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Review

Kainate Receptors and Mossy Fiber LTP

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Abstract

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. There are two distinct forms of LTP. One type is triggered by synaptic activation of NMDA receptors and the other is NMDA receptor-independent. The latter type of LTP has been mostly studied at mossy fiber/CA3 synapses. Here we summarise some of our recent studies concerning the mechanisms of the induction of the NMDA receptor-independent form of LTP at these CA3 synapses. This form of LTP is triggered by the synaptic activation of kainate receptors. We also address the importance of Ca^{2+} availability in the extracellular environment and the release of Ca^{2+} from intracellular stores for this form of LTP.

Keywords: Mossy fiber LTP; Kainate receptor; CA3 synapses; Ca^{2+} stores

Contents

INTRODUCTION

The ability of synapses to modify their function in response to an appropriate stimulus is a fundamental property of the nervous system network and it may represent an essential component of learning and memory (Bliss and Collingridge, 1993). Since its discovery, long-term potentiation (LTP) has been the most accepted and used model to explore synaptic functions at excitatory synapses underlying learning and memory processes.

L-Glutamate is the main excitatory neurotransmitter in the vertebrate nervous system. Pharmacological studies have identified three distinct classes of ionotropic glutamate receptors known as: AMPA, NMDA and kainate receptors (Watkins and Evans, 1981). Recent work using molecular cloning revealed the existence of five kainate receptor subunits (Bettler and Mulle, 1995). According to the IUPHAR nomenclature the subunits are named as: GLU_{K5}, GLU_{K6}, GLU_{K7}, GLU_{K1} and GLU_{K2} (Lodge and Dingledine, 2000). They are more commonly known as GluR5, GluR6, GluR7, KA-1 and KA-2. They can form homomeric and heteromeric assemblies. While AMPA and NMDA receptors are predominantly located postsynaptically, kainate receptors are also located presynap-

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tically at many synapses, where they can modulate transmitter release (Lerma, 2003).

Kainate receptors have been shown to have a variety of roles in the hippocampus; both in excitatory and inhibitory transmission (Lerma, 2003). Nevertheless, very little is known about the role of kainate receptors in LTP. Key roles in synaptic plasticity have been identified for two of the ionotropic glutamate receptors. At many synapses in the brain, transient activation of NMDA receptors leads to a persistent modification in the strength of synaptic transmission mediated by AMPA receptors (Bear and Abraham, 1996; Bliss

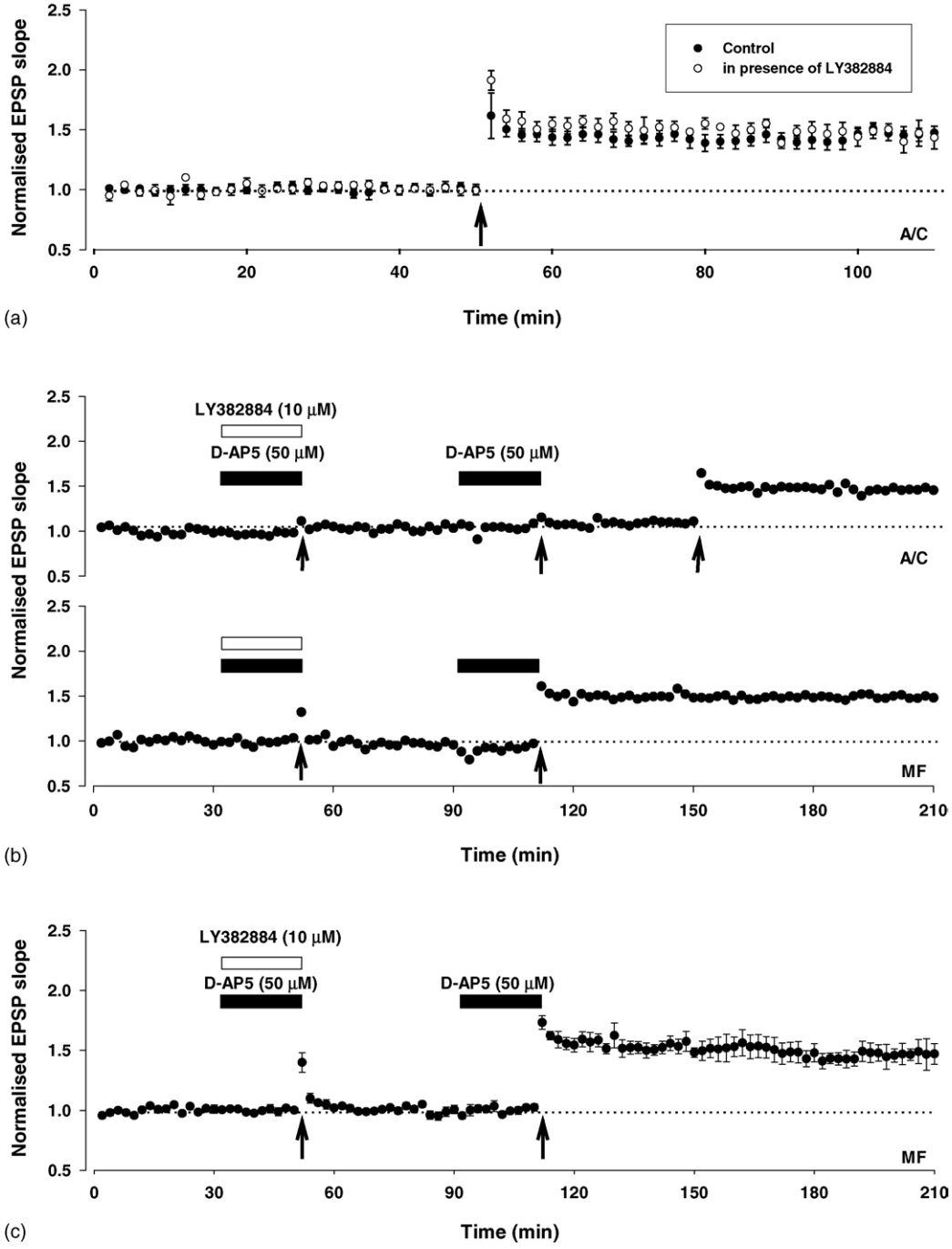

Fig. 1. LY382884 selectively blocks mossy fiber LTP. (a) Pooled data showing NMDA receptor-dependent LTP at CA3 synapses, evoked by tetanic stimulation of the associational commissural (A/C) fibers, under control conditions ($n = 6$) and in the presence of the Glu_{K5} antagonist LY382884 ($n = 3$). In this and subsequent plots, each point represents the slope average of four successive field EPSP responses. Note, in this and subsequent figures, that tetanic stimulation (100 Hz, 1s, test intensity, arrows) at mossy fiber pathway was always delivered in the presence of the NMDA receptor antagonist D-AP5. The duration of drug administration is indicated by the bars. (b) A single example to illustrate the reversible block of mossy fiber LTP by LY382884. (c) Pooled data (mean ± S.E. mean) of seven experiments showing that LY382884 fully blocks the induction of LTP in a reversible manner (from: Bortolotto et al., 1999).

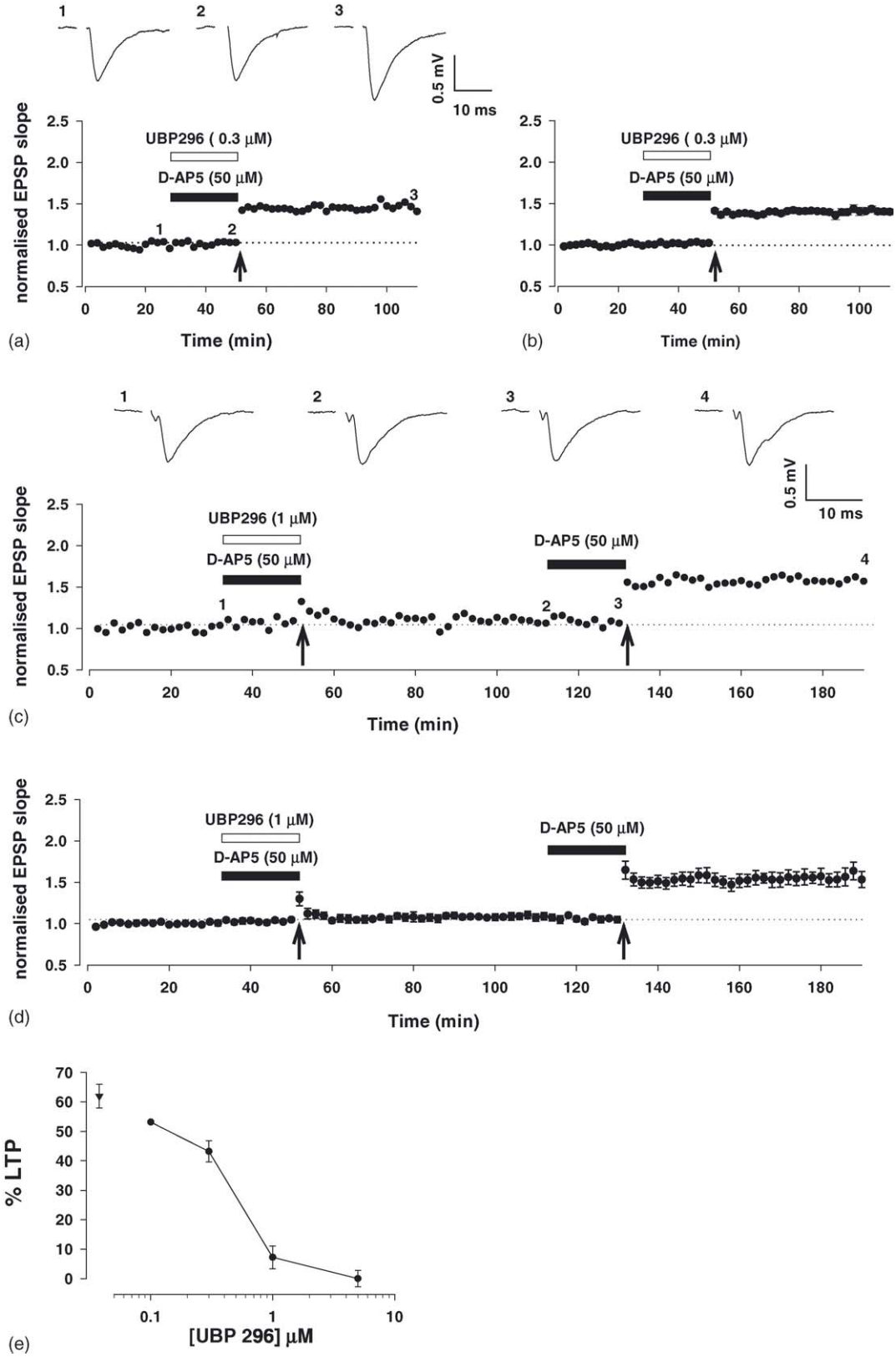

Fig. 2. Dose-dependent block of mossy fiber LTP by UBP296. (a) Single example showing that a low concentration of UBP296 does not block the induction of mossy fiber LTP. (b) Pooled data from six similar experiments. (c) Single experiment to show the reversible block of the mossy fiber LTP by 1 μM UBP296. (d) Pooled data for six equivalent experiments showing that mossy fiber LTP is blocked by UBP296 in a reversible manner. (e) The plot shows the level of mossy fiber LTP under control conditions (triangle) and in the presence of different concentrations of UBP296 (circles) (from: More et al., 2004).

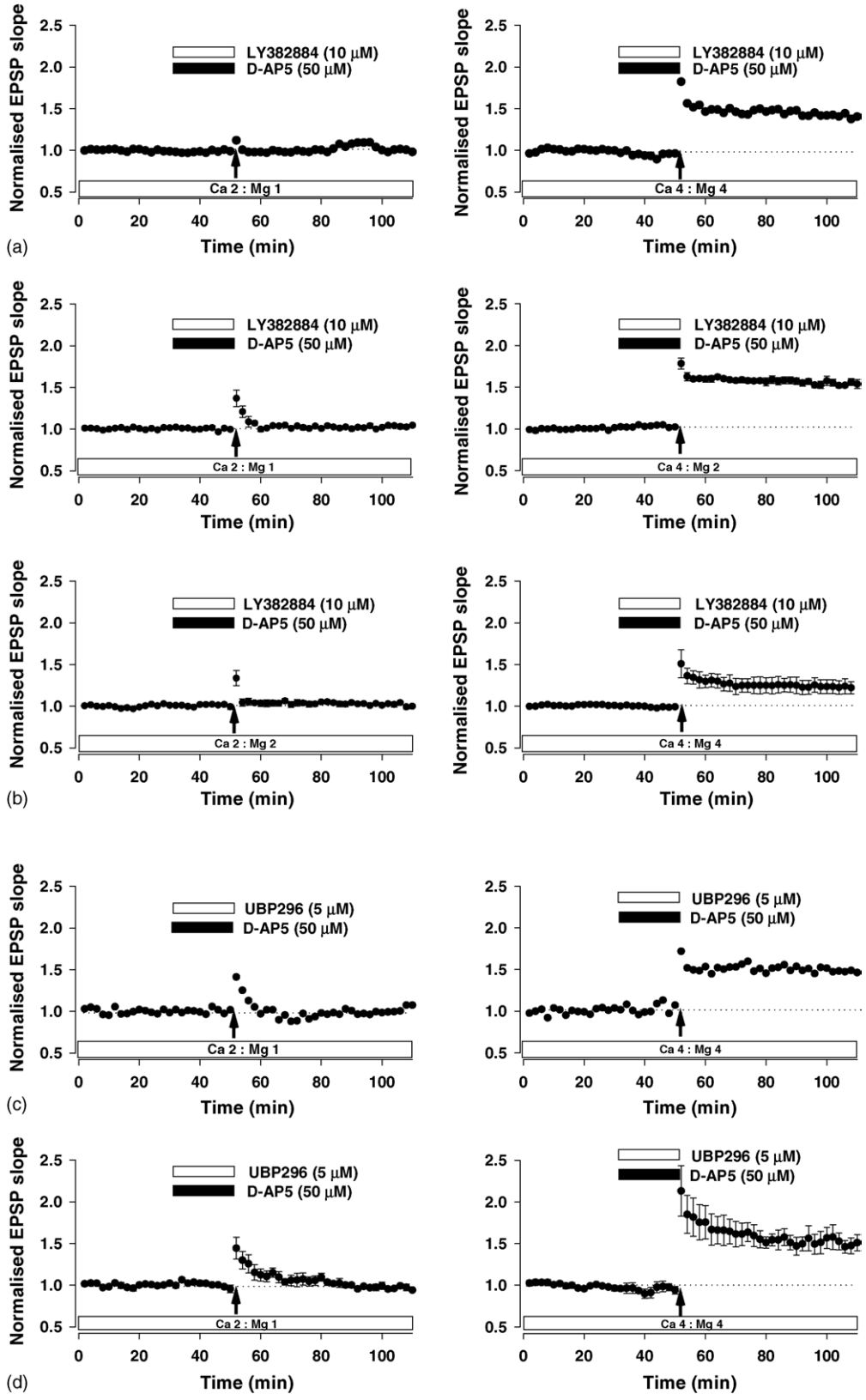

Fig. 3. Divalent cation concentration can affect the induction of mossy fiber LTP. The ability of LY382884 and UBP296 to block mossy fiber LTP is dependent on Ca^{2+} concentration. (a) A single example showing that Ca^{2+} concentration affects the ability of LY382884 to block the induction of mossy fibre LTP. (b) Pooled data showing the effects of LY382884 under four different divalent cation concentrations ($n = 4$ for each plot). (c) Single example to show the effects of UBP296 on the induction of mossy fiber LTP in Ca 2:Mg 1 (left) and in Ca 4:Mg 4 (right). (d) Pooled data from four experiments as in (c). Note that it is the absolute Ca^{2+} concentration not the divalent cation ratio that is the critical factor (from: Lauri et al., 2003; More et al., 2004).

and Collingridge, 1993). In contrast to NMDA receptor-dependent LTP, much less is known about the mechanism of induction of NMDA receptor-independent LTP. The most studied form of NMDA receptor-independent LTP is the mossy fiber LTP at CA3 synapses (Harris and Cotman, 1986).

Originally, it was believed that the induction of mossy fiber LTP did not require the activation of glutamate receptors since it could be induced in the presence of broad spectrum glutamate receptor antagonists, such as kynurenic acid or CNQX (Ito and Sugiyama, 1991; Castillo et al., 1994; Weisskopf

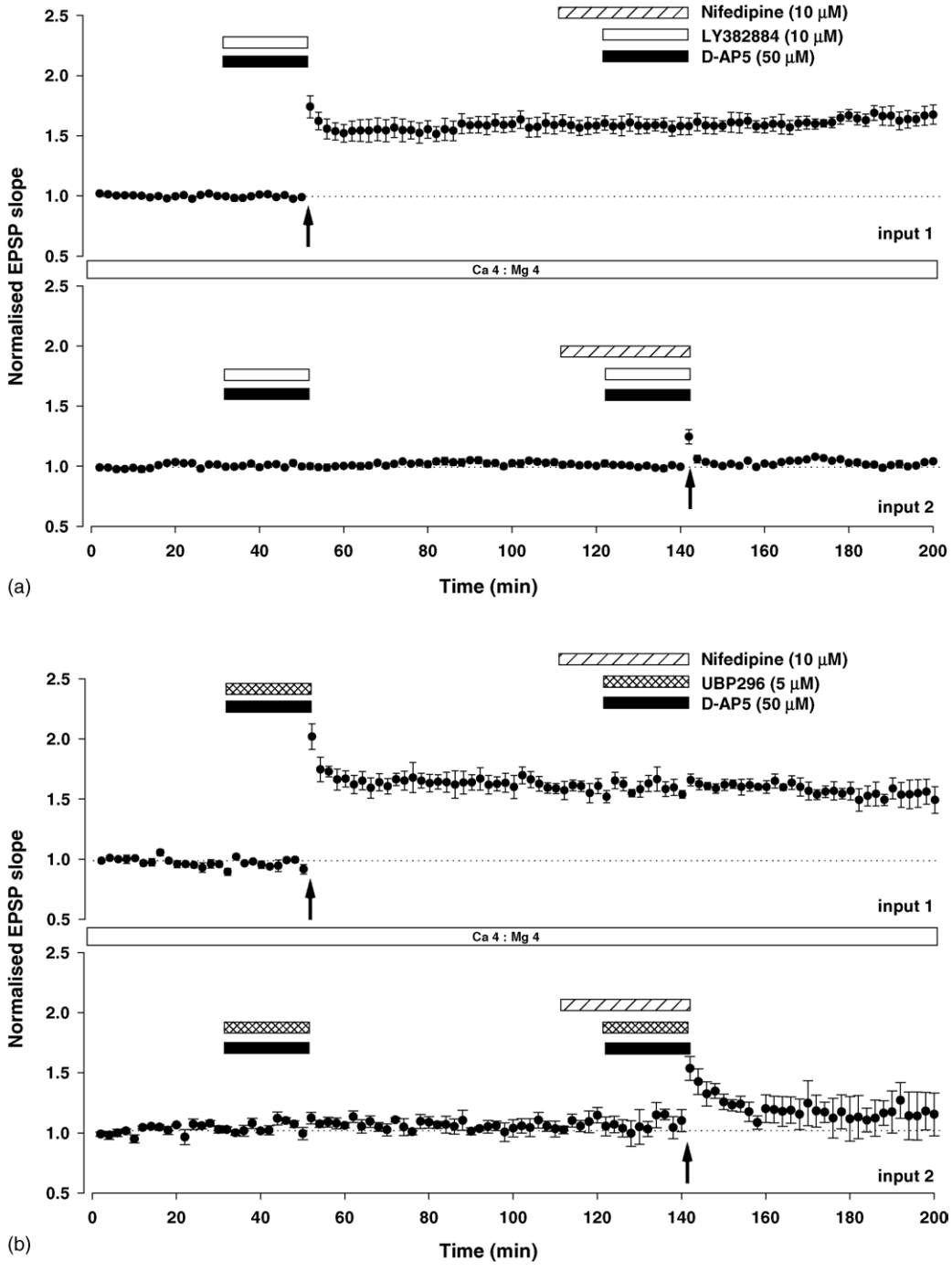

Fig. 4. L-type Ca^{2+} channels can compensate for Ca^{2+} entry through kainate receptors during mossy fiber LTP induction in elevated divalent cation concentration. (a and b – input 2) Pooled data showing that nifedipine rescued the ability of LY382884 and UBP296 to block the induction of mossy fiber LTP in elevated divalent cation concentration ($n = 5$ and $n = 4$, respectively). Observe that the application of nifedipine did not affect a pre-established LTP (a and b – input 1) (from: Lauri et al., 2003; More et al., 2004).

and Nicoll, 1995; Harris and Cotman, 1986; Yeckel et al., 1999). For a considerable amount of time it has also been known that mossy fiber synapses show a very high density of kainate receptor binding sites compared to NMDA receptors (Monaghan and Cotman, 1982). This observation raised the possibility that kainate receptors, at mossy fiber synapses, could play a similar role to the NMDA receptor at CA1 synapses by acting as the trigger for the induction of mossy fiber LTP.

However, to investigate this possibility the development of selective antagonists was necessary. The first selective antagonists directly interacting with kainate receptors were derivatives from a series of decahydroisoquinolines screened against AMPA receptors (Bleakman et al., 1996). Subsequent compounds, such as LY294486 (Clarke et al., 1997), its active isomer LY377770 and LY382884 (Smolders et al., 2002) showed greater selectivity towards GLU_{K5} (O'Neill et al., 1998). We found that LY382884 selectively blocked the induction of mossy fiber LTP in a reversible manner (Bortolotto et al., 1999). The original data are summarized in Fig. 1. Recently we confirmed our previous findings using a more potent and selective GLU_{K5} receptor antagonist, UBP296 (More et al., 2004). Those data are summarized in Fig. 2. These two sets of results suggest that GLU_{K5} receptors play an important role in the induction of mossy fiber LTP. Since our original findings were published the role of

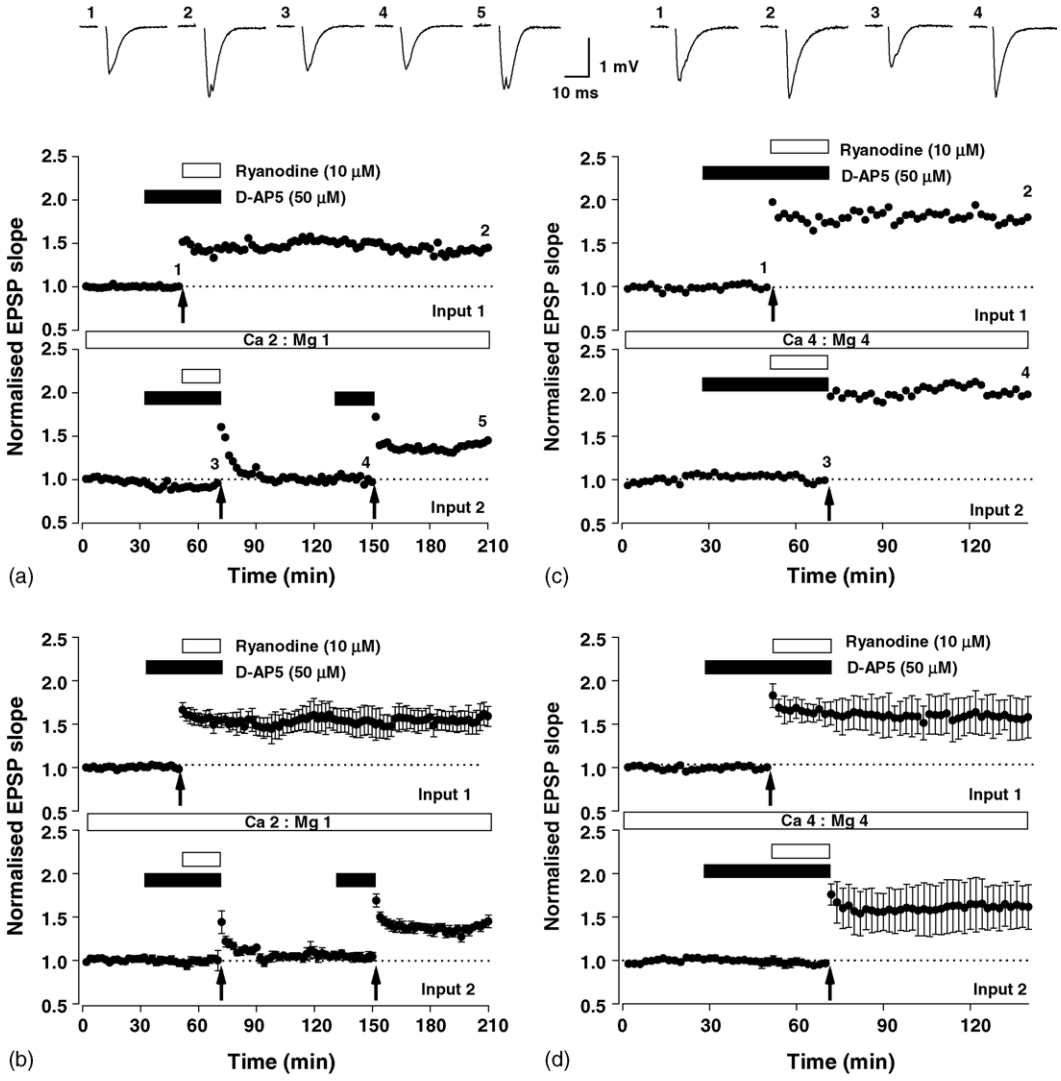

Fig. 5. Intracellular Ca^{2+} stores play a role in mossy fiber LTP induction. (a) A single example of an experiment to show that ryanodine blocks the induction of mossy fiber LTP, in Ca 2:Mg 1 (input 2). In input 1, the tetanus was delivered immediately before application of ryanodine and in input 2, the first tetanus was delivered in the presence of the drug. In this input, the second tetanus shows that after washing out ryanodine its effects were reversible. Note that ryanodine did not affect the pre-established LTP in input 1. (b) Pooled data from four experiments as presented in (a). (c) A single experiment to show that under elevated concentration of calcium and magnesium (Ca 4:Mg 4) ryanodine does not block the induction of mossy fiber LTP. (d) Pooled data of all experiments performed as in (c) $n = 4$ (from: Lauri et al., 2003).

GLU_{K5} receptors in mossy fiber LTP has been disputed by other groups (see Contractor et al., 2001; Schmitz et al., 2001; Breustedt and Schmitz, 2004). We suspect that differences in the experimental conditions employed could account for these discrepancies.

One significant difference between our work and several others is the concentration of calcium and magnesium in the medium. Normally we use 2 mM of Ca^{2+} and 1 mM Mg^{2+}, whilst other labs often use higher divalent cation concentrations (see Yeckel et al., 1999). Surprisingly, in our hands LY382884 did not block the induction of mossy fiber LTP in elevated concentrations of calcium and magnesium [Ca 4:Mg 4], suggesting that some form of compensatory mechanism takes over under these conditions. Our results addressing this point are summarized in Fig. 3. However, the divalent cation concentration is not the only factor that determines the involvement of GLU_{K5} kainate receptors (Breustedt and Schmitz, 2004).

Following the discovery that kainate receptors were involved in the induction of mossy fiber LTP we wanted to know how they operated and how their role may be compensated for. We found that in elevated concentrations of divalent ions (Ca 4:Mg 4), the ability of LY382884 to block mossy fiber LTP was restored in the presence of the L-type Ca^{2+} channel blocker nifedipine (Fig. 4). This result suggests that, in the presence of elevated levels of divalent cations, Ca^{2+} entry via L-type Ca^{2+} channels can compensate for Ca^{2+} entry via kainate receptors following their activation. Therefore, under elevated divalent cation conditions, mossy fiber LTP is only blocked when both L-type Ca^{2+} channels and kainate receptors are simultaneously inhibited. In summary, L-type Ca^{2+} channels compensate for the role of kainate receptors in the induction of mossy fiber LTP under high concentration of divalent ions.

Another source of Ca^{2+} involved in mossy fiber LTP could be the intracellular Ca^{2+} stores. Indeed previous work has shown that Ca^{2+} stores are involved in synaptic plasticity at a variety of synapses in the CNS (Frenguelli and Irving, 1996; Berridge, 1997). For example, at CA1 neurons, NMDA receptor-dependent LTP is inhibited by dantrolene (Obenaus et al.,

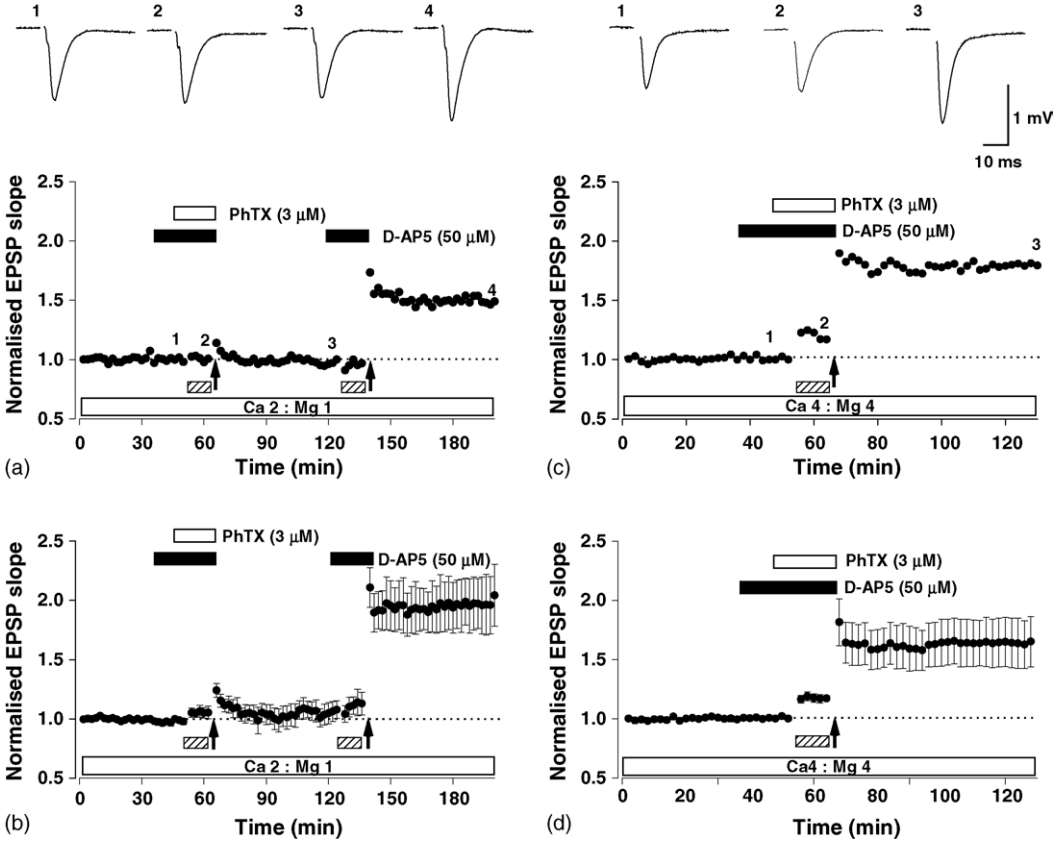

Fig. 6. Evidence that Ca^{2+} permeable kainate receptors are involved in the induction of mossy fiber LTP. (a) A single example to show the reversible block of mossy fiber LTP by PhTx in Ca 2:Mg 1. Note that additional stimuli (30 shocks at 50 Hz, delivered at 2-min intervals, hatched bars) were delivered prior to the tetanus due to the use-dependent nature of PhTx. (b) Pooled data as presented in (a) $n = 4$. (c) A single example showing that PhTx did not block the induction of mossy fiber LTP if the concentration of calcium and magnesium is raised to Ca 4:Mg 4. (d) Pooled data of all four experiments performed as in (c) (from: Lauri et al., 2003).

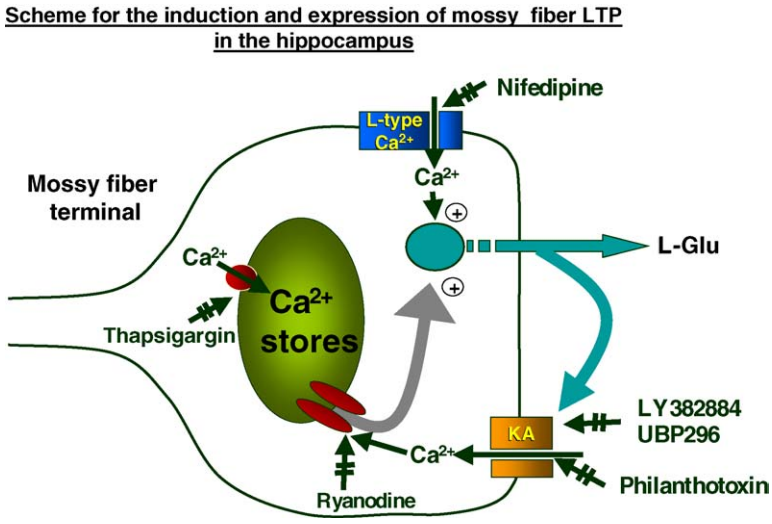

Fig. 7. A schematic model for the role of kainate receptors in mossy fiber LTP. Once glutamate has been released it can diffuse in the synaptic cleft back onto GLU_{K5}-containing, calcium-permeable presynaptic kainate receptors. Ca^{2+} permeating these receptors triggers Ca^{2+} release from internal stores that, in turn, initiates processes that results in LTP. In the presence of elevated Ca^{2+} in the medium, sufficient Ca^{2+} can enter via L-type Ca^{2+} channels during repetitive stimulation to provide an alternative Ca^{2+} source that triggers the induction of LTP. This parallel pathway means that both L-type Ca^{2+} channels and kainate receptors need to be blocked to prevent the induction of LTP under these conditions. This scheme is a modified version from Lauri et al. (2003).

1989), thapsigargin (Harvey and Collingridge, 1992; Bortolotto and Collingridge, 1993; Behnisch and Reymann, 1995) and ryanodine (Raymond and Redman, 2002). These effects are likely to be due to the magnification of the synaptic Ca^{2+} transient by release of Ca^{2+} from intracellular stores (Alford et al., 1993; Emptage et al., 1999). Ca^{2+} stores have also been shown to contribute to Ca^{2+} transients during brief high frequency activation of mossy fiber terminals (Liang et al., 2002). However, their role in mossy fiber plasticity was not known. Therefore, we investigated the roles of Ca^{2+} release from intracellular stores in mossy fiber LTP under conditions of lower and elevated divalent cations. We found that in Ca 2:Mg 1, ryanodine reversibly blocked the induction of mossy fiber LTP (Fig. 5a and b). In contrast, under elevated cation conditions (Ca 4:Mg 4) mossy fiber LTP was readily induced in the presence of ryanodine (Fig. 5c and d). In both conditions, pre-established LTP was not affected by ryanodine. These results show that intracellular Ca^{2+} stores play an important role in the induction process of mossy fiber LTP under certain experimental conditions.

The most direct way in which activation of kainate receptors could lead to Ca^{2+} release from intracellular stores would be direct permeation of Ca^{2+} through unedited kainate receptors. We tested this possibility using philanthotoxin (PhTx) which blocks Ca^{2+}-permeable receptors (Toth et al., 2000). Consistent with this possibility, PhTx under Ca 2:Mg 1, completely prevented the induction of mossy fiber LTP in a reversible manner (Fig. 6a and b). However, in Ca 4:Mg 4 PhTx was ineffective (Fig. 6c and d). These results suggest that Ca^{2+} released from intracellular stores is the element that triggers an intracellular cascade involved in the induction of mossy fiber LTP under certain experimental conditions.

CONCLUDING REMARKS

Our results suggest a model for the induction of mossy fiber LTP: the synaptic activation of presynaptic GLU_{K5}-containing kainate receptors leads to Ca^{2+} entry through these kainate receptors, which triggers Ca^{2+} release from intracellular stores, which then triggers the induction of mossy fiber LTP. Our scheme (Fig. 7) integrates earlier observations concerning the role of voltage-gated Ca^{2+} channels, cAMP and PKA (Nicoll and Malenka, 1995).

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