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Distinct mechanisms contribute to agonist and synaptically induced metabotropic glutamate receptor long-term depression

Timothy Connelly [a], Yuan Fan [b], Paul E. Schulz [c,*]

[a] University of Pennsylvania, Department of Neuroscience, United States
[b] University of Chicago, Department of Neurology, United States
[c] The University of Texas Health Science Center at Houston, Department of Neurology, United States

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ABSTRACT

Metabotropic glutamate receptor mediated long-term depression (mGlu receptor LTD) is evoked in hippocampal area CA1 chemically by the agonist 3,5-Dihydroxyphenylglycine (DHPG, *agonist* LTD) and synaptically by paired-pulse low frequency stimulation (PP-LFS, *synaptic LTD*). We tested the hypothesis that different expression mechanisms regulate mGlu receptor LTD in 15–21 day old rats through neurophysiologic recordings in CA1. Several findings, in fact, suggest that *agonist* and *synaptic* mGlu receptor LTD are expressed through different mechanisms. First, neither LTD occluded the other. Second, a low calcium solution enhanced *agonist* LTD but did not alter *synaptic* LTD. Third, application of the mGlu receptor antagonist LY341495 (2S-2-amino-2-(1S,2S-2-carboxycyclopropyl-1-yl)-3-(xanth-9-yl)propanoic acid) reversed *agonist* LTD expression, but did not alter *synaptic* LTD. Finally, an N-type, voltage-dependent calcium channel antagonist, ω-conotoxin GVIA (CTX), reduced *agonist* LTD expression by 35%, but did not alter *synaptic* LTD. CTX also blocked the increase in the paired-pulse ratio associated with *agonist* LTD. This study cautions against assuming that *agonist* and *synaptic* LTD are equivalent as several components underlying their expression appear to differ. Moreover, the data suggest that *agonist* LTD, but not *synaptic* LTD, has a presynaptic, N-channel mediated component.

1. Introduction

Metabotropic glutamate receptor dependent long-term depression (mGlu receptor LTD) is evoked through two methods in area CA1 of the hippocampus. It is induced *chemically* (*agonist* LTD) by application of the group I metabotropic glutamate receptor agonist (RS)-DHPG (Fitzjohn et al., 1999; Huber et al., 2000; Palmer et al., 1997). Alternatively, it is induced *synaptically* (*synaptic* LTD) by application of paired-pulse low-frequency stimulation (PP-LFS): a 1 Hz, 15 min stimulation with a 50 ms interpulse interval in the presence of the NMDA receptor antagonist DL-2-Amino-5-phosphonopentanoic acid (APV) (Kemp and Bashir, 1999).

Agonist and *synaptic* mGlu receptor LTD are assumed to be equivalent. In fact, they do share some induction mechanisms, including activation of group I metabotropic glutamate receptors, the G-protein G_q (Kleppisch et al., 2001), protein tyrosine phosphatases (Huang et al., 2004; Moult et al., 2008), extra-cellular signal-regulated kinase (Gallagher et al., 2004), Jun N-terminal kinase 1 (Li et al., 2007), and protein synthesis (Huber et al., 2000). Both forms of LTD are mediated by activation of eEF2K, which initiates translation of the immediate early gene Arc/Arg3.1 resulting in AMPA receptor endocytosis (Park et al., 2008; Waung et al., 2008). Additionally, *agonist* LTD has been found to occlude *synaptic* LTD in adult rats (Huber et al., 2001).

In juvenile animals, mGlu receptor LTD has been shown to be expressed by different mechanisms (Kemp et al., 2000; Overstreet et al., 1997), which are presynaptic and independent of protein synthesis (Nosyreva and Huber, 2005). It is unclear whether the mechanisms of *agonist* and *synaptic* LTD differ at this age, or if the developmental switch in LTD mechanisms explains differences found in previous studies. For example, *agonist* LTD is independent of postsynaptic calcium increases in juvenile animals (Fitzjohn et al., 2001), while *synaptic* LTD may require calcium (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Otani and Connor, 1998). Moreover, a Group I mGlu receptor knockout causes a deficit in *agonist* LTD in juvenile mice (Huber et al., 2001; Volk et al., 2006). It is interesting to note that even in adults differences have been found between the two methods of LTD induction. In particular, both the role of Homer protein (Ronesi and Huber, 2008) and the requirement for either pre- or post-synaptic muscarinic acetylcholine receptors differ (Kamsler et al., 2010; Volk et al., 2007).

Since *agonist* LTD is more commonly studied than *synaptic* LTD, some observations have not been confirmed for *synaptic* LTD in adults

* Corresponding author at: Department of Neurology, UTHealth: The University of Texas Health Science Center at Houston, 6410 Fannin, Suite 1014, Houston, TX 77030, United States.
E-mail address: Paul.E.Schulz@uth.tmc.edu (P.E. Schulz).

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or juveniles. For example, *agonist* LTD expression is enhanced in low-calcium solutions (Watabe et al., 2002) and is reversed by metabotropic glutamate receptor antagonists (Palmer et al., 1997; Watabe et al., 2002).

The goals of this study were to investigate whether *agonist* and *synaptic* LTD share common mechanisms of induction and expression in juvenile rats and to investigate specific mechanisms underlying each. We found several important differences between *agonist* and *synaptic* LTD at this stage of development. We also propose that *agonist* mGlu receptor LTD induction involves inhibition of N-type voltage-dependent calcium channels.

2. Materials and methods

All experimental procedures followed the guidelines and had the approval of the Institutional Animal Care and Use Committee of Baylor College of Medicine.

2.1. Slice preparation

Transverse hippocampal slices (400 μm thick) were prepared from 2–3 weeks old Sprague–Dawley rats (Harlan, Indianapolis, IN) using standard procedures described previously (Kasten et al., 2007). Rats were anesthetized with a combination of ketamine (42.8 mg/ml), xylazine (8.6 mg/ml), and acepromazine (1.4 mg/ml) at 1 ml/kg *i.p.* Rats were then transcardially perfused with ice-cold saline containing (in mM): 110 choline chloride, 2.5 KCl, 1.25 NaH_2PO_4, 28 $NaHCO_3$, 0.5 $CaCl_2$, 7 dextrose, 7 $MgCl_2$, 3 pyruvic acid, and 1.3 ascorbic acid. After decapitation, brain block containing hippocampus was sliced with a Vibratome (Technical Products International, Inc., O'Fallon, Missouri) in the same saline. A surgical cut was made between CA3 and CA1 to prevent the propagation of epileptiform activity. Slices were incubated in external saline (see below) for 15 min at 32 °C, and then stored at room temperature for >1 h before transferring to recording chamber.

2.2. Extracellular recordings

Slices were placed in an interface recording chamber (BSC Haas Top, Harvard Apparatus, Holliston, MA) at 32.5 °C. The external saline was perfused at 1–2 ml/min and contained (in mM): 119 NaCl, 2.5 KCl, 26.2 $NaHCO_3$, 30 sucrose, 10 dextrose, 2.5 $CaCl_2$, 1.3 $MgSO_4$, and 1 NaH_2PO_4. All chemicals were purchased from Sigma (St. Louis, MO). In some experiments, as indicated, calcium chloride was reduced to 1.3 mM and magnesium sulfate was reduced to 1 mM. Field excitatory post-synaptic potentials (fEPSPs) were recorded using pipettes made of capillary glass (A-M Systems Carlsborg, WA) filled with 750 mM NaCl with a resistance of 1–3 M. Microelectrodes were pulled from 1.5 mm OD glass tubing using a Flaming/Brown micropipette puller (Sutter Instruments, Novato, CA). Stimulation was for 50 μs at 20 s intervals via bipolar, Teflon-coated platinum electrodes. Electrodes were placed in stratum radiatum of area CA1. A single stimulus intensity that initially yielded a 1-mV field excitatory postsynaptic potential (fEPSP) was used throughout each experiment. To measure paired-pulse ratio (PPR), two synaptic stimuli were given in succession with an inter-pulse interval of 55 ms. For PP-LFS, paired-pulses with a 50 ms inter-pulse interval were given at 1 Hz for 15 min.

(R,S)-3,5-DHPG, anisomycin, ω-conotoxin GVIA, DL-2-Amino-5-phosphonopentanoic acid (APV), and LY341495 (2S-2-amino-2-(1S,2S-2-carboxycyclopropyl-1-yl)-3-(xanth-9-yl)propanoic acid) were purchased from Tocris (Ellisville, MO). DHPG and APV were added directly to the extracellular solution. Conotoxin (with 0.1 mg/mL cytochrome C, bovine heart) was aliquoted into the extracellular solution, and frozen before use. Anisomycin and LY341495 were dissolved in DMSO and sodium hydroxide, respectively, aliquoted, and frozen before use.

2.3. Data analysis

Data was acquired and analyzed on either a Mac running the DAM program written by Costa Colbert, or on PC using pClamp 9 for acquisition and Clampfit for analysis (Molecular Devices, Sunnyvale, CA). LTD was quantified by measuring the initial fEPSP slope. PPR was calculated by dividing the slope of the second fEPSP by the slope of the first fEPSP and subtracting 1. All results were expressed as the mean LTD ± S.E.M. Statistical analysis was performed using Student's independent or paired t-tests.

3. Results

3.1. Protein synthesis independence in juvenile rats

We tested the hypothesis that *synaptic* and *agonist* mGlu receptor LTD requires protein synthesis in 2–3 weeks old Sprague–Dawley rat hippocampi. Juvenile rats (<2 weeks) exhibit protein synthesis independent mGlu receptor LTD, while LTD in adult rats (>3 weeks) requires protein synthesis (Nosyreva and Huber, 2005). It was therefore necessary to establish the form of mGlu receptor LTD that we are observing in 2–3 weeks old rats. After obtaining a stable baseline for at least 20 min, 20 μM of the protein synthesis inhibitor anisomycin was applied for another 20 min before inducing either *agonist* or *synaptic* LTD.

To induce *agonist* LTD, 100 μM (R,S)-DHPG was applied for 20 min. This concentration is based on a previous study which resulted in robust, NMDA-independent LTD (Faas et al., 2002). The magnitude of *agonist* LTD was not affected by anisomycin. There was 45 ± 3% LTD ($n = 5$) in anisomycin treated slices versus 51 ± 5% ($n = 11$) in control slices ($P = 0.71$, Fig. 1A). There was a significant increase in the paired-pulse ratio (PPR) for both control and anisomycin LTD. The PPR in the anisomycin slices increased 50.9% from 0.57 ± 0.05 at baseline to 0.86 ± 0.07 at 45 min after induction ($P = 0.01$, $n = 5$). Control PPR increased 60.4% from 0.48 ± 0.06 at baseline to 0.77 ± 0.11 at 45 min after induction ($P = 0.003$, $n = 11$, Fig. 1B). These results suggest that there is a presynaptic modification with *agonist* mGlu receptor LTD (Foster and McNaughton, 1991; Schulz et al., 1994).

To induce *synaptic* mGlu receptor LTD, D,L-APV (100 μM) was washed in for 20 min before recording a baseline, after which PP-LFS was applied. *Synaptic* LTD was compared between anisomycin and control slices. The magnitude of *synaptic* LTD was similar for anisomycin treated slices (23 ± 4%) and controls (21 ± 5%) measured 45 min after induction (Fig. 1C). Both sets of slices showed significant increases in the PPR at 45 min after PP-LFS. Control slices had a 41.2% increase (0.34 ± 0.07 at baseline to 0.48 ± 0.06, $P = 0.001$, $n = 8$) and anisomycin-treated slices had a 22.9% increase (0.48 ± 0.10 at baseline to 0.59 ± 0.10 after induction, $P = 0.004$, $n = 6$, Fig. 1D) in agreement with a previous study (Nosyreva and Huber, 2005). In summary, *agonist* and *synaptic* mGlu receptor LTD have a presynaptic component and are protein synthesis independent at this stage of development, consistent with juvenile mGlu receptor LTD.

3.2. Failure of agonist and synaptic LTD to occlude each other

A common method to test whether two forms of plasticity employ the same mechanisms is to induce one and test whether it occludes induction of the other. We used this method to test whether *agonist* and *synaptic* LTD employ similar mechanisms.

In the first experiments, *synaptic* LTD was induced (Fig. 2A). A baseline was obtained and one round of PP-LFS was administered. After waiting 20 min, a second round was administered. The response was then allowed to stabilize for 40 min before inducing *agonist* LTD