Published in final edited form as:

Nat Neurosci. 2008 February ; 11(2): 170–177. doi:10.1038/nn2041.

SK2 channel plasticity contributes to LTP at Schaffer collateral-CA1 synapses

Mike T Lin^{1,5}, Rafael Luján^{2,5}, Masahiko Watanabe³, John P Adelman¹, and James Maylie⁴

¹Vollum Institute, Oregon Health & Science University, Portland, Oregon 97239, USA

²Departamento de Ciencias Médicas, Universidad de Castilla-La Mancha, Albacete, Spain, 02006

³Department of Anatomy, Hokkaido University School of Medicine, Sapporo 060-8638, Japan

⁴Department of Obstetrics and Gynecology, Oregon Health & Science University, Portland, Oregon 97239, USA

Abstract

Long-term potentiation (LTP) of synaptic strength at Schaffer collateral synapses has largely been attributed to changes in the number and biophysical properties of AMPA receptors (AMPA receptors). Small-conductance Ca^{2+} -activated K^{+} channels (SK2 channels) are functionally coupled with NMDA receptors (NMDARs) in CA1 spines such that their activity modulates the shape of excitatory postsynaptic potentials (EPSPs) and increases the threshold for induction of LTP. Here we show that LTP induction in mouse hippocampus abolishes SK2 channel activity in the potentiated synapses. This effect is due to SK2 channel internalization from the postsynaptic density (PSD) into the spine. Blocking PKA or cell dialysis with a peptide representing the C-terminal domain of SK2 that contains three known PKA phosphorylation sites blocks the internalization of SK2 channels after LTP induction. Thus the increase in AMPARs and the decrease in SK2 channels combine to produce the increased EPSP underlying LTP.

Activity-dependent changes in synaptic strength are widely believed to underlie the cellular mechanisms of learning and memory. This view has gained significant support from recent studies showing that learning induces long-lasting changes in synaptic strength¹⁻³. At Schaffer collateral-to-CA1 synapses in the hippocampus, stimulation protocols that coordinate presynaptic and postsynaptic activity to induce LTP affect the postsynaptic cell through a process that is dependent upon NMDAR activity and Ca^{2+} influx into the stimulated dendritic spine⁴⁻⁶. LTP inducing protocols act through PKA and calcium/calmodulin-dependent kinase II (CaMKII) to alter the biophysical properties⁷⁻⁹ and increase the number of AMPARs in the PSD¹⁰⁻¹⁴.

SK2 channels are activated solely by intracellular Ca^{2+} ions, with submicromolar Ca^{2+} affinity¹⁵, and are selectively blocked by the peptide toxin apamin. SK2 channels are expressed throughout the dendritic arbor of CA1 neurons and in dendritic spines^{16,17}. Whole-cell current-clamp recordings and Ca^{2+} imaging revealed that spine SK2 channels are activated by synaptically driven Ca^{2+} influx¹⁶. The repolarizing effect of SK2 channel activity opposes the depolarizing effect of AMPAR activity, reducing the EPSP, favoring Mg^{2+} reblocking of

Correspondence should be addressed to J.P.A. (adelman@ohsu.edu) or J.M. (mayliej@ohsu.edu).

⁵These authors contributed equally to this work.

AUTHOR CONTRIBUTIONS

M.T.L. performed the electrophysiological experiments and R.L. performed the immuno-EM experiments. M.W. supplied SK2-specific antibody. J.P.A. and J.M. are the corresponding authors.

NMDARs and reducing the Ca^{2+} transient¹⁶. SK2 channels are therefore ideally suited to modulate the induction of synaptic plasticity. Indeed, field recordings in area CA1 showed that blocking SK2 channels facilitates the induction of synaptic plasticity. In addition, administration of apamin to mice facilitates hippocampal-dependent memory encoding¹⁸. In contrast, overexpression of SK2 channels in transgenic mice impairs the induction of synaptic plasticity and severely impairs hippocampal-dependent learning¹⁹.

Therefore, SK2 channels in CA1 modulate the induction of synaptic plasticity and the acquisition of learning, likely by influencing Ca^{2+} entry during neuronal activity that induces LTP. Because spine SK2 channels and AMPARs contribute to an EPSP in opposing ways, we investigated whether SK2 channels participate in synaptic plasticity by undergoing an activity-dependent decrease that contributes to LTP.

RESULTS

SK2 and NMDARs colocalize in PSD of CA1 neurons

Previous electrophysiological experiments using different Ca^{2+} buffers suggested that in CA1 spines, NMDARs and SK2 channels are colocalized within 25 nm. To verify their organization within the PSD, we carried out post-embedding immunogold electron microscopy (EM) using an antibody to SK2 (anti-SK2) (see Supplementary Fig. 1 online). In sections from wild-type mice, we found SK2 immunoparticles within the PSD in the strata radiatum and lacunosum-moleculare and along the extrasynaptic plasma membrane of dendritic shafts, as well as at cytoplasmic sites, where they were always associated with intracellular membranes (see Supplementary Fig. 2 online).

Double immunogold labeling for SK2 and the NR1 subunit of the NMDAR²⁰ revealed colocalization of SK2- and NR1-specific gold particles in the PSD (Fig. 1a,b). Quantitative analysis of the tangential distribution of SK2- and NR1-specific gold particles along the PSD showed a similar distribution, with the overlap being strongest toward the middle of the PSD (Fig. 1c), in agreement with serial reconstructions of the PSD (see Supplementary Fig. 3 online). Therefore, SK2 channels and NMDARs cohabit the same microdomain within the PSD.

LTP induction abolishes SK2 channel contribution to EPSPs

To determine whether SK2 channel activity undergoes activity-dependent changes, we used whole-cell current-clamp recordings of CA1 neurons. To measure control and evoked LTP in the same cell, we performed two-pathway experiments to stimulate independent populations of spines. We placed two stimulating electrodes in the CA1 apical dendrite of the strata radiatum $\sim 100\ \mu\text{m}$ from the soma and $\sim 20\ \mu\text{m}$ lateral to the main dendritic shaft of the recorded cell. We verified the independence of the two pathways by a paired-pulse protocol (see Methods). Baseline EPSP slopes, calculated between 10% and 20% of the EPSP rise time, were measured for ≥ 5 min in pathways 1 and 2, with stimulation alternating every 30 s. To induce LTP, we used a theta-burst pairing (TBP) protocol in which presynaptic stimulation of one of the pathways was paired with backpropagating action potentials (b-APs; see Methods) while the synapses of the control pathway experienced only b-APs. TBP induced a robust LTP only in the paired stimulated pathway. At 40 min after TBP, the relative EPSP slope of the stimulated pathway increased by $317 \pm 64\%$, as compared to $43 \pm 19\%$ in the control pathway (Fig. 2a; $P < 0.05$, $n = 10$). In many cells the amount of LTP was sufficient to induce action potentials in the evoked pathway only (Fig. 2). Therefore, before addition of apamin, the stimulus strength in the evoked pathway was reduced below the action potential threshold and a new baseline was established. Apamin (100 nM) was then applied to assess the contribution of SK2 channel activity to the EPSP in either pathway. The results showed that EPSPs evoked

from the potentiated pathway were not significantly altered by apamin application ($-3 \pm 6\%$), whereas blockage of SK2 channels increased EPSPs in the control pathway by $41 \pm 13\%$ (Fig. 2b; $P < 0.05$, $n = 10$). Averaged EPSPs of ten traces for each condition are shown from a representative cell in Figure 2c-h, with the apamin effect expanded in Figure 2e,h. The reduced stimulus intensity did not select for a subset of evoked synapses with different responses to apamin, as shown by the results from cells in which LTP did not result in action potential firing and require a reduction in stimulus intensity. In these cells TBP yielded an increase in EPSP slope of $197 \pm 54\%$, compared to an increase of $36 \pm 23\%$ ($P < 0.05$, $n = 7$) in the independent pathway. Addition of apamin did not increase the EPSPs in the TBP pathway compared to the independent pathway ($6 \pm 8\%$ and $38 \pm 8\%$, $P < 0.05$).

The loss of SK2 channel activity required paired pre- and post-synaptic stimulation, as neither presynaptic stimulations nor post-synaptic b-APs alone resulted in either LTP (TB EPSPs: slope increase, $43 \pm 22\%$, $n = 9$; TB b-APs: slope increase, $61 \pm 27\%$, $n = 8$) or a loss of SK2 channel activity (TB EPSPs: slope increase with apamin, $43 \pm 10\%$; TB b-APs: slope increase with apamin, $39 \pm 11\%$) (see Supplementary Fig. 4 online). Supplementary Table 1 online gives details regarding EPSP amplitude, rise time and half-width for the various conditions.

LTP is necessary for the loss of SK2 channel activity

To assure that the TBP protocol induced NMDAR-dependent LTP and to rule out the possibility that the loss of SK2 channel activity might be due to the TBP protocol but not require LTP, we performed experiments in which the slices were initially bathed in D-2-amino-5-phosphonopentanoate (D-AP5, 50 μ M), to block NMDARs, and nimodipine (10 μ M), an L-type Ca^{2+} channel blocker. In this case, no LTP was induced in response to TBP stimulation as measured 2-7 min after TBP (slope increased $-3 \pm 14\%$ of baseline, $n = 8$) (Fig. 3a,b). After removal of D-AP5 and nimodipine, the EPSP slope increased, as compared to baseline, by $42 \pm 22\%$ 40 min after TBP. Even though nimodipine does not wash out, subsequent application of apamin further increased the EPSP slope by $49 \pm 11\%$ (Fig. 3a,c). Representative traces are shown in Figure 3b,c.

LTP at Schaffer collateral synapses requires the activity of CaMKII in the postsynaptic spine (refs. 21,22). When CaMKII was blocked by including a dominant-negative peptide²³ in the patch pipette (1 μ M), LTP was blocked (slope increase = $44 \pm 19\%$; $P < 0.05$, $n = 8$), and subsequent application of apamin to block SK2 channels increased the slope of the EPSP by $52 \pm 17\%$ (see Supplementary Fig. 4). These results show that the induction of LTP via TBP stimulation is required for the loss of SK2 channel activity in dendritic spines, and this process is dependent on both NMDARs and CaMKII.

To show that the loss of SK2 channel activity after the induction of LTP did not reflect the loss of NMDARs as a Ca^{2+} source, we measured the effect of apamin on the isolated NMDAR component of the EPSP ($\text{EPSP}_{\text{NMDA}}$) after TBP induction of LTP in a two-pathway experiment (see Supplementary Fig. 5 online). Thirty minutes after the induction protocol, the bath was perfused with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μ M) to block AMPARs and 0 mM Mg^{2+} to reveal the $\text{EPSP}_{\text{NMDA}}$. Application of apamin to block SK2 channel activity increased the $\text{EPSP}_{\text{NMDA}}$ only in the control pathway ($90 \pm 20\%$ versus $9 \pm 12\%$ in stimulated pathway; $P < 0.05$, $n = 10$), and subsequent application of D-AP5 (50 μ M) abolished the EPSP in both pathways. Moreover, the ratio of the pure NMDA component to the initial mixed EPSP was not different for the two pathways (see Supplementary Fig. 5b). Therefore, the TBP protocol did not result in the loss of the NMDA component of the EPSP.

LTP-dependent loss of SK2 channel activity requires PKA

A recent study reveals that activation of PKA downregulates SK2 channel surface expression in transfected Cos cells²⁴. Because PKA critically regulates surface expression of AMPARs in hippocampal neurons^{14,25-27}, the role of PKA in regulating SK2 channel density in spines was examined. Two-pathway experiments were performed in the presence of either H89 (10 μ M) or KT5720 (1 μ M), two different PKA blockers. Blocking PKA with KT5720 did not prevent LTP induction, although the magnitude of the LTP was slightly reduced compared to control conditions ($278 \pm 86\%$, $n = 8$) (Fig. 4a). However, subsequent application of apamin to block SK2 channel activity revealed that the EPSPs from both control and potentiated pathways were significantly increased ($45 \pm 16\%$ for control pathway; $28 \pm 11\%$ for potentiated pathway) (Fig. 4b). Representative traces are shown in Figure 4c-f; similar results were obtained for H89 ($n = 10$; see Supplementary Table 1). Therefore, the loss of SK2 channel activity from potentiated synapses depends on PKA activity.

The regulation of SK2 surface expression in transfected cells is due to PKA phosphorylation of the SK2 protein at three consecutive serine residues in the intracellular C-terminal domain of the channel²⁴. To determine whether PKA acts by phosphorylating SK2 channels at Ser568-570 (ref. 24), we performed two-pathway experiments with the peptide SK2₅₆₃₋₅₇₆, representing amino acids 563-576 of SK2, included in the intracellular pipette solution (10 μ M) (Fig. 5a-d). If PKA phosphorylates the SK2 channel at Ser568-570, then the peptide should titrate PKA activity toward these residues and block the loss of SK2 channels after the induction of LTP. Under these conditions TBP induced robust LTP with a time course similar to that for control slices ($276 \pm 72\%$, $n = 8$), but the SK2 contribution to the EPSP was still present in both the potentiated and control synapses ($53 \pm 17\%$ for control pathway; $44 \pm 18\%$ for TBP pathway) (Fig. 5a,b). Representative traces for both pathways are shown in Fig. 5c,d. In control experiments using the same peptide but with Ser568-570 substituted by alanines (SK2_{563-576A}; 10 μ M), the LTP was $289 \pm 65\%$ and the SK2 contribution to the EPSP was abolished in potentiated synapses after the induction of LTP (Fig. 5e-h; EPSP slope increased $45 \pm 16\%$ for control pathway; $6 \pm 7\%$ for TBP pathway, $n = 8$). Representative traces for both pathways are shown in Fig. 5g,h.

LTP causes PKA-dependent endocytosis of SK2

The results presented above suggested that SK2 channels might undergo PKA-dependent endocytosis upon the induction of LTP. To investigate the location of SK2 channels after the induction of LTP, we used immunogold labeling on slices treated with a chemical potentiation protocol that results in widespread LTP with the major attributes of pathway-specific electrically-induced LTP²⁸. We first checked that the chemical LTP abolishes the SK2 channel contribution to EPSPs and that the PKA blocker KT5720 prevented the loss of SK2 channel activity. A 5-min exposure to chemical LTP treatment potentiated all synapses studied and abolished the SK2 channel contribution to the EPSP (**Fig. 6a,b**, black, $n = 12$). Application of KT5720 before chemical induction of LTP did not significantly affect the amount of LTP but prevented the loss of SK2 channel function (**Fig. 6a,b**, blue, $n = 8$). Representative traces are shown in Figure 6c-f. These results demonstrate that chemical induction of LTP retains the central feature of the effects of LTP on SK2 channel function.

We then performed immunogold labeling for SK2 or PSD95 on hippocampal slices that had been bathed in control artificial cerebrospinal fluid (ACSF), chemical LTP solution, or chemical LTP solution with KT5720. Representative sections are presented in Figure 7a-f. In control slices, SK2 gold particles are seen in the PSD and perisynaptic region, and on membranes within the spine (Fig. 7a). By contrast, in slices treated with the chemical potentiation protocol, no SK2 gold particles were observed in the PSD region, whereas an increased number of SK2 gold particles were intracellular, within the spine (Fig. 7c).

Quantification of the distribution of SK2- and PSD95-specific gold particles revealed that chemical induction of LTP shifted the distribution of SK2 gold particles from the PSD region to internal membranes within the spine (Fig. 7g,h). The distribution could be well described by a sum of two Gaussians that showed that 81% of the SK2-specific gold particles were present within 20 nm of the PSD under control conditions. In contrast, after chemical induction of LTP, immunogold particles for SK2 were predominantly internalized into the spine (92% between 30-120 nm from the PSD). When KT5720 was included during the chemical LTP induction, however, the majority of SK2 immunogold particles remained in the PSD (64% within 20 nm of the PSD, Fig. 7e). The distribution of immunogold particles for PSD95 was not different under any of these conditions (100% within 20 nm of the PSD, Fig. 7h). Similarly, treatment with KT5720 did not significantly alter the distribution compared to control (92% of SK2 particles within 20 nm of PSD, see Supplementary Fig. 6 online). Immunogold particle counts for each condition are presented in Supplementary Table 2 online. These results show that PKA mediates the endocytosis of SK2 channels in potentiated spines.

DISCUSSION

Considerable evidence supports the view that AMPARs undergo both constitutive and activity-dependent trafficking, and that the insertion of AMPARs from an intracellular pool of receptors contributes to the increased EPSPs after the induction of LTP^{11-13,22}. Here we show that SK2 channels in the spine are localized within the PSD region of CA1 neurons and that TBP induction of LTP results in an NMDAR-dependent and pathway-specific downregulation of spine SK2 channel activity. The loss of SK2 channel activity is PKA-dependent and results from the removal of SK2 channels from the PSD region. An estimate of the relative contribution of SK2 channel plasticity is obtained from Figure 2. On average, TBP induction of LTP was 317% of baseline, reflecting an increase in AMPARs and a decrease in SK2 (Fig. 2a). Apamin increased the EPSP slope in the independent control pathway by 41% (Fig. 2b). Therefore, the loss of SK2 channel activity contributes ~13% to the total increased EPSP slope in the potentiated synapses. In results similar to those previously published²⁹, we measured a small degree, ~43%, of potentiation in unpaired, control synapses. This might represent either overlap of the two pathways, a consequence of whole-cell recording, or heterosynaptic potentiation.

Global and spine-specific changes in a wide array of ion channels that affect intrinsic excitability after the induction of LTP have been reported for hippocampal and cortical pyramidal neurons³⁰⁻³³. SK2 channels similarly can affect membrane excitability but also affect the shape of synaptically evoked EPSPs and affect dendritic integration^{16,34,35}. Recently, R-type Ca^{2+} channels, $\text{CaV}_{2.3}$, have been shown to undergo spine-specific synaptic depression after brief trains of b-APs and, like NMDARs, have been shown to be necessary for spine SK2 channel activity^{30,36}. It is unlikely that the activity-dependent depression of $\text{CaV}_{2.3}$ is responsible for the LTP-dependent loss of spine-specific SK2 channel function, as the immunogold labeling of SK2 in the PSD reveals an almost complete removal of SK2 channels from the PSD. In hippocampal cultures, Kv4.2 subunits that underlie the A-type currents in CA1 neurons are internalized in a NMDAR-dependent manner³², and in hippocampal slices the induction of LTP results in a hyperpolarized shift in the inactivation profile of Kv4 A-type currents that will affect dendritic integration³⁷. Although the density of voltage-gated Na^+ channels is not affected by the induction of LTP, the activation curve undergoes a hyperpolarized shift, with important consequences for intrinsic excitability³³. The activity- and NMDA-dependent internalization of SK2 channels described here shows that SK2 channels have a dynamic role in shaping spine-specific excitatory potentials and add to the complexity of synaptic plasticity.

The loss of SK2 channels from the PSD consequent to the induction of LTP requires PKA. The most parsimonious explanation for the results from the experiments in which a low concentration of SK2₅₆₃₋₅₇₆ peptide was included in the patch pipette is that PKA directly phosphorylates the channels to mediate internalization. Although we cannot rule out a more global effect on PKA, the slow, time-dependent increase in the EPSPs after TBP is preserved when the SK2₅₆₃₋₅₇₆ peptide is perfused through the patch pipette, in contrast to the global inhibition of PKA with KT5720 and H89 when the slow increase is not present, suggesting that global PKA is not inhibited by the peptide. In experiments in which PKA is globally inhibited by either KT5720 or H89, the lack of slow increase in the EPSP after TBP may relate to the inhibition of AMPA phosphorylation by PKA and, therefore, the lack of continued AMPA insertion into the PSD^{14,38}. Although previous work with GlurA-null mice suggested that this typical time-dependent increase after TBP is GlurA independent²⁹, this does not necessarily rule out PKA dependence on the time-dependent increase in GlurA-null mice. The SK2₅₆₃₋₅₇₆ peptide result also shows that SK2 internalization is unlikely to account for the slow time-dependent increase in EPSPs after LTP. Further experiments will now be required to understand how PKA phosphorylation of Ser568-570 results in channel endocytosis.

The endocytosis of SK2 channels from the PSD in potentiated spines requires the induction of LTP and CaMKII, suggesting that there is a common intracellular signaling pathway for NMDAR-dependent AMPAR insertion and SK2 channel removal. It has long been recognized that synaptic plasticity in CA1 spines requires Ca²⁺ influx through NMDARs and activation of CaMKII³⁹⁻⁴¹, whereas the role of PKA in the process of synaptic plasticity and AMPAR trafficking has only more recently become appreciated^{14,26,42}. In the absence of neuronal activity, AMPARs undergo constitutive recycling between the PSD and a population of early endosomes. This process is PKA dependent, as surface receptors are phosphorylated at Ser845 and dephosphorylated as they are removed from the cell surface³⁸. Recent reports suggest that neuronal activity capable of inducing LTP drives AMPARs into the PSD in a two-stage process. The rates of AMPAR recycling are enhanced and the receptors are first deposited at extrasynaptic sites, a process that is dependent upon PKA phosphorylation of Ser845, whereas their subsequent lateral diffusion into the PSD additionally requires CaMKII²⁵⁻²⁷. In addition to the important role played by PKA in AMPAR trafficking, it has also been proposed that PKA gates synaptic plasticity by modulating CaMKII activity via regulation of protein phosphatase 1 (refs. 43,44). PKA also affects late-phase LTP, generating new sites of synaptic transmission and endowing specificity through synaptic tagging^{45,46}. Therefore, PKA modulates multiple aspects of synaptic plasticity through its effects on a wide range of molecular targets. The PKA dependence of SK2 channel endocytosis upon the induction of LTP suggests that AMPARs and SK2 channel trafficking are coordinately controlled by PKA.

SK2 channel plasticity at Schaffer collateral synapses contributes ~13% to LTP. Although this is a significant contribution, the increased AMPAR contribution to the EPSP accounts for the majority of the increased excitability. However, the functional consequences of SK2 channel endocytosis for the induction of LTP may be more significant for the effect on subsequent synaptic activity. SK2 channels in the spines of CA1 neurons provide a negative feedback that attenuates by ~40% the Ca²⁺ influx through NMDARs¹⁶. The lost repolarizing effect mediated by the SK2 channels will profoundly increase the amount of Ca²⁺ entering through NMDARs upon subsequent synaptic activity. This will effectively shift the modification threshold^{47,48} and facilitate the induction of plasticity¹⁸. Therefore, it will be important to determine what the fates of the SK2 channels are once they are removed from potentiated spines and whether their loss is compensated by homeostatic plasticity mechanisms.

METHODS

Specific electron microscopy details

Antibodies used were anti-SK2 raised in rabbit ($1\text{--}2\ \mu\text{g ml}^{-1}$), anti-SK2 raised in guinea pig ($1\text{--}2\ \mu\text{g ml}^{-1}$) and anti-NR1 raised in rabbit ($1\text{--}2\ \mu\text{g ml}^{-1}$; Chemicon). Ultrastructural analyses were performed with a TEM-JEOL 1010 electron microscope (Synaptic Structure Laboratory, School of Medicine, University of Castilla-La Mancha, Albacete, Spain). Electron photomicrographs were captured with a charge-coupled device (CCD) camera (Mega View III, Soft Imaging System). Digitized electron images were modified for color, brightness and contrast with Adobe Photoshop version 7.0. Reproducibility of immunolabeling was assessed with tissue from at least three mice of the same genotype. Labeled structures were classified based on unambiguous morphological information in each section. Axons were identified by the lack of synaptic input and the presence of neurotubules and occasional vesicles. Axon terminals were identified by the presence of synapses and small round and/or large granular vesicles. Synapses were identified as parallel membranes separated by widened clefts that were associated with membrane specializations. Synapses showing a prominent density on the postsynaptic side were characterized as asymmetrical, whereas synapses showing equivalent densities on both sides were characterized as symmetrical. Dendrites were identified by the presence of synaptic contacts, lack of small vesicles, and presence of diffuse filaments and numerous mitochondria. Dendritic spines were identified as small protrusions showing membrane continuity with the dendritic shaft.

Post-embedding immunohistochemistry

Control and LTP-induced hippocampal slices ($300\ \mu\text{m}$) were fixed in 4% paraformaldehyde, 0.1% glutaraldehyde and $\sim 0.02\%$ picric acid made up in 0.1 M phosphate buffer (PB; pH 7.4) for 10 h. Slices were incubated in 1 M sucrose/PB solution overnight, slammed onto copper blocks cooled in liquid nitrogen and processed for osmium-free embedding. Briefly, slices were incubated for 40 min in 1% uranyl acetate, dehydrated in methanol and embedded in Unicryl resin (Electron Microscopic Sciences).

Ultrathin sections ($70\text{--}90\ \text{nm}$) from Unicryl-embedded blocks were incubated for 45 min on pioloform-coated nickel grids with drops of blocking solution consisting of 2% albumin in 0.05 M TBS, 0.9% NaCl and 0.03% Triton X-100. The grids were transferred to solutions of SK2, PSD95 or NR1 antibodies at a final protein concentration of $10\ \mu\text{g ml}^{-1}$ diluted in blocking solution overnight at room temperature ($22\ ^\circ\text{C}$). After several washes in TBS, grids were incubated for 2 h in drops of goat anti-guinea pig IgG or goat anti-rabbit IgG conjugated to 10 nm-colloidal gold particles (BioCell International), each diluted 1:80 in a 0.05 M TBS solution containing 2% normal human serum and 0.5% polyethylene glycol. Grids were then washed in TBS for 30 min and counterstained for electron microscopy with saturated aqueous uranyl acetate and lead citrate. Ultrastructural analyses were performed in a Jeol-1010 electron microscope.

Tangential distribution of SK2 within the PSD

Ultrathin sections were picked up on pioloform-coated mesh grids, immunoreacted and photographed from two of three animals used in the quantification of SK2 and NMDAR1 content within synapses. Synapses were measured only if the synaptic cleft was visible; therefore, synapses cut very tangentially were omitted. All gold particles found in the synaptic junctions in each synapse were included. Tangential location of gold particles was measured from the midline of the PSD. The distance between the midline and the edge of the PSD was divided into five bins, each bin corresponding to 10% of the PSD length in a single section, and each gold particle was assigned to a bin.

Slice preparation

All procedures were performed in accordance with the guidelines of Oregon Health & Science University and University of Castilla-La Mancha, Albacete, Spain. Hippocampal slices were prepared from 3- to 8-week-old C57BL/6J mice. Animals were anesthetized with isoflurane and decapitated. Cerebral hemispheres were quickly removed and placed into cold ACSF (in mM: 125 NaCl, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 2.0 CaCl₂, 1.0 MgCl₂, 15 glucose) and equilibrated with 95% O₂/5% CO₂. Hippocampi were removed and transferred into a slicing chamber containing sucrose-ACSF (in mM: 75 sucrose, 87 NaCl, 2.5 KCl, 21.4 NaHCO₃, 1.25 NaH₂PO₄, 0.5 CaCl₂, 7 MgCl₂, 1.3 ascorbic acid, 20 glucose) and equilibrated with 95% O₂/5% CO₂. Transverse hippocampal slices (300 μ m) were cut with a Leica VT1000s (Leica Instruments, Nussloch, Germany) and transferred into a holding chamber containing regular ACSF and equilibrated with 95% O₂/5% CO₂. Slices were incubated at 34 °C for 30-45 min and allowed to recover at room temperature for 1 h before recordings were performed.

Electrophysiology

For synaptically evoked recordings, CA1 pyramidal cells were visualized with infrared differential interference contrast optics (Leica DMLFS) and a CCD camera. Whole-cell patch-clamp recordings were obtained from CA1 pyramidal cells using an Axopatch 200B amplifier (Axon Instruments), digitized using an ITC-16 analog-to-digital converter (InstruTech) and transferred to a computer using Pulse software (Heka Elektronik). Patch pipettes (2-4 M Ω) were filled with a solution containing (mM) 120 K-gluconate, 20 KCl, 2 MgCl₂, 10 HEPES, 4 ATP, 0.3 GTP, and 14 phosphocreatine, pH 7.3. Series resistance was not electronically compensated and recordings with series resistance that changed more than 20% during the experiment were discarded. Electrophysiological records were filtered at 5 kHz and sampled at 20 kHz. The input resistance was determined from a ~30-pA (500-ms) hyperpolarizing current injection pulse interspersed between events. All recordings were from cells with a resting membrane potential between -75 and -60 mV and a stable input resistance. A bias current was applied to maintain the membrane potential at -60 mV.

Synaptic stimulation

EPSPs were recorded in whole-cell current-clamp mode. Capillary glass pipettes filled with ACSF, with a tip diameter of ~5 μ m, connected to an Iso-Flex stimulus isolation unit (A.M.P.I.) were used to stimulate presynaptic axons in stratum radiatum. Two stimulation electrodes were placed at equal distances from the soma (~100 μ m) and ~20 μ m on opposite sides of the dendrite of the recorded cell. Pathway independence was verified by the lack of paired pulse facilitation to synaptic stimulation delivered in each electrode with 50-ms intervals. SR95531 (2 μ M) and CGP55845 (1 μ M) were present to reduce the contributions of GABA_A and GABA_B, respectively. To prevent epileptic discharges in the presence of GABAergic blockers, the CA3 region was microdissected out of some slices before recording.

Theta-burst pairing (TBP)

Backpropagating action potentials (b-APs) were initiated in whole-cell current-clamp mode by somatic current injection (1 ms, 1-2 nA). The standard TBP protocol consists of EPSPs paired with a single b-AP timed so that the b-AP occurred at the peak of the EPSP as measured in the soma. A single burst consists of five EPSP-b-AP pairs delivered at 100 Hz and ten bursts delivered at 5 Hz per sweep. Three TBP sweeps were delivered at 30-s intervals. Subthreshold EPSPs were elicited by 100- μ s current injections that were less than one-third of the stimulus required for evoking an action potential. The general induction protocol was to establish a control baseline of EPSPs evoked every 15 s (alternating between the two stimulation electrodes, 30 s for Fig. 2). After establishing a stable baseline for 5-10 min, TBP, theta-burst EPSPs or theta-burst b-AP firing protocols were applied. Theta-burst EPSP stimulation and

theta-burst b-AP firing protocols consisted of the same stimulation as TBP except that theta-burst EPSP is presynaptic stimulation alone and theta-burst b-AP firing is postsynaptic current injection alone to evoke b-AP. To assay for SK2 channel activity, apamin (100 nM) was added to the bath solution 40–50 min after induction, and EPSPs were recorded for another 20 min. In many cells the LTP was sufficient to induce action potential firing in the evoked pathway. In these cases, the stimulus strength was reduced below action potential threshold and a new 10-min baseline was established before addition of apamin.

Data analysis

Data were analyzed using IGOR (WaveMetrics). The slope of the rising phase of the EPSP was measured between 10% and 20% of the rising phase. Summary plots were generated by binning data at 1 min intervals. Data are expressed as mean $\pm$ s.e.m. Pair two-sample *t*-tests were used to determine significance of data in the same pathway, and ANOVA with Dunnett's *post hoc* test was used to determine significance between groups of data; $P < 0.05$ was considered significant.

Chemical and solution

D-AP5, CNQX, CGP55845 and SR95531 were obtained from Tocris Cookson. Apamin was obtained from Calbiochem. CaMKII inhibitory peptide was a generous gift from T.R. Soderling. SK2₅₆₃₋₅₇₆ and SK2_{563-576A} peptides were obtained from Global Peptide. All other chemicals were obtained from Sigma-Aldrich unless specified. All perfusing solutions were modified from regular ACSF unless otherwise noted. NMDA component of EPSP was isolated by perfusing ACSF with 10 μ M CNQX and without MgCl₂. Chemical LTP was induced by 5 min bath application of potentiation solution (in mM: 124 NaCl, 5 KCl, 1.25 KH₂PO₄, 0.1 MgCl₂, 24 NaHCO₃, 2 CaCl₂, 10 glucose, 25 tetraethylammonium,) and equilibrated with 95% O₂/5% CO₂. In the experiments in which KT5720 or H89 was used, the slices were preincubated in KT5720 or H89 solutions for 20 min before transferring to recording chamber.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank W.W. Wu and M. Ferking for helpful discussions. This work was funded by research grants from the US National Institutes of Health to J.P.A. and J.M. and from the Spanish Ministry of Education and Science (BFU-2006-01896) and Junta de Comunidades de Castilla-La Mancha (SAN-04-008-00, PAI05-040) to R.L.

References

1. Dragoi G, Harris KD, Buzsaki G. Place representation within hippocampal networks is modified by long-term potentiation. *Neuron* 2003;39:843–853. [PubMed: 12948450]
2. Whitlock JR, Heynen AJ, Shuler MG, Bear MF. Learning induces long-term potentiation in the hippocampus. *Science* 2006;313:1093–1097. [PubMed: 16931756]
3. Gruart A, Munoz MD, Delgado-Garcia JM. Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice. *J. Neurosci* 2006;26:1077–1087. [PubMed: 16436593]
4. Collingridge GL, Kehl SJ, McLennan H. The antagonism of amino acid-induced excitations of rat hippocampal CA1 neurones in vitro. *J. Physiol. (Lond.)* 1983;334:19–31. [PubMed: 6134823]
5. Lisman J. A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proc. Natl. Acad. Sci. USA* 1989;86:9574–9578. [PubMed: 2556718]
6. Malenka RC, Nicoll RA. NMDA receptor-dependent synaptic plasticity: multiple forms and mechanisms. *Trends Neurosci* 1993;16:521–527. [PubMed: 7509523]

7. Benke TA, Luthi A, Isaac JT, Collingridge GL. Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature* 1998;393:793–797. [PubMed: 9655394]
8. Lee HK, et al. Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* 2003;112:631–643. [PubMed: 12628184]
9. Derkach V, Barria A, Soderling TR. Ca^{2+} /calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc. Natl. Acad. Sci. USA* 1999;96:3269–3274. [PubMed: 10077673]
10. Song I, Huganir RL. Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci* 2002;25:578–588. [PubMed: 12392933]
11. Brecht DS, Nicoll RA. AMPA receptor trafficking at excitatory synapses. *Neuron* 2003;40:361–379. [PubMed: 14556714]
12. Kennedy MJ, Ehlers MD. Organelles and trafficking machinery for postsynaptic plasticity. *Annu. Rev. Neurosci* 2006;29:325–362. [PubMed: 16776589]
13. Collingridge GL, Isaac JT, Wang YT. Receptor trafficking and synaptic plasticity. *Nat. Rev. Neurosci* 2004;5:952–962. [PubMed: 15550950]
14. Esteban JA, et al. PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nat. Neurosci* 2003;6:136–143. [PubMed: 12536214]
15. Köhler M, et al. Small-conductance, calcium-activated potassium channels from mammalian brain. *Science* 1996;273:1709–1714. [PubMed: 8781233]
16. Ngo-Anh TJ, et al. SK channels and NMDA receptors form a Ca^{2+} -mediated feedback loop in dendritic spines. *Nat. Neurosci* 2005;8:642–649. [PubMed: 15852011]
17. Sailer CA, Kaufmann WA, Marksteiner J, Knaus HG. Comparative immunohistochemical distribution of three small-conductance Ca^{2+} -activated potassium channel subunits, SK1, SK2, and SK3 in mouse brain. *Mol. Cell. Neurosci* 2004;26:458–469. [PubMed: 15234350]
18. Stackman RW, et al. Small conductance Ca^{2+} -activated K^{+} channels modulate synaptic plasticity and memory encoding. *J. Neurosci* 2002;22:10163–10171. [PubMed: 12451117]
19. Hammond RS, et al. Small-conductance Ca^{2+} -activated K^{+} channel type 2 (SK2) modulates hippocampal learning, memory, and synaptic plasticity. *J. Neurosci* 2006;26:1844–1853. [PubMed: 16467533]
20. Watanabe M, et al. Selective scarcity of NMDA receptor channel subunits in the stratum lucidum (mossy fibre-recipient layer) of the mouse hippocampal CA3 subfield. *Eur. J. Neurosci* 1998;10:478–487. [PubMed: 9749710]
21. Lisman J, Schulman H, Cline H. The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat. Rev. Neurosci* 2002;3:175–190. [PubMed: 11994750]
22. Derkach VA, Oh MC, Guire ES, Soderling TR. Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat. Rev. Neurosci* 2007;8:101–113. [PubMed: 17237803]
23. Pereda AE, et al. Ca^{2+} /calmodulin-dependent kinase II mediates simultaneous enhancement of gap-junctional conductance and glutamatergic transmission. *Proc. Natl. Acad. Sci. USA* 1998;95:13272–13277. [PubMed: 9789078]
24. Ren Y, et al. Regulation of surface localization of the small conductance Ca^{2+} -activated potassium channel, SK2, through direct phosphorylation by cAMP-dependent protein kinase. *J. Biol. Chem* 2006;281:11769–11779. [PubMed: 16513649]
25. Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD. Recycling endosomes supply AMPA receptors for LTP. *Science* 2004;305:1972–1975. [PubMed: 15448273]
26. Oh MC, Derkach VA, Guire ES, Soderling TR. Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. *J. Biol. Chem* 2006;281:752–758. [PubMed: 16272153]
27. Passafaro M, Piech V, Sheng M. Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. *Nat. Neurosci* 2001;4:917–926. [PubMed: 11528423]
28. Roth-Alpermann C, Morris RG, Korte M, Bonhoeffer T. Homeostatic shutdown of long-term potentiation in the adult hippocampus. *Proc. Natl. Acad. Sci. USA* 2006;103:11039–11044. [PubMed: 16829578]

29. Hoffman DA, Sprengel R, Sakmann B. Molecular dissection of hippocampal theta-burst pairing potentiation. *Proc. Natl. Acad. Sci. USA* 2002;99:7740–7745. [PubMed: 12032353]
30. Yasuda R, Sabatini BL, Svoboda K. Plasticity of calcium channels in dendritic spines. *Nat. Neurosci* 2003;6:948–955. [PubMed: 12937422]
31. Fan Y, et al. Activity-dependent decrease of excitability in rat hippocampal neurons through increases in I_h . *Nat. Neurosci* 2005;8:1542–1551. [PubMed: 16234810]
32. Kim J, Jung SC, Clemens AM, Petralia RS, Hoffman DA. Regulation of dendritic excitability by activity-dependent trafficking of the A-type K^+ channel subunit Kv4.2 in hippocampal neurons. *Neuron* 2007;54:933–947. [PubMed: 17582333]
33. Xu J, Kang N, Jiang L, Nedergaard M, Kang J. Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. *J. Neurosci* 2005;25:1750–1760. [PubMed: 15716411]
34. Faber ES, Delaney AJ, Sah P. SK channels regulate excitatory synaptic transmission and plasticity in the lateral amygdala. *Nat. Neurosci* 2005;8:635–641. [PubMed: 15852010]
35. Cai X, et al. Unique roles of SK and Kv4.2 potassium channels in dendritic integration. *Neuron* 2004;44:351–364. [PubMed: 15473972]
36. Bloodgood BL, Sabatini BL. Nonlinear regulation of unitary synaptic signals by $CaV_{2.3}$ voltage-sensitive calcium channels located in dendritic spines. *Neuron* 2007;53:249–260. [PubMed: 17224406]
37. Frick A, Magee J, Johnston D. LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. *Nat. Neurosci* 2004;7:126–135. [PubMed: 14730307]
38. Ehlers MD. Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* 2000;28:511–525. [PubMed: 11144360]
39. Malenka RC, et al. An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. *Nature* 1989;340:554–557. [PubMed: 2549423]
40. Bliss TV, Collingridge GL. A synaptic model of memory long-term potentiation in the hippocampus. *Nature* 1993;361:31–39. [PubMed: 8421494]
41. Malenka RC, Nicoll RA. Long-term potentiation—a decade of progress? *Science* 1999;285:1870–1874. [PubMed: 10489359]
42. Otmakhova NA, Lisman JE. Contribution of I_h and $GABA_B$ to synaptically induced afterhyperpolarizations in CA1: a brake on the NMDA response. *J. Neurophysiol* 2004;92:2027–2039. [PubMed: 15163674]
43. Blitzer RD, et al. Gating of CaMKII by cAMP-regulated protein phosphatase activity during LTP. *Science* 1998;280:1940–1943. [PubMed: 9632393]
44. Brown GP, et al. Long-term potentiation induced by theta frequency stimulation is regulated by a protein phosphatase-1-operated gate. *J. Neurosci* 2000;20:7880–7887. [PubMed: 11050107]
45. Bolshakov VY, Golan H, Kandel ER, Siegelbaum SA. Recruitment of new sites of synaptic transmission during the cAMP-dependent late phase of LTP at CA3-CA1 synapses in the hippocampus. *Neuron* 1997;19:635–651. [PubMed: 9331354]
46. Barco A, Alarcon JM, Kandel ER. Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell* 2002;108:689–703. [PubMed: 11893339]
47. Bear MF. Mechanism for a sliding synaptic modification threshold. *Neuron* 1995;15:1–4. [PubMed: 7619513]
48. Bienenstock EL, Cooper LN, Munro PW. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *J. Neurosci* 1982;2:32–48. [PubMed: 7054394]

Figure 1.

Colocalization of SK2 and NR1 within the PSD of CA1 spines. **(a)** Double immunogold labeling for SK2 and NR1 in a representative asymmetric synapse in the CA1 region of the hippocampus. Colocalization of SK2 channels (10-nm particles) and NMDAR NR1 (20-nm particles) in the PSD of individual spines(s) establishing synapses with axon terminals ('b') in the stratum radiatum. Scale bars, 0.2 μ m. **(b,c)** Quantitative analysis showing the tangential distribution of immunoparticles for SK2 **(b)** and NR1 **(c)** across the PSD. Both SK2 and NR1 are preferentially located at the middle of the PSD, and their densities decrease toward the edges of the synapse. 285 immunoparticles for NR1 were identified in 89 synapses and 170 immunoparticles for SK2 in 78 synapses.

Figure 2.

TBP induction of LTP abolishes SK2 channel activity. EPSPs evoked from two independent pathways were measured from the same cell. **(a)** Time course of the normalized EPSP slope (mean $\pm$ s.e.m.) from control (closed, black symbols, $n = 10$) and TBP-stimulated pathway (open, blue symbols). The TBP protocol was delivered at time 0. In most cells the amount of LTP was sufficient to induce action potentials in the evoked pathway only. Therefore, before addition of apamin the stimulus strength in the evoked pathway was reduced below action potential threshold and a new baseline was established. **(b)** Continued time course of the renormalized EPSP slope from control (black) and stimulated pathway (blue) before and after apamin application. **(c,d)** TBP pathway. Representative average of ten EPSPs, mean $\pm$ s.e.m. (shaded area), taken from the indicated shaded time points in **a** for baseline (black) and after TBP induction of LTP (blue) and **b** for before (blue) and after apamin (red) in the TBP pathway. **(f,g)** Control pathway. Representative average of ten EPSPs, mean $\pm$ s.e.m. (shaded area), taken from the indicated shaded time points in **a** for baseline (black) and after TBP (blue) and **b** for before (blue) and after apamin (red) in the control pathway. Scale bars, 1 mV and 10 ms for **c,d,f,g**. **(e,h)** Expanded time course of traces in **d** and **g**. Scale bars, 1 mV and 5 ms.

Figure 3.

NMDAR activity is necessary for loss of SK2 channel activity. EPSPs were evoked in the presence of D-AP5 (50 μ M) and nimodipine (10 μ M) to block LTP induction by a TBP protocol. (a) Time course of the normalized EPSP slope ($n = 8$). Slices were initially bathed in D-AP5 and nimodipine, and baseline EPSPs were recorded for 10 min. The TBP protocol was delivered at time 0, and D-AP5 and nimodipine were removed from the bath solution 4 min after TBP. Apamin was then applied 40 min after TBP. (b) Representative average EPSPs $\pm$ s.e.m. taken as average of ten EPSPs from the indicated shaded time points on the diary plot in a from baseline (black, time point 1) and 4 min after TBP (blue, time point 2). (c) Representative average EPSPs $\pm$ s.e.m. taken as average of 10 EPSPs from the indicated shaded time points on the diary plot in a from baseline before addition of apamin (black, time point 3) and after apamin (red, time point 4).

Figure 4.

PKA activity is necessary for the LTP-dependent loss of SK2 channel activity. EPSPs evoked from two independent pathways in the presence of KT5720 (1 μ M) to block PKA activity.

(a) Time course of the normalized EPSP slope from control (closed, black symbols, $n = 8$) and TBP pathway (open, blue symbols). The TBP protocol was delivered at time 0. (b) Continued time course of the renormalized EPSP slope from control (black) and TBP pathway (blue) before and after apamin application. Apamin increased the slope of EPSPs in both evoked ($28 \pm 11\%$) and control ($45 \pm 16\%$) pathways in the presence of PKA inhibitor. (c,e) Representative average EPSPs $\pm$ s.e.m. (shaded area) taken as average of five EPSPs from the indicated shaded time points in a for baseline (black) and after TBP induction of LTP (blue) in the TBP (c) and control (e) pathways. Scale bars, 2 mV and 10 ms. (d,f) Representative EPSPs $\pm$ s.e.m. taken as average of five EPSPs from the indicated time points in b before (blue) and after apamin application (red) in the evoked (d) and control (f) pathways. Scale bars, 2 and 1 mV for d and f, respectively, and 10 ms for time scale.

Figure 5.

PKA acts on spine SK2 channels. EPSPs evoked from two independent pathways with the SK2₅₆₃₋₅₇₆ (**a-d**) or SK2₅₆₃₋₅₇₆A peptide (**e-h**) in the patch pipette solution. (**a**) Time course of the normalized EPSP slope from TBP (blue) and control pathway (black) in the presence of 10 μ M SK2₅₆₃₋₅₇₆ peptide (means $\pm$ s.e.m., $n = 8$). (**b**) Continued time course of the renormalized EPSP slope 40 min after TBP stimulation before and after apamin application. (**c**) Representative average of ten EPSPs, mean $\pm$ s.e.m., taken from the indicated shaded time points in **a** for baseline (black) and 40 min after TBP (blue) in the presence of SK2₅₆₃₋₅₇₆ for TBP (top) and control pathway (bottom). Vertical scale bars, 2 and 0.5 mV, respectively. (**d**) Representative average of ten EPSPs $\pm$ s.e.m. taken from the indicated time points in **b** before (blue) and after apamin application (red) in the TBP (top) and control pathway (bottom). Scale bars, 2 and 0.5 mV, respectively. (**e,f**) Time course of normalized EPSP slope for TBP and independent pathway, as described for **a** and **b** in the presence of 10 μ M SK2₅₆₃₋₅₇₆A peptide. (**g**) Representative averages of TBP and control pathway from **e**, as described in **c**. Vertical scale bars, 2 and 1 mV, respectively. (**h**) Representative averages of TBP and control pathway from **f**, as described in **d**. Vertical scale bar, 2 and 1 mV, respectively. Horizontal scale bar, 10 ms.

Figure 6.

Chemical induction of LTP abolishes SK2 channel function in a PKA-dependent manner. **(a)** Time course of the normalized EPSP slope in the absence (closed, black symbols, $n = 10$) and presence (blue) of KT5720 (open, blue symbols, $n = 12$). At time 0 slices were bathed for 5 min in chemical potentiation solution to induce chemical LTP. EPSPs were continuously measured during and after re-perfusion with normal ACSF. The EPSPs were clearly potentiated with or without KT5720. **(b)** Continued time course of the renormalized EPSP slope before and after apamin application in the absence and presence of KT5720. **(c,e)** Representative average EPSPs $\pm$ s.e.m. (shaded area) taken as average of five EPSPs from the indicated shaded time points in **a** for baseline (black) and after induction of chemical LTP (blue) in the absence **(c)** and presence **(e)** of KT5720. **(d,f)** Representative EPSPs $\pm$ s.e.m. taken as average of five EPSPs from the indicated time points in **b** before (blue) and after apamin application (red) in the absence **(d)** and presence **(f)** of KT5720. Scale bars, 2 mV and 10 ms for time scale.

Figure 7.

Chemical induction of LTP results in a PKA-dependent decrease of SK2 immunoreactivity in the PSD. Immunoreactivity for SK2 and PSD95 in control slices (**a,b**) after induction of chemical LTP (**c,d**) and after induction of chemical LTP with KT5720 (**e,f**) as detected using post-embedding immunogold EM in the hippocampal CA1 region. (**a,b**) In control slices, immunoparticles for SK2 were found within the PSD of asymmetrical synapses (for example, arrow, **a**) in the stratum radiatum, as well as along the extrasynaptic plasma membrane (for example, crossed arrow) of spines (**s**) establishing synapses with axon terminals (**b**). Immunogold particles for PSD95 were always observed within the PSD of asymmetrical synapses (for example, arrows, **b**). (**c,d**) After induction of chemical LTP, immunoparticles for SK2 were not detected within the PSD of asymmetrical synapses, but instead they were observed associated with intracellular membranes (for example, arrowheads, **c**). Immunogold particles for PSD95 were always observed within the PSD of asymmetrical synapses (for example, arrows, **d**). (**e,f**) After induction of chemical LTP in slices pre-treated with KT5720, immunoparticles for SK2 were mainly found within the PSD of asymmetrical synapses (for example, arrow, **e**), as well as associated with intracellular membranes (for example, arrowheads, **e**). Den, dendritic shafts. Scale bars, 0.5 mm. (**g,h**) Quantification of particles with radial distance from the PSD for SK2 (**g**) and PSD95 (**h**). Solid traces represent fits of a sum of two Gaussians to each distribution for SK2 and one Gaussian for PSD95.