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Regulation of synaptic structure and function by palmitoylated AMPA receptor binding protein

Charu Misra [1], Sophie Restituito, Jainne Ferreira, Gerald A. Rameau [2], Jie Fu [3], Edward B. Ziff *

Department of Biochemistry, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA

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ABSTRACT

AMPA receptor binding protein (ABP) is a multi-PDZ domain scaffold that binds and stabilizes AMPA receptor (AMPAR) GluR2/3 subunits at synapses. A palmitoylated N-terminal splice variant (pABP-L) concentrates in spine heads, whereas a non-palmitoylated form (ABP-L) is intracellular. We show that postsynaptic Sindbis viral expression of pABP-L increased AMPAR mediated mEPSC amplitude and frequency and elevated surface levels of GluR1 and GluR2, suggesting an increase in AMPA receptors at individual synapses. Spines were enlarged and more numerous and nerve terminals contacting these cells displayed enlarged synaptophysin puncta. A non-palmitoylated pABP-L mutant (C11A) did not change spine density or size. Exogenous pABP-L and endogenous GRIP, a related scaffold, colocalized with NPRAP (δ-catenin), to which ABP and GRIP bind, and with cadherins, which bind NPRAP. Thus postsynaptic pABP-L induces pre and postsynaptic changes that are dependent on palmitoylation and likely achieved through ABP association with a multi-molecular cell surface signaling complex.

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Introduction

AMPA receptors are hetero-tetrameric combinations of GluR1-4 subunits that provide the major fast excitatory inputs in the CNS (Hollmann and Heinemann, 1994; Rosenmund et al., 1998). Regulation of AMPA receptor number at the postsynaptic membrane contributes to changes in synapse strength and to synaptic plasticity (Barry and Ziff, 2002; Bredt and Nicoll, 2003; Malinow and Malenka, 2002; Sheng and Hyoung Lee, 2003). Such regulation requires a synaptic infrastructure that controls the trafficking and localization of receptors, such as through the interaction of receptors with synaptic scaffolding proteins.

The extreme C-termini of the GluR2 and GluR3 subunits are host to interactions with three PDZ (postsynaptic density-95/discs large/zona occludens) domain-containing proteins: ABP (AMPA receptor binding protein) (Srivastava et al., 1998), PICK1 (protein interacting with C kinase) (Dev et al., 1999; Xia et al., 1999) and GRIP (glutamate receptor interacting protein) (Dong et al., 1997). Two isoforms of ABP have been cloned: a six (ABP-S) PDZ domain-containing form, and a seven (ABP-L) PDZ containing variant, both of which interact with GluR2 through their fifth PDZ domain (Srivastava et al., 1998). PDZ domain six of ABP binds to liprin-α (Wyszynski et al., 2002), as well as to Eph receptor tyrosine kinases and ephrin ligands (Torres et al., 1998). This provides ABP with the capacity to link AMPA receptors to a large complex of proteins that function in receptor trafficking or synapse modification. ABP-L is substantially homologous to GRIP and while their expression overlaps in many CNS neurons, they are also independently expressed (Burette et al., 2001). A variant of ABP-L differing in its first 18 amino acids and exhibiting palmitoylation of the cysteine residue at position 11 has been described (pABP-L), as has a palmitoylated GRIP variant (Yamazaki et al., 2001). pABP-L targets specifically to spine heads, localizing with exogenous GluR2 (DeSouza et al., 2002). The non-palmitoylated variant, ABP-L, is conspicuously absent in spines when exogenously expressed and targets to intracellular clusters, where it partially localizes with exogenous GluR2 at intracellular membranes (DeSouza et al., 2002; Fu et al., 2003). ABP-L PDZ 2 can interact with the ARM domain protein, NPRAP/δ-catenin (Ochiishi et al., 2008; Silverman et al., 2007), which interacts with the synaptic cell adhesion proteins, the cadherins (Lu et al., 1999). The ABP-L complex with NPRAP is directed to the plasma membrane by the cadherin interaction. Dominant negative constructs that disrupt the NPRAP-ABP interaction, decrease the levels of GluR2 at the plasma membrane (Ochiishi et al., 2008; Silverman et al., 2007), confirming the role of this interaction in plasma membrane targeting of GluR1 and GluR2.

Disruption of the GluR2-ABP/GRIP interaction by phosphorylation of the PDZ binding site in the GluR2 C-terminal domain impairs

* Corresponding author. Fax: +1 212 263 8214.
E-mail address: edward.ziff@nyumc.org (E.B. Ziff).
[1] Present address: Schering Plough Research Institute, 2000 Galloping Hill Road, Kenilworth, NJ 07033, USA.
[2] Present address: Dept. of Biology, Morgan State University, 1700 E. Cold Spring Lane, Baltimore MD 21251, USA.
[3] Present address: The City of New York Department of Health, 455 First Avenue, New York, NY 10016, USA.

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AMPA receptor clustering, increases the rate of GluR2 endocytosis and generates LTD (Chung et al., 2003; Kim et al., 2001; Lu and Ziff, 2005; Matsuda et al., 2000; Osten et al., 2000; States et al., 2008). The trafficking destination of endocytosed GluR2 is not firmly established (States et al., 2008), but several studies suggest a role for ABP/GRIP in tethering AMPARs intracellularly (Daw et al., 2000; Fu et al., 2003).

Although functions for ABP in receptor anchoring and stabilization on the cell surface and intracellularly have been proposed, the roles of the different ABP isoforms in receptor tethering or synaptic function are not known. Also unclear is the significance of the observation that palmitoylation selectively targets and enriches pABP-L in spines (DeSouza et al., 2002), critical structures at which most excitatory synapses in the brain are found (Nimchinsky et al., 2002).

Here, we have expressed exogenous GFP-tagged versions of ABP in hippocampal neurons to gain insights into their functions. We demonstrate that pABP-L expression selectively induces multiple changes relative to ABP-L, including enhancement of synaptic transmission and increase of AMPA receptor abundance at synapses, as well as pre- and postsynaptic development. We also show that ABP/GRIP colocalize with cadherin and NPRAP at synapses. These observations suggest a role for pABP-L, which is localized at the plasma membrane, in establishment and control of synaptic function. Because similar observations have been made for other scaffolds (El-Husseini Ael et al., 2002; Sala et al., 2001; Schluter et al., 2006; Waites et al., 2009), these functions may be more general.

Results

pABP-L expression increases mEPSC frequency and amplitude

To study the roles of pABP-L and ABP-L in synaptic function, we expressed the proteins tagged at the C-terminus with GFP from Sindbis virus vectors in cultured hippocampal neurons at DIV 18–21. Fig. 1A shows a schematic representation of the structures of the proteins used in this study. Expression of pABP-L-GFP (henceforth pABP-L) or ABP-L-GFP (henceforth ABP-L) resulted in the formation of puncta that were localized to spines and intracellular clusters respectively (Fig. 1B), as previously described (DeSouza et al., 2002).

Whole-cell recordings were made from pABP-L or ABP-L infected hippocampal neurons, 18–25 h after infection. Recordings were made from infected pyramidal-shaped neurons of similar size. Miniature excitatory postsynaptic currents (mEPSCs) were isolated in the presence of tetrodotoxin to inhibit action potential evoked events. Fig. 1C shows representative mEPSC traces at a holding potential of −70 mV, from pABP-L and ABP-L infected neurons. The downward deflections depict individual miniature synaptic currents. mEPSCs in pABP-L and ABP-L infected neurons were compared with those from uninfected neurons or GFP infected neurons. Mean mEPSC frequencies in GFP infected neurons (1.3 ± 0.4 Hz, $n = 6$) were not significantly different from those in uninfected neurons (0.91 ± 0.2 Hz, $n = 7$), and were subsequently pooled and deemed as controls (mean frequency 1.0 ± 0.2 Hz ($n = 13$)). pABP-L infected neurons displayed an approximate four-fold increase in mEPSC frequency to 5.56 ± 1 Hz, ($n = 7$; range 3.6–10.3 Hz). In contrast, ABP-L infected neurons displayed mEPSC frequencies that were similar to those in control cells (1.39 ± 0.3 Hz, $n = 9$); note greater frequency of downward deflections in mEPSC traces for pABP-L compared to ABP-L infected neurons (Fig. 1C). This difference persisted at different holding potentials (data not shown). Further, mEPSCs in neurons overexpressing either pABP-L or ABP-L could be inhibited by CNQX at a holding potential of −70 mV, confirming they were AMPAR mediated (Fig. 1C; $n = 5$ each). Fig. 1D shows data from a number of cells in the form of a bar graph ($p < 0.05$).

We next analyzed the amplitude of mEPSCs from infected neurons. Fig. 1E shows amplitude histograms from an individual pABP-L and ABP-L infected cell. There was a greater distribution of high amplitude events in the pABP-L infected cell (gray, background histogram), than the ABP-L infected cell (blue, forefront histogram). On average, pABP-L infected neurons displayed higher mean amplitude of mEPSCs (−65.1 ± 4 pA), than ABP-L (−51 ± 4 pA) or GFP (−41 ± 5 pA) infected neurons ($p < 0.05$, $n = 8$, 13 and 12 respectively). Fig. 1F shows the scatter of the data, from a number of cells for pABP-L, ABP-L and GFP infected neurons; a significant difference in the amplitude of mEPSCs in pABP-L expressing cells was observed.

These data suggest that pABP-L plays a role in the regulation of synaptic transmission. Since one interpretation of changes in frequency involves changes in quantal release (Murthy et al., 1997) we asked whether there were any changes in the presynaptic terminal subsequent to postsynaptic overexpression of pABP-L.

Large synaptophysin puncta contact pABP-L expressing neurons

We assayed for the effects of pABP-L or ABP-L expression on the levels of synaptophysin, a vesicle associate protein used as a presynaptic marker. We stained for synaptophysin at 18 h after infection, the same time at which electrophysiological recordings were made. Synaptophysin staining was measured in puncta associated with dendrites of neurons expressing the exogenous ABP. These synaptophysin puncta lie within axons that are presynaptic to the neuron expressing the exogenous ABP and do not reside in the same neuron as the exogenously expressed ABP. Synaptophysin staining density associated with pABP-L expression was markedly greater than the intensity associated with ABP-L infected neurons (Figs. 2A, B, right panels). Synaptophysin staining of puncta proximal to nearby uninfected cells (controls) is shown in the left panels of Fig. 2, for comparison. Synaptophysin staining associated with pABP-L infected neurons was 149.3 ± 14.5% of control ($p < 0.05$; $n = 4$; 24 cells) and that associated with ABP-L infected neurons, 110 ± 10% of control ($p > 0.05$; $n = 4$; 24 cells). We also investigated whether there was a difference in the number or size of the associated synaptophysin puncta for pABP-L versus ABP-L infected neurons. A 20 μm length of primary dendrite was selected and the number of synaptophysin puncta contacting the dendrite was counted. Also quantified was the number of synaptophysin puncta with a diameter greater than 1 μm. Whereas the number of synaptophysin puncta contacting infected neurons per 20 μm length of dendrite was not significantly different between pABP-L, ABP-L and GFP infected neurons (15 ± 1.1 puncta, 12.7 ± 1.2 puncta and 12.1 ± 1.7 puncta respectively; $n = 4$; 24 cells), the number of puncta with a diameter greater than 1 μm was significantly more numerous in pABP-L infected neurons (2.74 ± 0.4; $p < 0.05$) than in ABP-L (0.66 ± 0.2) or GFP infected neurons (0.78 ± 0.3). The mean synaptophysin puncta diameter was: 0.83 ± 0.1, for pABP-L ($n = 15$) and 0.55 ± 0.06 for ABP-L ($n = 12$). Fig. 2A depicts this difference indicating the larger and more intense synaptophysin puncta in pABP-L infected neurons; Fig. 2C shows the average data from the synaptophysin experiments in the form of a bar graph. Taken together, these observations suggest that pABP-L infected neurons are contacted by presynaptic terminals with a larger synaptophysin content, which is consistent with the electrophysiological data that demonstrates increased mEPSC frequency.

Surface AMPA receptor abundance increases in pABP-L expressing neurons

While changes in mEPSC frequency often have a presynaptic basis, the results thus far do not exclude a postsynaptic component to the increased mEPSC frequency in pABP-L infected neurons, arising from the insertion of receptors into synapses could not be excluded. Furthermore, ABP plays a role in retaining AMPA receptors at the neuron surface (Kim et al., 2001; Ochiishi et al., 2008; Osten et al., 2000; Perez et al., 2001; Silverman et al., 2007; Xia et al., 1999) and at intracellular sites (Braithwaite et al., 2002; Daw et al., 2000; Fu et al.,

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