Metabotropic Glutamate Receptor Antagonist AIDA Blocks Induction of Mossy Fiber-CA3 LTP In Vivo


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Thompson, Kenira J., Mario L. Mata, James E. Orfita, Edwin J. Barea-Rodriguez, and Joe L. Martinez Jr. Metabotropic glutamate receptor antagonist AIDA blocks induction of mossy fiber-CA3 LTP in vivo. J Neurophysiol 93: 2668–2673, 2005. First published November 17, 2004; doi:10.1152/jn.00901.2004. Metabotropic glutamate receptors (mGluRs) are implicated in long-term memory storage. mGluR-I and mGluR-II antagonists impede various forms of learning and long-term potentiation (LTP) in animals. Despite the evidence linking mGluRs to learning mechanisms, their role in mossy fiber-CA3 long-term potentiation (LTP) is not yet clear. To explain the involvement of mGluR-I in memory mechanisms, we examined the function of the mGluR-I antagonist 1-aminoindan-1, 5-dicarboxylic acid (AIDA) on the induction of mossy fiber-CA3 LTP in vivo in male Sprague Dawley and Fischer 344 (F344) rats. Acute extracellular mossy fiber (MF) responses were evoked by stimulation of the MF bundle and recorded in the stratum lucidum of CA3. The excitatory postsynaptic potential (EPSP) magnitude was measured by using the initial slope of the field EPSP slope measured 2–3 ms after response onset. After collection of baseline MF-CA3 responses at 0.05 Hz, animals received either ((±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (N-methyl-D-aspartate-R antagonist, 10 mg/kg ip), naloxone (opioid-R antagonist, 10 mg/kg ip), or AIDA (mGluR antagonist, 1 mg/kg ip or 37.5 nmol ic). LTP was induced by two 100-Hz trains at the intensity sufficient to evoke 50% of the maximal response. Responses were collected for an additional 1 h. AIDA blocked induction of LTP in the mossy fiber pathway (P < 0.05) in both strains of rats after systemic and in Sprague Dawley rats after intrahippocampal injection.

INTRODUCTION

Metabotropic glutamate receptors (mGluRs) comprise a family of G-coupled receptors known to modify Ca2+ and K+ channels, GABA A receptors, and the N-methyl-D-aspartate (NMDA)-R and AMPA-R (Nicoletti et al. 1999; Pin and Duvoisin 1995; Skeberdis et al. 2001), all of which underlie learning and memory-related mechanisms. To date, eight subtypes of the mGluRs have been identified and placed into three distinct classes (mGluR1–8). Class I includes mGluR1 and mGluR5, both of which are coupled to phospholipase C (PLC). Classes II (mGluR2 and mGluR3) and III (mGluR4,6,7,8) are negatively coupled to adenyly cyclase (Pin and Duvoisin 1995). Because of the role mGluRs play in the modulation of such significant neuronal channels, multiple studies have addressed the role of the various mGluR subclasses on learning and memory.

Intra-peritoneal (ip) injections of the class I selective mGluR antagonist -aminooindan-1, 5-dicarboxylic acid (AIDA) imp-
tained in and released by the MFs and has been confirmed both in vivo and in vitro (Derrick et al. 1991, 1992; Derrick and Martinez 1994; Jin and Chavkin 1999). Further evidence suggests that Ca$^{2+}$ entry is necessary for MF-LTP induction (Bashir et al. 1993; Ito and Sugiyama 1991; Williams and Johnston 1996; Yeckel et al. 1999). However, little is known regarding the specific receptor systems that interact with opioid receptors to result in LTP induction at the MF pathway.

In this study, we examined the role of the mGluR1 antagonist AIDA on LTP induction at the MF-CA3 pathway in vivo using Sprague Dawley (Mata et al. 2000) and F344 rats. Although there is evidence against the role of mGluR in MF LTP (Hsia et al. 1995), all of these previous studies were conducted in vitro, and there is extensive evidence documenting differences in LTP induction and maintenance at the MF pathway in vivo and in vitro (Barea-Rodriguez et al. 2000; Derrick and Martinez 1996; Nicoll et al. 1994). We report that AIDA significantly impairs induction of MF LTP in both of the strains tested.

**METHODS**

**Electrophysiology**

All experimental procedures were approved in advance by the Institutional Animal Care and Use Committee at the University of Texas at San Antonio and are in accordance with National Institutes of Health guidelines. Adult male Sprague Dawley and F344 rats (300–450 g; Charles River Laboratories, Indianapolis, IN) were anesthetized with pentobarbital sodium (Nembutal solution; 50 mg/kg ip, Henry Schein Veterinary Supply) and placed on a stereotaxic frame. Electrodes were placed in the MF pathway at coordinates corresponding to the s. lucidum. The first electrode was initially placed in the granule cell layer of the dentate gyrus with the aid of stereotaxic coordinates and audio monitoring of CA1 pyramidal and granule cell unit discharges due to injury. The second electrode was stereotaxic coordinates and audio monitoring of CA1 pyramidal and corresponding to the s. lucidum. The first electrode was initially placed in the MF pathway at coordinates listed in the preceding text. The combination of cannula-recording electrode was constructed by insulating (Epoxylite, Irvine, WA) the outside of the cannula, except for the tip and a 3-cm portion at the top. A stainless steel wire (A-M Systems) was wrapped around the cannula at the noninsulated top, and connected to an amplifier using an amphenol connector. Plastic tubing (PE 20) was attached to the top opening of the cannula to allow for drug delivery into the area in which evoked responses were collected. Responses were recorded, amplified, filtered at 0.1 Hz to 10 kHz, and recorded in the s. lucidum of area CA3 (Fig. 1). Teflon-coated twisted tungsten wire (A-M Systems, Carlsborg, WA) exposed only at the tip was used to make stimulating electrodes. Each electrode was connected to a Grass Stimulus Isolation Unit to deliver stimulation at 10–50 μA for 0.2 ms. MF-CA3 responses were recorded, amplified, filtered at 0.1 Hz to 10 kHz, and stored for off-line analysis. The EPSP magnitude was measured by using the initial slope of the field EPSP slope measured 2–3 ms after response onset. After collection of baseline MF-CA3 responses for ≥20 min at 0.05 Hz, animals received either ((±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP; NMDA-R antagonist, 10 mg/kg ip; Sigma RBI, St. Louis, MO), naloxone hydrochloride (opioid receptor antagonist, 10 mg/kg ip; Sigma RBI) or AIDA (mGluR1 antagonist, 1 mg/kg ip, Sigma). CPP was used to ensure that the recordings were from MF-evoked responses, and was given at a dose previously found to be effective at blocking NMDA-dependent LTP in vivo (Hernandez et al. 1994). One hour or 30 min (respectively) after drug administration, LTP was induced by two 100-Hz, 1-s trains delivered at the same intensity sufficient to evoke 50% of the maximal MF response. Evoked MF responses were collected for an additional 60 min in all groups (n = 3, F344; n = 7, Sprague Dawley). In control animals, low-frequency MF responses were collected at 0.05 Hz for 1 h, but 100-Hz trains were not delivered. Electroencephalogram (EEG) recordings were monitored for 1 min after high-frequency train and no seizures or after discharge activity was noted in the animals. MF LTP was measured at 1 h by obtaining the percent change in the field EPSP slope as calculated using a 20% trimmed mean from the last 5 min of baseline and the last 5 min of the 1-h collection period. All animals were killed using Nembutal solution (100 mg/kg ip) 1 h after delivery of the high-frequency train.

**Intrahippocampal injections**

A 33-gauge stainless steel cannula-recording electrode was placed above the CA3 pyramidal cell layer of the dorsal hippocampus using the coordinates listed in the preceding text. The combination of cannula-recording electrode was constructed by insulating (Epoxylite, Irvine, WA) the outside of the cannula, except for the tip and a 3-cm portion at the top. A stainless steel wire (A-M Systems) was wrapped around the cannula at the noninsulated top, and connected to an amplifier using an amphenol connector. Plastic tubing (PE 20) was attached to the top opening of the cannula to allow for drug delivery into the area in which evoked responses were collected. Responses were recorded via direct stimulation of the MFs using a stainless steel bipolar electrode. Evoked responses were collected using the protocol mentioned previously. After a 20-min baseline period, AIDA (25, 37.5, or 50 nmol; 1 μl total volume; Sigma, n = 3) or saline (1 μl total, n = 3) were delivered unilaterally into the s. lucidum of hippocampal area CA3 via pressure injection using a syringe pump (Harvard Apparatus, Indianapolis, IN). The drugs were delivered over a 5-min period of time. The drug infusion period was followed by a 30-min post infusion period, followed by delivery of high-frequency stimulation of two 100-Hz trains, with an intertrain interval of 20 s (200 total pulses). Evoked responses were collected for 1 h post high-frequency stimulation.

**Verification of electrode placement**

Verifications were performed using electrophysiological criteria for all animals. Electrophysiological criteria involved audio localization of CA1, CA3, and granule cells in the dentate, localization of antidromic response in the dentate gyrus, and the presence of MF evoked responses preceded by a presynaptic volley with an onset of 2.5 ms (orthodromic response). Histological verification of electrode placement was performed on 10% of subjects and correct electrode placement was observed in all of these brains.
RESULTS

For F344 rats, one-way repeated-measures ANOVA was used to examine percent change values in the field EPSP slope for each group. Post hoc Tukey tests were used to determine whether posttreatment and post high-frequency stimulation responses were different from responses recorded during the baseline period. Student’s t-test was used to determine whether postinjection responses differed from baseline in all groups. We found that treatment with (±)-CPP did not block MF LTP induction because there was a significant increase in the field EPSP slope after high-frequency stimulation \( [F(2, 8) = 49.461; P = 0.002] \) compared with baseline (Fig. 2A). No significant differences were found between the baseline period and post-CPP injection period. In the naloxone and AIDA-treated animals, no LTP was observed because no significant differences were found among baseline, posttreatment, and posttetanization fEPSP. However, a nonsignificant depression in the fEPSP was evident for naloxone (Fig. 2B). t-test revealed no significant differences between baseline and postsaline injection fEPSP in the control group \( (P = 0.119, \text{Fig. 2C}) \), indicating that responses remained stable throughout the duration of evoked MF responses for this group. A significant increase was found in the field EPSP for the LTP group \( [F(2, 6) = 28.13, P < 0.001] \) compared with the saline control group.

Figure 3A shows a summary of control experiments in Sprague Dawley rats in which robust MF LTP was elicited by high-frequency stimulation \( (2 \times, 100 \text{ Hz}, 20 \text{ s inter-trial interval (ITI)} \) at test intensity; control, \( 142.4 \pm 5.2\% \), \( n = 5 \)). MF LTP induction was not blocked by the NMDA-R antagonist CPP \( (122.7 \pm 14\%, \text{n = 3, Fig. 2B}) \). Stimulation delivered in the presence of naloxone blocks MF LTP induction \( (97.5 \pm 9.6\%, \text{n = 3, Fig. 2C}) \). Finally, MF LTP is blocked by AIDA \( (114.0 \pm 4.4\%, \text{n = 7, Fig. 2D}) \). Intracranial administration of AIDA in Sprague Dawley rats also blocked MF LTP induction, as seen in Fig. 4B \( (104 \pm 6\%, \text{n = 3}) \). Figure 4A shows a summary of the saline control group in which robust LTP was elicited \( (115.03 \pm 6.4\%, \text{n = 3}) \).

DISCUSSION

Our results indicate that the mGluR antagonist AIDA (systemic injection) blocks induction of MF LTP in vivo in both Sprague Dawley (Mata et al. 2000) and F344 rats. MF-CA3 LTP was also blocked after intrahippocampal infusion of AIDA in Sprague Dawley rats.

In the MF synaptic pathway, class I mGluRs have been localized to CA3 dendritic spines in s. radiatum (Lujan et al. 1996). Activation of these mGluR1s evoke \( Ca^{2+} \) release in CA3 neurons (Kapur et al. 2001), supporting the idea that

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**FIG. 2.** 1-aminoindan-1, 5-dicarboxylic acid (AIDA) blocks in vivo long-term potentiation (LTP) induction in the mossy fiber pathway of Fischer 344 (F344) rats. Plot of normalized field excitatory postsynaptic potential (fEPSP) slope magnitude of mossy fiber-CA3 responses over time at current intensities eliciting 50% of the maximal response. A: in the presence of \((\pm)\)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP; 10 mg/kg ip, \( n = 3 \)), delivery of high-frequency stimulation (HFS) trains \( [2 \times 1-s, 100-Hz \text{ trains, } 20-s \text{ inter-trial interval (ITI)}] \) to the mossy fibers induced LTP \( (134.9 \pm 9.2\%, P < 0.05) \). B: in the presence of naloxone (10 mg/kg ip, \( n = 3 \)), HFS stimulation \( [2 \times 1-s, 100-Hz \text{ trains, } 20-s \text{ ITI}] \) to the mossy fibers blocked LTP induction \( (94.0 \pm 11.5\%, P < 0.05) \). C: the class 1 mGluR antagonist AIDA (1 mg/kg ip, \( n = 3 \)) blocked LTP induction \( (102.2 \pm 4.6\%, P < 0.05) \). Calibration: 0.5 mV, 5 ms.
mGluRs are activated by MF bursts, and initiate Ca\(^{2+}\)/H\(_{11001}\) release in CA3 pyramidal cells (Kapur et al. 2001). Our findings reiterate the importance of postsynaptic Ca\(^{2+}\)/H\(_{11001}\) in LTP induction at the MF synapse. Various studies show that any experimental

**FIG. 3.** AIDA blocks in vivo LTP in the mossy fiber pathway of Sprague Dawley rats. Plot of normalized fEPSP slope magnitude of mossy fiber-CA3 responses over time at current intensities eliciting 50% of the maximal response. A: MF LTP is induced in the presence of saline (115.03 ± 6.4%, n = 3). B: LTP induction is blocked in the presence of AIDA (37.5 nM; 1 µl total volume, delivered for 5 min; 104 ± 6%, n = 3). Calibration: 0.5 mV, 5 ms.

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procedure that impedes an increase in postsynaptic Ca\(^{2+}\) concentration disrupts LTP at this synapse (Kapur et al. 2001; Yeckel et al. 1999). Our work in vivo with AIDA shows an mGluR-mediated blockade of LTP induction at the MF synapse that is potentially due to an mGluR effect on Ca\(^{2+}\)-mediated transmission at the MF-CA3 synapse.

The MFs contain and release prodynorphin and proenkephalin-derived opioid peptides (Chavin et al. 1993a,b; Gall et al. 1981; McGinty and Bloom 1983; McGinty et al. 1983), and the release of endogenous opioid peptides by MF synapses requires high-frequency synaptic activity (Derrick and Martinez 1996). In our in vivo preparation, MF-CA3 LTP is blocked by the opioid antagonist naloxone. Our findings agree with previous literature showing that activation of endogenous opioid peptides is essential for LTP induction at the MF-CA3 pathway (Derrick and Martinez 1994a,b, 1996; Derrick et al. 1992). Blockade of NMDA-R by CPP and the presence of LTP in the MFs observed in these experiments also rules out the possibility of this receptor type being involved in this form of synaptic plasticity.

In our preparation, no strain differences were seen, as the AIDA-induced blockade of LTP induction was observed in both Sprague Dawley and F344 rats. The lack of between strain differences in our experiment is noteworthy because strain differences in spatial learning among Long Evans, F344, Dark-Agouti, Wistar, and Sprague Dawley rats have been documented (Troy Harker and Whishaw 2002). These studies indicate more dramatic differences on spatial learning acquisition but also occur in matching to place performance (Troy Harker and Whishaw 2002). In general, both spatial and nonspatial deficits can be associated with inbreeding. Despite these differences in spatial learning among Long Evans, F344, Dark-Agouti, Wistar, and Sprague Dawley rats, the processes for LTP induction at the MF pathway appear to be quite similar. Both strains show an NMDA receptor-independent form of LTP that is blocked by the opioid-receptor antagonist naloxone as well as by the mGluR antagonist AIDA. Even with the mounting evidence supporting NMDA receptor-independent LTP in the MF-CA3 pathway, the specific mechanisms by which opioid peptides, and more recently, mGluRs, mediate LTP in this region are not completely understood.

Interestingly, the concentration of AIDA used for intrahippocampal injections in this study (37.5 nmol) is relatively low when compared with the values typically used in vitro preparations (Brandrowski et al. 2003; Yeckel et al. 1999). This is not surprising as a complete understanding of the relationship between in vitro and in vivo drug metabolism/drug-drug interaction is still emerging. In fact, some literature suggests that using in vitro-in vivo correlation models (IVIVC) to predict in vivo concentration profiles given the in vitro release characteristics of a drug is not always accurate (Pitsiou et al. 2001). Multiple systematic differences must be considered, such as temporal variations between in vitro and in vivo release, the mechanics involved in slice preparation, and overall drug pharmacokinetics differences due to exposure in a controlled bath versus the intact animal. We are confident that the concentration of AIDA used in this study corresponds with typical doses used for in vivo intracranial administration of the drug. In fact, concentrations as low as 0.4 nmol successfully reduce acute neuronal degeneration and behavioral deficits after traumatic brain injury in rats (Lyeth et al. 2001). Similarly low concentrations of intrahippocampal AIDA (50 nmol) significantly alter fear conditioning (Maciejak et al. 2003). We found that other concentrations of AIDA (25 and 50 nmol, data not shown) did not result in significant changes in LTP induction. The dramatic differences that exist between in vitro and in vivo preparations further reiterate the importance of validating results using both in vitro and in vivo models.

Earlier reports indicate that rodent long-term retention is impaired by AIDA (Christoffersen et al. 1999; Nielsen et al. 1997) and that this impairment is not due to drug-induced behavioral effects (Nielsen et al. 1997). LTP, as the widely accepted model for hippocampally dependent reference memory, has also been inhibited by the class I/II mGluR antagonist MCPG (Riedel et al. 1996a,b). In this experiment, we find that in addition to opioid peptides, mGluRs are also involved in the induction of MF-LTP in vivo. As mentioned earlier, there is extensive work documenting differences in LTP induction and maintenance at the MF pathway in vivo and in vitro (Barea-Rodriguez et al. 2000; Derrick and Martinez 1994a,b 1996; Derrick et al. 1992; Nicoll et al. 1994; Zalutsky et al. 1992). Despite some evidence against the role of mGluRs in MF LTP in vivo (Hsia et al. 1995; Mellor and Nicoll 2001), our results indicate a blockade of in vivo MF LTP induction in the presence of AIDA, thus supporting the literature linking mGluRs to hippocampally dependent memory mechanisms (Conquet et al. 1994; Kapur et al. 2001).


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