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Subunit-specific surface mobility of differentially labeled AMPA receptor subunits

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Abstract

Lateral mobility of AMPA-type glutamate receptors as well as their trafficking between plasma membrane and intracellular compartments are major mechanisms for the regulation of synaptic plasticity. Here we applied a recently established labeling technique in combination with lentiviral expression in hippocampal neurons to label individual ACP-tagged AMPA receptor subunits specifically at the surface of neurons. We show that this technique allows the differential labeling of two receptor subunits on the same cell. Moreover, these subunits are integrated into heteromeric receptors together with endogenous subunits, and these labeled receptors are targeted to active synapses. Sequential labeling experiments indicate that there is basal surface insertion of GluR1, GluR2 and GluR3, and that this insertion is strongly increased following potassium depolarization. Moreover, we found that ACP-labeled GluR3 shows the highest surface mobility among GluR1, GluR2, and GluR3. In double-infected neurons the diffusion coefficient of labeled GluR2 at the surface of living neurons is significantly higher in GluR2/GluR3-infected neurons compared to GluR1/GluR2-infected neurons suggesting a higher mobility of GluR2/3 receptors compared to GluR1/2 receptors. These results indicate that surface mobility is regulated by different subunit compositions of AMPA receptors.

Keywords: Trafficking; Lateral movement; Endocytosis; Receptor trafficking

Introduction

Fast synaptic transmission at excitatory glutamatergic synapses is principally mediated by AMPA-type glutamate receptors (AMPAR). AMPAR rapidly cycle between internal compartments and the neuronal surface by exocytosis/endocytosis. In addition, there is shuttling of receptors between extrasynaptic plasma membrane sites and the post-synaptic density (PSD). These two dynamic events are believed to be major components that determine efficacy of synaptic transmission. During synaptic potentiation additional receptors are recruited to the synapse, while upon synaptic depression receptors are removed from the PSD (Beattie et al., 2000; Lu et al., 2001; Luscher et al., 1999; Man et al., 2000; Matsuda et al., 2000; Shi et al., 2001; Xia et al., 2000). AMPAR are heterotetramers composed of the four subunits GluR1-4 (or GluR-A-D),

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with GluR1/2 and GluR2/3 as the major forms in the hippocampus (Wenthold et al., 1996). These different subunit compositions show differential trafficking behavior. During long-term potentiation (LTP) GluR1/2 receptors have been proposed to be inserted into synapses in an activity-dependent fashion via calcium-calmodulin-dependent kinase II-dependent phosphorylation (CaMKII) (Hayashi et al., 2000; Shi et al., 1999). This regulation depends on the GluR1 subunit (Passafaro et al., 2001; Shi et al., 2001). In contrast to LTP, induction of long-term depression (LTD) acts on GluR2 in order to internalize receptors by endocytosis into endosomes (Lee et al., 2004). GluR2/3 receptors cycle also in an activity-independent manner between the neuronal surface and endosomal compartments (Passafaro et al., 2001; Shi et al., 2001). These endosomes comprise important sorting stations between endosomal recycling and degradation pathways (Ehlers, 2000; Kulangara et al., 2007; Lee et al., 2004; Park et al., 2004; Steiner et al., 2005).

Although a recent study on the implication of the exocyst complex in AMPAR recruitment showed insertion of AMPAR directly at the PSD (Gerges et al., 2006), physiological recordings using a photoactivatable AMPAR antagonist suggested fast insertion of receptors at distant sites of synapses (Adesnik et al., 2005). In accordance with the latter possibility, GluR2 tagged with the pH-sensitive variant of GFP is inserted along the dendritic shaft (Ashby et al., 2006; Yudowski et al., 2007). Such an insertion distant to the synapse requires lateral movement along the plasma membrane. Shuttling of antibody-labeled surface GluR2 in and out of synapses has been shown in cultured hippocampal neurons (Bats et al., 2007; Borgdorff and Choquet, 2002), but little information is available on the subunit-specific characteristics of receptor movement at the surface.

In the present study we analyzed surface mobility of different AMPAR subunits by a recently developed technique that allows to specifically label a given surface receptor with synthetic probes. The technique involves expressing the surface protein of interest as a fusion protein with an acyl carrier protein (ACP; 77 residues) or a mutant thereof (ACP9) at its extracellular domain (George et al., 2004; Sielaff et al., 2006). These tags can then be specifically derivatized with synthetic probes using the bacterial phosphopantetheine transferases (PPTases) AcpS (which labels only ACP) or Sfp (which labels both ACP and ACP9), and synthetic CoA derivatives (Fig. 1). As the PPTases and the CoA derivatives are membrane impermeable, the approach allows to label only the cell-surface subpopulation of a given receptor, whereas internal receptors are not modified. In addition, two receptors or subunits can be labeled simultaneously, and the tags are of much smaller size than antibody conjugates.

Here we single- or double-infected hippocampal neurons with lentiviral vectors expressing ACP- or ACP9-tagged AMPAR subunits. We found that this system allows for differential labeling of two subunits of GluR1/2- or GluR2/3-infected cells. These exogenously expressed GluRs form heteromeric receptors with

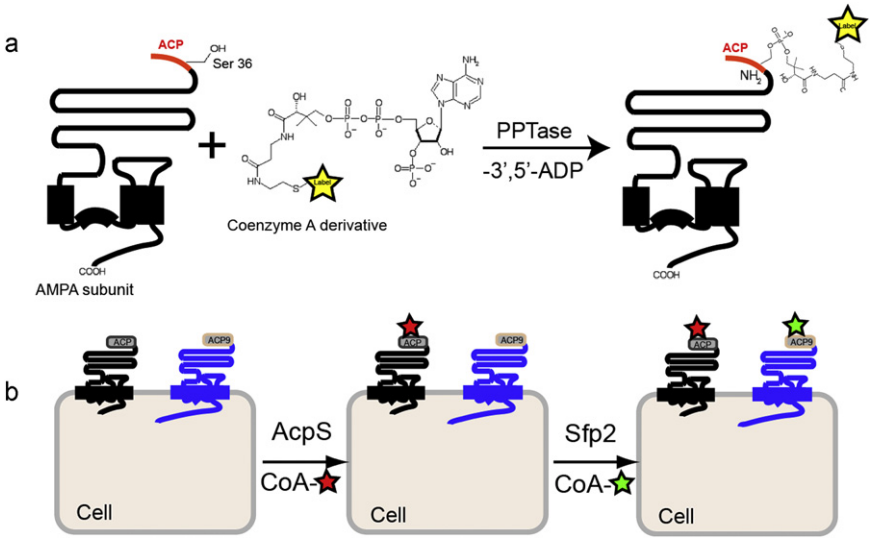

Fig. 1. Labeling of surface AMPAR subunits with the ACP technique. (a) Scheme of the labeling reaction. The 77 amino acid long acyl carrier protein (ACP) tag is fused to the extracellular aminoterminus of the GluR subunit (downstream of the signal peptide). The coenzyme A (CoA) derivative is covalently linked to serine 36 of the ACP by a phosphopantethein transferase (PPTase). (b) Principle of sequential two-color labeling with two carrier protein tags, ACP and its mutant ACP9, on the same cell. In a first step the PPTase AcpS links CoA to the ACP tag, but not to the ACP9 tag. In a second step another PPTase, Sfp, links CoA specifically to the ACP9 tag, because ACP was saturated in the first step by AcpS.