The pore-forming α-subunit Kv4.2 is a key constituent of the A-type channel and critically involved in the regulation of dendritic excitability and plasticity. Here we show that Kv4.2 is enriched in the postsynaptic density (PSD) fraction and specifically interacts with synapse-associated protein 97 (SAP97). This interaction requires an intact C terminus of Kv4.2 and occurs via the PDZ domains of SAP97. Pharmacologically induced translocation of SAP97 to spines also drives Kv4.2 to the PSD, whereas SAP97 lentivirally based RNA interference reduces Kv4.2 in the PSD. In addition, calcium/calmodulin-dependent protein kinase II (CaMKII)-dependent SAP97 phosphorylation regulates the subcellular localization of Kv4.2. These results show that SAP97-CaMKII pathway plays an important role for the trafficking of Kv4.2 to dendrites and spines.

The efficient transmission and processing of information in neurons depend on the precise subcellular localization and distribution of ion channels in different compartments and at sites of synaptic communication (1, 2). Dendrites and spines are the primary sites of synaptic input and express several ligand- and voltage-gated ion channels. Among those are the transient, fast-inactivating A-type channels. The density of A-type channels increases at least 5-fold from the soma to about 350 μm in dendrites (3) and appears to be high in the oblique dendrites as well. The A-type current plays an important role in regulating dendritic excitability by reducing the amplitude of excitatory postsynaptic potentials and back propagating action potential and by regulating the induction and expression of long term potentiation. These A-type channels are also modulated by a variety of neurotransmitters such as norepinephrine, acetylcholine, and dopamine (4).

Pharmacological and molecular biological studies have implicated Kv4.2 as a key subunit of the A-type channel (5). Biochemical studies have demonstrated the phosphorylation of Kv4.2 by various kinases in agreement with functional studies; for example, phosphorylation of Kv4.2 by CaMKII (12) was shown to increase the surface expression of Kv4.2 without affecting its biophysical properties (6). Despite this observation, very little is known about how K⁺ channels, and in particular A-type channels, are targeted and expressed in dendrites and spines. In heterologous expression systems, K⁺ channels have been shown to interact with members of the membrane-associated guanylate kinase (MAGUK) family. In particular, interaction with PSD-95 has been shown to facilitate surface expression and clustering of the K⁺ channels (7–9). SAP97 and PSD-95 have been shown to exhibit distinct mechanisms for regulating the surface expression and clustering of Kv1-type channels. SAP97 is also of importance for the surface expression of Kv1.5 (10) and the inwardly rectifying channel Kir2.1 (11). In addition, SAP97 regulates biosynthesis and surface expression of AMPA receptors (12, 13). SAP97 localization to spines appears to be regulated by CaMKII (14). In particular, we have previously shown that CaMKII can in vivo phosphorylate SAP97 into two different residues, Ser-39 and Ser-232. These two residues are located in crucial domains responsible for regulating SAP97 protein trafficking as well as binding of SAP97 to interacting proteins (15).

Here, we have investigated the molecular details of Kv4.2 interaction with MAGUK proteins in organotypic slice cultures of the hippocampus and the role of this interaction for the subcellular localization of Kv4.2 in hippocampal neurons. In addition, we have found evidence for the SAP97-CaMKII pathway in regulating the subcellular localization of Kv4.2.

**EXPERIMENTAL PROCEDURES**

**DNA Constructs**—The enhanced green fluorescent protein Kv4.2 fusion (EGFP Kv4.2) has been described previously (16). A truncation mutant of Kv4.2 lacking the entire C terminus was constructed by introducing a stop codon at position 409 of Kv4.2. The EGFP Kv4.2 fusion construct and the EGFP Kv4.2 truncation mutant lacking the C terminus were subcloned into the pSinRep5 (17; Invitrogen). Both EGFP fusion constructs...
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were expressed at similar expression levels (data not shown). The Kv4.2 IRES constitutively active calmodulin-dependent kinase II α (IRES tCaMKII) was generated by transferring the IRES tCaMKII construct kindly provided by Y. Hayashi (Massachusetts Institute of Technology) into EGFP Kv4.2 in pSinRep 5. The integrity of these constructs was verified by automatic sequencing.

Antibodies—The monoclonal antibody to Kv4.2 (K57/1) was purchased from Neuromab and has been described previously (18). The monoclonal antibody was developed by and/or obtained from the University of California, Davis, NINDS/ NIMH NeuroMab Facility. Monoclonal antibody to synaptophysin was purchased from Roche Applied Science; mouse αCaMKII antibody was purchased from Chemicon International, Inc. (Temecula, CA); polyclonal SAP97 antibody, polyclonal SAP102 antibody, and monoclonal PSD-95 antibody were purchased from Affinity BioReagents Inc. (Golden, CO); monoclonal SAP97 antibody was purchased from StressGen (Victoria, British Columbia, Canada); polyclonal anti-GFP and AlexaFluor 488, 555, 568, and 633 secondary antibodies were purchased from Molecular Probes (Eugene, OR).

Neuronal Culture and Transfection—Hippocampal neuronal cultures were prepared from embryonic day 18 to 19 rat hippocampi as described previously, with minor modifications (19). Neurons were transfected using the calcium phosphate precipitation method at 7 days in vitro (DIV).

Organotypic Slice Cultures and Sindbis Virus Infections—Organotypic slice cultures were prepared from postnatal day 7 (P7) animals and cultured on Millicell inserts (Millipore) as described previously in detail (17). Typically, slice cultures were infected after 5–7 DIV. Sindbis pseudovirions were prepared using a modified less cytotoxic variant. The cDNAs were subcloned into a modified form of pSinRep5 and packaged using the DHBB helper. Pseudovirions were harvested typically after 72 h and used to focally infect the slice cultures as described previously (17).

Lentiviral RNA Interference of SAP97—For the siRNA expressing lentivirus vector, an RNAi stem-loop (12) has been cloned in the lentivirus-based vector pLL3.7 (20; Massachusetts Institute of Technology Center for Cancer Research, Cambridge), and an empty pLL3.7 vector has been used to generate the control lentivirus vectors. The lentiviral infecting particles were prepared as described previously (21). Neurons were infected with SAP97i-lentivirus or control virus at DIV6, and TIF preparation was performed at DIV10.

Subcellular Fractionation—TIF were isolated from neurons harvested at 10–14 DIV or hippocampal slices as described previously (15). PSDs from rat hippocampus were purified as described previously (22).

COS-7 Cell Culture and Transfection—COS-7 cells at 20–50% confluence (24 h after plating on glass coverslips in 12-well plates) were transfected by Superfect® transfection reagent (Qiagen, Valencia, CA) with cDNA expression constructs (1–1.5 μg of DNA/well) for 3 h at 5% CO2, 37 °C. Cells were washed twice with phosphate-buffered saline, fed with Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum, and 1% penicillin/streptomycin, and grown for 24–48 h before fixation for immunocytochemistry or before metabolic labeling experiments. Mutated products were obtained by using the QuickChange™ site-directed mutagenesis kit (Stratagene, La Jolla, CA).

Immunofluorescence Labeling, Image Acquisition, Quantification, and Statistical Analysis—Hippocampal neurons were fixed in 100% methanol at −20 °C for 15 min. Primary and secondary antibodies were applied in GDB buffer (30 mM phosphate buffer (pH 7.4) containing 0.2% gelatin, 0.5% Triton X-100, and 0.8 M NaCl). Confocal images were obtained using a Nikon ×60 objective with sequential acquisition setting at 1024 ×1024 pixel resolution. Each image was a 2 series projection of −8–12 images taken at 0.5–1-μm depth intervals. Transfected COS-7 cells, transfected neurons, and pharmacologically treated neurons were chosen randomly for quantification from two to five coverslips from three to five independent experiments. Quantification of confocal experiments was performed using Lasershop software (Bio-Rad). Image acquisition, quantification of the fluorescence signal, and co-localization analysis were performed by investigators who were ‘blind’ to the experimental condition. Quantification of Western blot analysis was performed by means of computer-assisted imaging (Quantity-One® System; Bio-Rad), and statistical evaluations were performed according to one-way analysis of variance followed by Bonferroni as post hoc comparison test; if the experiment includes only two experimental conditions, paired Student’s t test was used. All data are presented as mean ± S.E. and, if not indicated otherwise, as percentage of control derived from three to six independent experiments.

Cloning, Expression, and Purification of GST Fusion Protein—SAP97 fragments were subcloned downstream of gluthathione S-transferase (GST) in the BamHI and HindIII sites of the expression plasmid pGEX-KG by PCR using plaque-forming unit polymerase (Promega). The inserts were fully sequenced with the ABI Prism 310 genetic analyzer (ABI Prisma). SAP97-GST fusion proteins were expressed in Escherichia coli, purified on glutathione-agarose beads (Sigma), and eluted as described previously (23).

Pulldown Assay—Aliquots of PSD containing 10 μg of proteins were diluted with Tris-buffered saline, 0.1% SDS to a final volume of 200 μl and incubated (1 h, 37 °C) with glutathione-agarose beads saturated with GST fusion proteins or GST alone. The beads were extensively washed with Tris-buffered saline, 0.1% Triton X-100. Bound proteins were resolved by SDS-PAGE and subjected to immunoblot analysis with a monoclonal Kv4.2 antibody.

RESULTS

SAP97 Co-localization and Interaction with Kv4.2 from Rat Hippocampus—We first examined the relative abundance of voltage-gated potassium channel Kv4.2 in rat hippocampal subcellular compartments by means of a biochemical fractionation method, as described previously (15). Postsynaptic densities (PSD) were purified from rat hippocampus, and the expression of Kv4.2 as well as of pre- and postsynaptic markers was investigated in various subcellular compartments through Western blotting analysis. As shown in Fig. 1a, Kv4.2 channel was enriched in the PSD fraction and in the Triton-insoluble “PSD-enriched” fraction (TIF). Kv4.2 was present with a similar
distribution in the total homogenate and in the crude membrane fraction (P2) and at a low level in the synaptosomal membrane and in the low speed supernatant (S1) fractions. In the same samples, we examined the subcellular distribution of synaptophysin (presynaptic marker), αCaMKII, PSD-95, SAP102, and SAP97. As expected, synaptophysin was present in all subcellular compartments analyzed but not in the PSD and in the TIF-purified fractions, whereas PSD-95 and αCaMKII have a similar distribution pattern being enriched in synaptosomes and PSD fractions (Fig. 1a). The partition pattern of Kv4.2 channel was similar to that of SAP97 and SAP102.

Previous works described the presence of a specific binding between Kv4.2 and PSD-95 (7, 8). Because we found a comparable subcellular localization of Kv4.2 with both SAP97 and SAP102, we investigated whether Kv4.2 forms complexes with different members of the MAGUK protein family. First of all, co-immunoprecipitation experiments were performed from homogenate of rat hippocampus (Fig. 1b). As shown in Fig. 1b (top panel), homogenates were incubated with antibodies directed against PSD-95, SAP97, and SAP102, and the precipitates were probed with the Kv4.2 antibody. Anti-SAP97 and anti-PSD-95, but not anti-SAP102, co-immunoprecipitated Kv4.2 (Fig. 1b, top panel); the absence of any Kv4.2 signal in the No Ab lane tends to exclude that in our experimental conditions, and the co-precipitation of Kv4.2 reflects an unspecific immunoprecipitation of insoluble proteins. Accordingly, anti-Kv4.2 was able to co-precipitate SAP97 and PSD-95 but not SAP102 (Fig. 1b, bottom panel). Furthermore, we performed Kv4.2-MAGUK co-immunoprecipitation experiments from different subcellular fractions, i.e. synaptosomes and PSD to evaluate the capability of SAP97 and PSD-95 to bind Kv4.2 in specific subcellular compartments. Fig. 1c (top panel) shows that, in all tested compartments, Kv4.2 co-precipitates with SAP97 suggesting the subsistence of the binding between the proteins also at synaptic sites. On the other hand, the PSD-95-Kv4.2 interaction was more pronounced in the PSD fraction (Fig. 1c, bottom panel).

It is known that PSD-95 binding to Kv4.2 requires the VSAL motif at the C-terminal domain of the channel indicating a PDZ-mediated interaction (7, 8). To determine which SAP97 domain is involved in the association to Kv4.2, fusion proteins of GST with SAP97-N terminal domain-(7–223), SAP97-PDZ1-(217–318), SAP97-PDZ3-(465–545), and SAP97-SH3-(580–650) were prepared and incubated with aliquots of PSD purified from rat hippocampi for a pulldown assay. Only GST-
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**FIGURE 2.** **Kv4.2-SAP97 interaction in virally infected organotypic slices.** a, organotypic slices were infected with a modified Sindbis virus expressing EGFP-SAP97, EGFP-PSD-95 (left panel), or EGFP-SAP97 ΔPDZ (right panel) constructs, and homogenates were immunoprecipitated (i.p.) by means of an EGFP antibody. Western blotting (WB) for Kv4.2 was performed in the immunocomplex. b, organotypic slices were infected with a virus expressing EGFP-Kv4.2 or EGFP-Kv4.2 C-terminal deletion construct. Co-immunoprecipitation assay was performed in the homogenate using an EGFP antibody. Western blotting analysis was performed for SAP97 in the co-immunoprecipitated material. No binding of SAP97 to the Kv4.2 2168 deletion construct was observed. c, organotypic slices were infected with virus expressing EGFP-Kv4.2 in the absence or presence of active truncated αCaMKII (1-290).

SAP97-PDZ1 and GST-SAP97-PDZ3 were able to pull down Kv4.2, and neither GST alone nor other SAP97 domains tested showed positive results (Fig. 1d).

Interaction between SAP97 and Kv4.2 in Virally Infected Organotypic Slices—To further confirm a Kv4.2/SAP97 interaction, active αCaMKII (1–290 truncation) was co-transfected with SAP97 and Kv4.2 (Fig. 2a). The co-immunoprecipitation assay showed the presence of Kv4.2-SAP97 co-precipitation also in presence of the active form of the kinase (Fig. 2c), suggesting that the constitutively active CaMKII does not qualitatively alter the interaction between Kv4.2 and SAP97.

**SAP97-mediated Trafficking of Kv4.2 in Cultured Hippocampal Neurons**—To address the role of SAP97 in modulating Kv4.2 localization to the postsynaptic compartment, we infected primary hippocampal cultures with SAP97 RNAi expressing lentivirus. Nearly 100% of neurons in culture can be infected with lentivirus with minimal cytotoxicity (data not shown); the effect of SAP97 RNAi could therefore be quantified through a biochemical approach across the entire population of neurons in culture. TIF was obtained from control and SAP97 RNAi neurons (15, 24), and protein levels were measured in the homogenate and TIF. The same amount of proteins from homogenate and TIF was loaded on the SDS-PAGE for Western blot analysis. Compared with empty virus infection at the same viral titer, lentivirus expressing SAP97 RNAi caused profound and specific knockdown of SAP97 as shown by immunoblotting of total homogenate of hippocampal cultures (Fig. 3a). As shown in Fig. 3a, siRNA knockdown of SAP97 leads to significant reduction in Kv4.2 localization in the postsynaptic compartment (p < 0.05, −30.0 ± 7.4% SAP97 RNAi versus control) suggesting a specific role for SAP97 in the correct Kv4.2 localization at synaptic sites. No alterations of SAP102 or αCaMKII levels were observed in total lysates as well as in TIF fractions, confirming the specificity of SAP97 RNAi knockdown (Fig. 3a).

Recent observations indicate that activation of ryanodine receptors in the hippocampus, through caffeine treatment, can play a role in synaptic plasticity events by means of elevation of CaMKII activity (14), addressing CaMKII as a potential enzymatic target of the calcium-induced calcium release from ER ryanodine stores. Interestingly, calcium-induced calcium release from ryanodine receptors, as induced by caffeine treatment, has been shown necessary to trigger CaMKII-dependent action.
SAP97 trafficking (14, 24). Based on these observations, we tested whether caffeine treatment (10 mM, 15 min) was sufficient to induce not only SAP97 but also Kv4.2 trafficking from the ER. These experiments were performed in the presence of D-2-amino-5-phosphopentanoic acid (APV) to block NMDA receptors as a source of extracellular calcium. As shown in Fig. 3b, caffeine treatment significantly increased Kv4.2 immunostaining in TIF without affecting the total Kv4.2 protein level in the homogenate (*, p < 0.01; +60.2 ± 13.1%, caffeine versus control expressed as Kv4.2 ratio TIF/homogenate). As expected, treatment of hippocampal cultures with caffeine also leads to a higher staining of SAP97 in TIF (*, p < 0.01; +51.1 ± 8.3%, caffeine versus control expressed as SAP97 ratio TIF/homogenate) confirming previous observations (14). On the other hand, no significant effect of caffeine treatment on αCaMKII, SAP102 and PSD-95 localization in the TIF was found (Fig. 3b).

We have very recently described that treatment of hippocampal neurons with ryanodine (100 μM), to block calcium-induced calcium release from intracellular ER stores, and with a brief pulse of NMDA (50 μM), to promote a raise in calcium levels into the postsynaptic compartment, does not influence SAP97-Ser-39 phosphorylation and consequently does not modify SAP97 subcellular distribution (24). Based on these data, we treated primary hippocampal neurons with NMDA + ryanodine to check whether not only SAP97 but also Kv4.2 localization was unaffected by this experimental treatment. As shown in Fig. 3c, no significant effect of both SAP97 (p < 0.05; −3.7 ± 9.3%, NMDA + ryanodine versus control expressed as SAP97 ratio TIF/homogenate) and Kv4.2 (p > 0.05; −9.1 ± 13.2%, NMDA + ryanodine versus control expressed as Kv4.2 ratio TIF/homogenate) localization in the TIF was induced by NMDA + ryanodine treatment.

To further study the role of SAP97-CaMKII pathway on subcellular localization of native Kv4.2 in neurons, we analyzed by confocal microscopy the effect of caffeine treatment on the endogenous distribution of Kv4.2 in primary hippocampal cultures. It has been shown that Kv4.2 channels in neurons, particularly in dendrites, are preferentially localized in the cell soma; immunoreactivity was also detectable in dendrites where a diffuse staining was present. Interestingly, caffeine (Fig. 3d, right panel) was able to affect Kv4.2 immunostaining leading to a higher signal in the dendritic shafts paralleled to a decreased immunofluorescence in the cell soma. Co-immunoprecipitation experiments were performed to check the possible modulation of the MAGUK-Kv4.2 interaction by caffeine. A significant increase of SAP97-Kv4.2 co-precipitation was found (Fig. 3e) both in homogenate (*, p < 0.05; +30.2 ± 6.9%, caffeine versus control) and TIF (*, p < 0.01; +76.1 ± 10.5%, caffeine versus control expressed as SAP97 ratio TIF/homogenate) confirming previous observations (14). On the other hand, no significant effect of caffeine treatment on αCaMKII, SAP102 and PSD-95 localization in the TIF was found (Fig. 3b).
versus control) confirming the hypothesis of a Kv4.2 SAP97-mediated trafficking induced by caffeine (Fig. 3e). PSD-95-Kv4.2 complex was detected in the TIF only after caffeine treatment, confirming the increased localization of Kv4.2 in the postsynaptic compartment. No PSD-95-Kv4.2 interaction was found in the homogenate under these experimental conditions (Fig. 3e). No interaction of SAP102 to Kv4.2 was found confirming the specificity of the binding of SAP97 and PSD-95 to the channel (Fig. 3e).

**CaMKII-dependent Phosphorylation of SAP97-Ser-39 Affects Kv4.2 Trafficking**—A co-localization assay in COS-7 cells was used to study interactions of wild type and mutated forms of SAP97 and Kv4.2 in intact cells and to check whether CaMKII phosphorylation of SAP97 and Kv4.2 phospho-sites could affect Kv4.2-SAP97 protein-protein interaction. In single transfections, both Kv4.2wt (Fig. 4a) and SAP97wt (Fig. 4b) showed a characteristic perinuclear accumulation as reported previously (15, 28). Co-transfection of SAP97wt and Kv4.2wt (Fig. 4, f–h) displayed a high co-localization value between the two proteins (87.1 ± 6.3%) confirming an elevated affinity of Kv4.2 channel for SAP97, as described above.

Recent data showed that CaMKII-dependent SAP97 Ser-39 phosphorylation, induced by caffeine treatment, regulates the subcellular localization of SAP97, providing a fine molecular mechanism responsible for the synaptic delivery of SAP97 itself as well as SAP97-interacting proteins (14, 15). Based on these observations, we co-transfected in COS-7 cells Kv4.2wt with SAP97(S39D) mutation construct mimicking CaMKII-dependent SAP97 Ser-39 phosphorylation (Fig. 4, c–e). Co-expression of Kv4.2wt with SAP97(S39D) caused a redistribution of Kv4.2 channel staining throughout the cell with a concomitant reduction of the strong fluorescence around the nucleus. Thus, SAP97 mutant mimicking the CaMKII phospho-site appears to facilitate release of the Kv4.2 channel from the internal compartments and recruitment toward the cell surface, without affecting SAP97 clustering with Kv4.2. Conversely, co-transfection of Kv4.2 with SAP97 (S39A) showed a more

![FIGURE 4. SAP97 modulates Kv4.2 localization in transfected COS-7 cells.](image-url)
observations in COS-7 cells (see Fig. 4, f–h) indicating a high co-localiza-
tion between the two wild-type pro-
teins without any specific effect on
Kv4.2 distribution. On the other
hand, hippocampal neurons trans-
fected with GFP-SAP97 S39D
showed an increased staining of
Kv4.2 in “spine-like” structures
where the Kv4.2 channel co-local-
izes precisely with the S39D mutant
form of SAP97 (14) (Fig. 5c). In addi-
tion, quantification of the dendritic
shaft versus somatic signal of Kv4.2,
measured by the relative fluores-
cence intensity within these struc-
tures, revealed that GFP-SAP97
S39D transfected neurons displays
an increased Kv4.2 fluorescent sig-
nal in dendritic structures (Fig. 5, c
and d, p < 0.005, GFP-SAP97 S39D
versus untransfected). Conversely,
transfection of GFP-SAP97 S39A
(Fig. 5a) resulted in a redistribution
of Kv4.2 signal toward the cell soma
with a decreased dendritic staining
(Fig. 5d, **, p < 0.001, GFP-SAP97
S39A versus untransfected) suggest-
ning that CaMKII phosphorylation
of SAP97-Ser-39 phospho-site can be
necessary for synaptic trafficking of
SAP97 interacting proteins, such as
Kv4.2.

DISCUSSION

The somatodendritic A-type
potassium current, I_{sA}, is particu-
larly important for regulating action potential back propaga-
tion as well as the local excitability of the dendrite. Modula-
tions in the expression and functional properties of the I_{sA}
current have been observed during long term potentiation
and epilepsy, and increasing evidence suggests that abnor-
mal regulation of these channels may be acquired during
epilepsy (4), leading to an increased excitability of the
pyramidal neuron dendrite that contributes to the initiation
and prolongation of seizures. It is thereby of importance
to understand the regulatory mechanism underlying the traf-
icking of key constituents of I_{sA}, such as Kv4.2, into
dendrites and spines.

Here we show that Kv4.2 is enriched in rodent purified PSDs
by means of SAP97-mediated trafficking from the ER. Indeed,
we have observed that Kv4.2 interacts with SAP97 and PSD-95,
although in different cell compartments suggesting the binding
with SAP97 as being instrumental for SAP97 trafficking to and
with PSD-95 for localization in the postsynaptic compartment.
Based on co-immunoprecipitation experiments, the interac-
tion of Kv4.2 with SAP97 appears to require an intact C termi-
nus of Kv4.2. In GST pulldown assays, we have identified the

pronounced perinuclear accumulation of the two proteins (Fig.
4, i–k). No effect on Kv4.2 distribution was observed by mutation
into aspartate of the two Kv4.2 CaMKII-dependent phospho-sites
(6) both in Kv4.2 single transfections (data not shown) and in co-
transfection with SAP97wt (Fig. 4, l–n). No significant effect on the
degree of co-localization between the two transfected proteins
was produced by any mutation constructs used, confirming the idea
that CaMKII phosphorylation of Kv4.2 or SAP97 does not affect
the co-localization between the two proteins.

To confirm the results obtained in transfected COS-7 cells
and to address the role of SAP97 Ser-39 phosphorylation in
modulating Kv4.2 localization in neurons, we transfected pri-
mary hippocampal cultures with GFP-SAP97wt, GFP-SAP97-
S39D, or GFP-SAP97-S39A constructs (Fig. 5, a–d) (14, 15).
Interestingly, SAP97 staining overlapped Kv4.2 distribution in
cultured hippocampal neurons (Fig. 5b); co-localization analy-
sis revealed a high co-localization pattern between the two pro-
teins (76.0 ± 9.2%). Analysis of endogenous Kv4.2 staining in
neurons transfected with GFP-SAP97wt revealed no major dif-
fences compared with untransfected cells (data not shown;
for quantification see Fig. 5d) in agreement with previous

FIGURE 5. Kv4.2 localization in neurons is influenced by SAP97 Ser-39 phosphorylation. Hippocampal
cultures were either left untransfected (b), transfected with constructs for GFP-SAP97(S39A) (a) or GFP-
SAP97(S39D) (c), fixed, and stained. Transfection of SAP97(S39A) determines a different spatial distribution
of Kv4.2 with a marked staining of cell soma compared with untransfected neurons. Conversely, an increased
dendritic Kv4.2 signal is found as consequence of SAP97(S39D) transfection; scale bar, 10 μm. Representative
dendrite at higher magnification is present on the bottom of each panel. d, quantification of experiments
shown in a–c. To this aim, the ratio of dendrites to cell soma average fluorescence was computed and averaged
(*, p < 0.005 versus controls; **, p < 0.001 versus control).
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PDZ domains of SAP97 as being a key constituent for the binding to Kv4.2. We have previously shown that CaMKII phosphorylates SAP97 and causes an enrichment of SAP97 in the postsynaptic compartment (14, 15). In addition, it has been demonstrated that CaMKII phosphorylation of Kv4.2 increases the surface expression of Kv4.2 in fibroblasts or cultured hippocampal neurons without altering its biophysical properties (6). Interestingly, here we show that CaMKII-dependent phosphorylation of Kv4.2 in the two previously identified sites (6) does not affect Kv4.2 interaction with SAP97 as well as Kv4.2 distribution in transfected cells.

Interestingly, knockdown of SAP97 significantly reduces the localization of Kv4.2 in the Triton-insoluble postsynaptic compartment addressing a specific role of SAP97 in driving Kv4.2 to synaptic sites. Using site-specific mutants of SAP97 mimicking either its phosphorylation in Ser-39 by CaMKII or abolishing it, we show that the SAP97 Ser-39 phosphorylation increased the presence of endogenous Kv4.2 in dendrites compared with wild type, whereas the mutant with abolished phosphorylation site decreased it.

In addition, we dissected the intracellular biochemical pathways governing trafficking of endogenous Kv4.2 from the ER. In particular, treating neurons with caffeine, a pharmacological tool capable of activating ER-associated CaMKII and consequently driving the exit of SAP97 from the ER (14), we saw an increase in the staining of Kv4.2 in dendrites, as well as its level in a TIF fraction, resembling the composition of the postsynaptic density. On the other hand, a pharmacological strategy in a TIF fraction, resembling the composition of the postsynaptic density, is capable of activating ER-associated CaMKII and consequently addressing a possible differential role of the two isoforms of SAP97 and PSD-95. Although the isoforms influence AMPA receptor-dependent trafficking and distribution of endogenous Kv4.2 in transfected fibroblasts by co-expressed PSD-95, but we did not notice any apparent differences in the localization of endogenous Kv4.2 upon expression of PSD-95 in transfected neurons. Our co-immunoprecipitation experiments clearly suggest the presence of a PSD-95-Kv4.2 complex only at synaptic sites, suggesting that SAP97 is a key protein responsible for Kv4.2 trafficking, whereas PSD-95 represents an important anchoring element for Kv4.2 once the protein has been delivered at membrane/synaptic compartment.

Finally, our study has clarified the molecular details of Kv4.2 interaction with MAGUK proteins in hippocampal neurons addressing a key role of CaMKII in the regulation of SAP97-mediated localization of Kv4.2 at synaptic sites.

Expression of PSD-95 did not change the localization of Kv4.2 in dendrites or spines in cultured neurons (data not shown). A previous report had suggested clustering of Kv4.2 in transfected fibroblasts by co-expressed PSD-95, but we did not notice any apparent differences in the localization of endogenous Kv4.2 upon expression of PSD-95 in transfected neurons. Our co-immunoprecipitation experiments clearly suggest the presence of a PSD-95-Kv4.2 complex only at synaptic sites, suggesting that SAP97 is a key protein responsible for Kv4.2 trafficking, whereas PSD-95 represents an important anchoring element for Kv4.2 once the protein has been delivered at membrane/synaptic compartment.

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