HHS Public Access

Author manuscript

FEBS Lett. Author manuscript; available in PMC 2017 September 01.

Published in final edited form as:

FEBS Lett. 2016 September ; 590(17): 2934–2939. doi:10.1002/1873-3468.12334.

CaMKII–mediated displacement of AIDA-1 out of the postsynaptic density core

Ayse Dosemeci[a,*], **Dana Toy**[a], **Amelia Burch**[a], **K. Ulrich Bayer**[b], and **Jung-Hwa Tao-Cheng**[c]

[a]Laboratory of Neurobiology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA

[b]Department of Pharmacology, University of Colorado Denver, School of Medicine, Aurora, CO

[c]EM Facility, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA

Abstract

Ankyrin repeat and sterile alpha motif domain-containing protein 1B (ANKS1B, also known as AIDA-1) is a major component of the postsynaptic density (PSD) in excitatory neurons where it concentrates at the electron-dense core under basal conditions and moves out during activity. The present study investigates the molecular mechanism underlying activity-induced displacement of AIDA-1. Experiments with PSD fractions from brain indicate phosphorylation of AIDA-1 upon activation of endogenous CaMKII. Immuno-electron microscopy studies show that treatment of hippocampal neurons with NMDA results in an ~30nm shift in the median distance of the AIDA-1 label from the postsynaptic membrane, an effect that is blocked by the CaMKII inhibitor tatCN21. CaMKII-mediated redistribution of AIDA-1 is similar to that observed for SynGAP. CaMKII-mediated removal of two abundant PSD-95 binding proteins from the PSD core during activity is expected to initiate a molecular reorganization at the PSD.

Keywords

AIDA-1; CaMKII; postsynaptic density

1. Introduction

The postsynaptic density (PSD) at the glutamatergic synapse is a large protein complex lining the postsynaptic membrane and contains neurotransmitter receptors, signaling and scaffold elements. At the electron-dense core of the PSD, PSD-95 and related MAGUKs form a scaffold that binds multiple components, including glutamate receptors of the NMDA and AMPA types as well as other major PSD components, GKAPs, SynGAP and AIDA-1 (for review see [1]). Changes in the amounts and organization of the molecules that make up the PSD are likely to underlie changes in synaptic efficacy.

*Corresponding Author: Ayse Dosemeci, 301-435-2795, dosemeca@mail.nih.gov.

ImmunoEM studies reveal that, during activity, certain proteins move into the PSD whereas others move out or redistribute within the PSD. The earliest finding was the accumulation of CaMKII to the deeper ‘pallial’ layer of the PSD [2]. Subsequent studies demonstrated that scaffold proteins of the Shank family and the deubiquitinase CYLD also move into the PSD [3], [4] while SynGAP moves out of the PSD core [5] under excitatory conditions. The movement of these components depends on the accumulation and/or activation of CaMKII [6], [7], [8], suggesting that this kinase is the upstream regulator of activity-induced re-organization at the PSD.

Short forms of AIDA-1 (AIDA1d and AIDA1e) are major components of the PSD and bind directly to PSD-95 through their C-terminal TTIF sequence [9]. Quantitative mass spectrometry studies estimated a molar ratio of about one AIDA-1 for every two PSD-95 molecules in isolated PSD fraction from brain [10]. ImmunoEM studies revealed a concentration of the protein within the dense core of the PSD in adult brain hippocampus and cerebral cortex [11]. Recent immunoEM studies using cultured hippocampal neurons indicated similar distribution and demonstrated that, under excitatory conditions, including application of NMDA and depolarization with high K^+, AIDA-1 moves out of the PSD core [12]. Considering its high stoichiometry and strategic positioning by PSD-95, redistribution of AIDA-1 is expected to promote profound changes in the organization of the PSD complex. In the present study we investigated the molecular mechanism of AIDA-1 movement under excitatory conditions, focusing on the potential involvement of CaMKII.

2. Materials and Methods

Antibodies

Two antibodies were used for AIDA-1. Antibody1 (Ab1), polyclonal rabbit antibody against the peptide LKRFPVHPVTGPR corresponding to the N-terminal of Q8BZM2 was custom-generated by Affinity Bioreagents. Antibody2 (Ab2), polyclonal rabbit antibody against the peptide RLHDDPPQKPPRSIT corresponding to residues 946–960 of ANS1B_HUMAN was obtained from Zymed (Cat # 36-7000). Rabbit polyclonal antibody for SynGAP (Cat # 06900) was from Millipore.

Preparation of PSD fractions, phosphorylation and Western immunoblotting

PSD fractions from cerebral cortex were prepared using brains from Sprague Dawley rats collected and frozen in liquid nitrogen within 2 min of decapitation by Pel-Freez Biologicals (Rogers, AR) as described previously [13]. To assess phosphorylation of AIDA-1 in isolated PSDs by endogenous CaMKII activity, PSD samples were pre-incubated in 0.1 M DTT for 1–2 h and then incubated for 20 min at 37°C in media containing 5 mM $MgCl_2$, 50 μg/ml leupeptin, 20 mM DTT, 0.8 μM microcystinLR in 20 mM HEPES pH 7.4 with or without 1 mM ATP, 1 mM CaCl2, 40 μg/ml calmodulin, 2.5 μM CN21 or 2.5 μM control peptide as indicated. The reaction was stopped by the addition of SDS-containing PAGE sample buffer. PSD samples were resolved by SDS-PAGE using 10% Mini PROTEAN TGX precast polyacrylamide gels (BioRad), transferred to PVDF membranes using the Trans-Blot Turbo Transfer System (BioRad), blocked, incubated with primary (1:5000 dilution for SynGAP

antibody and 1:500 dilution for AIDA-1 antibody1) and secondary antibodies. Immunoblots were visualized by chemiluminescence (BioRad).

Preparation and treatment of dissociated hippocampal cultures

The animal protocol was approved by the National Institute of Neurological Disorders and Stroke/National Institute of Deafness and Communication Disorders/National Center for Complementary and Integrative Health Animal Use and Care Committee and conforms to NIH guidelines. Hippocampi from 20–21 day embryonic Sprague-Dawley rats were dissociated and grown on a feeder layer of glial cells for 3 weeks as detailed before [14]. Experiments were carried out with culture dishes placed on a floating platform in a water bath maintained at 37°C. Incubation media contained 124 mM NaCl, 2mM KCl, 1.24 mM KH_2PO_4, 1.3 mM $MgCl_2$, 2.5 mM $CaCl_2$, 30 mM glucose in 25 mM HEPES at pH 7.4 with or without the addition of NMDA (50 μM), tatCN21 (20 μM) or control peptide (tatCtrl, 20 μM) as indicated. Cell cultures were pre-incubated for 20 min in basal medium with or without peptides and then exposed for 2 min to basal or NMDA-containing media.

Pre-embedding immunogold labeling and electron microscopy

Cells were fixed with 4% paraformaldehyde (EMS, Fort Washington, PA) in PBS for 25–35 min (35 min for AIDA1 antibody1 at 1:100 dilution and 25 min for AIDA1 antibody2 at 1:50 dilution), and thoroughly washed in PBS and stored at 4°C before immunolabeling. Samples were permeablized and blocked with 0.1% saponin and 5% normal goat serum in PBS for 30–60 min, incubated with primary and then secondary antibodies (Nanogold, Nanoprobes, Yaphand, NY) for 1 h each at room temperature, fixed with 2% glutaraldehyde in PBS and stored at 4°C. Samples were then washed in deionized water, silver enhanced (HQ kit, Nanoprobes), treated with 0.2% osmium tetroxide in 0.1M phosphate buffer at pH 7.4 for 30 min on ice, treated with 0.25% uranyl acetate in acetate buffer at pH 5.0 for 1 h at 4°C, dehydrated in graded ethanols, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate, examined in a JEOL 1200 electron microscope, and images were collected with a digital CCD camera (AMT XR-100, Danvers, MA, USA).

Morphometry

Asymmetric excitatory synapses were identified by clusters of synaptic vesicles in the presynaptic terminal, rigidly apposed pre- and post-synaptic membranes forming a synaptic cleft, and a characteristic dense material underneath the postsynaptic membrane. Every cross-sectioned synaptic profile labeled for AIDA-1 was photographed for morphometry and at least five randomly selected grid openings were examined for each sample.

The PSD complex was outlined by the postsynaptic membrane, two parallel lines dropped perpendicular to the postsynaptic membrane, and an arbitrary border 120 nm deep to the postsynaptic membrane [5]. As described before [5], the PSD complex was further divided into two compartments: (1) the PSD core, an area immediately beneath the postsynaptic membrane containing characteristic dense material with the cytoplasmic border arbitrarily set at 40 nm; (2) the PSD pallium, or the contiguous network, extending deeper into the cytoplasm.

The distance from the center of the gold particle to the outer edge of the postsynaptic membrane was measured for every particle in the marked PSD complex area and plotted into histograms. Because the laminar distribution of AIDA-1 under stimulated conditions was typically skewed, values for median instead of mean were used for a nonparametric statistical test (Wilcoxon test; KaleidaGraph, Synergy Software, Reading, PA).

Results

Redistribution of AIDA-1 under excitatory conditions reported previously [12] shows a striking similarity to that of SynGAP, another major PSD constituent that moves out of the PSD core during activity [5]. SynGAP is phosphorylated by CaMKII and CaMKII-mediated phosphorylation is necessary for its translocation [6], [15]. In order to test whether AIDA-1 is similarly phosphorylated upon activation of CaMKII at the PSD, in vitro experiments were carried out using PSD fractions.

As reported previously, isolated PSDs contain CaMKII, as well as other Ca^{2+}-dependent and -independent kinases [16], [17]. Incubation with ATP in the absence of Ca^{2+} promotes a change in the electrophoretic mobilities of SynGAP and of the two isoforms of AIDA-1 at ~50 KDa and ~60 KDa (Fig. 1, lane2 vs lane1) suggesting some phosphorylation by Ca^{2+}-independent kinases. However, in the presence of Ca^{2+} and calmodulin, a further shift in electrophoretic mobilities of these proteins is observed (Fig. 1, lane3) and this secondary shift is blocked by the inclusion of CN21, a peptide inhibitor specific for CaMKII [18] (Fig. 1, lane5). Thus, both AIDA-1 isoforms as well as SynGAP appear to be phosphorylated upon activation of CaMKII at the PSD.

In the next series of experiments by pre-embedding immunoEM, tatCN21, a membrane permeable version of the CaMKII inhibitor peptide [18], was used to investigate the involvement of CaMKII on the redistribution of AIDA-1 in cultured hippocampal neurons. Figure 2, upper panels are electron micrographs of synapses from hippocampal cultures immunogold labeled for AIDA-1. Histograms on the panels below show distribution of label from all synapses measured in a typical experiment (Experiment 3 on Table 1). As previously reported, under basal conditions (control), AIDA-1 immunolabel is concentrated within an area ~40 nm deep from the postsynaptic membrane that contains the electron-dense core of the PSD. Exposure to NMDA for 2 min promotes a dramatic shift in the localization of AIDA-1 label away from the postsynaptic membrane, with a significant fraction of the label localized outside the electron dense core. Although previous studies [12] established that, upon NMDA treatment, AIDA-1 label density decreases within the area 0–40 nm from the membrane and increases within the adjacent area 40–120 nm from the postsynaptic membrane, data is inconclusive on whether any label spills beyond the area of observation (0–120 nm from the postsynaptic membrane). Upon pre-incubation with the CaMKII inhibitor tatCN21, exposure to NMDA fails to promote movement of AIDA-1 out of the PSD core and the label distribution remains very similar to that in control cultures that were not exposed to NMDA.

The median distances of label from the postsynaptic membrane following four different treatment protocols (Control, NMDA, NMDA after pre-incubation with tatCN21 and

NMDA after pre-incubation with control peptide) were assessed in four experiments using two different antibodies (Table 1). NMDA promoted an average of 32 nm increase in the median distance of label, statistically significant in all four experiments. The NMDA-induced increase was blocked by the inclusion of CaMKII inhibitor tatCN21. As observed previously for the translocation of other proteins [7] control peptide had only a slight effect that may be attributed to the tat sequence.

Discussion

The present study indicates that CaMKII activation is necessary for the NMDA-induced movement of AIDA-1 out of the PSD core. Previous findings demonstrated a very similar CaMKII-mediated movement of SynGAP [6] as well as CaMKII-mediated accumulation of Shanks at the pallial level of the PSD [8]. AIDA-1, SynGAP and Shanks are all major constituents of the PSD and their redistribution is expected to result in major re-organization of the protein complex. Thus, activation of CaMKII at the PSD appears to be the primary event that triggers structural changes induced by synaptic activity.

CaMKII activation is necessary for the induction of certain types of long-term synaptic modification including NMDA-receptor dependent LTP (see reviews: [19]; [20]) and LTD [21]. Although several proteins at the PSD have been implicated in these processes, precise mechanisms are still unclear. A recent study [22] added AIDA-1 to the list of PSD proteins involved in long-term synaptic modification: Tindi et al [22] demonstrated that loss of AIDA-1 causes impairment of both NMDA-dependent LTP and NMDA-dependent LTD. CaMKII-mediated movement of AIDA-1 at the PSD described in the present study provides a potential mechanism for the involvement of AIDA-1 in synaptic modification.

The PSD core is a crowded array, tightly organized around a PSD-95 scaffold. It can be envisaged that abundant PSD-95-binding proteins such as AIDA-1 and SynGAP add stability to the complex and prevent the insertion or removal of elements such as AMPA receptors. CaMKII-mediated exit of AIDA-1 and SynGAP during activity could render the PSD core more flexible and vacate PDZ domains on PSD-95 that are also the binding sites for TARPs, the auxiliary subunits of AMPA receptors. Indeed, loss of SynGAP, like AIDA-1, is known to affect LTP [23]. However, it should be noted that loss of AIDA-1 does not cause a change in basal levels of AMPA receptors at the synapse [22], suggesting possible compensation by SynGAP.

The above discussion implies that SynGAP and AIDA-1 at the PSD fulfill similar roles of maintaining a relatively rigid core structure under basal conditions only to move out under stringent excitatory conditions that promote CaMKII activation. The exit of these abundant proteins would facilitate molecular re-organization at the core. We hypothesize that CaMKII-mediated translocations of SynGAP and AIDA-1 represent a preliminary step in synaptic modification, a window of opportunity that can lead to either LTP or LTD. The stimulation protocol applied here (50μM NMDA for 2 min), optimized for our studies on the redistribution of PSD components, is not known to induce maintained changes in synaptic efficacy. Also, while both SynGAP and AIDA-1 exit the PSD core under this protocol, their levels at the core return to basal within 30 min after the removal of NMDA [5], [12]. Thus,

an interesting possibility to be tested by future immunoEM studies is whether protocols known to induce LTP or LTD can promote maintained changes in the levels and distributions of AIDA-1 and SynGAP at the PSD.

SynGAP and AIDA-1 may exhibit differences in their structural roles at the PSD core as well as differences in their regulation in response to different activity states. Thus the stoichiometry and localization of SynGAP and AIDA-1 at a particular synapse could define a unique core organization and also allow fine-tuning of the postsynaptic response to incoming stimuli.

Acknowledgments

We thank Dr. Thomas Reese for support and discussion, Christine A. Winters for hippocampal cultures, Rita Azzam and Virginia Crocker for EM technical assistance. Supported by the Intramural Research Program of the NIH, NINDS, and by RO1NS081248 (to K.U.B.).

Abbreviations

PSD	postsynaptic density
EM	electron microscopy

References

1. Kim E, Sheng M. PDZ domain proteins of synapses. Nat Rev Neurosci. 2004; 5:771–781. [PubMed: 15378037]
2. Dosemeci A, Tao-Cheng JH, Vinade L, Winters CA, Pozzo-Miller L, Reese TS. Glutamate-induced transient modification of the postsynaptic density. Proc Natl Acad Sci U S A. 2001; 98:10428–10432. [PubMed: 11517322]
3. Tao-Cheng JH, Dosemeci A, Gallant PE, Smith C, Reese T. Activity induced changes in the distribution of Shanks at hippocampal synapses. Neuroscience. 2010; 168:11–17. [PubMed: 20347015]
4. Dosemeci A, Thein S, Yang Y, Reese TS, Tao-Cheng JH. CYLD, a deubiquitinase specific for lysine63-linked polyubiquitins, accumulates at the postsynaptic density in an activity-dependent manner. Biochem Biophys Res Commun. 2013; 430:245–249. [PubMed: 23146630]
5. Yang Y, Tao-Cheng JH, Reese TS, Dosemeci A. SynGAP moves out of the core of the postsynaptic density upon depolarization. Neuroscience. 2011; 192:132–139. [PubMed: 21736925]
6. Yang Y, Tao-Cheng JH, Bayer KU, Reese TS, Dosemeci A. Camkii-Mediated Phosphorylation Regulates Distributions of Syngap-alpha1 and -alpha2 at the Postsynaptic Density. PloS one. 2013; 8:e71795. [PubMed: 23967245]
7. Thein S, Tao-Cheng JH, Li Y, Bayer KU, Reese TS, Dosemeci A. CaMKII mediates recruitment and activation of the deubiquitinase CYLD at the postsynaptic density. PloS one. 2014; 9:e91312. [PubMed: 24614225]
8. Tao-Cheng JH, Yang Y, Bayer KU, Reese TS, Dosemeci A. NMDA-induced accumulation of Shank at the postsynaptic density is mediated by CaMKII. Biochem Biophys Res Commun. 2014; 450:808–811. [PubMed: 24952157]
9. Jordan BA, Fernholz BD, Khatri L, Ziff EB. Activity-dependent AIDA-1 nuclear signaling regulates nucleolar numbers and protein synthesis in neurons. Nat Neurosci. 2007; 10:427–435. [PubMed: 17334360]
10. Lowenthal MS, Markey SP, Dosemeci A. Quantitative mass spectrometry measurements reveal stoichiometry of principal postsynaptic density proteins. J Proteome Res. 2015; 14:2528–2538. [PubMed: 25874902]

11. Jacob AL, Jordan BA, Weinberg RJ. Organization of amyloid-beta protein precursor intracellular domain-associated protein-1 in the rat brain. J Comp Neurol. 2010; 518:3221–3236. [PubMed: 20575057]
12. Dosemeci A, Toy D, Reese TS, Tao-Cheng JH. AIDA-1 Moves out of the Postsynaptic Density Core under Excitatory Conditions. PloS one. 2015; 10:e0137216. [PubMed: 26356309]
13. Dosemeci A, Reese TS, Petersen J, Tao-Cheng JH. A novel particulate form of Ca(2+)/calmodulin-dependent [correction of Ca(2+)/CaMKII-dependent] protein kinase II in neurons. J Neurosci. 2000; 20:3076–3084. [PubMed: 10777771]
14. Lu Z, McLaren RS, Winters CA, Ralston E. Ribosome association contributes to restricting mRNAs to the cell body of hippocampal neurons. Mol Cell Neurosci. 1998; 12:363–375. [PubMed: 9888989]
15. Araki Y, Zeng M, Zhang M, Huganir RL. Rapid dispersion of SynGAP from synaptic spines triggers AMPA receptor insertion and spine enlargement during LTP. Neuron. 2015; 85:173–189. [PubMed: 25569349]
16. Dosemeci A, Jaffe H. Regulation of phosphorylation at the postsynaptic density during different activity states of Ca2+/calmodulin-dependent protein kinase II. Biochem Biophys Res Commun. 2010; 391:78–84. [PubMed: 19896464]
17. Yamauchi T. Molecular constituents and phosphorylation-dependent regulation of the post-synaptic density. Mass Spectrom Rev. 2002; 21:266–286. [PubMed: 12533800]
18. Vest RS, Davies KD, O'Leary H, Port JD, Bayer KU. Dual mechanism of a natural CaMKII inhibitor. Mol Biol Cell. 2007; 18:5024–5033. [PubMed: 17942605]
19. Lisman J, Yasuda R, Raghavachari S. Mechanisms of CaMKII action in long-term potentiation. Nat Rev Neurosci. 2012; 13:169–182. [PubMed: 22334212]
20. Coultrap SJ, Bayer KU. CaMKII regulation in information processing and storage. Trends Neurosci. 2012; 35:607–618. [PubMed: 22717267]
21. Coultrap SJ, Freund RK, O'Leary H, Sanderson JL, Roche KW, Dell'Acqua ML, Bayer KU. Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. Cell Rep. 2014; 6:431–437. [PubMed: 24485660]
22. Tindi JO, Chavez AE, Cvejic S, Calvo-Ochoa E, Castillo PE, Jordan BA. ANKS1B Gene Product AIDA-1 Controls Hippocampal Synaptic Transmission by Regulating GluN2B Subunit Localization. J Neurosci. 2015; 35:8986–8996. [PubMed: 26085624]
23. Komiyama NH, Watabe AM, Carlisle HJ, Porter K, Charlesworth P, Monti J, Strathdee DJ, O'Carroll CM, Martin SJ, Morris RG, O'Dell TJ, Grant SG. SynGAP regulates ERK/MAPK signaling, synaptic plasticity, and learning in the complex with postsynaptic density 95 and NMDA receptor. J Neurosci. 2002; 22:9721–9732. [PubMed: 12427827]

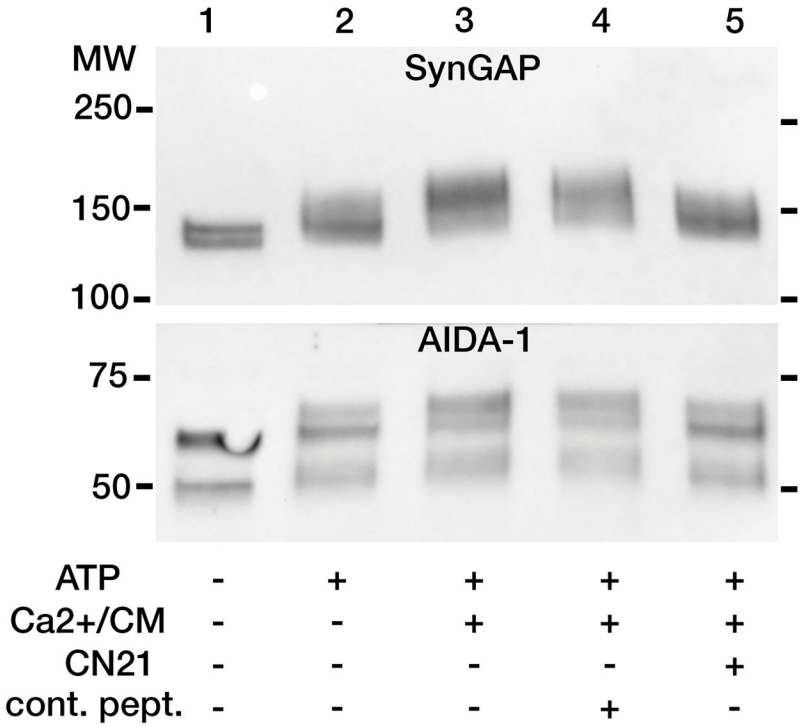

Fig. 1. Phosphorylation of SynGAP and AIDA-1 in isolated PSD fractions

PSD fraction was incubated in basic medium with additions as indicated. Following SDS-PAGE and electrophoretic transfer, membranes were cut horizontally and the upper portion was probed with an antibody for SynGAP, whereas the lower portion was probed with an antibody for AIDA-1. In the presence of ATP (lane2), both SynGAP and AIDA-1 displayed a shift in electrophoretic mobility characteristic of phosphorylation. When Ca^{2+} and calmodulin were added, together with ATP (lane3), a further shift in mobility was observed. This Ca^{2+}/calmodulin –dependent shift was completely reversed by CN21 (lane5) a specific inhibitor of CaMKII, but not by a control peptide (lane4).

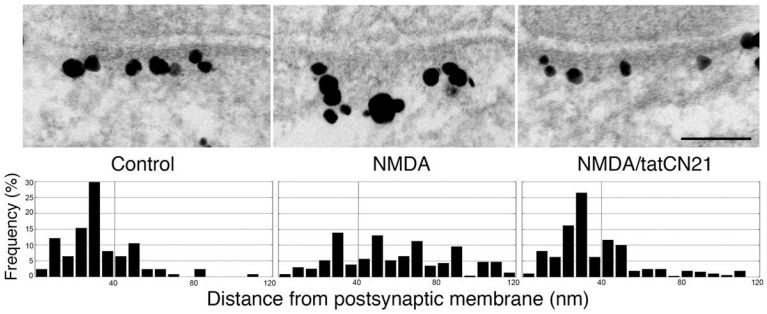

Fig. 2. Activity-induced redistribution of AIDA-1 is blocked by tatCN21, a specific inhibitor of CaMKII

Hippocampal cultures were pre-incubated with or without tatCN21 (20 μM) for 20 min before the application of NMDA (50 μM, 2 min). *Upper panel:* electronmicrographs of asymmetric synapses in dissociated hippocampal cultures immunolabeled for AIDA-1 using antibody1. Silver enhanced gold label appears as dark grains of irregular size. *Lower panel:* histograms from a representative experiment depicting the distribution of AIDA-1 label within the PSD complex. While NMDA promotes a distinctive shift in the distribution of AIDA-1 label, pre-incubation of cultures with tatCN21 blocks this effect.

Table 1

The NMDA-induced increase in median distance of AIDA-1 label from the postsynaptic membrane is blocked by the CaMKII inhibitor, tatCN21.

Median distance of label from postsynaptic membrane in nm (n=number of gold particles; number of PSDs)					
	Exp #	**Control**	**NMDA**	**NMDA/tatCN21**	**NMDA/tatCtrl**
Ab1	1	26.7 (162; 36)	56.7 (96; 30)	26.7 (149; 34)	46.7 (179; 27)
	2	30.0 (154; 36)	63.3 (214; 46)	33.3 (223; 45)	53.3 (286; 39)
	3	30.0 (124; 32)	56.7 (236; 43)	30.0 (373; 75)	53.3 (339; 59)
Ab2	4	26.7 (216; 55)	66.7 (108; 28)	30.0 (109; 33)	56.7 (174; 40)
Combined Mean ± SEM		**28.4±0.9**	**60.9±2.5**	**30.0±1.3**	**52.5±2.1**

Statistical significance evaluated by Wilcoxon test within each experiment and by one-way ANOVA with Tukey's post test among the four experiments: $P<0.0001$ for the following comparisons: Control vs. NMDA, NMDA vs. NMDA/tatCN21.