glass (Garner Glass, Claremont, CA) and had resistances of 1–3 MΩ. Series resistances were 1–5 MΩ prior to electronic compensation of 80%. Whole-cell patch-clamp recordings were made with an EPC-7 (Heka Elektronik, Germany) or Axon 200B (Molecular Devices, Sunnyvale, CA) patch clamp amplifier. Voltage protocol generation and data acquisition were performed using custom software (courtesy Stephen R. Ikeda, NIAAA, Rockville, MD) on a Macintosh G3 or G4 computer (Apple Computer, Cupertino, CA) with an InstruTech (Heka Elektronik) ITC-16 or ITC-18 data acquisition board. Currents were low-pass filtered at 3–5 kHz using the 4-pole Bessel filter in the patch clamp amplifiers, digitized at 2–5 kHz and stored on the computer for later analysis. Experiments were performed at 21–24 °C (room temperature). Patch-clamp data analysis was performed using the Igor Pro software package (Wavemetrics, Lake Oswego, OR).

The external (bath) recording solution contained (in mM): 155 tris hydroxymethyl aminomethane, 20 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10 glucose, 10 $CaCl_2$, and 0.0003 tetrodotoxin (TTX), pH 7.4. The internal (pipette) solution contained: 120 N-methyl-D-glucamine (NMG) methanesulfonate, 20 TEA, 11 EGTA, 10 HEPES, 10 sucrose, 1 $CaCl_2$, 4 MgATP, 0.3 Na_2GTP, and 14 tris creatine phosphate, pH 7.2. L-Glutamate (Sigma) was used as the agonist for mGluRs. SNX482 (SNX) and ω-conotoxin GVIA (CTX) were obtained from Tocris Bioscience (Ellisville, MO). L-Glutamate was obtained from Sigma–Aldrich (St. Louis, MO). All drugs and control solutions were applied to cells using a custom, gravity-driven perfusion system positioned ~100 μm from the cell, allowing rapid solution exchange (≤250 ms). The degree of calcium current inhibition was calculated as the maximum current inhibition in the presence of drug compared to the last current measurement prior to drug application.

2.2. Primary culture preparation, cDNA injection, antibodies, and plasmid generation

A description of cell isolation and cDNA injection is found elsewhere (Ikeda et al., 1995; Lu et al., 2009). Animal protocols were approved by the university committee on animal resources (UCAR). Briefly, SCGs were removed from adult male Wistar rats (175–225 g) after CO_2 euthanasia and decapitation, then incubated in Earle's balanced salt solution (InVitrogen, Life Technologies Carlsbad, CA) containing 0.6 mg/ml trypsin (Worthington Biochemicals, Freehold, NJ) & 0.8 mg/ml collagenase D (Boehringer Mannheim Biochemicals, Indianapolis, IN) for 60 min at 35 °C. Cells were transferred to minimum essential medium (InVitrogen/Gibco), plated on poly-L-lysine (Sigma Chemical Co., St. Louis, MO) coated culture dishes and incubated at 37 °C for 2–4 h before cDNA injection. Injected cells were incubated overnight at 37 °C (95% air and 5% CO_2; 100% humidity) and patch clamp experiments were performed the next day. Anti-CaV2.1 antibody was obtained from Abcam (Cabbridge, MA). Anti-HA was obtained from Covance (Princeton, NJ). Both were used at a dilution of 1:500 for experiments shown in Fig. 1.

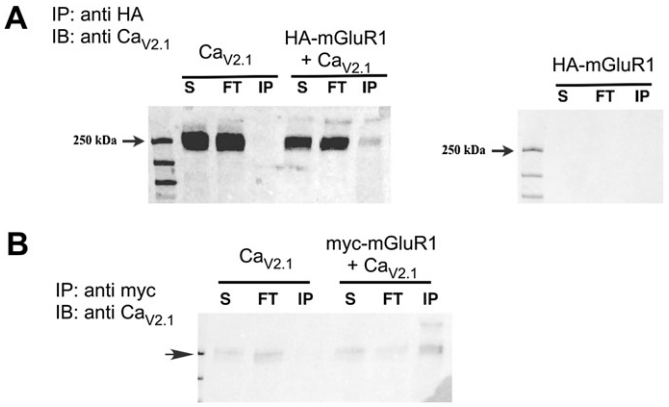

Fig. 1. $Ca_{V2.1}$ co-immunoprecipitates with mGluR1 in HEK293 cells. A, Anti-$Ca_{V2.1}$ (1:500) immunoblot showing protein from supernatant (S), flow-through (FT) and from the anti-HA immunoprecipitation (1:500) experiment (IP), with expression conditions indicated above: $Ca_{V2.1}$ alone and HA-mGluR1 + $Ca_{V2.1}$ (*left*) and HA-mGluR1 alone (*right*). B, Similar to A, but using an anti-myc antibody paired with myc-mGluR1 for the IP. Arrow indicates 250 kDa marker.

Injection of cDNA was performed with an Eppendorf 5247 microinjector and InjectMan NI 2 micromanipulator (Madison, WI) 3–5 h following cell isolation. Injection electrodes were made with a Sutter P-97 horizontal electrode puller (Novato, CA) from thin-walled, borosilicate glass (World Precision Instruments, Sarasota, FL). Plasmids were stored at −20 °C as a 0.4–1 μg/μl stock solution in TE buffer (10 mM TRIS, 1 mM EDTA, pH 8). All mGluR constructs were injected at 100–130 ng μl^{-1} (pCDNA3.1$^+$; InVitrogen). All neurons were co-injected with "enhanced" green fluorescent protein cDNA (0.02 μg/μl; pEGFPN1; BD Biosciences-Clontech, Palo Alto, CA) for identification of successfully injected cells.

Constructs were all sequence confirmed, and PCR products were purified with Qiagen (Valencia, CA) silica membrane spin columns prior to restriction digestion and ligation. Midipreps were prepared using Qiagen anion exchange columns, and amplified in either Top10 or DH5α *E. coli* (InVitrogen).

3. Results

3.1. mGluR1 and $Ca_{V2.1}$ interact

To verify that an interaction between mGluR1 and $Ca_{V2.1}$ could be detected, we expressed in HEK293 cells a hemagglutinin (HA)-tagged mGluR1, $Ca_{V2.1}$ (with β2A and α2δ), or both constructs together and performed co-immunoprecipitation experiments. Cell lysates were immunoprecipitated (IP) with an anti-HA antibody and blots probed using an anti $Ca_{V2.1}$ antibody. Results of these experiments are shown in Fig. 1, which also illustrates samples from the supernatant (S) and flow-through (FT), as well as the IP for each expression condition. A prominent band just under 250 kDa, the expected size of $Ca_{V2.1}$, was evident in the lysates of $Ca_{V2.1}$ expressing cells and also appeared (although less prominently) in the IP only when the channel was coexpressed with HA–mGluR1 (Fig. 1A, HA-mGluR1a + $Ca_{V2.1}$, "IP" lane). Analogous experiments were performed using myc-tagged mGluR1 with $Ca_{V2.1}$ with similar results (Fig. 1B). These data confirm the findings of Kitano et al. (Kitano et al., 2003) of an interaction between mGluR1 and $Ca_{V2.1}$.

3.2. $Ca_{V2.1}$ channels can be expressed in rat sympathetic neurons and modulated by mGluR1

The long forms of the Homer scaffolding proteins (Homer-1b, 1c, 2 and 3) bind to a proline rich motif near the C-terminus of mGluR1 and 5 (Brakeman et al., 1997; Xiao et al., 1998; Tu et al., 1999), causing these receptors to form clusters (Kammermeier et al., 2000; Kammermeier, 2006), and organize around the post-synaptic density (Ango et al., 2000). As a result of this interaction, mGluR1/5-mediated modulation of voltage dependent, N-type calcium channels ($Ca_{V2.2}$) and M-type potassium channels (K_{V7}) is