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Assessing the Role of GLU_{K5} and GLU_{K6} at Hippocampal Mossy Fiber Synapses

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It has been suggested recently that presynaptic kainate receptors (KARs) are involved in short-term and long-term synaptic plasticity at hippocampal mossy fiber synapses. Using genetic deletion and pharmacology, we here assess the role of GLU_{K5} and GLU_{K6} in synaptic plasticity at hippocampal mossy fiber synapses. We found that the kainate-induced facilitation was completely abolished in the $GLU_{K6}^{-/-}$ mice, whereas it was unaffected in the $GLU_{K5}^{-/-}$. Consistent with this finding, synaptic facilitation was reduced in the $GLU_{K6}^{-/-}$ and was normal in the $GLU_{K5}^{-/-}$. In agreement with these results and ruling out any compensatory effects in the genetic deletion models, application of the GLU_{K5}-specific antagonist LY382884 [(3*S*,4a*R*,6*S*,8a*R*)-6-(4-carboxyphenyl)methyl-1,2,3,4,4a, 5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid] did not affect short-term and long-term synaptic plasticity at the hippocampal mossy fiber synapses. We therefore conclude that the facilitatory effects of kainate on mossy fiber synaptic transmission are mediated by GLU_{K6}-containing KARs.

Key words: **hippocampus; kainate receptors; long-term potentiation; mossy fiber synapses; short-term potentiation; synaptic plasticity**

Introduction

Glutamatergic transmission in the CNS is mediated postsynaptically by ionotropic and metabotropic receptors. Ionotropic glutamate receptors are widely known to mediate the majority of fast excitatory synaptic transmission in the CNS, primarily through the activation of postsynaptic AMPA and NMDA receptors (Hollmann and Heinemann, 1994). Kainate receptors (KARs) were then first shown to contribute to excitatory postsynaptic transmission at mossy fiber synapses onto CA3 pyramidal neurons in the hippocampus (Castillo et al., 1997; Vignes and Collingridge, 1997) and have since been described at several other synapses throughout the CNS as well (Frerking and Nicoll, 2000; Lerma, 2003). In addition to their conventional postsynaptic role, KARs can also act presynaptically to modulate neurotransmitter release (Frerking and Nicoll, 2000; Lerma, 2003). Until recently, it was generally believed that activation of presynaptic KARs reduces neurotransmitter release independent of the type of synapse. For example, it has been shown that kainate downregulates excitatory synaptic transmission in areas CA1 and CA3 of the hippocampus (Chittajallu et al., 1996; Vignes et al., 1998). However, we found recently that exogenous kainate application at low doses rather facilitates transmission at mossy fiber synapses (Schmitz et al., 2001), whereas higher concentrations of kainate depress transmitter release (Kamiya and Ozawa, 2000; Schmitz et al., 2000, 2001). These findings have been repeated by several other groups, and there is now a general agreement about this view (Lauri et al., 2001a; Ji and Staubli, 2002; Contractor et al., 2003; Rodriguez-Moreno and Sihra, 2004). The effect is not restricted to hippocampal mossy fiber synapses but can also be observed at parallel fiber synapses in the cerebellum (Delaney and Jahr, 2002). More importantly, synaptic activation of presynaptic KARs is at least in part responsible for short-term plasticity (Contractor et al., 2001; Lauri et al., 2001a; Schmitz et al., 2001) and the induction of long-term potentiation (LTP) (Bortolotto et al., 1999; Contractor et al., 2001; Schmitz et al., 2003) at the mossy fiber synapse.

The subunit composition of presynaptic KARs on mossy fiber terminals remains obscure. Pharmacological studies using LY382884 [(3*S*,4a*R*,6*S*,8a*R*)-6-(4-carboxyphenyl)methyl-1,2,3,4,4a, 5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid] have suggested a role for GLU_{K5}-containing KARs in short-term as well as long-term plasticity at mossy fiber synapses (Bortolotto et al., 1999; Lauri et al., 2001a,b, 2003; Bortolotto et al., 2003). However, GLU_{K5} is poorly expressed in the granule cells that act as presynaptic afferents at mossy fiber synapses (Bahn et al., 1994; Paternain et al., 2000), and other studies using knock-out (KO) mice have concluded that GLU_{K6}-containing KARs are critically involved (Contractor et al., 2001; Schmitz et al., 2003). In the current study, we investigated the role of GLU_{K5}- and GLU_{K6}-containing KARs in synaptic plasticity at hippocampal mossy fiber synapses using both genetic deletion models as well as GLU_{K5}-specific pharmacological agents.

Materials and Methods

Preparation. Hippocampal slices were prepared from wild-type rats (Sprague Dawley or Wistar rats) or GLU_{K5}-deficient, GLU_{K6}-deficient, and wild-type mice (postnatal days 18–40) as described previously (Schmitz et al., 2003). In brief, the animals were anesthetized with halothane and decapitated, and the brains were removed. Tissue blocks containing the subicular area and hippocampus were mounted on a Vi-

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bratome in a chamber filled with ice-cold artificial CSF (ACSF) containing the following (in mM): 50 NaCl, 150 sucrose, 25 $NaHCO_3$, 2.5 KCl, 1 NaH_2PO_4, 0.5 $CaCl_2$, 7 $MgSO_4$, and 10 glucose, pH 7.4 (saturated with 95% O_2 and 5% CO_2). Transverse slices were cut at 300–500 μm thickness and heated to 35°C for 30 min. Slices were then cooled to room temperature and transferred to ACSF containing the following (in mM): 124 NaCl, 26 $NaHCO_3$, 10 glucose, 3 KCl, 2 $CaCl_2$, 1 $MgSO_4$, and 1.25 NaH_2PO_4. In some experiments (see Figs. 1, 2), a slightly modified cation solution was used (2.5 mM $CaCl_2$ and 1.3 mM $MgSO_4$). All ACSF was equilibrated with 95% O_2 and 5% CO_2. The slices were stored in a submerged chamber in which they were held for 1–7 hr before being transferred to the recording chamber, in which they were perfused with ACSF at a rate of 3–4 ml/min.

Electrophysiology. Whole-cell recording electrodes were filled with the following (in mM): 120 Cs-gluconate, 5 CsCl, 10 tetraethylammonium-Cl, 8 NaCl, 10 HEPES, 5 EGTA, 4 MgATP, 0.3 Na_3GTP, 5 HEPES, and 5 QX-314 (lidocaine *N*-ethyl bromide), pH adjusted to 7.3 with CsOH. Access resistances ranged between 5 and 18 MΩ, were continuously checked during the recording, and were not allowed to vary >15% during the course of the experiment. No series resistance compensation was used. Electrode resistances ranged from 2 to 6 MΩ. Field potential recordings were performed with low-resistance patch pipettes filled with external solution placed in stratum lucidum. Bipolar tungsten electrodes or patch pipettes filled with external solution were placed in the granule cell layer or in the hilus region to stimulate mossy fibers. One of the group II metabotropic glutamate receptor agonists, DCG-IV [2*S*,2′*R*,3′*R*-2-(2′,3′-dicarboxycyclopropyl)glycine] (0.5–1 μM) or L-CCG1 [(2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine] (10 μM), was applied at the end of each experiment to verify that the signal was generated by mossy fiber synapses. In addition, slices were only accepted that had >350% synaptic facilitation when stimulus frequency was changed from 0.05 to 1 Hz. Field EPSPs in area CA1 were recorded in stratum radiatum after stimulation of the Schaffer collaterals.

Average values are expressed as mean ± SEM. Drugs used were CNQX, NBQX (2,3-dihydroxy-6-nitro-7-sulfonyl-benzo[*f*]quinoxaline), D-AP-5, DCG-IV, L-CCG1, ATPA [(*RS*)-2-amino-3-(3-hydroxy-5-tertbutylisoxazol-4-yl)propanoic acid], and GYKI53655 [1-(4-aminophenyl)-3-methylcarbamyl-4-methyl-7,8-methylenedioxy-3,4-dihydro-5H-2,3-benzodiazepine] and were obtained from Tocris Cookson (Ellisville, MO).

Fluorescence measurements. Mossy fibers were locally labeled with a pressure stream of the low-affinity calcium indicator magnesium green AM (Molecular Probes, Eugene, OR) (Regehr and Tank, 1991; Breustedt et al., 2003). Recordings were started 3–7 hr after labeling of the slices. Mossy fibers were stimulated extracellularly, and epifluorescence was measured with a single photodiode from a spot a few hundred micrometers away from the loading site. The signals from the photodiode were digitized (National Instruments, Austin, TX) at 5 kHz and captured with IGOR Pro software (WaveMetrics, Lake Oswego, OR). The change in fluorescence intensity (ΔF) relative to the initial intensity of fluorescence was calculated ($\Delta F/F$). Mossy fiber-evoked fast calcium transients were insensitive to blockers of ionotropic glutamate receptors (CNQX and APV) but were reduced by group 2 metabotropic glutamate receptor agonists such as L-CCG1 or DCG-IV, thereby confirming the selectively of presynaptic loading and recording.

Results

Previous studies have reported that low doses of kainate facilitate transmitter release from rat hippocampal mossy fiber synapses (Schmitz et al., 2001). Lauri et al. (2001a,b) reported an antagonistic effect of LY382884, a GLU_{K5}-specific antagonist, on the kainate-induced facilitation. Here, we revisit the kainate-induced facilitation of mossy fiber transmission in different types of genetic deletion models and by using GLU_{K5}-specific compounds.

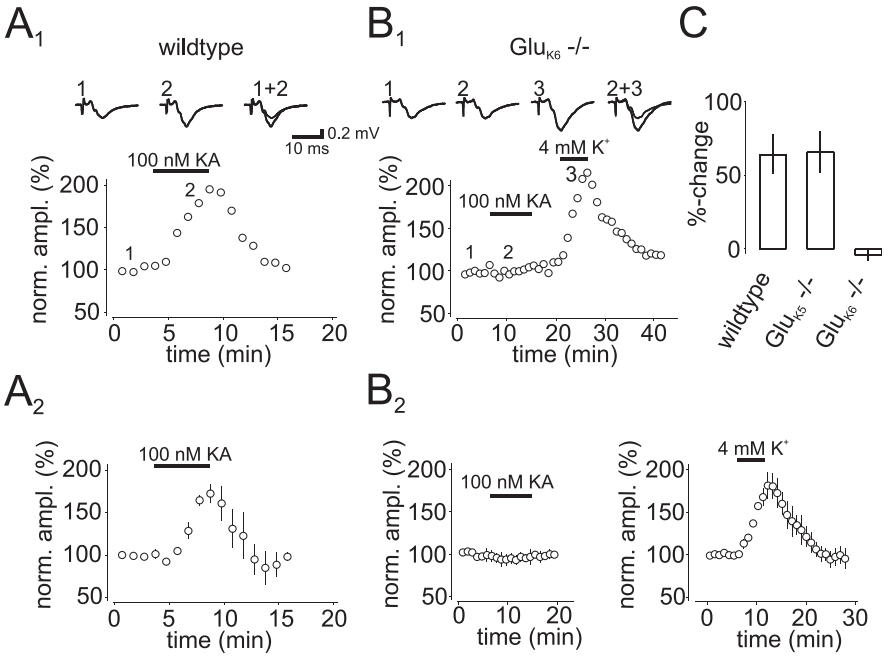

Figure 1. Kainate-induced increase in mossy fiber synaptic transmission is absent in $Glu_{K6}^{-/-}$ knock-out mice. Application of low concentrations of kainate (100 nM) induces an increase in the mossy fiber field EPSP response in CA3 in wild-type mice as shown in the representative example in A_1. A summary of seven such experiments is depicted in A_2. In $GLU_{K6}^{-/-}$ mice, application of kainate has no effect on mossy fiber transmission (B_1). Subsequent application of K^+ still induces an increase in transmission in the $GLU_{K6}^{-/-}$ mice. Summary data of nine and four experiments are shown, respectively (B_2). *C*, The bar plot summarizes the percentage of increase in mossy fiber transmission after kainate application in wild-type, $GLU_{K5}^{-/-}$, and $GLU_{K6}^{-/-}$ slices. Note that the kainate-induced increase in transmission is unaffected in the GLU_{K5}-KO mouse. norm. ampl., Normalized amplitude.

Short-term synaptic plasticity is reduced in the $GLU_{K6}^{-/-}$

Similar to rat hippocampal slices, mossy fiber synaptic transmission in wild-type mice was increased approximately twofold by low doses of kainate (100 nM). A typical example is shown in Figure 1A_1. In some of the recordings, transmission was biphasically modulated by 100 nM kainate: an initial increase was followed by a decrease in transmission, as has been demonstrated previously in rat slices (Schmitz et al., 2001). A summary of seven such experiments is shown in Figure 1A_2. Similar kainate applications were repeated in slices of GLU_{K5}- and GLU_{K6}-deficient mice. There was a complete block of the kainate effects on mossy fiber synaptic transmission in the GLU_{K6}-KO mice ($n = 9$) (Fig. 1*B*), but the kainate-induced enhancement of transmission was normal in the GLU_{K5}-KO mice ($n = 5$) (Fig. 1*C*). Importantly, the basic mechanism through which a presynaptic depolarization causes an increase of synaptic transmission at the mossy fiber synapse (Lauri et al., 2001a; Schmitz et al., 2001) was still present in the GLU_{K6}-KO mouse, because low doses of K^+ (4 mM) evoked an approximately twofold increase in transmission ($n = 4$) (Fig. 1*B*).

The synaptic activation of KAR in wild-type, GLU_{K5}-KO, and GLU_{K6}-KO mice was also compared by stimulating mossy fibers repetitively with five pulses at 25 Hz. Although we observed a large facilitation in wild-type mice ($n = 7$) and GLU_{K5}-KO mice ($n = 5$), this facilitation was significantly attenuated in the GLU_{K6}-deficient mice ($n = 7$; $p < 0.01$) (Fig. 2*A*). Similar results were also observed at a stimulus frequency of 100 Hz (Fig. 2A_3)

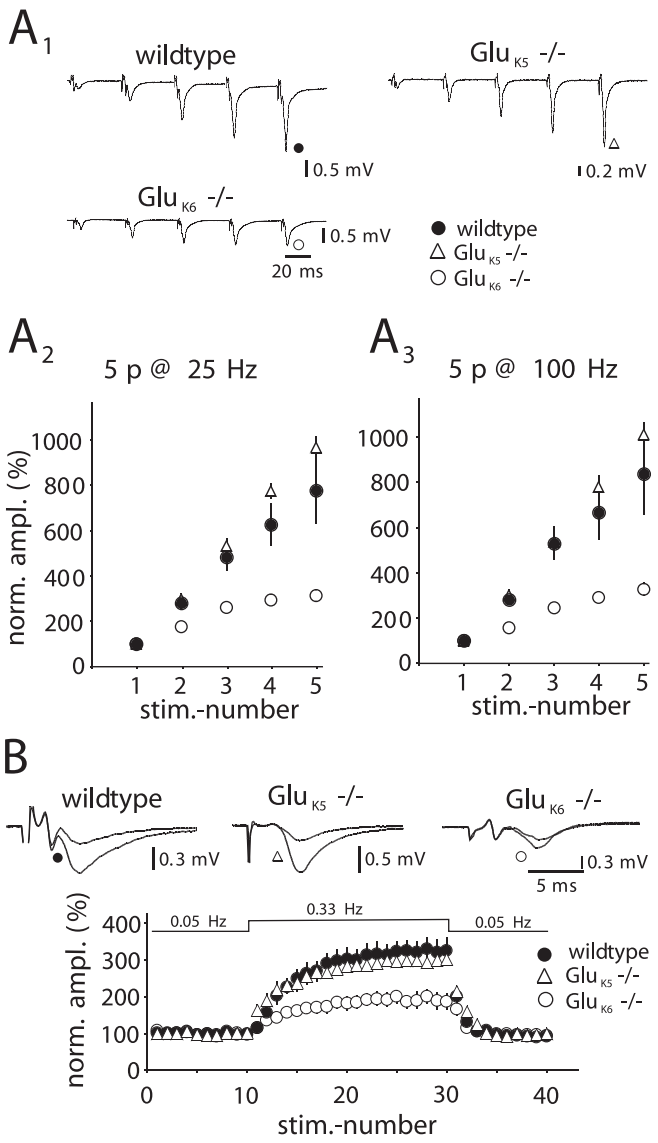

Figure 2. Impaired short-term synaptic plasticity in $GLU_{K6}^{-/-}$ mice. Sample traces in A_1 show field potential responses recorded in stratum lucidum of CA3 elicited by repetitive stimulation of mossy fibers at 25 Hz. Wild-type and $GLU_{K5}^{-/-}$ mice show pronounced facilitation, whereas the facilitation is significantly reduced in $GLU_{K6}^{-/-}$ mice. A_2, A_3, The normalized amplitude (norm. ampl.) is plotted against the stimulus (stim.) number for five pulses (5 p) at 25 Hz and for five pulses at 100 Hz, respectively. *B*, Increasing the stimulation frequency from 0.05 to 0.33 Hz for 20 pulses leads to an enhancement of mossy fiber transmission. This enhancement is reduced in $GLU_{K6}^{-/-}$ mice compared with wild-type and $GLU_{K5}^{-/-}$ knock-out mice.

($n = 5$). Mossy fiber synaptic transmission is not only dynamically modulated by high-frequency activity but is also sensitive to changes in low-frequency stimulation, a phenomenon termed "frequency facilitation" (Regehr et al., 1994; Salin et al., 1996). A change in stimulation frequency from 0.05 to 0.33 Hz results in an increase of transmission of 300% in wild-type mice. In agreement with previous results in GLU_{K6}-deficient mice, as well as with pharmacological studies using CNQX, this frequency facilitation was reduced in the GLU_{K6}-KO (Fig. 2*B*) but was again normal in the GLU_{K5}-deficient mice.

GLU_{K5} is not involved in short-term synaptic plasticity

The results thus far gained from the different genetic deletion models suggest that GLU_{K6} and not GLU_{K5}-containing KAR is critical for kainate-induced synaptic facilitation. However, it has been argued that the normal role of GLU_{K5} is exactly compensated for in the $GLU_{K5}^{-/-}$ mice (Bortolotto et al., 2003; Lauri et al., 2003). More importantly, using a GLU_{K5}-specific antagonist in rat hippocampal slices, data have been presented that GLU_{K5}-containing KAR are crucial for mossy fiber short-term plasticity and LTP (Bortolotto et al., 2003). We therefore performed a new series of experiments in which we used the previously used GLU_{K5}-specific agonist ATPA and the GLU_{K5}-specific antagonist LY382884 in rat hippocampal slices. All of the experiments were done in 2 mM $CaCl_2$ and 1 mM $MgSO_4$ to exactly mimic recently used methods (Lauri et al., 2003).

Initially, to make sure that ATPA and LY382884 (10 μM) work in our hands, we performed several control experiments. First, we confirmed that the ATPA-induced depression of synaptic transmission in area CA1 of the hippocampus was blocked in the $GLU_{K5}^{-/-}$ mice ($n = 4$; data not shown). Second, we determined that LY382884 did indeed block the ATPA-induced depression of synaptic transmission in area CA1 of the hippocampus ($n = 4$) (Fig. 3*A*). Third, ATPA is known to activate GLU_{K5} on interneurons and thereby increase the frequency of spontaneous IPSCs (sIPSCs) (Cossart et al., 1998; Schmitz et al., 2000). We found that LY382884 greatly reduced the ATPA-induced increase in sIPSCs recorded in interneurons in stratum lucidum of area CA3 ($n = 6$) (Fig. 3*B*). Fourth, we examined the effect of LY382884 on the ATPA-induced inward currents in stratum lucidum interneurons of area CA3. As can be seen in Figure 3*C*, LY382884 antagonized the ATPA-induced inward current as well the ATPA-induced conductance change ($n = 4$).

Having unequivocally proven that both ATPA as an agonist and LY382884 as an antagonist act on GLU_{K5}-containing KARs, we then examined the role of GLU_{K5} KARs in mossy fiber synaptic plasticity.

In noticeable contrast to kainate (Fig. 1), we could not find any facilitatory effect of the GLU_{K5}-specific agonist ATPA on mossy fiber synaptic transmission. We tested several concentrations of the agonist, starting from 10 nM and ending at 2 μM ($n \geq 5$ for each concentration). In no case could we find any enhancement of transmission (data not shown). With higher concentrations of ATPA (>0.5 μM), we found a depression of transmission (Fig. 4*A*).

We then tested the effect of LY382884 on low-frequency-induced facilitation at the mossy fiber synapses. An example of such an experiment is shown in Figure 4*A*. We first established the stability of the typically pronounced frequency facilitation and then applied the antagonist. As can be seen in the example, as well as in the summary plots in Figure 4*A*, there was no effect of LY382884 on frequency facilitation, whereas in the same experiment, the ATPA-induced depression was completely abolished, further supporting the efficacy of LY382884.

Subsequently, we synaptically activated KARs by using a short repetitive stimulation of five pulses at 25 Hz. After a stable baseline, we then applied the GLU_{K5}-specific antagonist LY382884. As shown in Figure 4*B*, there was no effect of LY382884 on any of the responses during the train. Even the fifth response, when activation is maximal, was not affected by LY382884. A summary of five such experiments is shown in Figure 4*B*, in which the ratio of the fifth over the first response is illustrated.

Assessing the role of Ca^{2+} stores in synaptic facilitation

Ca^{2+} permeation through GLU_{K5}-containing KARs as a trigger for Ca^{2+}-induced Ca^{2+} release has been suggested as a novel mechanism for synaptic facilitation and LTP at mossy fiber synapses (Lauri et al., 2003). We therefore tested the effects of thapsigargin, which depletes intracellular Ca^{2+} stores, on synaptic

facilitation. To our surprise, we could not detect any significant effect of thapsigargin on mossy fiber synaptic facilitation (Fig. 5*A*) ($n = 4$). We also tested cyclopiazonic acid (CPA), another blocker of Ca^{2+}–ATPase at the sarcoendoplasmatic reticulum. However, CPA was also without any effect on synaptic facilitation ($n = 5$). Consistent with the lack of effects of thapsigargin and CPA, ryanodine, which selectively blocks Ca^{2+}-induced Ca^{2+} release, also failed to have an influence on synaptic facilitation (Fig. 5*A*) ($n = 6$).

Controversy exists whether Ca^{2+} transients at hippocampal mossy fiber synapses are affected by compounds that interfere with Ca^{2+} stores. Carter et al. (2002) found no effect of ryanodine and thapsigargin on mossy fiber Ca^{2+} transients. However, it has been argued that this might be attributable to a slightly different extracellular Ca^{2+} concentration (3 vs 2 mM). We therefore made use of an optical method to study the influence of Ca^{2+} stores on mossy fiber Ca^{2+} transients in the presence of 2 mM Ca^{2+} and 1 mM $MgSO_4$ (Lauri et al., 2003). Figure 5*B* shows an example in which the mossy fiber tract was labeled with a low-affinity Ca^{2+}-sensitive fluorescent dye (magnesium green), and Ca^{2+} transients were imaged after repetitive stimulation (five pulses at 25 Hz). Consistent with our previous results on synaptic facilitation and in agreement with Carter et al. (2002), we were unable to detect any significant effect of ryanodine on the Ca^{2+} transients (Fig. 5*B*) ($n = 6$).

Mossy fiber LTP is independent of GLU_{K5}

Previous studies have shown that mossy fiber LTP is reduced in $GLU_{K6}^{-/-}$ mice, whereas it is normal in $GLU_{K5}^{-/-}$ mice (Contractor et al., 2001; Schmitz et al., 2003). These results are in striking contrast to pharmacological experiments in which the GLU_{K5}-specific antagonist LY382884 completely blocked mossy fiber LTP induction, independent of the induction protocol used (Bortolotto et al., 2003). It has been argued that the absence of an effect in the $GLU_{K5}^{-/-}$ is attributable to a compensatory mechanism (Bortolotto et al., 2003; Lauri et al., 2003). We therefore tested the GLU_{K5}-specific antagonist LY382884 on mossy fiber LTP. Surprisingly, mossy fiber LTP was not affected by LY382884 (Fig. 6*A*). In control slices, mossy fiber synapses were potentiated to 206 ± 1.1% ($n = 7$), whereas in the presence of LY382884, synaptic strength was increased to 200 ± 1.9% ($n = 6$). However, we found recently that KARs are not

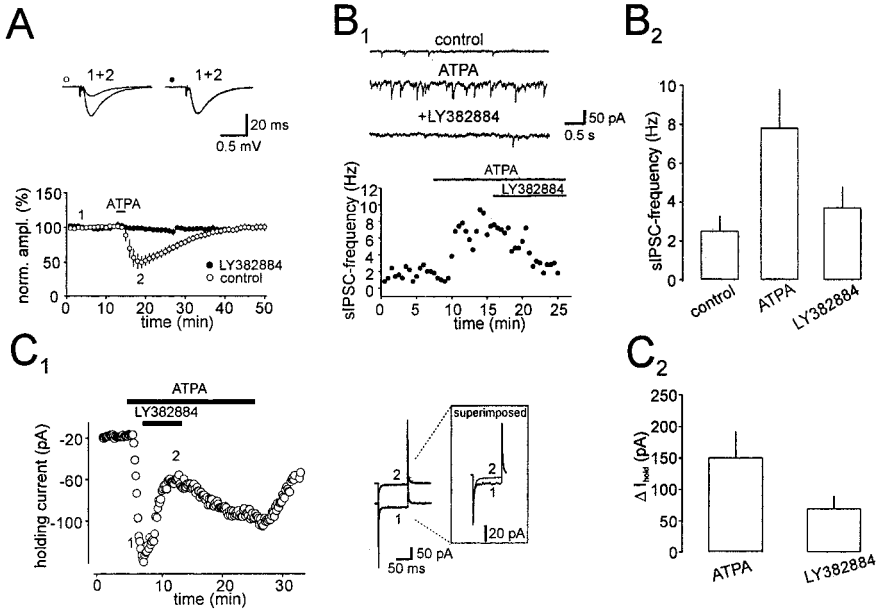

Figure 3. LY382884 is a specific GLU_{K5} receptor antagonist. *A*, The GLU_{K5} receptor-specific agonist ATPA induced a reversible depression of field EPSPs in area CA1 of the hippocampus (open circle). Preapplication of LY382884 (10 μM) completely abolished this effect of ATPA (filled circle). norm. ampl., Normalized amplitude. B_1, ATPA increased the frequency of sIPSCs in stratum lucidum interneurons of area CA3. The GLU_{K5} receptor-specific antagonist LY382884 greatly reduced the ATPA-induced increase in sIPSCs. B_2, A summary of six such experiments. C_1, Whole-cell recording of an interneuron in stratum lucidum of area CA3. Application of the GluR5-specific agonist ATPA (2 μM) induced an inward current that was reversibly reduced by LY382884 (10 μM). The experiment was done in the presence of the AMPA receptor antagonist GYKI53655. C_2, A summary of four such recordings.

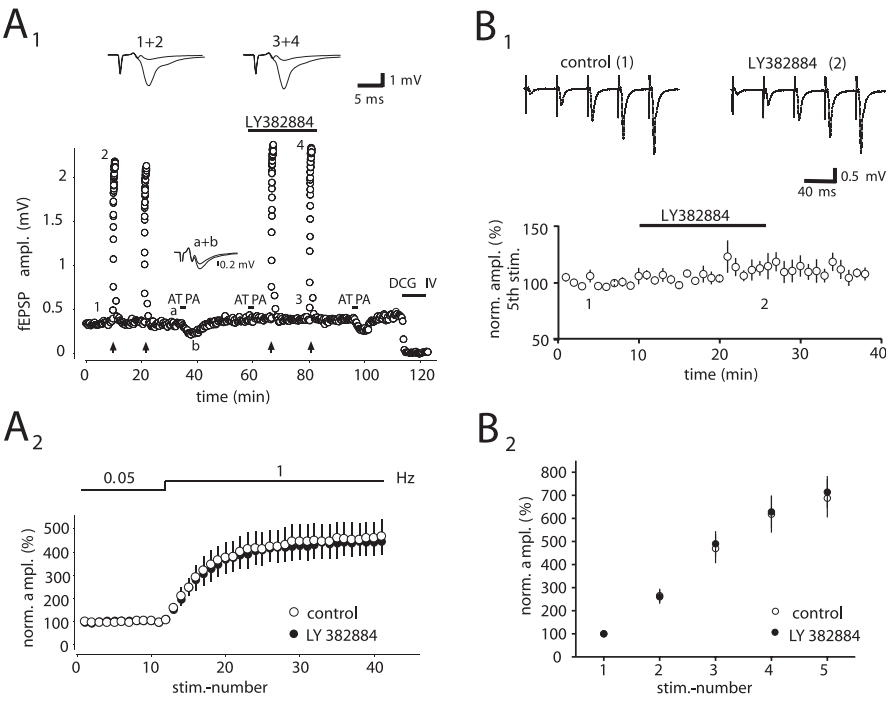

Figure 4. The GLU_{K5}-specific antagonist LY382884 does not affect mossy fiber short-term synaptic plasticity. *A*, Increasing the stimulation frequency from 0.05 to 1 Hz caused a large increase in mossy fiber synaptic transmission, which was not affected by the GLU_{K5}-specific antagonist LY382884. In the same experiment, the efficacy of LY382884 on the ATPA-induced depression was demonstrated. A_2, Five such experiments are summarized. fEPSP ampl., Field EPSP amplitude. *B*, Sample traces showing CA3 field responses elicited by repetitive stimulation (stim.) of mossy fibers (5 pulses at 25 Hz) in control conditions (1) and after the application of 10 μM LY382884 (2). Bottom, The time course for the fifth amplitude in the stimulus train is plotted against time, during and after the application of LY382884. B_2, The corresponding field EPSP amplitudes under control conditions and in the presence of LY382884 are plotted against the stimulus (stim.) number. norm. ampl., Normalized amplitude.

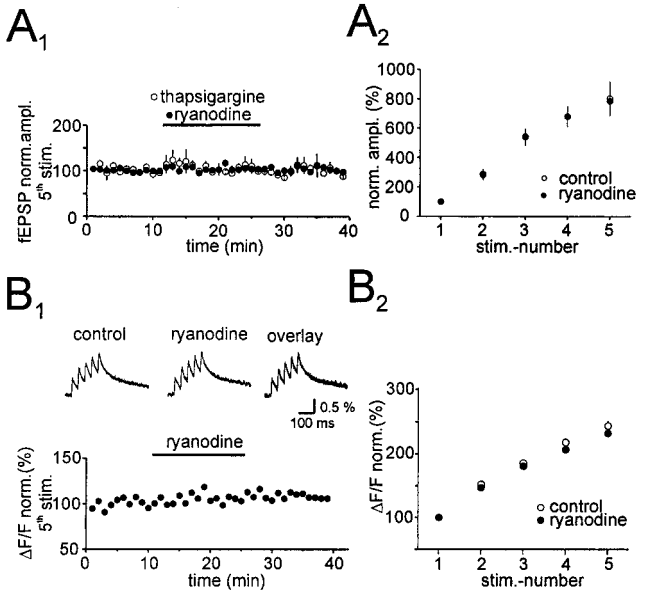

Figure 5. Calcium-induced calcium release does not contribute to mossy fiber synaptic transmission. *A*, Mossy fibers were stimulated with five pulses at 25 Hz and CA3 field EPSPs (fEPSPs) were recorded. The graph in A_1 depicts the normalized amplitude of the fifth stimulus (5^{th} stim.) in the train. Application of 10 μM ryanodine or 4 μM thapsigargin has no influence on the amplitude of the field EPSP. A_2, The amplitudes of the five stimuli normalized with respect to the first stimulus for control and during perfusion with ryanodine. *B*, Presynaptic calcium transients were elicited by repetitive stimulation (5 pulses at 25 Hz) of mossy fibers. B_1, Application of 10 μM ryanodine has no effect on the fifth stimulus in the train. The graph shows a representative experiment; traces at the top are averages of five responses. B_2, The amplitudes of the stimuli are normalized with respect to the first pulse for control and during application of ryanodine. Summary data for six experiments. norm. ampl., Normalized amplitude.

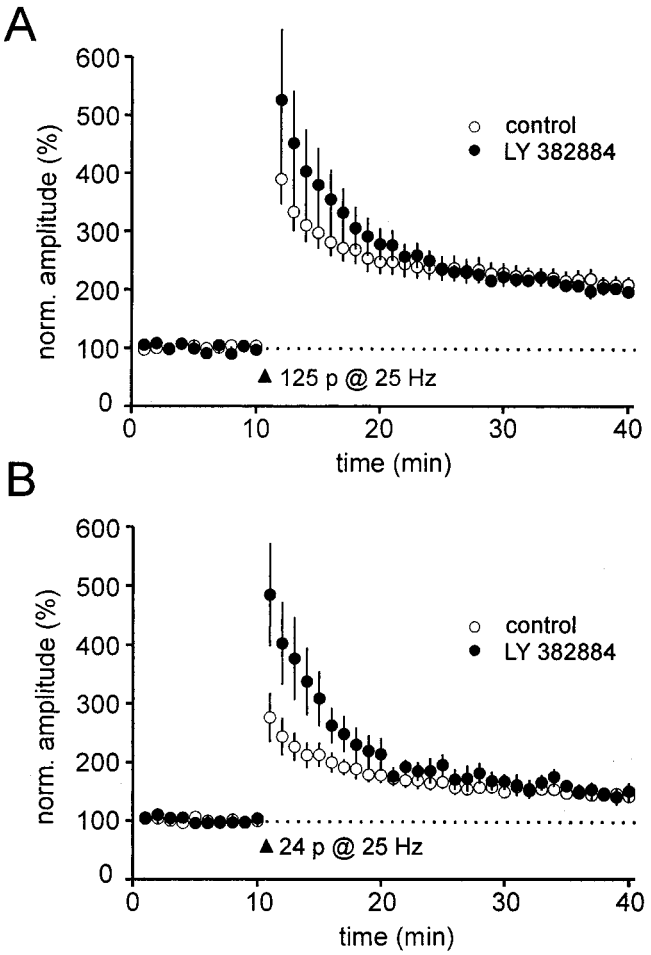

Figure 6. Mossy fiber LTP is not affected by the Glu_{K5} antagonist LY382884. *A*, The magnitude of LTP, induced with a strong tetanus consisting of 125 pulses at 25 Hz, is unaltered by previous application of 10 μM LY382884. Depicted are summary plots for seven (control) and six (LY382884) experiments. The triangle indicates the time of tetanization. Data points corresponding to the first minute after the tetanus are omitted for clarity. *B*, LTP induction with a low-intensity stimulus of 24 pulses at 25 Hz is also not affected by LY382884 (10 μM). Summary plots are shown for five (control) and six (LY382884) experiments. Norm., Normalized.

an absolute requirement for mossy fiber LTP but rather set the induction threshold (Schmitz et al., 2003). In previous experiments, KAR-mediated effects on mossy fiber LTP were maximal when using an induction protocol composed of 24 pulses at 25 Hz. We also examined LY382884 on LTP induced with this weaker induction protocol. As can be seen in Figure 6*B*, this attempt also failed to show any involvement of GLU_{K5} KARs in mossy fiber LTP (control, 144 ± 1.0%, $n = 5$; LY382884, 147 ± 2.2%, $n = 6$). We noted, in contrast, an increase in posttetanic potentiation in the LY382884-treated slices compared with control. This effect might be a result of a block of GLU_{K5}-containing receptors on interneurons, which could lead to a reduction of disynaptic inhibition after stimulation.

Discussion

Recent experiments have suggested functions for kainate receptors as mediators and modulators of synaptic transmission in area CA3 of the hippocampus (Frerking and Nicoll, 2000; Lerma, 2003). The specific KAR subunits that mediate the presynaptic and postsynaptic roles at the mossy fiber synapse have been the subject of much interest (Bortolotto et al., 2003). Using GLU_{K5} subunit-selective antagonists, it was first argued that GLU_{K5} subunits critically contribute to postsynaptic kainate currents (Vignes et al., 1997, 1998). This finding was surprising, because GLU_{K5} expression in the postsynaptic CA3 pyramidal cells is weak at best (Bahn et al., 1994; Paternain et al., 2000). However, GLU_{K5}-specific agonists could not mimic the effects of kainate onto CA3 pyramidal neurons (Vignes et al., 1998; Schmitz et al., 2000), and kainate currents were not affected in GLU_{K5} knock-out mice (Contractor et al., 2000), whereas kainate currents were absent in GLU_{K6} knock-out mice (Mulle et al., 1998; Contractor et al., 2000). In a subsequent reappraisal, it was reported that a more specific GLU_{K5} antagonist, LY382884, does not antagonize postsynaptic KARs on CA3 pyramidal cells (Lauri et al., 2001a; Bortolotto et al., 2003). From this data, it seems likely that GLU_{K6} is a critical component of the postsynaptic KARs at mossy fiber synapses, whereas GLU_{K5} is not. Despite this initial controversy (Vignes et al., 1997, 1998; Mulle et al., 1998; Lauri et al., 2001a), there is now a consensus that postsynaptic KARs on CA3 pyramidal cells do not contain GLU_{K5} (Lauri et al., 2001a; Bortolotto et al., 2003); however, uncertainty still exists about whether GLU_{K5} or GLU_{K6} subunits are functionally important at the mossy fiber terminals (Bortolotto et al., 2003). A recent elegant study unequivocally demonstrated the presence of GLU_{K6}, GLU_{K1}, and GLU_{K2} subunits at mossy fiber terminals by using newly developed antibodies (Darstein et al., 2003). These anatomical results fit with our present data, demonstrating that KAR activation by bath-applied kainate enhances transmitter release from hippocampal mossy fiber terminals in wild-type and GLU_{K5}-KO mice, an effect that is absent in the GLU_{K6}-deficient mice. We also demonstrated that GLU_{K6}-containing KARs can be

activated synaptically and contribute to the pronounced short-term plasticity at the mossy fiber synapse, because short-term plasticity is clearly impaired in the GLU_{K6}- but not the GLU_{K5}-deficient mice. Our results are comparable with those of Contractor et al. (2001), with the exception that we observe effects in GLU_{K6}-KO mice even on high-frequency stimulation (e.g., 100 Hz) (Fig. 2*A*). These results suggest that GLU_{K6} is an important subunit in presynaptic KARs at mossy fiber terminals, whereas GLU_{K5} is not.

This conclusion conflicts with previously published reports using GLU_{K5}-selective agonists and antagonists (Lauri et al., 2001a,b, 2003; Bortolotto et al., 2003). However, using these agents, we have been unable to reproduce the key results that implicate GLU_{K5} in glutamate release from mossy fiber terminals. In our hands, the GLU_{K5}-specific agonist ATPA does not mimic the effects of kainate at mossy fiber synapses. Furthermore, the GLU_{K5}-specific antagonist LY382884 failed to mimic the inhibitory effects seen with CNQX–NBQX or in the GLU_{K6}-KO mouse (Contractor et al., 2001; Schmitz et al., 2001, 2003) on either synaptic facilitation or mossy fiber LTP. The reason for the discrepancy between the present study and previous ones using LY382884 are unclear, but we think it is unlikely to be attributable to pharmacological considerations, because we found that ATPA and LY382884 are indeed a GLU_{K5}-selective agonist and antagonist, respectively (Figs. 3, 4*A*).

In conclusion, we report here that the facilitatory effects of KARs in synaptic plasticity at hippocampal mossy fiber synapses are mediated by KARs containing GLU_{K6} but not GLU_{K5}. This conclusion is based on both genetic and pharmacological evidence and is consistent with both expression and anatomical data (Bahn et al., 1994; Paternain et al., 2000; Darstein et al., 2003). The apparent absence of GLU_{K5} from mossy fiber synapses suggests that behavioral effects of GLU_{K5}-selective drugs (Smolders et al., 2002) are unlikely to be mediated by effects on mossy fiber transmission and may indicate a molecular basis for strategies to selectively affect distinct KAR subpopulations in the hippocampus.

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