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REVIEW

REGULATION OF AMPA RECEPTORS AND SYNAPTIC PLASTICITY

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Abstract—Neuronal activity controls the strength of excitatory synapses by mechanisms that include changes in the postsynaptic responses mediated by AMPA receptors. These receptors account for most fast responses at excitatory synapses of the CNS, and their activity is regulated by various signaling pathways which control the electrophysiological properties of AMPA receptors and their interaction with numerous intracellular regulatory proteins. AMPA receptor phosphorylation/dephosphorylation and interaction with other proteins control their recycling and localization to defined postsynaptic sites, thereby regulating the strength of the synapse. This review focuses on recent advances in the understanding of the molecular mechanisms of regulation of AMPA receptors, and the implications in synaptic plasticity.

Key words: long-term potentiation, long-term depression, glutamate, receptor trafficking, receptor phosphorylation.

Contents

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Abbreviations: ABP, AMPA receptor-binding protein; AMPAR, AMPA receptor; Arc, activity-regulated cytoskeleton-associated protein; Aβ, beta-amyloid; BAR, Bin/amphyphysin/Rvs; BDNF, brain-derived neurotrophic factor; CAM, cell adhesion molecule; CaMKII, Ca^{2+}- and calmodulin-dependent protein kinase II; cGKII, cGMP-dependent protein kinase II; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FRAP, fluorescence recovery after photobleaching; GFP, green-fluorescent protein; GluRδ2, δ2 glutamate receptor; GRIP, glutamate receptor interacting protein; LBD, ligand-binding domain; LTD, long-term depression; LTP, long-term potentiation; MAGUK, membrane-associated guanylate kinase; MAPK, mitogen-activated protein kinase; mEPSC, miniature excitatory postsynaptic current; mGluRs, metabotropic glutamate receptors; Narp, neuronal-activity-regulated pentraxin; NCAM, neural cell adhesion molecule; NMDA, *N*-methyl-D-aspartate; NMDAR, *N*-methyl-D-aspartate receptor; NPR, neuronal pentraxin receptor; NPRAP, neural plakophilin-related arm protein; NP1, neuronal pentraxin 1; PDZ, postsynaptic density 95/disc large/zonula occludens-1; PICK1, protein interacting with C-kinase-1; PI3-K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PSA, polysialic acid; PSD, postsynaptic density; SEP, superecliptic pHluorin; Ser, serine; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors; TARP, transmembrane AMPA receptor regulatory proteins; TM, transmembrane; TNFα, tumor necrosis factor-α.

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Glutamate is the major excitatory neurotransmitter in the brain, and its effects are mediated by activation of ionotropic and metabotropic receptors, differing in their molecular, biochemical pharmacological and physiological properties (Hollmann and Heinemann, 1994; Kew and Kemp, 2005). The ionotropic glutamate receptors have been classified into three major subtypes, AMPA, kainate, and *N*-methyl-D-aspartate (NMDA) receptors, named after their most selective agonist (Watkins et al., 1981). AMPA receptors (AMPARs) are responsible for the primary depolarization in glutamate-mediated neurotransmission and play key roles in synaptic plasticity. Long-lasting and activity-dependent changes in synaptic strength (long-term potentiation, LTP, or long-term depression, LTD) are associated with changes in the phosphorylation and cellular distribution of AMPAR, and are thought to underlie learning and memory formation (Rumpel et al., 2005; Morris, 2006; Pastalkova et al., 2006; Whitlock et al., 2006). Deregulation of AMPAR activity is also involved in pathology [e.g. (Kwak and Weiss, 2006; Liu et al., 2006)]. This review will concentrate on the molecular mechanisms of regulation of AMPARs, and their implications in synaptic plasticity.

EXPRESSION OF AMPARs

AMPARs consist of four closely related genes, with about 70% sequence homology (Collingridge et al., 2004), that encode the four subunits GluR1-4 or A–D (Laube et al., 1998; Mano and Teichberg, 1998; Rosenmund et al., 1998). *In situ* hybridization studies, receptor autoradiography using [^{3}H]AMPA and [^{3}H]glutamate as ligands, and immunocytochemistry with antibodies raised against GluR1–GluR4 subunits [reviewed in (Hollmann and Heinemann, 1994; Petralia et al., 1999)] showed a widespread distribution of AMPARs in the brain, as expected from their key role in excitatory neurotransmission. GluR1–GluR3 subunits are enriched in the outer layers of the cerebral cortex, hippocampus, olfactory regions, basal ganglia, lateral septum and amygdala (Kein-

anen et al., 1990; Beneyto and Meador-Woodruff, 2004). The GluR4 subunit is present in lower amounts throughout the CNS, except in the reticular thalamic nuclei and the cerebellum, where this subunit is also abundant (Petralia and Wenthold, 1992; Martin et al., 1993; Spreafico et al., 1994). The expression of AMPAR subunits is also differentially regulated during development (Petralia et al., 1999; Palmer et al., 2005b; Talos et al., 2006), and although they are regarded as neuronal receptors, they have also been detected in glial cells (Gallo and Russell, 1995; Janssens and Lesage, 2001; Lin and Bergles, 2004).

A recent study using quantitative fluorescence *in situ* hybridization demonstrated that endogenous mRNAs encoding AMPAR subunits GluR1 and GluR2 are localized to proximal and distal dendrites of hippocampal neurons and that a substantial fraction of synaptic sites contain GluR2 mRNA clusters (Grooms et al., 2006). The presence in dendrites of the machinery necessary for protein synthesis, together with the mRNA for AMPAR subunits, suggests that local synthesis of AMPAR subunits regulates local receptor abundance and composition (Steward and Levy, 1982; Kacharmina et al., 2000; Tang and Schuman, 2002; Asaki et al., 2003; Ju et al., 2004; Grooms et al., 2006). Accordingly, chronic activity blockade increases the synthesis of GluR1 in dendrites, and acute activation of group I metabotropic glutamate receptors (mGluRs) or acute depolarization with KCl increases the synthesis of both GluR1 and GluR2 (Ju et al., 2004). Also, dopamine receptor activation promotes transport of endogenous mRNAs, including those from GluR1 and GluR2 in hippocampal neurons (Smith et al., 2005). Interestingly, *in situ* hybridization studies also demonstrate the presence of mRNA encoding proteins relevant in the regulation of the excitatory synapses, such as microtubule-associated protein 2 (MAP2), the α-subunit of Ca^{2+}- and calmodulin-dependent protein kinase II (CaMKII-α), brain-derived neurotrophic factor (BDNF), activity-regulated cytoskeleton-associated protein (Arc), TrkB receptor, inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) receptor, the atypical protein kinase Mζ, the NMDA receptor (NMDAR) NR1 subunit, and glycine receptor α subunit in dendritic layers of hippocampus and in dendrites of hippocampal neurons *in vivo* and *in vitro* [for reviews, see (Martin and Zukin, 2006; Schuman et al., 2006)]. Localization of mRNAs and regulated translation in dendrites have recently gained widespread acceptance as mechanisms fundamental to synaptic plasticity (Eberwine et al., 2001; Steward and Schuman, 2003; Martin and Zukin, 2006; Schuman et al., 2006).

AMPAR STRUCTURE AND DIVERSITY

AMPARs are largely Ca^{2+}-impermeable, display exceptionally fast kinetics and mediate moment-to-moment synaptic signaling (Jonas, 2000). These characteristic functional properties depend on the subunit composition and on subunit modifications introduced by alternative splicing.

The AMPAR GluR1–GluR4 subunits combine in tetramers in different stoichiometries (Hollmann and Heinemann, 1994), which determine channel function (i.e. desensitization/resensitization kinetics and conductance properties) (Ozawa et al., 1998) and trafficking to synapses (Malinow et al., 2000). Stargazin and other transmembrane (TM) AMPA receptor regulatory proteins (TARPs) also coassemble stoichiometrically with native AMPARs. The TARPs act as auxiliary subunits that are required for AMPAR maturation and trafficking, and modulate channel function (see below) (Korber et al., 2007; Ziff, 2007).

Each AMPAR subunit comprises about 900 amino acids and has a molecular weight of about 105 kDa. The GluR1–GluR4 subunits share 68–74% amino acid sequence identity (Collingridge et al., 2004) and contain four hydrophobic domains: TM1, TM3, and TM4 transverse the membrane, while M2 faces the cytoplasm as a reentered loop that forms part of the channel pore (Fig. 1A). The N-terminal segment is homologous to the bacterial leucine-isoleucine-valine binding protein (LIVBP), while the adjacent ligand-binding domain (LBD) is homologous to glutamine binding protein (Madden, 2002). The LBD is split into the S1 and S2 segments by TM segments. Ligand binding to the LBD initiates conformational changes that are transduced to the TM segments and trigger opening of the channel's gate (Gouaux, 2004; Mayer, 2005). All three modules—the N-terminus, the LBD, and the ion-channel domain—are engaged in distinct, interdependent subunit-subunit interactions (Greger et al., 2006). The C-terminal part of S2 is not directly involved in agonist binding and, due to alternative RNA splicing, is expressed in two forms, flip and flop, that differ in a few amino acids only, but which result in receptors with different desensitization and endoplasmic reticulum (ER) export kinetics (Sommer et al., 1990; Mosbacher et al., 1994; Coleman et al., 2006). A recent study from our laboratory identified versions of the AMPAR subunits that lack both the flip and flop exons, and play a dominant negative role (Gomes et al., 2007b).

Finally, the C-terminus of AMPAR subunits is intracellular and shows differences between the subunits. GluR1, GluR4, and an alternative splice form of GluR2 (GluR2L) have longer cytoplasmic tails with high homology (Fig. 1B). In contrast, the predominant splice form of GluR2, GluR3, and an alternative splice form of GluR4 (GluR4c) have shorter, homologous cytoplasmic tails (Fig. 1B). Receptors composed of subunits with short cytoplasmic C-termini (GluR2/3) cycle continuously in and out of the synapse, with a time constant of about 15 min (Passafaro et al., 2001; Shi et al., 2001), whereas receptors containing long C-termini (GluR1/2 and GluR2/4) are added into synapses in an activity-dependent manner (Hayashi et al., 2000; Shi et al., 2001). Each subunit binds specific intracellular proteins through the C-terminal tail, and these interactions play important roles in controlling the trafficking of AMPARs and/or their stabilization at the synapses.

AMPAR POST-TRANSLATIONAL MODIFICATIONS

Phosphorylation is a key post-translational modification in regulating AMPAR function (Carvalho et al., 2000). It can