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Kv4.2 block of long-term potentiation is partially dependent on synaptic NMDA receptor remodeling

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Abstract

Proper expression of synaptic NMDA receptors (NMDARs) is necessary to regulate synaptic Ca^{2+} influx and the induction the long-term potentiation (LTP) in the mammalian hippocampus. Previously we reported that expressing the A-type K^+ channel subunit Kv4.2 in CA1 neurons of organotypic slice cultures reduced synaptic NR2B-containing NMDAR expression and completely blocked LTP induced by a pairing protocol. As pretreatment with an NMDAR antagonist (APV) overnight blocked the reduction of NR2B-containing receptors in neurons expressing EGFP-labeled Kv4.2 (Kv4.2g), we hypothesized that LTP would be rescued in Kv4.2g neurons by overnight treatment with APV. We report here that the overnight APV pretreatment in Kv4.2g-expressing neurons only partially restored potentiation. This partial potentiation was completely blocked by inhibition of the CAMKII kinase. These results indicate that A-type K^+ channels must regulate synaptic integration and plasticity through another mechanism in addition to their regulation of synaptic NR2 subunit composition. We suggest that dendritic excitability, which is regulated by Kv4.2 expression, also contributes to synaptic plasticity.

Keywords

Synaptic plasticity; A-type K^+ channel; Kv4.2; LTP; NMDA receptor; CaMKII

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.brainresbull.2010.09.012.

Conflict of interest statement

None.

1. Introduction

In CA1 pyramidal neurons of mammalian hippocampi, Kv4.2 channels, underlying A-type K^+ currents, have important roles in dendritic signal processing, including the regulation of action potential (AP) back-propagation, synaptic integration and filtering [4,6,7,12,16,17], and in various types of synaptic plasticity [5,9,11,19]. These modulatory functions of Kv4.2 channels in dendritic information processing can be mediated directly through changes in membrane excitability as for AP back-propagation [5,7,12,19]. Additionally, we have found that the expression level of Kv4.2 is reduced after long-term potentiation (LTP) induction [8,11] and that Kv4.2 functional expression level in turn affects synaptic N-methyl-D-aspartate receptor (NMDAR) composition through a Ca^{2+} signaling cascade involving Ca^{2+}/Calmodulin kinase II (CaMKII) [9]. These secondary effects on synaptic NMDAR composition are dependent on spontaneous activation of NMDARs, as pretreatment with APV to block NMDARs in EGFP-labeled Kv4.2 (Kv4.2g) expressing neurons, but not action potential block by TTX, completely abolishes Kv4.2-dependent synaptic remodeling [9]. Atype channels are therefore able to modulate synaptic plasticity induction and expression through both direct and indirect mechanisms.

While NMDARs are required to induce many forms of synaptic plasticity [14], the relative importance of synaptic and extrasynaptic subunit composition has been debated [3,13,15]. In our previous report, recordings in CA1 hippocampal neurons from Kv4.2$^{-/-}$ mice showed an enhanced NR2B/NR2A ratio but normal levels of LTP with a strong induction protocol. However, LTP was blocked by the NR2B antagonist in recordings from Kv4.2$^{-/-}$ but not wild type mice, demonstrating that LTP induction and/or expression was dependent on the expression level of total synaptic NMDARs but not necessarily on the type of NMDAR subunits [9]. Consistent with these data, Kv4.2g expression in CA1 hippocampal neurons resulted in a complete block of LTP in organotypic slice cultures (Fig. 1). We therefore wondered whether restoration of normal NMDAR expression in CA1 neurons, but expressing Kv4.2g would allow for the induction of normal synaptic LTP, or if there is a direct effect of Kv4.2 expression level on synaptic plasticity. To test this, we examined NMDAR-dependent LTP in Kv4.2g expressing neurons, which were pretreated with the NMDAR antagonist, APV to prevent Kv4.2-dependent NMDAR remodeling.

Neurons expressing Kv4.2g but pretreated with APV overnight to block remodeling of synaptic NMDAR subunit composition exhibited partially restored LTP. This partial potentiation, restored by APV pretreatment, was abolished by blocking CaMKII activity. These results indicate that A-type K^+ channels regulate synaptic integration and plasticity through another mechanism, in addition to their regulation of synaptic NR2 subunits. We propose that Kv4.2 channel regulation of dendritic excitability therefore directly contributes to the expression of LTP, perhaps through their internalization after LTP induction [8,11].

2. Materials and methods

2.1. Tissue preparation and viral infection

Organotypic hippocampal slices (350 μm thick) were prepared from postnatal day 7–8 Sprague–Dawley rats. An attenuated Sindbis virus system was employed to express Kv4.2g. All electrophysiological measurements from organotypic slices were made 1–2 days after viral infection of CA1 pyramidal neurons (4–6 DIV). More detailed protocols for tissue preparation and viral infections are available in our previous paper [12]. All animal protocols were approved by both Animal Care and Use Committees of the National Institute of Child Health and Human Development in USA and of Jeju National University in Korea.

2.2. Electrophysiology

For patch-clamp recordings from organotypic slices, slices were transferred to a submerged recording chamber with continuous flow of ACSF containing (in mM): 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 25 Glucose, 2 $CaCl_2$, 1 $MgCl_2$. 5 μM 2-chloroadenosine and 5 μM bicuculine were added in some recordings to block recurrents and inhibitory inputs from CA3 neurons. Patch electrodes (4–6 MΩ) were filled with (in mM); 20 KCl, 125 Kglu, 10 HEPES, 4 NaCl, 0.5 EGTA, 4 ATP, 0.3 TrisGTP and 10 Phosphocreatin. pH and osmolarity were adjusted to 7.2–7.3 and 280–300 mOsm, respectively, in all experiments.

Excitatory EPSCs were induced by stimulation of Schaffer-collateral pathway via bipolar electrodes located 100–200 μm from the recorded cell soma. The test stimulation in all EPSC experiments was set at 0.1 Hz and 0.2 ms duration and its intensity (100–900 μA) was adjusted to induce ~100 pA EPSCs at a −65 mV holding potential. Whole-cell recording parameters were monitored throughout each experiment and recordings where series resistance varied by more than 10% were rejected.

2.3. LTP induction and drug application

In this study the pairing protocol for LTP induction consisted of low frequency stimulation (2 Hz, 0.2 ms duration) paired with depolarization to 0 mV for 1 min. Potentiation of EPSCs was measured at a −65 mV holding potential, using the same 0.1 Hz testing stimulation protocol for EPSCs mentioned above. All recordings were performed at 31–32 °C. The NMDA antagonist, APV (100 μM) or voltage-gated sodium channel blocker, TTX (0.5 μM) was added to slice culture media right after viral infection (18–24 h). All drugs were washed out for 20–30 min after transferring slices to a recording chamber before starting the experiment. Preincubation with drugs did not affect LTP induction and expression in uninfected neurons (see Fig. 2).

2.4. Recordings and statistical analysis

All recordings were low-pass filtered at 5 kHz and digitized at 10 kHz by an Instrutech ITC-18 A/D board controlled by software written for Igor Pro (WaveMetrics). An Axopatch-200B amplifier was employed for whole-cell patch recordings. Command pulse generation, data acquisition and analysis were performed using IGOR Pro (Wavemetrics). SPSS (SPSS Inc.) and Excel (Microsoft) software were used for further data and statistical analysis. One-way ANOVA, and Student's *t* tests were used to examine statistical significance, set to $p < 0.05$.

3. Results

3.1. Effects of APV and TTX pretreatment on LTP induction in uninfected control neurons

Previously, we reported that enhancing total Kv4.2 expression level by viral manipulation in CA1 neurons of organotypic cultured slices resulted in synaptic remodeling by altering the level of NR2B-containing NMDARs in synaptic sites. In Kv4.2g expressing cells, EPSC potentiation was gradually reduced and completely blocked 20 min after LTP stimulation even though initial potentiation was comparable to control (Fig. 1) [9]. NMDAR and CaMKII activation, but not action potential firing, were found to be necessary for synaptic NMDAR remodeling by Kv4.2g. Those results indicated that the reduction of NR2B subunit may contribute to the abolition of LTP in Kv4.2g neurons through the downregulation of CaMKII activation, which is necessary to maintain LTP expression [1]. Therefore, we reasoned that overnight (18–24 h) APV treatment, blocking synaptic NMDAR remodeling [9], would restore the LTP expression in Kv4.2g neurons.

As a control experiment, we first tested if APV or TTX overnight pretreatment had any effect on LTP induced in uninfected control neurons (Fig. 2). After transferring slices to a recording chamber, APV (100 μM) or TTX (0.5 μM) was washed out for 20–30 min before recording in normal external solution. CA1 neurons treated with either APV or TTX overnight exhibited about a 70% increase in EPSC amplitude over baseline that was maintained for the duration of the recording (Fig. 2; $p > 0.05$). These results are similar to the normal potentiation in control neurons (Fig. 1) and consistent with our previous finding that neither APV nor TTX overnight treatment had an effect on synaptic NMDAR subunit composition in uninfected control neurons [9].

3.2. Effects APV and TTX pretreatment on LTP induction in Kv4.2g neurons

We previously found that overnight pretreatment of TTX did not block the remodeling of synaptic NMDAR composition in Kv4.2g expressing neurons [9]. After first confirming that expression of Kv4.2g did not affect whole-cell clamp conditions (Supplemental Fig. 1), we investigated LTP in Kv4.2g neurons pretreated with TTX. Consistent with our previous report, LTP stimulation resulted in a pattern of initial potentiation that was slightly reduced compared to uninfected neurons (Figs. 2 and 3; 5–10 min post-LTP; uninfected + TTX: 73.85 ± 5.83; Kv4.2g + TTX: 60.81 ± 3.64%; $p < 0.05$). However, potentiation was gradually lost such that average EPSC amplitude was back to baseline levels by 20 min after induction (Fig. 2A–C; 40–50 min post-LTP, 2.07 ± 5.02%; $p < 0.01$ compared with “uninfected + TTX”, 64.61 ± 10.95%). This time course and magnitude of potentiation matched that observed in Kv4.2g neurons without drug pretreatment as previously reported (Fig. 1; 40–50 min post-LTP, −3.03 ± 12.93%). Again, this result is consistent with a role for synaptic NMDAR remodeling in LTP as TTX treatment did not block the reduction of synaptic NR2B fraction in Kv4.2g neurons [9].

Eight of 10 Kv4.2g neurons pretreated with APV did, however, show a significant and critical rescue of LTP (Fig. 3A–C; 40–50 min post-LTP, 38.01 ± 8.86%; $p < 0.01$ compared with “Kv4.2g + TTX”). In these recordings, while initial EPSC amplitude was similar with those of the TTX-treated Kv4.2g neurons, it leveled-out to about at 40% potentiation above baseline around 20 min after LTP induction. This level of potentiation is both significantly greater than “Kv4.2g” and “Kv4.2g + TTX” and significantly less than “uninfected” (Figs. 1 and 3; $p < 0.01$). These findings indicate that blocking the reduction of NR2B subunits by APV pretreatment in Kv4.2g neurons acts as a critical factor in the induction or maintenance of LTP. However, because the potentiation recovered by APV pretreatment was only partial compared with that in uninfected control neurons, there must exist another factors acting to reduce potentiation in Kv4.2g neurons.

3.3. The role of CaMKII activity for rescuing LTP in Kv4.2g

We tested if the rescued LTP in Kv4.2g neurons was dependent on CaMKII activity, according to the hypothesis that the restoration of synaptic NMDARs and Ca^{2+} signaling cascade including CaMKII activity lead the partial rescue of EPSC potentiation in Kv4.2g neurons [9]. NMDA-dependent synaptic plasticity requires CaMKII activation; perhaps through its binding with NR2B [1,2,16]. For this experiment, we tested the effect of KN93 (20 μM), a blocker of CaMKII, on LTP in Kv4.2g + APV and control, uninfected groups (Fig. 4). In both groups, KN93 completely blocked the expression of LTP within 20 min (Fig. 4) with a time course similar to that found for Kv4.2g expressing neurons shown in Fig. 1 [9] and in the presence of TTX (Fig. 3B). This result indicates that the partial recovery of LTP in Kv4.2g neurons by APV pretreatment requires CaMKII activation, which is dependent on NMDAR subunit composition.

4. Discussion

The results in the present study clearly demonstrate that sufficient expression of synaptic NMDARs is necessary for lasting synaptic potentiation in CA1 neurons of organotypic slices. This is evidenced, in Fig. 3, by (1) the abolition of LTP in Kv4.2g neurons with TTX pretreatment, and (2) partial recovery of LTP in Kv4.2g neurons with APV pretreatment. We have previously shown that Kv4.2g expressing neurons exhibit a decreased NR2B/NR2A synaptic fraction, and that this remodeling of synaptic NMDARs was blocked by preventing synaptic NMDR activation but not by preventing action potential firing [9]. Therefore, Kv4.2g neurons with TTX pretreatment have less NMDARs and do not support lasting LTP (Fig. 3). However, APV pretreatment induces here a partial recovery of LTP in Kv4.2g neurons, demonstrating that sufficient expression of synaptic NMDARs is important for the induction or maintenance of LTP (Figs. 1 and 2) [9]. We also show here that this rescued potentiation in Kv4.2g expression neurons, by APV pretreatment, requires activation of CaMKII, as KN93 completely blocked the expression of LTP in Kv4.2g neurons with APV pretreatment. This dependence on CaMKII activity indicates that the partially recovered potentiation shares a common mechanism of LTP induced in control conditions, which requires CaMKII-mediated GluR1 phosphorylation and insertion. In the present study, our purpose was to test if normal synaptic NMDAR expression in Kv4.2g neurons would restore synaptic long-term plasticity. We expected that APV pretreatment would fully recover LTP in Kv4.2g neurons, as APV pretreatment can fully restore the synaptic expression of NR2B-containing NMDARs to control (uninfected) levels in Kv4.2g neurons [9]. Yet, results from Fig. 3 show that Kv4.2g expressing neurons, upon APV pretreatment, only exhibit partially recovered LTP, suggesting the existence of another mechanism important to the induction or maintenance of LTP that is altered in Kv4.2g expressing neurons.

Previously, we reported that Kv4.2g is internalized from the cell membrane with LTP induction [11] and that blocking A-type channel internalization abolished LTP in CA1 neurons of organotypic slices [8]. Moreover, there is extensive evidence that A-type channels can shape synaptic events [7,11,10,16]. When considered together these results indicate that Kv4.2 internalization during LTP might directly contribute to enhanced synaptic efficacy after LTP induction [18]. Alternatively, the existence of more A-type channels (~2-fold in Kv4.2g neurons [12]) may suppress dendritic and synaptic membrane excitability, inhibiting NMDAR activation to reduce CaMKII activation, leading to only a partial recovery of potentiation in Kv4.2g neurons (Fig. 3). A more detailed analysis of the direct role of A-type channels to regulate synaptic plasticity will be greatly aided by the development of specific Kv4.2 channel blockers and/or constructs which specifically regulate Kv4.2 trafficking.

5. Conclusion

In summary, NMDAR- and Ca^{2+}-mediated expression and trafficking of glutamate receptors at postsynaptic sites have been predominately considered to underlie long-term changes of synaptic plasticity. Based on the results of this and previous work [9], we suggest that the expression level and trafficking of A-type K^+ channels is also a contributing factor to determine plasticity and integration of synaptic inputs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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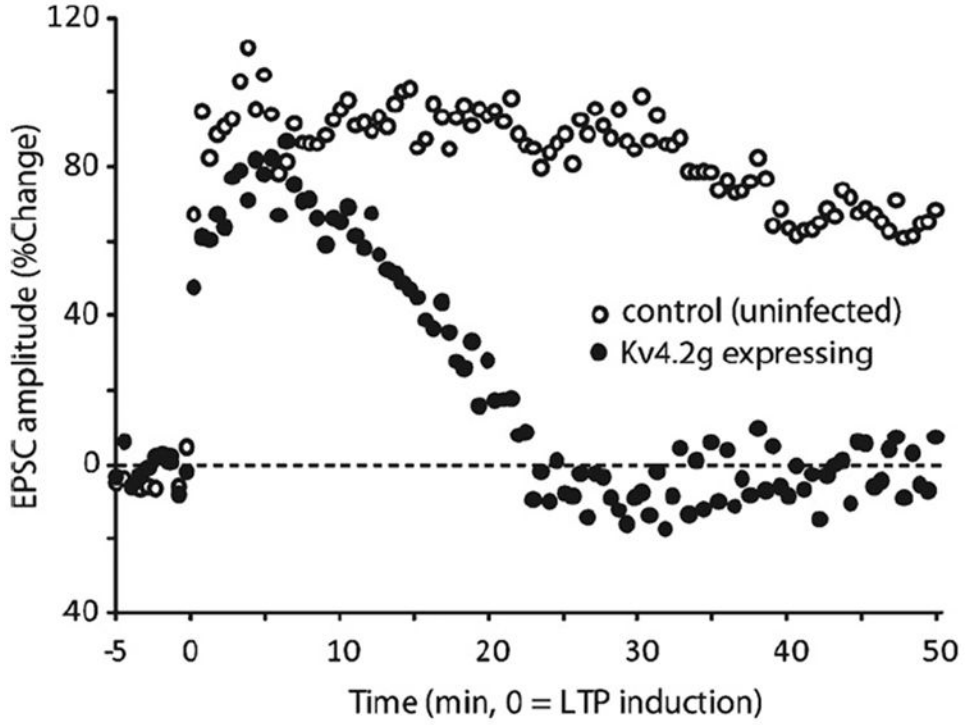

Fig. 1.

EPSC amplitudes, recorded in CA1 neurons of organotypic slices, are enhanced by applying a pairing LTP protocol (control uninfected). However, Kv4.2g expression completely blocked potentiation within 20 min after LTP induction. Each point was averaged from 9 control and 11 Kv4.2g neurons. This data is modified from data previously reported in Jung et al. [9]. Significant differences ($p < 0.05$) of potentiation between two groups were observed from 15 min after LTP induction.

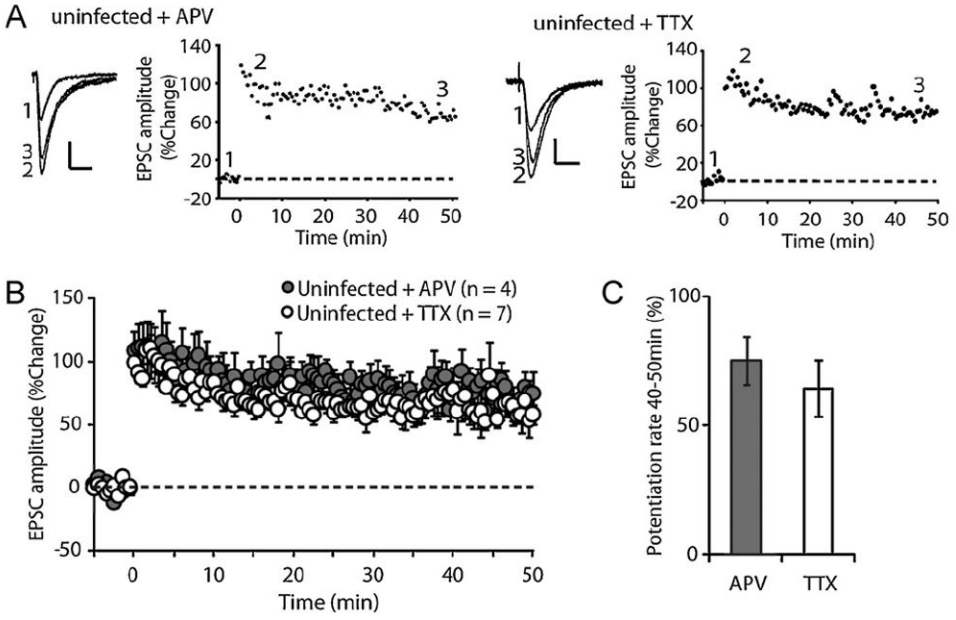

Fig. 2.

Pretreatment of either APV or TTX overnight shows no effects on LTP induced by a pairing protocol in uninfected neurons. Either APV (100 μM) or TTX (0.5 μM) was added to culture media of organotypic slices during overnight (18–24 h) and washed out 20–30 min before recording. (A) Examples of pairing-induced LTP in individual uninfected neurons of each group. For LTP experiments, EPSCs were recorded at a −65 mV holding potential. Scale bars: 50 pA, 20 ms. LTP was induced at the point of 0 min. (B) Pooled LTP data. (C) Potentiation at 40–50 min after LTP induction. EPSC amplitude was still potentiated 30–50 min after pairing in uninfected neurons pretreated with either APV or TTX, showing no significant difference from with non-pretreated control neurons [9]. Error bars represent SEM.

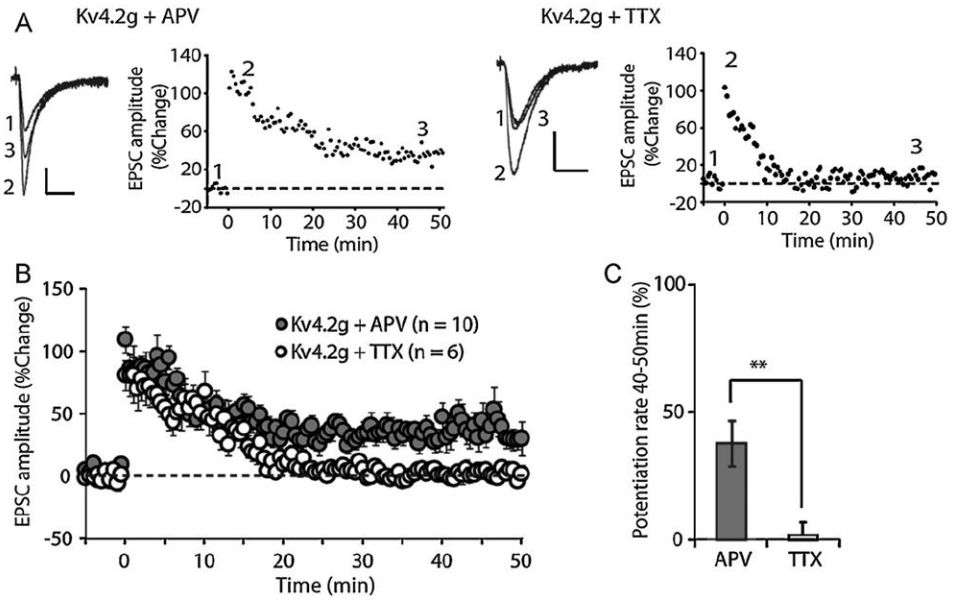

Fig. 3.

Pretreatment of Kv4.2g neurons with APV (100 μM) overnight induces a partial recovery of LTP. (A) Examples of pairing-induced LTP in individual Kv4.2g neurons of with APV (100 μM) or TTX (0.5 μM) pretreatment. Scale bars: 50 pA, 20 ms. LTP was induced at the point of 0 min. (B) Pooled LTP data. APV overnight pretreatment induces the partial but significant potentiation of EPSC amplitude 30–50 min after LTP induction, while neurons pretreated with TTX still show the abolition of LTP. (C) Potentiation rate at 40–50 min after LTP induction. The difference in potentiation between APV and TTX groups shows statistical significance (** $p < 0.01$). Error bars represent SEM.

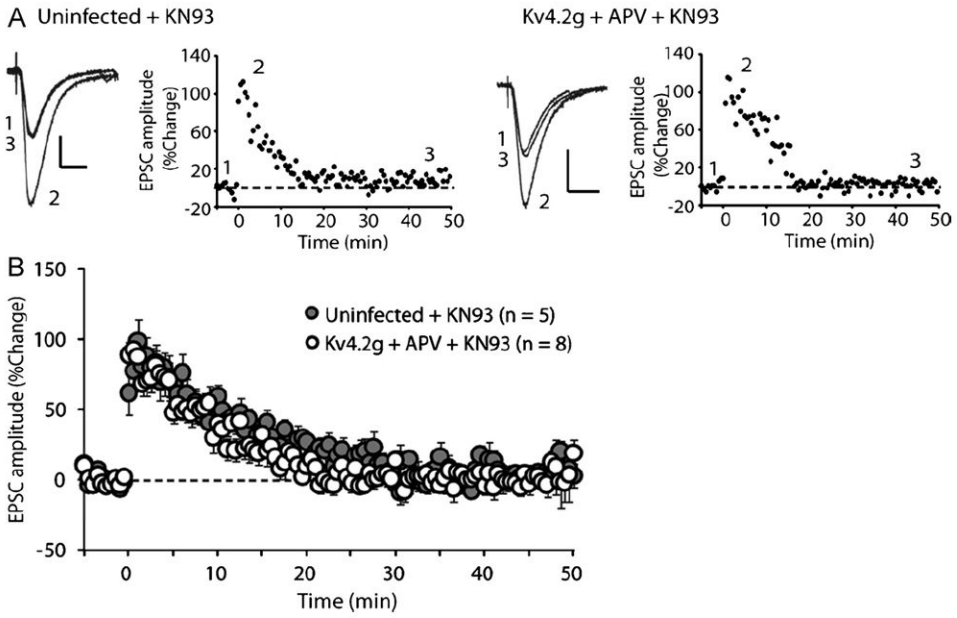

Fig. 4.

Blocking of CaMKII activation abolishes the partial recovery of LTP by APV pretreatment in Kv4.2g neurons. (A) Examples of pairing-induced LTP in individual neurons of each group. KN93 (20 μM) to block CaMKII activation was continuously added to an external solution from 20 min before recording. Scale bars: 50 pA, 20 ms. LTP was induced at the point of 0 min. (B) Pooled LTP data showing the complete abolition of LTP by KN93 in both groups. Error bars represent SEM.