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Differential roles of GRIP1a and GRIP1b in AMPA receptor trafficking

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ABSTRACT

Regulated trafficking controls AMPA receptor (AMPAR) number at the postsynaptic membrane to modify the efficiency of synaptic transmission. The PDZ proteins GRIP1 and the related ABP-L/GRIP2 bind AMPAR subunit GluA2, and have been proposed to play a role in AMPAR trafficking associated with Long Term Depression (LTD) of synaptic transmission. Both GRIP1 and ABP-L/GRIP2 exist in different splice isoforms, including alternative 18 amino acid domains at the extreme N-terminus, which determine whether the protein can be palmitoylated. The implications of this differential splicing for AMPAR trafficking is unknown. Here, we use surface biotinylation and quantitative Western blotting to show that the N-terminal splice variants GRIP1a and GRIP1b have differential effects in NMDA-induced AMPAR internalization in cultured hippocampal neurons. GRIP1a inhibits, but GRIP1b enhances this trafficking event. We further demonstrate that GRIP1a and GRIP1b have dramatically different subcellular distributions in cultured neurons and exhibit NMDA-dependent colocalisation with early endosomes. We propose that GRIP1 palmitoylation modulates NMDA-induced AMPAR internalisation by differential regulation of the early endosomal system.

AMPA receptors mediate most fast synaptic excitation in the brain and their activity-dependent trafficking is a major determinant of synaptic strength. AMPAR expression at the synaptic plasma membrane is regulated by endocytosis, exocytosis, recycling and lateral diffusion events that contribute to reduced (Long Term Depression, LTD) or increased synaptic strength (Long Term Potentiation, LTP) [5,3,21,17]. Multiple protein interactions with AMPAR subunits mediate these trafficking events [19,11]. Among these the ABP/GRIP family of proteins are multi-PDZ domain proteins that interact with the extreme C-terminus of AMPAR subunits GluA2 and GluA3 [8,23]. GRIP1 and ABP (also known as GRIP2) are expressed from two separate genes and differential RNA splicing gives rise to multiple isoforms of each of these proteins [26,9].

ABP/GRIP proteins are be involved in anchoring AMPARs either at the synapse [18] or at an intracellular location [6,2]. One possible explanation for these distinct subcellular sites of action is that both GRIP1 and ABP exist in two different N-terminal splice forms, only one of which contains a consensus sequence for the posttranslational modification palmitoylation [27,7]. Conjugation of the fatty acid palmitate facilitates substrate protein association with specific cellular membrane compartments [14]. GRIP1b and pABP-L splice variants can be palmitoylated, whereas GRIP1a and ABP-L cannot.

Members of the ABP/GRIP family of proteins have been implicated in hippocampal [6], and more recently in cerebellar LTD [25]. Their precise function in AMPAR trafficking remains unclear, although it has been reported that GRIP1 interacts with the endosomal protein NEEP21, and regulates the recycling of internalised AMPARs back to the plasma membrane [24]. ABP/GRIP may also play a role in stabilising the pool of AMPARs internalised during LTD [6]. However, the function in AMPAR trafficking and synaptic plasticity of specific GRIP1 N-terminal splice variants that govern palmitoylation has not been explored.

Here, we investigated the specific roles of GRIP1a and GRIP1b in the regulation of AMPAR surface expression and NMDA-induced AMPAR trafficking. We show that overexpression of GRIP1b enhances, and GRIP1a inhibits NMDA-induced AMPAR internalisation, most likely via a re-organisation of the endosomal system by GRIP1. Our results demonstrate a previously unsuspected divergence in the roles of different GRIP1 isoforms in AMPAR trafficking, and also suggest important differences between GRIP1 and the closely related ABP-L.

Every attempt was made to minimise distress and suffering in animals used to produce neuronal cultures and all procedures were in compliance with the Animal [Scientific Procedures] Act 1986. Hippocampal and cortical primary neuronal cultures were prepared from E18 Wistar rats as previously described [15]. For imaging studies, neurons were plated on glass coverslips at a density of 75,000 per 24 mm coverslip. For biotinylation assays, neurons were plated at a density of 1×10^6 per 60 mm dish. Neurons were maintained in Neurobasal medium with B27, Glutamine and Pen/Strep for 14–21 days before use. Sindbis viruses were prepared according to the Sindbis Expression System manual

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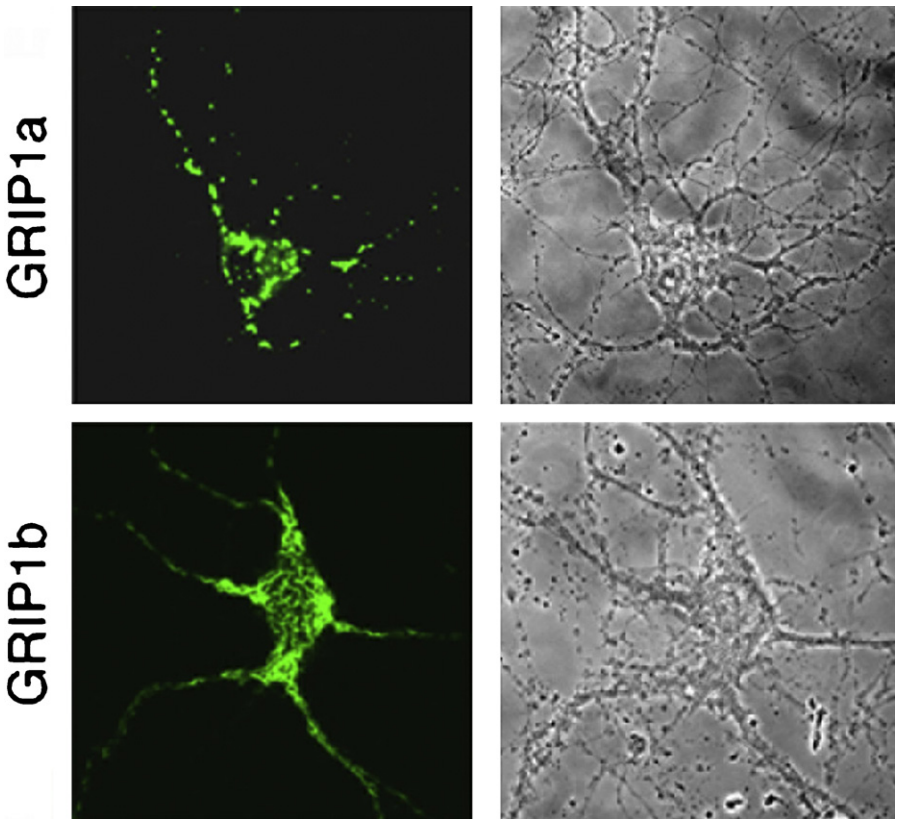

Fig. 1. Differential subcellular distributions of GRIP1a and GRIP1b isoforms. Dissociated hippocampal neurons (18 DIV) were infected with Sindbis virus expressing GFP-GRIP1a or GFP-GRIP1b. Cells were fixed and imaged by confocal microscopy 24 h later. Left panels show GFP/YFP fluorescence, right panels show transmission images.

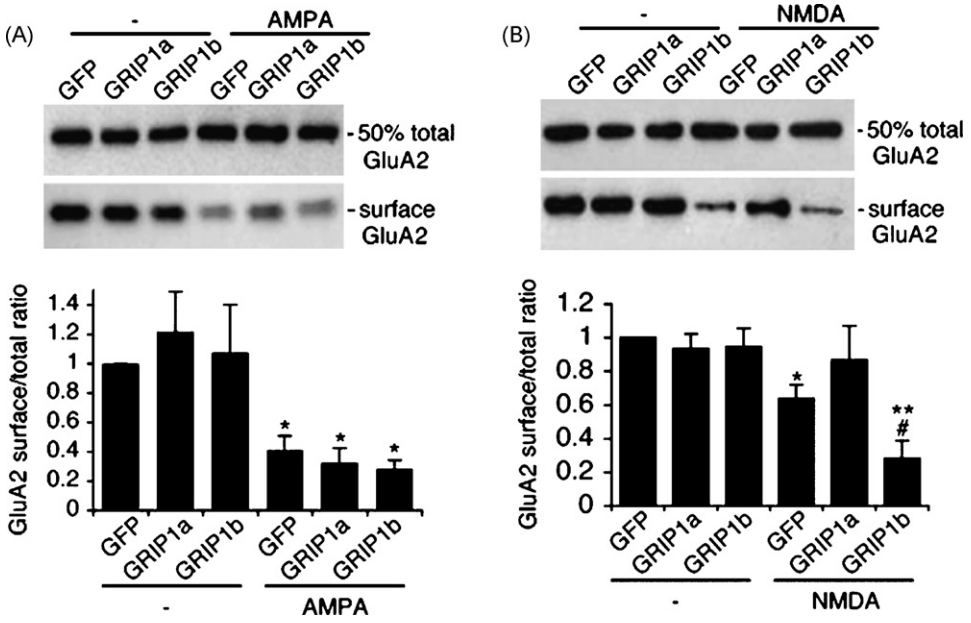

Fig. 2. Effects of GRIP1a and GRIP1b overexpression on regulated AMPAR internalisation. (A) GRIP1a/GRIP1b overexpression does not affect AMPA-induced internalisation of GluA2-containing AMPARs. Dissociated hippocampal neurons infected with Sindbis virus expressing GFP-GRIP1a or GFP-GRIP1b were pretreated with TTX for 60 min, and stimulated with 100 μM AMPA for 10 min. Top panel shows representative western blots for total and surface GluA2. Graph shows pooled data presented as ratios of surface over total GluA2. $n = 6$, $^{*}p < 0.05$, compared to equivalent condition without AMPA treatment. (B) GRIP1a and GRIP1b have differential effects on NMDA-induced internalisation of GluA2-containing AMPARs. Dissociated hippocampal neurons infected with Sindbis virus expressing GFP-GRIP1a or GFP-GRIP1b were pretreated with TTX for 60 min, and stimulated with 25 μM NMDA for 3 min, with 10 min incubation following drug washout. Top panel shows representative western blots for total and surface GluA2. Graph shows pooled data presented as ratios of surface over total GluA2. $n = 5$, $^{*}p < 0.05$, $^{**}p < 0.01$, compared to equivalent condition without NMDA treatment. $\#p < 0.05$, compared to GFP + NMDA condition.