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A Critical Role of a Facilitatory Presynaptic Kainate Receptor in Mossy Fiber LTP

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Summary

The mechanisms involved in mossy fiber LTP in the hippocampus are not well established. In the present study, we show that the kainate receptor antagonist LY382884 (10 μM) is selective for presynaptic kainate receptors in the CA3 region of the hippocampus. At a concentration at which it blocks mossy fiber LTP, LY382884 selectively blocks the synaptic activation of a presynaptic kainate receptor that facilitates AMPA receptor-mediated synaptic transmission. Following the induction of mossy fiber LTP, there is a complete loss of the presynaptic kainate receptor-mediated facilitation of synaptic transmission. These results identify a central role for the presynaptic kainate receptor in the induction of mossy fiber LTP. In addition, these results suggest that the pathway by which kainate receptors facilitate glutamate release is utilized for the expression of mossy fiber LTP.

Introduction

There is considerable interest in understanding long-term potentiation (LTP) of glutamatergic synaptic transmission, because the molecular mechanisms involved in its induction and expression are believed to be critical for learning and memory and other plastic synaptic processes (Bliss and Collingridge, 1993). There are two primary forms of LTP expressed in the mammalian CNS (Nicoll and Malenka, 1995), which are distinguished on their requirement for the synaptic activation of NMDA receptors. Whereas the mechanism of induction of NMDA receptor-dependent LTP is well established (Bliss and Collingridge, 1993), considerable controversy surrounds the mechanism of induction of the NMDA receptor-independent form. Thus, at mossy fiber synapses in the hippocampus, where NMDA receptor-independent LTP (Harris and Cotman, 1986) has been most extensively investigated, it is not known whether its induction is presynaptic (Zalutsky and Nicoll, 1990; Katsuki et al., 1991; Weisskopf and Nicoll, 1995; Mellor and Nicoll, 2001), postsynaptic (Williams and Johnston, 1989; Jaffe and Johnston, 1990; Yeckel et al., 1999), or involves contributions from both sides of the synapse (Urban and Barrionuevo, 1996).

A clue to the mechanisms of the induction of mossy fiber LTP has been provided recently by the finding that a kainate receptor is involved in the induction process (Bortolotto et al., 1999; Contractor et al., 2001). At mossy fiber synapses, kainate receptors are located both pre- (Kehl et al., 1984; Represa et al., 1987; Vignes et al., 1998; Contractor et al., 2000; Kamiya and Ozawa, 2000; Schmitz et al., 2000; Contractor et al., 2001; Schmitz et al., 2001) and postsynaptically (Robinson and Deadwyler, 1981; Westbrook and Loftman, 1983; Castillo et al., 1997; Vignes and Collingridge, 1997; Mulle et al., 1998). It has been shown that synaptically released L-glutamate can activate these receptors (Castillo et al., 1997; Vignes and Collingridge, 1997; Vignes et al., 1997; Mulle et al., 1998; Contractor et al., 2000; Schmitz et al., 2000; Contractor et al., 2001). However, the physiological role of the presynaptic kainate receptor is unclear, since both inhibition (Schmitz et al., 2000) and facilitation (Schmitz et al., 2001) of transmission have been reported.

In the present study, we show that the selective kainate receptor antagonist LY382884 blocks presynaptic but not postsynaptic kainate receptors at a concentration that also blocks mossy fiber LTP. This effect is associated with antagonism of the synaptic activation of a presynaptic kainate receptor that facilitates AMPA receptor-mediated synaptic transmission. Furthermore, the expression of mossy fiber LTP is associated with a complete loss of the presynaptic kainate receptor-mediated facilitation of synaptic transmission. These findings demonstrate a critical role for the presynaptic kainate receptor in the induction of mossy fiber LTP. Furthermore, these data indicate that the facilitatory presynaptic mechanism used by these receptors is also utilized for the expression of mossy fiber LTP.

Results

Synaptic Activation of Kainate Receptors during the Induction of Mossy Fiber LTP

We have reported that the kainate receptor antagonist LY382884 reversibly blocks the induction of mossy fiber LTP but not NMDA receptor-dependent LTP in the hippocampus (Bortolotto et al., 1999). Although these experiments suggested that kainate receptors are a trigger for the induction of some forms of NMDA receptor-independent synaptic plasticity, they did not identify the locus of the induction. We therefore analyzed the synaptic responses during the high-frequency train used to induce mossy fiber LTP. The NMDA receptor antagonist D-AP5 (50 μM) was present to prevent the potentially confounding influence of the synaptic activation of NMDA receptors (Weisskopf and Nicoll, 1995). As reported previously, 10 μM LY382884 had no significant effect on basal, AMPA receptor-mediated synaptic responses (Bortolotto et al., 1999). However, 10 μM

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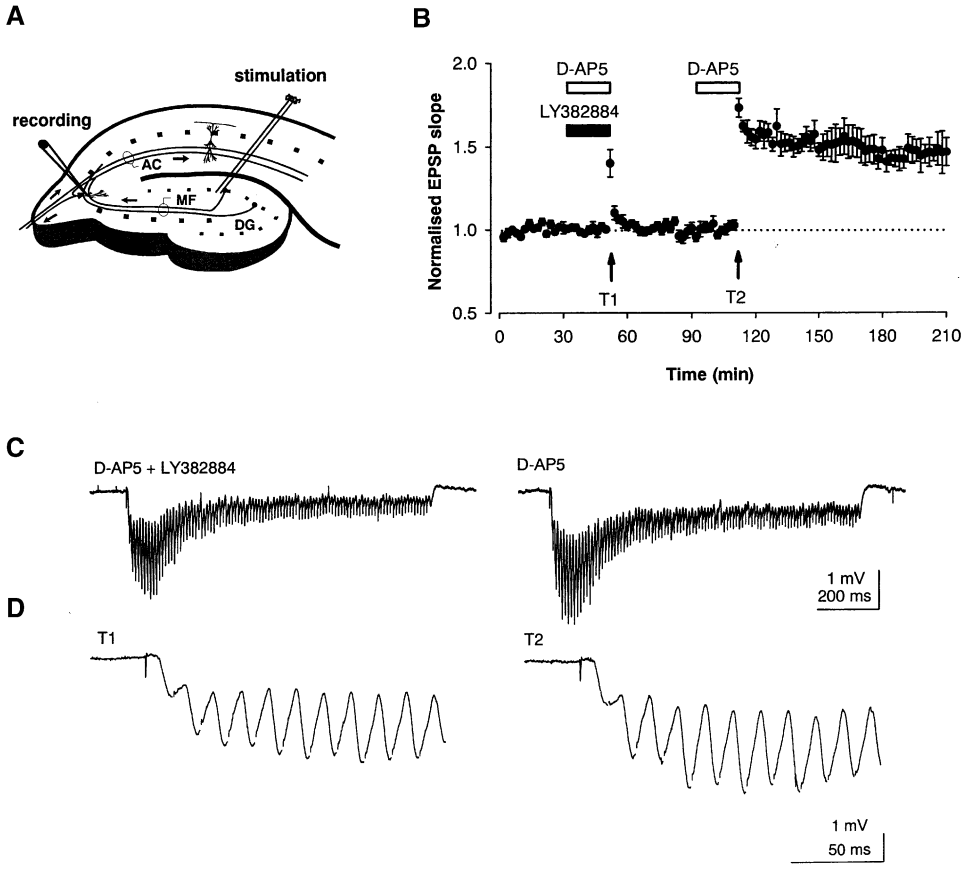

Figure 1. The Kainate Receptor Antagonist LY382884 Depresses Mossy Fiber Synaptic Transmission during High-Frequency Stimulation

(A) Schematic of the electrode placements for activating mossy fibers.

(B) Plot of fEPSP slope versus time for seven experiments in which a tetanus (T1; 100 Hz, 1 s, test intensity) did not induce mossy fiber LTP when delivered in the presence of LY382884 (10 μM) plus D-AP5 (50 μM). A second tetanus (T2) delivered in D-AP5 alone, following washout of LY382884, induced mossy fiber LTP (data replotted from Bortolotto et al., 1999).

(C) Field EPSPs evoked by mossy fiber stimulation (100 Hz, 1 s) in the presence of D-AP5 (50 μM) to show the effects of LY382884 (10 μM) on the synaptic response.

(D) First ten fEPSPs (from [C]) on an expanded timescale.

LY382884 significantly reduced the peak amplitude of the fEPSPs during the high-frequency train (100 Hz, 1 s; Figure 1). The LY382884-sensitive facilitation was evident by the peak of the second fEPSP and persisted for the duration of the high-frequency train.

To investigate the mechanism of this effect, we made patch-clamp recordings from CA3 neurons and studied the synaptic response during brief, high-frequency stimulation of mossy fibers, following blockade of NMDA receptor and $GABA_A$ receptor-mediated conductances. Consistent with the extracellular data, 10 μM LY382884 had no effect on the EPSC evoked by low-frequency stimulation or on the first EPSC in a high-frequency train (of five shocks delivered at 100 Hz). However, it caused a substantial, reversible reduction in the amplitude of the subsequent EPSCs in the train (Figure 2). This effect was rapid and pronounced since the facilitation of the second EPSC in the train was inhibited by 67% ± 12% ($n = 9$; $p < 0.01$).

One potential problem in studying mossy fiber synaptic transmission is the possibility of the recruitment of recurrent excitatory synapses made between CA3 neurons. An effect via polysynaptic pathways is very unlikely to explain the effects of LY382884 described here, due to its very rapid effect on frequency facilitation. Indeed, we believe that a significant involvement of polysynaptic pathways in the synaptic responses is unlikely under the conditions of the present experiments. For example, we saw no change in the decay kinetics of AMPA receptor-mediated components during high-frequency transmission, which would be expected if polysynaptic components were progressively recruited (τ_D of AMPA receptor-mediated EPSCs: single shock = 14.4 ± 1.5 ms; fifth EPSC in 100 Hz train = 13.1 ± 0.7 ms; $p > 0.2$; $n = 8$). However, to formally exclude the possibility of a polysynaptic involvement in the effect, we performed additional experiments under both current and voltage-clamp conditions using modified divalent cations, which suppress the activation of polysynaptic pathways. Altering the Ca^{2+}:Mg^{2+} ratio from 2:1 to 1:3 led to a substantial reduction in the size of EPSPs (depression = 73% ± 5%; $n = 7$), and the trains of five shocks did not evoke action potentials. However, in the same cells under voltage-clamp conditions using the same stimulus intensity,

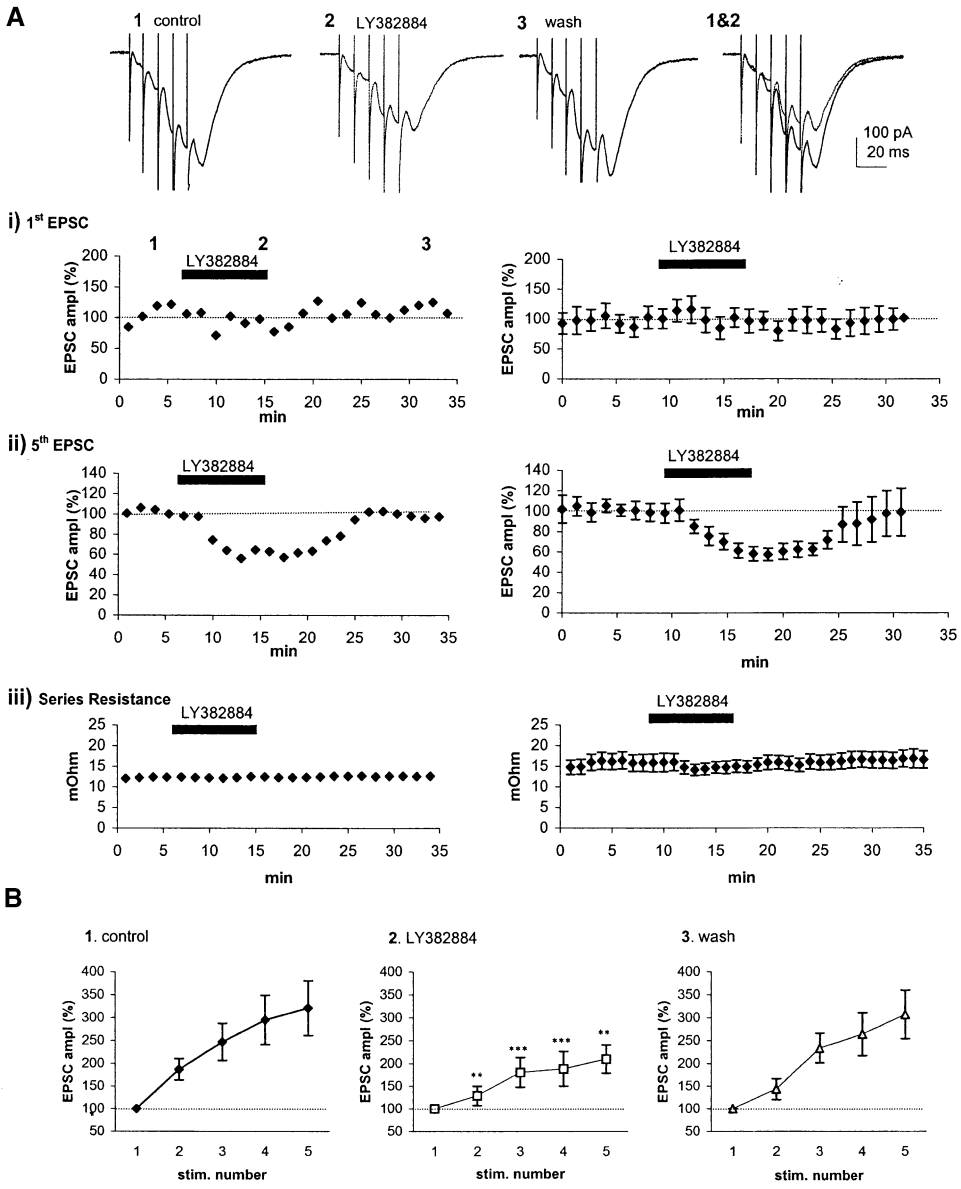

Figure 2. LY382884 Blocks the Frequency-Dependent Facilitation of Mossy Fiber EPSCs

(A) Effects of LY382884 (10 μM) on the first (i) and fifth (ii) EPSCs in a five shock 100 Hz train and series resistance (iii) for a single example (left) and pooled data (right; n = 9). Traces are averages of five responses obtained at the times indicated (1–3).

(B) Summary data for nine experiments showing effects of LY382884 on the peak amplitude of each EPSC during the 100 Hz train.

LY382884 still blocked frequency facilitation. This inhibition (55% ± 15% inhibition; n = 7; Figures 3A–3D) was as large as that observed under our standard conditions (2 Ca^{2+}:1 Mg^{2+}; $p > 0.6$). Therefore, the effects of LY382884 cannot be explained by the involvement of polysynaptic circuits.

It has been suggested that $GABA_B$ receptors indirectly mediate some of the effects of kainate receptor activation at mossy fiber synapses (Schmitz et al., 2000). It is formally possible that LY382884 could be working via an indirect effect involving $GABA_B$ receptors, for example, by reducing synaptic excitation of GABAergic neurons. Although this is extremely unlikely due to the rapid effect of LY382884 on frequency facilitation, we also included the $GABA_B$ receptor antagonist CGP 55845A (1 μM; Davies et al., 1993) during the above experiments. $GABA_B$ receptor blockade did not prevent the LY382884 antagonism of frequency facilitation (Figures 3A–3D).

It has been shown that a kainate receptor with characteristics of a metabotropic receptor can mediate some of the effects of kainate (Rodríguez-Moreno and Lerma, 1998; Freking et al., 2001). To test whether the kainate receptor responsible for frequency facilitation has similar metabotropic properties, we used calphostin C, a protein kinase C inhibitor that blocks the activation of the metabotropic kainate receptor (Rodríguez-Moreno and Lerma, 1998). However, addition of calphostin C (1 μM) did not significantly affect the level of frequency

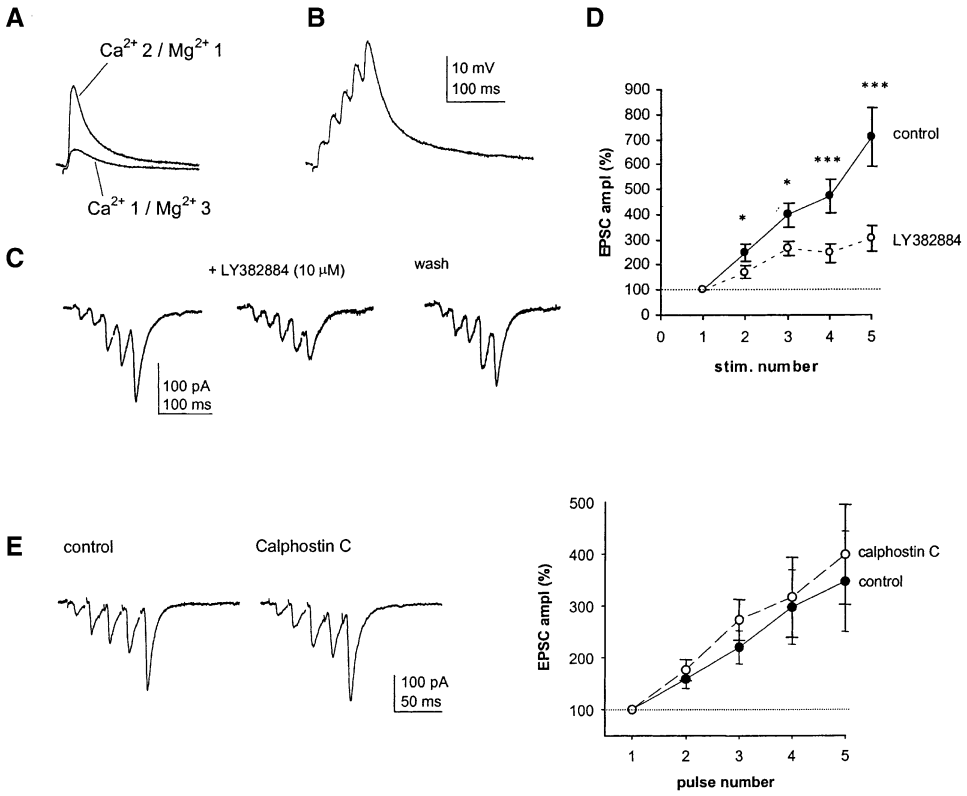

Figure 3. Evidence that LY382884 Blocks Frequency Facilitation of Mossy Fiber EPSCs via a Direct Action on Ionotropic Kainate Receptors

(A) The effects of changing from standard (2 mM Ca^{2+}, 1 mM Mg^{2+}) to modified (1 mM Ca^{2+}, 3 mM Mg^{2+}) medium on the EPSP in response to single-shock stimulation.

(B) The effects of five stimuli delivered at 50 Hz in modified medium. Note the lack of action potentials.

(C) The effect of LY382884 (10 μM) on frequency facilitation of EPSCs in modified medium.

(D) Pooled data for seven neurons. The $GABA_B$ receptor antagonist CGP55845A (1 μM) was present throughout.

(E) Frequency facilitation is unaffected by calphostin C (1 μM). The graph plots pooled data for ten neurons.

facilitation induced by a 50 Hz train (Figure 3E). Furthermore, LY382884 (10 μM) inhibited frequency facilitation in the presence of calphostin C (43% ± 9% inhibition; $n = 10$; $p < 0.05$) to an extent that was not significantly different to that observed under control conditions.

The effect of LY382884 (10 μM) was selective for mossy fibers, since high-frequency stimulation of associational-commissural fibers produced very little facilitation (Salin et al., 1996), and this was not significantly affected by LY382884 (Figures 4A and 4B). This further demonstrates that the effects of LY382884 are directly on mossy fiber synapses.

To determine how long the effects of activation of the facilitatory kainate receptor lasted, we delivered test pulses at various intervals following a train of five shocks, delivered at 100 Hz. Facilitation of the test EPSC was evident at all intervals tested, between 100 ms and 2.5 s. LY382884 (10 μM) caused a substantial inhibition of the facilitation at all intervals between 100 ms and 2 s ($n = 8$; Figure 4C). These data suggest therefore that this receptor might also contribute to the augmentation of mossy fiber synaptic transmission that is induced by repetitive stimulation at low frequencies (Salin et al., 1996). Consistent with this possibility, LY382884 (10 μM) antagonized augmentation, induced by the delivery of 30–50 stimuli delivered at 0.3–0.5 Hz, by 53% ± 5% ($n = 7$; data not shown).

LY382884 (10 μM) Is a Selective Antagonist of Presynaptic Kainate Receptors at Mossy Fiber Synapses

Since LY382884 substantially antagonizes frequency facilitation within 10 ms (i.e., by the time of the second EPSC in the 100 Hz train), the effect cannot be due to antagonism of the underlying kainate receptor-mediated EPSC, which is activated much more slowly at mossy fiber synapses (Castillo et al., 1997; Vignes and Collingridge, 1997). Also, the kainate receptor-mediated EPSC is far too small to account for the large effects observed in the present study, even if it was fully blocked by LY382884 (Castillo et al., 1997; Vignes and Collingridge, 1997). However, it is possible that antagonism of postsynaptic kainate receptors could underlie a small part of the depression of frequency facilitation toward the end of the high-frequency train.

We therefore examined the ability of LY382884 to inhibit kainate currents in CA3 neurons. We used the application of low concentrations of kainate (50–200 nM), in the presence of GYKI53655 (50 μM), to specifically activate high-affinity kainate receptors that are concen-

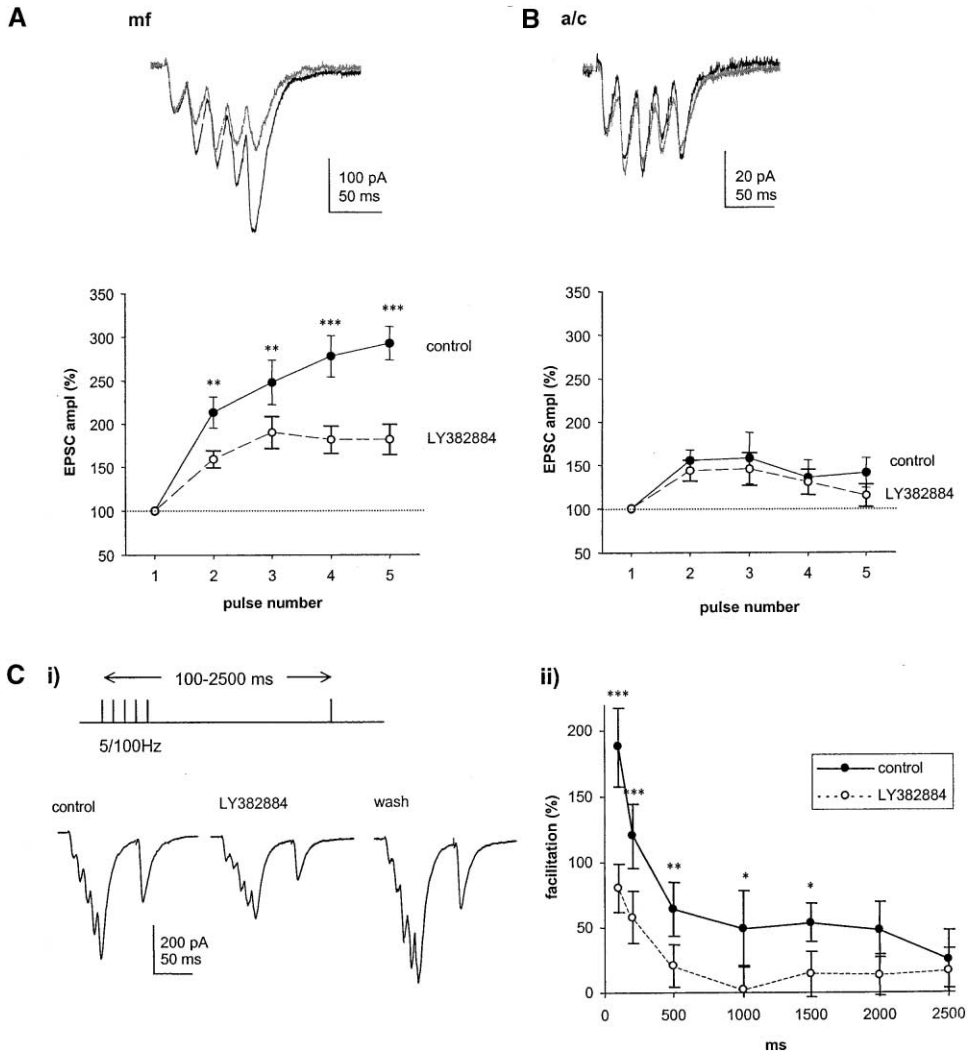

Figure 4. Selectivity of the Actions of LY382884 for the Mossy Fiber Pathway and Properties of Frequency Facilitation

(A) Shows that LY382884 (10 μM) inhibits frequency facilitation of mossy fiber (mf) EPSCs evoked at 50 Hz.

(B) Compared with mossy fibers, associational/commissural (a/c) synapses in the same neurons show a much less pronounced frequency facilitation and are insensitive to LY382884. The traces are from a single neuron, and the graphs are summary data (n = 6).

(C) Single AMPA receptor-mediated EPSCs were evoked at various times following a five shock, 100 Hz train to determine the duration of frequency facilitation and its sensitivity to LY382884 (10 μM). (i) Shows an example neuron where test EPSCs were evoked 100 ms after the start of the train. (ii) Pooled data for eight neurons.

trated pre- and postsynaptically in the terminal fields of mossy fibers (Monaghan and Cotman, 1982; Represa et al., 1987). LY382884 (10 μM) had no effect on kainate-induced currents, whereas 10 μM LY294486 was effective (Figures 5A and 5D), as described previously (Vignes et al., 1997). Kainate-induced currents were also antagonized by the AMPA/kainate receptor antagonist NBQX (20 μM) but not by the application of an additional 50 μM GYKI53655 (Figures 5B and 5D). This confirms that the use of nanomolar concentrations of kainate, plus the potent noncompetitive AMPA receptor antagonist GYKI53655, limits the actions of kainate to high-affinity kainate receptors. In contrast to area CA3, kainate currents in granule cells were antagonized by 10 μM LY382884 (Figures 5C and 5D). NBQX was also found to antagonize kainate currents in these neurons (Figure 5D). The main conclusion from these experiments is that 10 μM LY382884 does not antagonize kainate receptors located postsynaptically on CA3 neurons.

The effects of LY382884 on frequency facilitation are most simply explained by inhibition of an autoreceptor that acts to facilitate AMPA receptor-mediated synaptic transmission directly. It has been shown that low doses of kainate facilitate mossy fiber synaptic transmission (Kehl et al., 1984; Schmitz et al., 2001). We therefore tested whether the kainate receptor mediating this effect is indeed sensitive to LY382884. Kainate (25–50 nM) facilitated the EPSC in the majority of neurons tested (n = 12), and this effect was blocked by LY382884 (10 μM; n = 4; Figure 5E). Higher concentrations of kainate (100–200 nM) depressed transmission (data not shown), as previously described (Kamiya and Ozawa, 2000; Schmitz et al., 2000, 2001).

Collectively, these data demonstrate that 10 μM

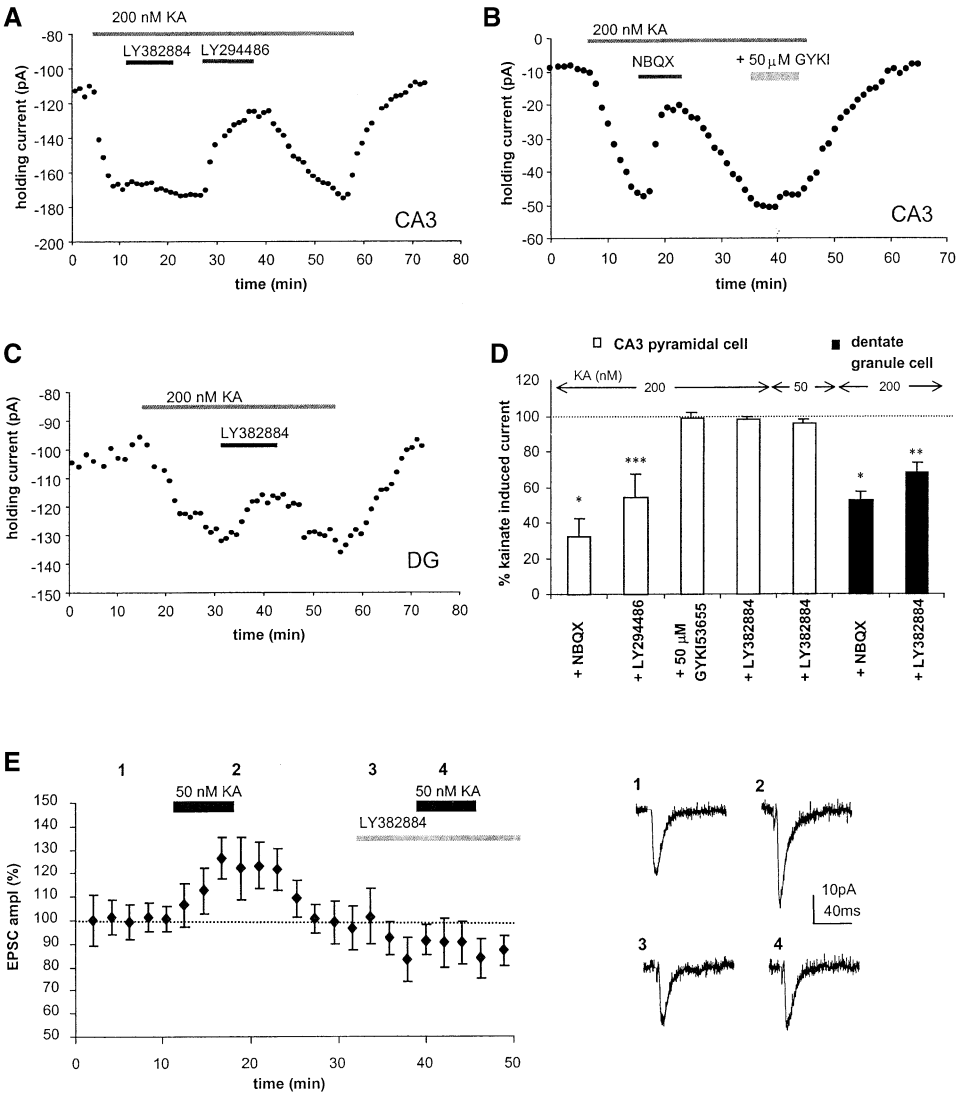

Figure 5. LY382884 (10 μM) Is a Selective Presynaptic Kainate Receptor Antagonist at Mossy Fibers

(A) Lack of effect of 10 μM LY382884 but antagonism by 10 μM LY294486 of a sustained current induced by 200 nM kainate, in the presence of 50 μM GYKI53655.

(B) Antagonism of a kainate current by 20 μM NBQX but no effect of the addition of a further 50 μM GYKI53655.

(C) Antagonism of a kainate current in a dentate gyrus (DG) neuron by LY382884.

(D) Data pools from between three and seven experiments.

(E) Pooled data showing the effect of 50 nM kainate on AMPA receptor-mediated EPSCs, before and in the presence of LY382884 (10 μM; n = 4). To the right of the graph are shown example EPSCs from a single experiment obtained at the times indicated (1–4).

LY382884 is a selective antagonist of presynaptic kainate receptors at mossy fiber synapses. At a concentration of 10 μM, LY382884 blocks frequency facilitation and also the induction of mossy fiber LTP (Bortolotto et al., 1999). Therefore, this strongly suggests that frequency facilitation, through this presynaptic kainate receptor mechanism, is necessary for the induction of kainate receptor-dependent mossy fiber LTP.

LTP Expression Selectively Occludes the Action of the Facilitatory Presynaptic Kainate Receptor

Previously it has been shown that frequency facilitation is reduced (by ~45%) following the induction of mossy fiber LTP (Toth et al., 2000), consistent with a presynaptic locus of expression (e.g., Zalutsky and Nicoll, 1990). Since only half the frequency facilitation is lost during LTP, this raises the possibility that there are two mechanistically distinct components to frequency facilitation. These could be kainate receptor-dependent and -independent, and only one of these occludes with mossy fiber LTP. To determine whether the LTP expression mechanism occludes with the action of the presynaptic kainate receptor, we simultaneously studied the level of frequency facilitation of potentiated and control mossy fiber inputs in two-pathway experiments (Figures 6A–6C). Induction of LTP caused an input-specific decrease in the amount of frequency facilitation of ~60%, consistent with previous observations (Toth et al., 2000). This

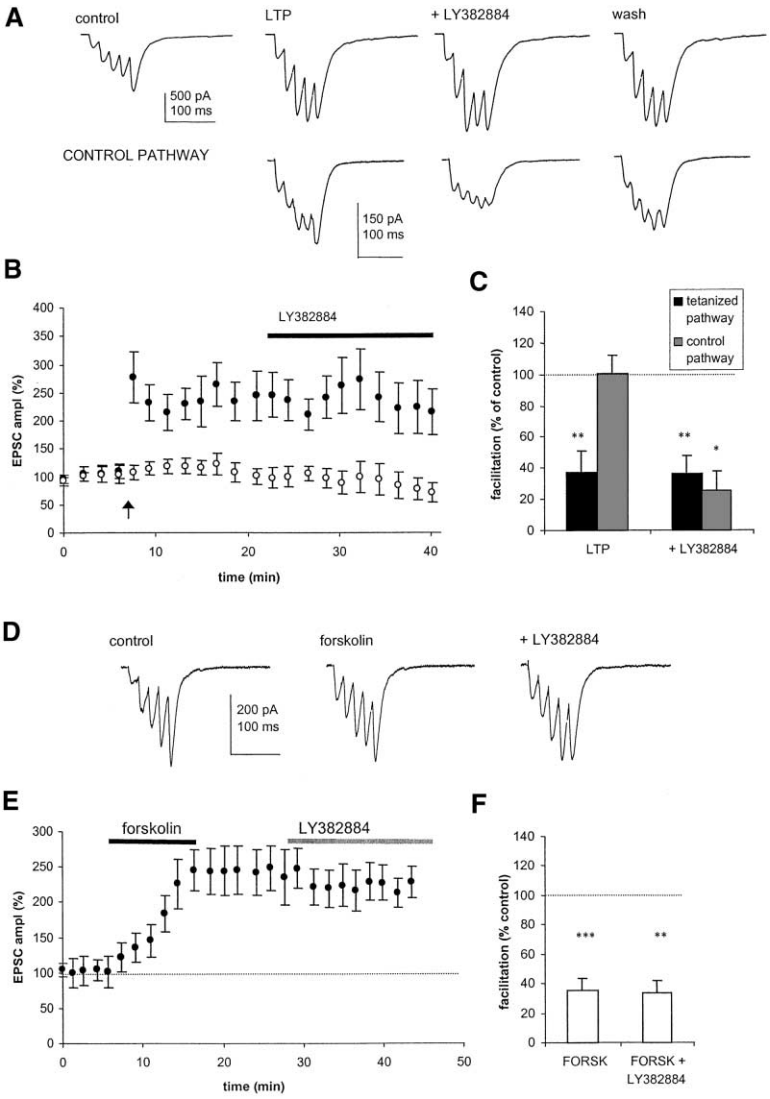

Figure 6. Mossy Fiber LTP Occludes the Action of the Presynaptic Facilitatory Kainate Receptor

(A) Representative traces showing the responses to five shocks at 50 Hz before and after the induction of LTP, in the presence of D-AP5 (50 μM), and the lack of effect of LY382884 (10 μM). The lower traces are from a control (i.e., nontetanized) input, showing the typical effect of LY382884 on frequency facilitation.

(B) Pooled data showing input-specific mossy fiber LTP for six experiments and the timing of the application of LY382884.

(C) Amount of frequency facilitation (amplitude of fifth as percentage of first EPSC in the train) expressed as a percentage of the frequency facilitation during baseline recordings for these six neurons. ** and * indicate significant differences compared to the same pathway during baseline.

(D–F) Equivalent presentation for seven experiments showing that potentiation of mossy fiber transmission induced by forskolin (50 μM) also occludes the action of the facilitatory presynaptic kainate receptor.

decrease in facilitation was accompanied with a complete loss of sensitivity to LY382884, indicating that the facilitatory presynaptic kainate receptor mechanism was selectively occluded by LTP.

Forskolin has been shown to potentiate mossy fiber transmission via the same expression mechanism as mossy fiber LTP (Weisskopf et al., 1994; Bortolotto et al., 1999). Like tetanus-induced LTP, forskolin-induced potentiation was also associated with a loss of ~60% of the frequency facilitation, and this was associated with a complete loss in sensitivity to LY382884 (Figures 6D–6F). These data show that, although mossy fiber synapses can still exhibit a component of frequency facilitation after LTP, the presynaptic facilitatory kainate receptor mechanism is selectively occluded.

The ability to occlude the action of the facilitatory autoreceptor by inducing mossy fiber LTP enabled us to test directly our conclusion that LY382884 (10 μM) does not directly affect postsynaptic kainate receptors at mossy fiber synapses. Thus, if the effects of LY382884 are solely via inhibition of the facilitatory presynaptic kainate receptor, then LY382884 should have no effect on the kainate receptor-mediated EPSC evoked by high-frequency trains, following the induction of mossy fiber LTP. Consistent with this prediction, LY382884 (10 μM) antagonized kainate receptor-mediated EPSCs in control inputs, as reported previously (Bortolotto et al., 1999), but had no significant effect on kainate receptor-mediated EPSCs in tetanized inputs in two-pathway LTP experiments performed in seven slices (Figure 7).

The occlusion experiment leads to a further prediction if the facilitatory kainate receptor is indeed the induction trigger for mossy fiber LTP. Since the induction of mossy fiber LTP results in complete occlusion of the facilitatory presynaptic kainate receptor mechanism, it follows that kainate receptor-dependent mossy fiber LTP should, unlike NMDA receptor-dependent LTP, be fully saturated by a single tetanus, under the conditions of our experiments. Consistent with this prediction, we found that mossy fiber LTP was always saturated by a single tetanus (Figure 8).

To further investigate the mechanism of interaction between kainate receptors and the expression of mossy fiber LTP, we performed one final series of experiments.

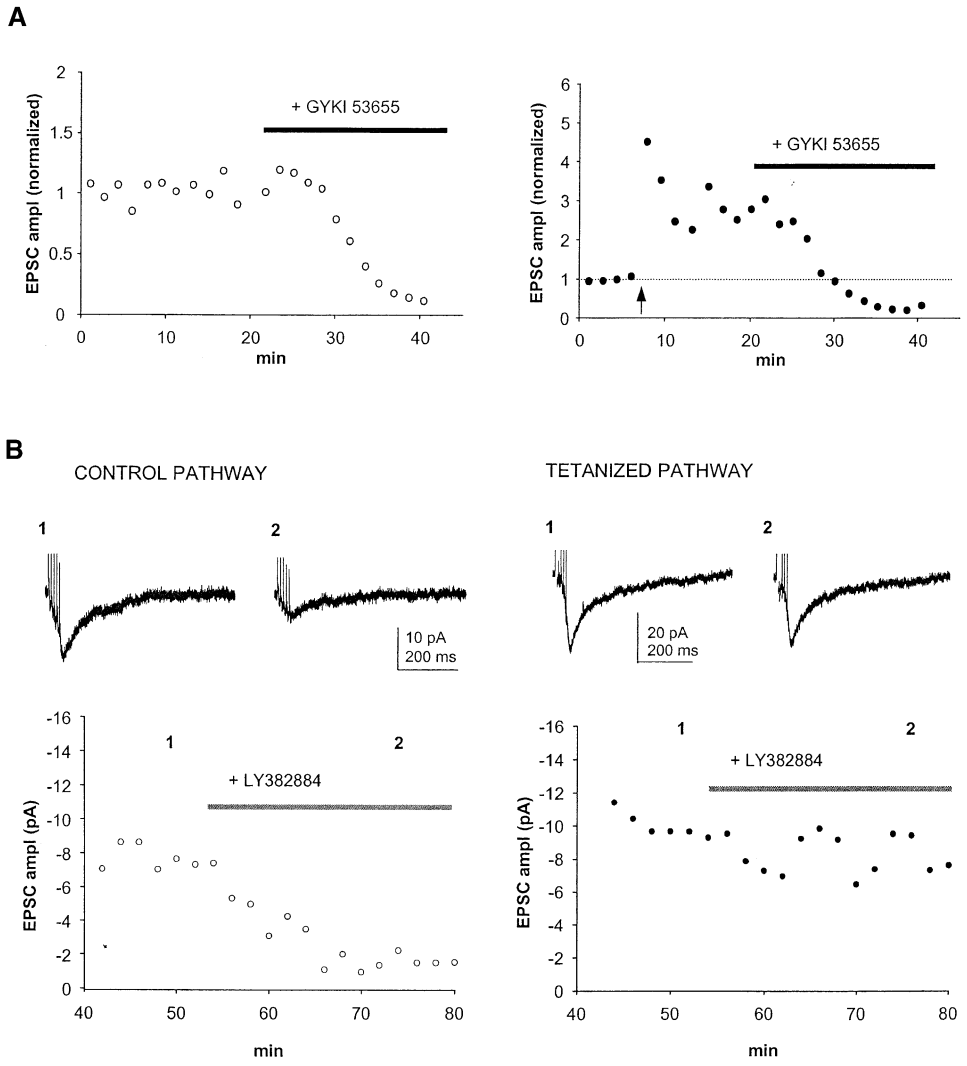

Figure 7. LY382884 Does Not Antagonize Kainate Receptor-Mediated EPSCs following the Induction of LTP

(A) The effects of 50 μM GYKI53655 on EPSCs evoked by single-shock stimulation of mossy fibers in a control pathway (left) and a tetanized pathway.

(B) Kainate receptor-mediated EPSCs were evoked in this neuron by five shocks delivered at 100 Hz, in the continued presence of GYKI53655, and the effects of 10 μM LY382884 compared in both inputs. LY382884 caused substantial inhibition of kainate receptor-mediated EPSCs in the control pathway (47% ± 12% inhibition; n = 7) but had no direct effect in the pathway that had undergone LTP (8% ± 9% inhibition). The small decrease in the tetanized pathway can be fully attributed to slight run-down of kainate receptor-mediated EPSCs that was also observed in the absence of LY382884.

In two-pathway experiments, we tested the sensitivity of mossy fiber synapses to low doses of kainate after the generation of LTP. In all experiments in which stable LTP was induced, the ability of kainate (50 nM) to facilitate synaptic transmission was lost in the LTP pathway, while, in the same cell, the control pathway still exhibited facilitation in response to kainate (Figure 9). These data show that LTP either prevents the activation of the presynaptic kainate receptor or uncouples its activation from the facilitation of L-glutamate release. In the same experiments, we also compared the sensitivity of LTP and control pathways to elevated K^+ (from 3 to 7 mM), since this treatment also facilitates L-glutamate release at mossy fiber synapses (Schmitz et al., 2001). The expression of mossy fiber LTP was also associated with loss of the K^+-evoked facilitation (Figure 9). Since elevating K^+ will cause depolarization, this shows that the occlusion with mossy fiber LTP is via a process after the depolarization of mossy fibers and hence downstream from the activation of kainate receptors.

Discussion

In this study, we have shown that the kainate receptor antagonist LY382884 is a selective antagonist for presynaptic kainate receptors at the hippocampal mossy fiber synapse. This antagonist blocks the synaptic activation of the facilitatory presynaptic kainate receptor and thereby reduces AMPA receptor-mediated synaptic

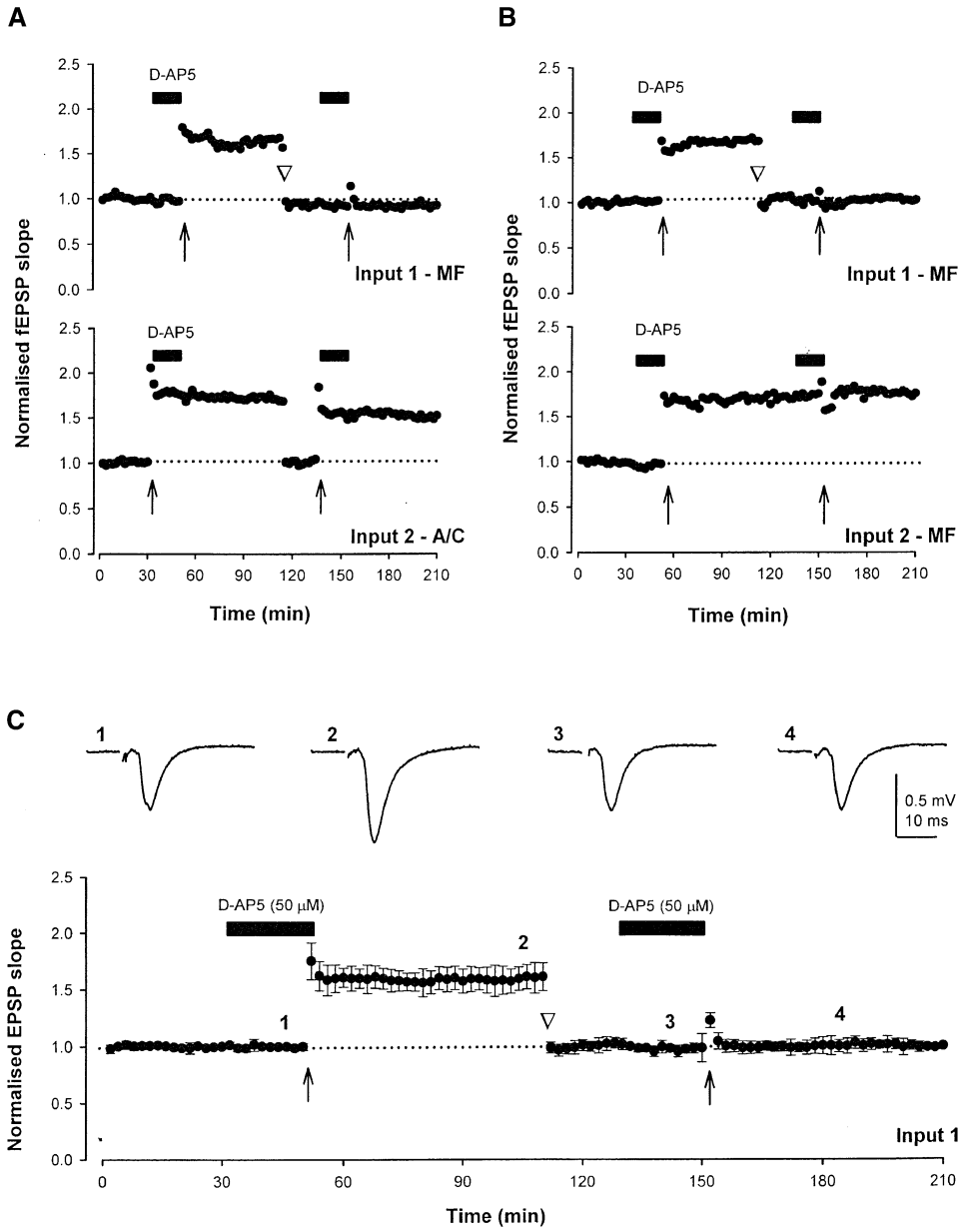

Figure 8. Mossy Fiber LTP Is Saturated by a Single Tetanus

(A) A two-input experiment to show that following the induction of mossy fiber LTP a second tetanus failed to induce any further potentiation in input 1. The stimulus intensity was reduced at the time indicated by the arrowhead. In input 2, a tetanus delivered to the assoc/comm. input, immediately prior to addition of D-AP5, induced LTP that was not saturated since a second tetanus induced further LTP in this input.

(B) In this two-input experiment, two separate mossy fiber inputs were studied. In the second, the stimulus intensity was not reduced following the induction of LTP. Mossy fiber LTP was saturated in both inputs.

(C) Pooled data for five slices, in which the stimulus intensity was reduced 1 hr following the induction of mossy fiber LTP. Note the complete saturation of mossy fiber LTP.

transmission during high-frequency stimulation. At this concentration, it also blocks the induction of mossy fiber LTP. In addition, mossy fiber LTP expression is associated with a selective occlusion of the presynaptic kainate receptor component of frequency facilitation. These data demonstrate that the synaptic activation of the facilitatory presynaptic kainate receptor is critically involved in the mechanisms of mossy fiber LTP.

LY382884 Is a Selective Antagonist for the Facilitatory Presynaptic Kainate Receptor at Mossy Fiber Synapses

The antagonist we have used, LY382884, is highly selective for kainate receptors. For example, at the concentration we have used (10 μM), it has no effect on the synaptic activation of AMPA, NMDA, $GABA_A$, or $GABA_B$ receptors (Bortolotto et al., 1999). Surprisingly, it was

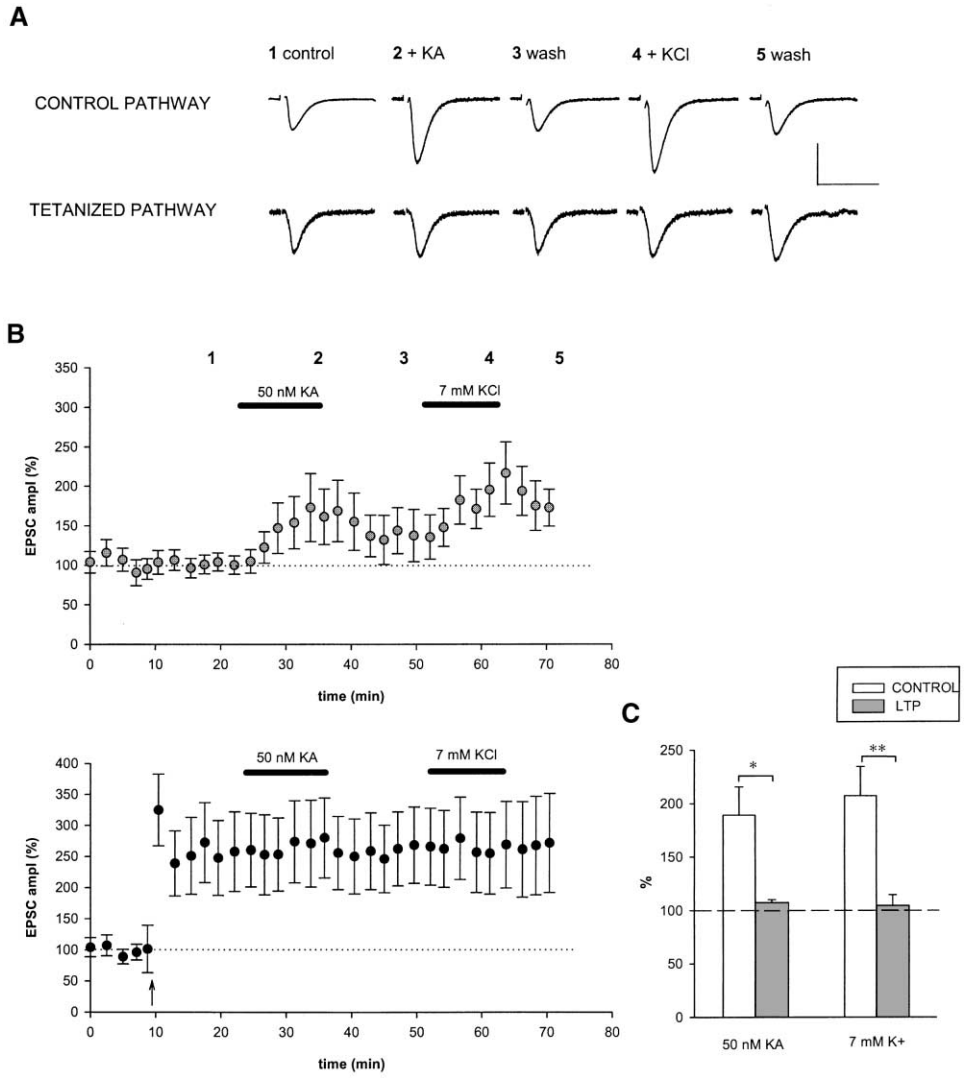

Figure 9. Mossy Fiber LTP Occludes the Facilitatory Action of Both Kainate and K^+

(A) Traces from a representative experiment to show that both kainate and elevated K^+ fail to facilitate mossy fiber transmission in an input in which LTP has been induced while still facilitating the control input. Scale bar, 100 pA (top), 50 pA, 50 ms.

(B) Pooled data from nine experiments, showing the effects of kainate and elevated K^+ on the control input (upper graph) and the potentiated input. The tetanus was delivered, in the presence of D-AP5 (50 μM), at the time indicated by the arrow.

(C) Quantification of the effects of kainate and elevated K^+ on EPSC amplitude in control and LTP pathways for these neurons. ** and * indicate a significant difference between the effects on the control and LTP pathways.

selective for pre *versus* postsynaptic kainate receptors at mossy fiber synapses. We demonstrated this selectivity in several ways. Thus, 10 μM LY382884 blocked kainate facilitation of mossy fiber synaptic transmission but did not antagonize postsynaptic kainate-induced currents in CA3 neurons. Furthermore, it did not affect mossy fiber-evoked kainate receptor-mediated EPSCs once the action of the presynaptic facilitatory kainate receptor had been occluded by the expression of mossy fiber LTP.

LY382884 showed a similar ability to inhibit frequency facilitation of AMPA receptor-mediated synaptic transmission at mossy fibers as do the AMPA/kainate receptor antagonists NBQX and CNQX of NMDA receptor-mediated synaptic transmission at this pathway (Schmitz et al., 2001; Lauri et al., 2001). The advantage of LY382884 is that it inhibits these presynaptic kainate receptors at a concentration that does not affect AMPA receptor-mediated synaptic transmission. Therefore, the involvement of the presynaptic kainate receptor in the frequency facilitation of AMPA receptor-mediated synaptic transmission can be studied directly. This is important for several reasons. Not only is the role of NMDA receptors at mossy fiber synapses unclear, but NMDA receptor-mediated synaptic transmission underestimates the true level of frequency facilitation at mossy fiber syn-

apses (Schmitz et al., 2001), presumably because NMDA receptors, due to their high affinity for L-glutamate, become saturated during high-frequency stimulation.

Properties of the Facilitatory Presynaptic Kainate Receptor

By studying facilitation of AMPA receptor-mediated synaptic transmission, we could determine some of the fundamental properties of this receptor mechanism. The activation was rapid (within 10 ms), the facilitation was substantial (greater than 100% facilitation), and long lasting (up to 2 s following the termination of a brief high-frequency train), suggesting the involvement of a high-affinity kainate receptor. Theoretically, presynaptic kainate receptors might act via a metabotropic function. However, our finding that calphostin C, an effective inhibitor of this form of kainate receptor, did not influence the level of frequency facilitation together with the rapid activation of the facilitatory autoreceptor mechanism means that this possibility is unlikely, at least for the rapid high-frequency facilitation characterized here.

Subunit Composition of the Facilitatory Presynaptic Kainate Receptor

Of the kainate receptor subunits, LY382884 is highly active at GluR5 homomers and GluR5-containing heteromers but is inactive at GluR6 and GluR7 homomers and GluR6-KA2 heteromers (Bortolotto et al., 1999). It is not known whether it is active at other heteromeric combinations of GluR5-lacking kainate receptors such as GluR6-KA1. Recently, on the basis of an analysis of knockout mice (Contractor et al., 2000), it has been suggested that GluR6 but not GluR5 kainate receptors are involved in the inhibition of mossy fiber synaptic transmission in response to high concentrations (3 μM) of kainate. However, a role of GluR5 in facilitation in area CA3 was suggested on the basis of an analysis of mEPSCs. A further analysis of these mice (Contractor et al., 2001) found that facilitation induced by low frequencies (0.2–5 Hz) was suppressed in GluR6 but not GluR5 knockout mice, which is in apparent contradiction to our findings that LY382884 suppressed frequency facilitation at this low-frequency range. However, facilitation at 100 Hz was not altered in the GluR6 knockout mice (no data were provided for the GluR5 knockout mice). Therefore, the subunit composition of kainate receptors at mossy fibers appears to be complex. Unambiguous identification of the precise subunit composition of the kainate receptors on mossy fibers will require, first, the development of genetic techniques that can distinguish between acute receptor loss and developmental/compensatory changes and, second, pharmacological agents that distinguish the various heteromeric kainate receptor combinations.

Role of the Facilitatory Presynaptic Kainate Receptor in Mossy Fiber LTP

The primary finding of the present paper is that the facilitatory presynaptic kainate receptor functions as a trigger for mossy fiber LTP, just as the postsynaptic NMDA receptor is a trigger for LTP at many other synapses in the hippocampus. However, unlike NMDA receptor-dependent LTP, kainate receptor-dependent LTP is an entirely presynaptic process. The role of the presynaptic kainate receptor may be to maintain a high level of release during the high-frequency transmission necessary for LTP induction, possibly due to depolarization of presynaptic elements (Kamiya and Ozawa, 2000; Schmitz et al., 2000). Such a mechanism could explain why it is possible under certain circumstances to negate the requirement for kainate receptor activation for mossy fiber LTP (Nicoll et al., 2000; see also Bortolotto et al., 2000), assuming that the necessary level of depolarization can be provided by other mechanisms.

In addition to providing presynaptic depolarization that would facilitate the activation of voltage-gated Ca^{2+} channels, presynaptic kainate receptors might gate Ca^{2+} directly, depending on their edited state. The Ca^{2+} provided by either of these routes may then activate adenylylcyclase (Weisskopf et al., 1994; Bortolotto et al., 1999). This mechanism does not exclude parallel or alternative mechanisms for mossy fiber LTP possibly involving, for example, mGlu receptors (Bashir et al., 1993; Yeckel et al., 1999), postsynaptic induction mechanisms (Yeckel et al., 1999), and glutamate receptor-independent induction processes (Castillo et al., 1994).

An unexpected observation is that the expression of mossy fiber LTP selectively and fully occludes with the facilitatory presynaptic kainate receptor mechanism. This is unlikely simply to be due to the change in probability of release, since approximately half of the frequency facilitation remains after LTP induction, showing that these synapses can still express an increase in release probability. One possibility therefore is that the mechanism accessed by the facilitatory presynaptic kainate receptor is the same one that is selectively used as the expression mechanism of mossy fiber LTP. Given that K^+-induced facilitation of mossy fibers was also occluded by LTP induction, one possibility is that the expression mechanism of mossy fiber LTP involves a long-term alteration in the sensitivity of the coupling of the release machinery to depolarization, perhaps by modification of a voltage-gated ion channel. Future investigations into this mechanism may enable further novel insights into to the molecular mechanisms of expression of mossy fiber LTP.

Experimental Procedures

Experiments were performed on transverse rat hippocampal slices (400 μm), using standard techniques (Vignes and Collingridge, 1997; Bortolotto et al., 1999). Slices were perfused with extracellular solution containing (mM) NaCl, 124; KCl, 3; NaH_2PO_4, 1.25; $MgSO_4$, 1; $NaHCO_3$, 26; D-glucose, 10–15; and $CaCl_2$, 2 at room temperature. In some experiments, where indicated, a modified divalent cation solution was used (1 mM $CaCl_2$, 3 mM $MgSO_4$). Field potential recordings were made using microelectrodes containing 4 M NaCl, and whole-cell recordings were made using patch electrodes (3–5 MΩ) containing (in mM) $CsMeSO_4$, 130; HEPES, 10; EGTA, 0.5; Mg-ATP, 4; Na-GTP, 0.3; QX-314, 5; NaCl, 8; 285 mOsm (pH 7.2). For experiments using current-clamp recordings, the filling solution was (in mM) $KMeSO_4$, 130; HEPES, 5; EGTA, 0.2; Mg-ATP, 4; Na-GTP, 0.5; NaCl, 8.5; 285 mOsm (pH 7.2). Synaptic responses were evoked by stimulation of the dentate granule cell layer (mossy fiber pathway) and in stratum radiatum of area CA1 (associational commissural pathway) at a baseline interval of 15–60 s. For whole-cell recordings, slices were obtained from 14- to 18-day-old animals, and neurons

were visualized using infrared microscopy, and ascorbate (1 mM), picrotoxin (PTX, 100 μM), and D-AP5 (50 μM) were included in the perfusate throughout recordings. In some experiments, we added CGP 55845A, at a concentration (1 μM) that completely blocks the synaptic activation of $GABA_B$ receptors (Davies et al., 1993). LTP was induced by between 1 and 3 tetani (100 Hz, 1 s, test intensity; 10 s intervals). For LTP experiments using whole-cell recordings, 4 mM Ca^{2+}, 2 Mg^{2+} was used in the perfusate. Data were collected and analyzed online using the LTP program (Anderson and Collingridge, 2001; http://www.ltp-program.com). Series resistance was estimated online as described previously (Kidd and Isaac, 1999). Postsynaptic summation of EPSCs during trains of high-frequency stimulation (25–100 Hz) was removed by scaling the EPSC in response to low-frequency stimulation to the peak of first EPSC in the train and subtracting (Kidd and Isaac, 2001). This process was repeated sequentially for all the EPSCs in the train. For each train, facilitation of peak EPSC amplitude was calculated as a percentage of the amplitude of the first EPSC. Data are expressed as mean ± SEM. Statistical significance was assessed using the Student's t test. (In the figures, statistical significance is denoted as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.005$). LY382884 can be synthesized according to Bleisch et al. (1997).

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