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Activation of NMDA receptors reduces metabotropic glutamate receptor-induced long-term depression in the nucleus accumbens via a CaMKII-dependent mechanism

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ABSTRACT

Glutamate is the major excitatory neurotransmitter in the brain and exerts its actions through two distinct types of receptors, ionotropic and metabotropic glutamate receptors (mGluR). Although functional interplay between ionotropic *N*-methyl-D-aspartate receptors (NMDAR) and mGluR has been convincingly demonstrated in native and recombinant systems, the mechanism by which NMDAR activation leads to modulation of mGluR function has yet to be elucidated. Using whole-cell patch-clamp recordings in mouse nucleus accumbens (NAc) slices, we found that tetanic stimulation (TS) of excitatory afferents with a naturally occurring frequency (10 min at 13 Hz) reliably induces a mGluR1/5-dependent long-term depression (mGluR1/5-LTD) of excitatory synaptic transmission. Blockade of NMDAR during but not after TS showed enhanced mGluR1/5-LTD induction, which is associated with its antagonism of TS-induced calcium/calmodulin-dependent protein kinase II (CaMKII) activation. The ability of NMDAR antagonists to promote mGluR1/5-LTD induction was mimicked by a selective CaMKII inhibitor KN-62. However, the induction of mGluR1/5-LTD by bath-applied agonist (*S*)-3,5-dihydrophenylglycine was not affected by NMDAR blockade. We also observed that NMDAR or CaMKII blockade during TS significantly blunted TS-induced increased serine/threonine phosphorylation of the scaffold protein Homer1b/c and resulted in an increased interaction of mGluR5 with the Homer1b/c. These results indicate that synaptically released glutamate during TS of excitatory afferents can activate both NMDAR and mGluR1/5 in NAc neurons concomitantly and that activation of NMDAR may stimulate CaMKII-mediated phosphorylation of Homer1b/c and impair the interaction between mGluR5 and Homer1b/c, thereby attenuating mGluR1/5-LTD induction. This study provides a novel molecular mechanism by which NMDAR could regulate mGluR5 function.

1. Introduction

Glutamate, the major excitatory neurotransmitter in the brain, elicits its actions by activation of both ionotropic glutamate (iGluR) and metabotropic glutamate receptors (mGluR). The iGluR, including *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) and kainate subtypes, are a class of heteromeric ligand-gated cation channels that mediate fast excitatory synaptic transmission (Dingledine et al., 1999). The mGluR includes eight subtypes that have been divided into group I (mGluR1 and 5), group II (mGluR2 and 3) and group III (mGluR4, 6, 7 and 8) based on sequence homology, signal transduction mechanisms and pharmacological properties (Conn and Pin, 1997; Niswender and Conn, 2010). The mGluR mediates slow glutamate responses through GTP-binding proteins coupling to various intracellular signaling cascades that can modulate a wide range of voltage- and ligand-gated ion channels. The functional interaction between iGluR and mGluR has long been investigated, especially focusing on the interaction between mGluR5 and NMDAR. For instance, previous studies have demonstrated that activation of mGluR5 can potentiate responses mediated by NMDAR in the hippocampus, striatum, cortex and spinal cord (Doherty et al., 1997; Ugolini et al., 1997; Attucci et al., 2001; Pisani et al., 2001; Kotecha et al., 2003). Reciprocally, activation of NMDAR has also been shown to enhance mGluR5-mediated responses in both native and recombinant systems (Lüthi et al., 1994; Challiss et al., 1994; Alagarsamy et al., 1999, 2005). Although compelling

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evidence exists to support the reciprocal positive-feedback interaction between these two glutamate receptor subtypes, there is evidence that mGluR5 and NMDAR can physically interact each other and undergo a reciprocal constitutive inhibition (Perroy et al., 2008; Bertaso et al., 2010). The reason for this discrepancy is unclear but could be related to differences in experimental conditions. Despite characterizing the crosstalk between mGluR5 and NMDAR, surprisingly little is known about the mechanism underlying the functional interaction between these two classes of glutamate receptors.

Homer proteins are a family of multifaceted scaffolding proteins that bind to the intracellular C-terminal tail of group I mGluR and are an integral part of the postsynaptic mGluR signaling complex (Brakeman et al., 1997; Xiao et al., 1998; Duncan et al., 2005). The constitutively expressed longer form of Homer proteins, Homer1b/c, Homer2a/b and Homer3, can multimerize through their C-terminal coiled-coil structure and link group I mGluR to specific cellular locations for the regulation of specific signaling activities (Shiraishi-Yamaguchi and Furuichi, 2007; Park et al., 2008). In contrast, Homer1a, a short form of Homer, lacks the coiled-coil domain and behaves as an endogenous dominant-negative inhibitor to block the function of long Homer (Xiao et al., 1998).

The nucleus accumbens (NAc), a central component of the mesolimbic dopamine system, has been implicated in functions ranging from motivation and reward to feeding and drug addiction. Pharmacological activation or synaptic stimulation of mGluR1/5 in medium spiny neurons (MSNs) of the NAc can lead to a postsynaptically induced but presynaptically expressed long-term depression (LTD) (Robbe et al., 2002; Fourgeaud et al., 2004; Huang et al., 2011). The induction of this LTD required postsynaptic activation of mGluR5 and an increase of intracellular Ca^{2+} concentrations, triggering the synthesis and release of endocannabinoids, which in turn travel retrogradely across the synaptic cleft to bind to presynaptic type 1 cannabinoid receptors and result in a long-lasting decrease in presynaptic glutamate release (Robbe et al., 2002). In addition, interactions of mGluR5 with longer form of Homer proteins are required for this LTD (Fourgeaud et al., 2004). In this study, we identified a novel functional interaction between NMDAR and mGluR5 when they were activated concomitantly by synaptic released glutamate. We found that synaptic activation of NMDAR may stimulate calcium/calmodulin-dependent protein kinase II (CaMKII)-mediated phosphorylation of Homer1b/c and blunt the interaction between mGluR5 and Homer1b/c, thereby attenuating the induction of mGluR1/5-dependent LTD (mGluR1/5-LTD).

2. Materials and methods

2.1. Slice preparations and electrophysiology

All experimental procedures were conducted in accordance with NIH guidelines for experiments with animals (Guide for the Care & Use of Laboratory Animals, NRC, 1996) and approved by the Institutional Animal Care and Use Committee at National Cheng Kung University. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Slice preparation and whole-cell patch-clamp recordings were performed as described previously (Huang et al., 2011). In brief, male C57BL/6J mice (28–35 days old) were killed by decapitation under deep isoflurane anesthesia and coronal slices (250 μm thick) containing the NAc were prepared using a vibrating microtome (VT1200S; Leica, Nussloch, Germany). The slices were placed in a storage chamber of artificial CSF (aCSF) oxygenated with 95% O_2/5% CO_2 and kept at room temperature (23–25 °C) for at least 1 h prior to recording. The composition of the aCSF solution was (in mM): 117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 1.2 NaH_2PO_4 and 11 glucose at pH 7.3–7.4. For recording, one slice was transferred to a recording chamber and fixed at the glass bottom of the chamber with a nylon grid on a platinum frame. The chamber consisted of a circular well of low volume (1–2 ml) and was perfused constantly at 32.0 ± 0.5 °C with a rate of 2–3 ml/min. Whole-cell patch-clamp recordings were performed from visualized MSNs located in the core region of the NAc. The MSNs were voltage-clamped at −70 mV. Recordings were made using a patch clamp amplifier (Axopatch 200B, Axon Instruments, Union City, CA) under infrared-differential interference contrast microscope. Electrical signals were low-pass filtered at 2 kHz, digitized at 10 kHz using a 12 bit analog-to-digital converter (Digidata 1320, Axon Instruments). An Intel Pentium-based computer with pCLAMP software (Version 8.0; Axon Instruments) was used for on-line acquisition and off-line analysis of the data. For measurement of synaptically evoked excitatory postsynaptic currents (EPSCs), a bipolar stainless steel stimulating electrode was placed 150–200 μm rostral to the recording electrode to stimulate excitatory afferents at 0.05 Hz and the superfusate routinely contained gabazine (10 μM) to block inhibitory synaptic responses. The strength of synaptic transmission was quantified by measuring the amplitude of EPSCs. The LTD was induced by tetanic stimulation (TS) delivered at 13 Hz for 10 min (Robbe et al., 2002; Fourgeaud et al., 2004) or a brief bath application of group I mGluR agonist (*S*)-3,5-dihydroxyphenylglycine (DHPG; 100 μM) for 10 min (Huang et al., 2011). The magnitude of LTD was measured by comparing the average of the responses obtained during the last 10 min of the recording with the average of the responses over a 10 min baseline period before LTD induction. The electrode resistance was typically 3–6 MΩ. The composition of intracellular solution was (mM): 115 K-gluconate; 20 KCl; 10 HEPES; 2 $MgCl_2$; 0.5 EGTA; 3 Na_2ATP; 0.3 Na_3GTP; 5 QX-314 and sucrose to bring osmolarity to 290–300 mOsM and pH to 7.3.

Spontaneous EPSCs (sEPSCs) were recorded from MSNs held in voltage-clamp at a potential of −70 mV in the presence of gabazine (10 μM) and analyzed off-line using a commercially available software (Mini Analysis 4.3; Synaptosoft, Leonia, NJ). sEPSCs were completely blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) plus D(−)-2-amino-5-phosphonovaleric acid (APV, 50 μM), indicating that these are glutamatergic events. The software detects events based on amplitudes exceeding a threshold set just above the baseline noise of the recording (−5 pA). All detected events were reexamined and accepted or rejected on the basis of subjective visual examination. Events with multiple overlapping sEPSCs were eliminated from the analysis and events were rejected if the time to the peak was >1 ms or the time to the decay point was >3 ms. The software then measured amplitudes and intervals between successive detected events. The sEPSC frequencies were calculated by dividing the total number of detected events by the total time sampled. Periods of 3–5 min were analyzed before or 20–30 min after 13 Hz TS. Events were ranked by amplitude and interevent interval for preparation of cumulative probability distribution. Amplitude histograms were binned in 1 pA intervals. Series resistance and input resistance were monitored on-line throughout the whole-cell recording with a 5 mV depolarizing step given after every afferent stimulus and data were discarded if access resistance changed by more than 20%.

2.2. Western blotting and coimmunoprecipitation

The microdissected NAc core tissue samples were lysed in ice-cold Tris–HCl buffer solution (TBS; pH 7.4) containing a cocktail of protein phosphatase and proteinase inhibitors (50 mM Tris–HCl, 100 mM NaCl, 15 mM sodium pyrophosphate, 50 mM sodium fluoride, 1 mM sodium orthovanadate, 5 mM EGTA, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 μM microcystin-LR, 1 μM okadaic acid, 0.5% Triton X-100, 2 mM benzamidine, 60 μg/ml aprotinin, and 60 μg/ml leupeptin) to avoid dephosphorylation and degradation of proteins, and ground with a pellet pestle (Kontes glassware, Vineland, NJ, USA). Samples were sonicated and spun down at 15,000 × *g* at 4 °C for 10 min. The supernatant was then assayed for total protein concentration using Bio-Rad Bradford Protein Assay Kit (Hercules, CA). Each sample from tissue homogenate was separated in 10% SDS-PAGE gel. Following the transfer on nitrocellulose membranes, blots were blocked in buffer solution containing 5% milk and 0.1% Tween-20 in PBS (124 mM NaCl, 4 mM KCl, 10 mM Na_2HPO_4, and 10 mM KH_2PO_4, pH 7.2) for 1 h and then blotted for 2 h at room temperature with the monoclonal antibody that recognizes phosphorylated CaMKIIα at threonine-286 site (1:2000; Affinity Bioreagents, Golden, CO). It was then probed with HRP-conjugated secondary antibody for 1 h and developed using the ECL immunoblotting detection system (Amersham Biosciences, Buckinghamshire, UK), according to manufacturer's instructions. The immunoblots using phosphorylation site-specific antibody were subsequently stripped and reprobed with an antibody that recognizes CaMKIIα (1:1000; Affinity Bioreagents). Coimmunoprecipitation was carried out essentially as previously reported (Huang and Hsu, 2006). Homogenates (250 μg) from each treatment group were incubated with anti-Homer1b/c antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA) in TBS for 4 h at 4 °C. The antibody–protein complexes were then pelleted with protein A-Sepharose beads. The complex was isolated by centrifugation and washed three times. Proteins eluted from the beads were subjected to separation by 4–12% NuPAGE Novex Bis–Tris gels (Invitrogen, Carlsbad, CA) and immunoblotting for anti-mGluR5 (1:2000; Millipore Bioscience Research Reagents, Bedford, MA) or anti-phosphoserine/threonine antibodies (1:2000; Cell Signaling Technology, Beverly, MA). For sequential probing of the same membrane, the membrane was stripped of antibody, washed, and reprobed. Immunoblots were analyzed by densitometry using Bio-profil BioLight PC software. Background correction values were subtracted from each lane to minimize variability across membranes.