HHS Public Access

Author manuscript

Nat Neurosci. Author manuscript; available in PMC 2011 March 01.

Published in final edited form as:

Nat Neurosci. 2010 September ; 13(9): 1053–1055. doi:10.1038/nn.2613.

A calcineurin/AKAP complex is required for NMDA receptor-dependent LTD

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Abstract

AKAP79/150 is a protein scaffold thought to position specific kinases (PKA, PKC) and phosphastases (calcineurin) in appropriate synaptic domains so that their activities can regulate excitatory synaptic strength. Using a viral-mediated molecular replacement strategy in rat hippocampal slices, we found that AKAP is required for NMDA receptor-dependent LTD solely due to its interaction with calcineurin.

Genetic, molecular and pharmacological manipulations have provided support for a critical role of AKAP79/150 (A-kinase anchoring protein) in regulating excitatory synaptic transmission and plasticity but the molecular mechanisms by which this occurs are confusing1-5. Using a lentivirus-mediated molecular replacement strategy targeting PSD-95, it was recently demonstrated that an AKAP150/PSD-95 complex is required for NMDA receptor (NMDAR)-dependent LTD as well as NMDAR-triggered endocytosis of AMPA receptors (AMPARs)6,7. Using the same strategy we addressed the roles of endogenous AKAP and its binding to calcineurin (PP2B), PKA and PKC in regulating basal synaptic transmission and several prominent forms of synaptic plasticity.

We found that expression of a highly effective short-hairpin RNA to AKAP150 (shAKAP) (Supplementary Fig. 1) in a subpopulation of CA1 pyramidal cells in rat hippocampal slice cultures (Fig. 1) produced a modest but significant increase in evoked AMPAR-mediated EPSCs (AMPAR EPSCs) when compared to neighboring, simultaneously recorded uninfected cells (Fig. 1a**:** uninfected −43.2 ± 4.2 pA, infected −58.4 ± 6.8 pA; n = 19 pairs). In contrast, NMDAR-mediated EPSCs (NMDAR EPSCs) were unaffected (Fig. 1a**:** uninfected 25.7 ± 7.1 pA, infected, 28.2 ± 5.8 pA; n = 13 pairs). Next, we examined

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AUTHOR CONTRIBUTIONS

V.B. generated and tested all of the molecular reagents. S.J. performed and analyzed all of the electrophysiology experiments. S.J. and R.C.M. designed the experiments, interpreted the results and wrote the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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NMDAR-dependent LTD (Supplementary Fig. 2) by performing experiments that were conducted and analyzed blindly without knowledge of whether cells expressed GFP alone or shAKAP with GFP. Acute knockdown of AKAP blocked LTD whereas control cells exhibited robust LTD (Fig. 1c,e,f; control 50 ± 2 % of baseline, n = 7; shAKAP 110 ± 10 %; n= 7). This block of LTD was unlikely to be due to an effect of shAKAP on NMDARs since NMDAR EPSCs were unaffected and cannot be attributed to occlusion since AMPAR EPSCs were enhanced by shAKAP, not reduced. Importantly, the block of LTD by shAKAP as well as the increase in AMPAR EPSCs were rescued by simultaneous expression of recombinant AKAP150 fused to GFP (Supplementary Fig. 3), experiments that we again performed blindly (Fig. 1b; AMPAR EPSC, uninfected −48.0 ± 8.5 pA, infected −52.5 ± 8.7 pA, n = 14 pairs; NMDAR EPSC, uninfected 39.5 ± 5.9 pA, infected, 31.8 ± 5.4 pA, n = 13 pairs; Fig. 1d,g,h; control LTD, 48 ± 8 %, n = 6; shAKAP+AKAP-GFP, 47 ± 11 %, n = 7).

We also examined the consequence of knocking down AKAP on two other prominent forms of synaptic plasticity: mGluR-dependent LTD (mGluR LTD) and NMDAR-dependent LTP. Expression of shAKAP had no detectable effect on mGluR LTD, elicited by application of the group I agonist 3,5-dihydroxyphenylglycine (DHPG, 25 μM) in the presence of D-AP5 (50 μM) (Fig. 1i-k; control 45 ± 5 %, n = 4; shAKAP 48 ± 8 %, n = 4). To examine LTP, we injected the shAKAP-expressing lentivirus stereotaxically into the hippocampus and prepared standard acute slices 7-10 days later (see **Supplementary Methods**). Expression of shAKAP had no effect on LTP when compared to control cells recorded from the same sets of slices (Fig. 1l,m; control, 212 ± 10 %, n = 6; shAKAP 200 ±8 %, n = 7). These results suggest that AKAP is required specifically for NMDAR-dependent LTD and not other forms of plasticity.

To examine which AKAP-binding partners are required for LTD, we simultaneously expressed shAKAP with AKAP mutants known to specifically disrupt the binding of either PP2B, PKA, or PKC (Supplementary Fig. 3 and Fig. 2). In contrast to wildtype AKAP (Fig. 1b), we found that expressing an AKAP mutant with a truncation of the PP2B binding domain8,9 did not rescue the enhancement of AMPAR EPSCs (Fig. 2a; uninfected −39.8 ± 6.5 pA, infected −63.5 ± 7.3 pA, n = 13 pairs) and that this manipulation still had no effect on NMDAR EPSCs (Fig. 2a; uninfected 42.3±7.4 pA, infected, 45.7 ± 5.8 pA, n = 12 pairs). Furthermore, unlike wildtype AKAP, the PP2B-binding mutant did not rescue LTD (Fig. 2c,e,f; control 48 ± 4 %, n = 6; shAKAP+ PP2B-GFP 95 ± 5 %, n = 7). The synaptic targeting of the mutant AKAP in dissociated cultured neurons, as defined by co-localization with the presynaptic active zone protein Bassoon, was indistinguishable from wildtype AKAP (Supplementary Fig. 4). These results suggest that PP2B bound to AKAP is required for LTD and also plays a role in tonically inhibiting basal AMPAR-mediated synaptic transmission.

In contrast to the lack of effects of expressing AKAP PP2B, the two AKAP mutants, which disrupt the binding to either PKA or PKC1,2,9.10 (PKA-GFP and PKC-GFP respectively; Supplementary Fig. 3**; Supplementary Methods**) behaved identically to wildtype AKAP. They both successfully rescued basal AMPAR-mediated transmission as well as LTD (Fig. 2b,d, g-l; **shAKAP+ PKA-GFP**: AMPAR EPSC, uninfected −50.8 ± 4.9 pA, infected −55.7 ± 6.2 pA, n = 10 pairs; NMDAR EPSC, uninfected 54.4 ± 8.1 pA,

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infected, 51.3 ± 7.9 pA, n = 10 pairs; LTD, control 48 ± 7 %, n = 7; shAKAP+ PKA-GFP 55 ± 8 %, n = 8. **shAKAP+ PKC-GFP**: AMPAR EPSC, uninfected −38.2 ± 6.0 pA, infected −37.5 ± 5.7 pA, n = 10 pairs; NMDAR EPSC, uninfected 42.9 ± 8.7 pA, infected, 41.2 ± 5.3 pA, n = 10 pairs; LTD control 49 ± 3 %, n = 7; shAKAP+ PKC-GFP 43 ± 4 %, n = 7). Again, we performed all experiments blindly without knowledge of the form of AKAP that was expressed in individual cells.

By replacing endogenous AKAP in single cells with AKAP mutants, we have demonstrated that AKAP binding solely to PP2B is required for NMDAR-dependent LTD and also plays a role in tonically inhibiting basal synaptic strength. Our results are consistent with previous findings that AKAP knockout mice lack LTD in hippocampal slices3 and that PP2B bound to AKAP regulates AMPAR-mediated whole cell currents in cultured neurons9. Although it has been suggested that PKA bound to AKAP tonically regulates basal synaptic strength1,2,11,12 and influences both LTD and LTP4,5, our results do not support these conclusions. They are instead consistent with the hypothesis that PKA spatial distribution is imposed by MAP2 within dendritic shafts and that PKA is translocated to spines during LTP induction protocols13. An attractive hypothesis consistent with prior results6,7,14 is that in the basal state synapses contain a pool of largely inactive PP2B bound to AKAP15. During the induction of LTD, AKAP binds to PSD-95 causing the release of PP2B which helps trigger an enhancement of synaptic AMPAR endocytosis. Subsequently, AKAP may leave the spine, a step that could contribute to shrinkage of spines10 that accompanies LTD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We thank members of the Malenka lab for constructive comments on the manuscript and during the course of the experiments. We especially thank Debanjan Goswami for performance of the experiments shown in Supplementary Figure 4. This work was supported by a gift from the Morby Charitable Fund for Alzheimer's Research and a grant from the US National Institutes of Health (MH63394). S.J. was supported by a Postdoctoral Fellowship from the Ramon Areces Foundation.

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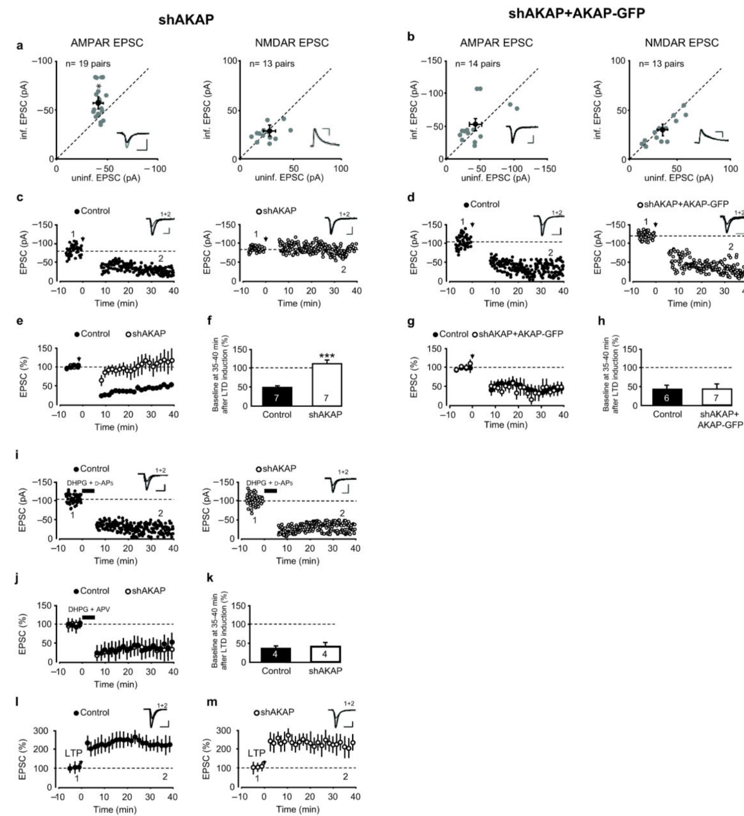

Figure 1.

AKAP knockdown enhances basal AMPAR EPSCs and blocks NMDAR-dependent LTD; effects that are rescued by simultaneous expression of wildtype AKAP-GFP. (**a**) Amplitudes of AMPAR EPSCs and NMDAR EPSCs expressing shAKAP (inf. EPSC) plotted as a function of the amplitudes of EPSCs simultaneously recorded from adjacent control cells (uninf. EPSCs). Bold symbols show mean ± s.e.m. (* indicates $p < 0.05$). In this and all subsequent panels, inserts show sample EPSCs (calibration bars: AMPAR EPSCs, NMDAR EPSCs; 50, 50 pA / 20, 50 ms). (**b**) Amplitudes of AMPAR EPSCs and NMDAR EPSCs from paired recordings of control neurons and neurons expressing shAKAP plus wildtype AKAP-GFP (calibration bars: AMPAR EPSCs, NMDAR EPSCs; 20, 50 pA / 10, 50 ms). Sample experiments (**c**) and summary graphs (mean ± s.e.m.) (**e,f**) of LTD in control cells (expressing GFP alone) and shAKAP expressing cells. (*** indicates $p < 0.001$). (calibration bars in c: left panel 20 pA/10 ms; right panel 40 pA/10ms). Sample experiments (**d**) and summary graphs (**g,h**) of LTD in uninfected control cells and cells expressing shAKAP plus AKAP-GFP (calibration bars in d: 20 pA / 20 ms). Sample experiments (**i**) and summary graphs (**j,k**) of mGluR LTD induced by DHPG (25 μM) application (in the presence of 50 μM APV) in uninfected control cells and shAKAP expressing cells (calibration bars in i: left panel, 25 pA / 20 ms; right panel, 50 pA / 10 ms). Summary graphs of LTP from control cells (**l**; n = 6) and shAKAP expressing cells (**m**; n = 7) (calibration bars: 10 pA / 20 ms).

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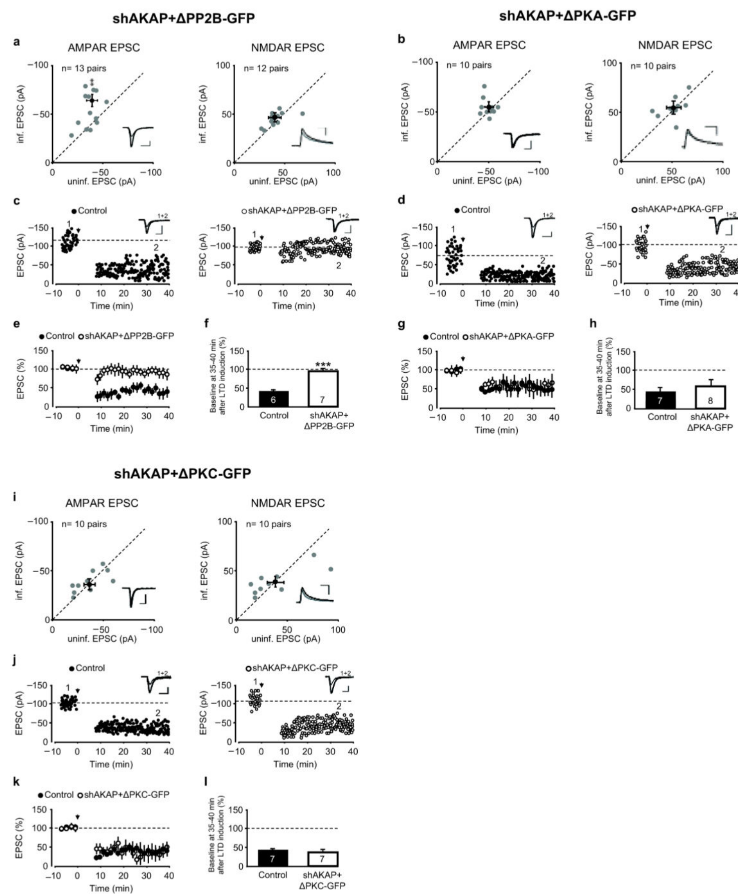

Figure 2.

Calcineurin (PP2B) binding to AKAP is required for NMDAR-dependent LTD. (**a**) Amplitudes of AMPAR EPSCs and NMDAR EPSCs from paired recordings of control neurons and neurons expressing shAKAP plus AKAP PP2B-GFP (* indicates $p < 0.05$; calibration bars: AMPAR EPSCs and NMDAR EPSCs: 10, 25 pA / 20, 50 ms). (**b**) Amplitudes of AMPAR EPSCs and NMDAR EPSCs from paired recordings of control neurons and neurons expressing shAKAP plus AKAP PKA-GFP (calibration bars: AMPAR EPSCs and NMDAR EPSCs: 30, 25 pA / 10, 50 ms). Sample experiments (**c**) and summary graphs (**e,f**) of LTD in control cells and cells expressing shAKAP plus AKAP PP2B-GFP (calibration bars in c: 60 pA / 10 ms). (*** indicates $p < 0.001$). Sample experiments (**d**) and summary graphs (**g,h**) of LTD in control cells and cells expressing shAKAP plus AKAP PKA-GFP (calibration bars in d: left panel 10 pA / 20 ms; right panel 60 pA / 10 ms) (**i**) Amplitudes of AMPAR EPSCs and NMDAR EPSCs from paired recordings of control neurons and neurons expressing shAKAP plus AKAP PKC-GFP (calibration bars: AMPAR EPSCs and NMDAR EPSCs: 30, 30 pA / 10, 50 ms). Sample experiments (**j**) and summary graphs (**k,l**) of LTD in control cells and cells expressing shAKAP plus AKAP PKC-GFP (calibration bars in j: left panel 25 pA / 20 ms; right panel 25 pA / 10 ms).