Kainate receptor-interacting proteins and membrane trafficking

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Abstract
Kainate receptors are composed of several subunits and splice variants, but the relevance of this diversity is still not well understood. The subunits and splice variants show great divergence in their C-terminal cytoplasmic tail region, which has been identified as a region of interaction with a number of protein partners. Differential trafficking of kainate receptors to neuronal compartments is likely to rely on interactions with distinct subsets of protein partners. This review summarizes our knowledge of the regulation of trafficking of kainate receptors and focuses on the identification and characterization of functions of interacting partners.

Kainate receptors are ionotropic glutamate receptors composed of various combinations of five subunits GluR5, GluR6, GluR7, KA1 and KA2. They form cationic ion channels that can flux Ca$^{2+}$ depending on mRNA editing of a glutamate in the channel pore region. At variance with AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) and NMDA (N-methyl-D-aspartate) receptors that mainly operate at postsynaptic sites and are the key agents of fast glutamatergic synaptic transmission, kainate receptors have different functions depending on their subcellular localization. At postsynaptic sites, kainate receptors are implicated in synaptic currents of small amplitude and slow decay kinetics that display prominent summation properties in response to repetitive stimulations. Presynaptic kainate receptors regulate the release of GABA (γ-aminobutyric acid) and glutamate at many different synapses, facilitating presynaptic forms of short- and long-term synaptic plasticity. Some of the functions of kainate receptors involve a metabotropic action through coupling with a G-protein, which does not require an ionotropic action. For instance, kainate receptors regulate neuronal excitability by inhibition of Ca$^{2+}$-dependent K$^+$ channels. The diverse functions of kainate receptors have recently been reviewed [1,2].

Functional studies suggest that segregated populations of kainate receptors are localized at diverse subcellular compartments. This raises the question of the polarized trafficking of kainate receptors to pre- and post-synaptic sites, and their stabilization in specific functional domains. The regulated targeting of kainate receptors is likely to depend on subunit composition and on specific interactions with subsets of interacting proteins. Elucidating the cellular mechanisms underlying these processes is important to unravel kainate receptor-mediated signalling in the brain. This review will focus on our current understanding of the molecular mechanisms that govern membrane trafficking of kainate receptors, with a special emphasis on the identification and the role of kainate receptor-interacting proteins.

Membrane delivery of recombinant kainate receptor subunits and splice variants
Studies with recombinant receptors in cell lines and cultured neurons have defined rules for the trafficking of kainate receptors to the plasma membrane. The relative level of their surface expression depends on subunits and alternative splicing of their C-terminal domain, and on subunit composition of heteromeric receptors. Some subunit splice variants are endowed with a forward trafficking motif, whereas others are retained in the ER (endoplasmic reticulum) due to retention signals.

KA2 was initially thought to require the presence of other subunits to form receptor complexes, but KA2 can in fact form homomeric assemblies in heterologous cells, although it is retained in the ER in the absence of GluR5, GluR6 or GluR7 [3–5]. This subunit possesses an ER retention motif in its C-terminus (RRRRR), and a di-leucine endocytic motif that may mediate its rapid, clathrin-dependent endocytosis and low steady-state plasma membrane expression. The ER retention/retrieval signal in KA2 is sterically shielded during heteromeric assembly, allowing delivery of functional heteromeric receptors to the plasma membrane [5]. The polyarginine ER retention motif functions as an interaction domain for COP1 (coatomer protein 1), a complex that plays a central role in the retrograde trafficking from the Golgi to the ER of misfolded or unassembled proteins [6]. The assembly of KA2 with GluR6 markedly reduces interaction between KA2 and COP1.

GluR5, GluR6 and GluR7 can form functional homomeric receptor channels and have multiple isoforms derived from alternative splicing and RNA editing. These isoforms display different patterns of expression at the plasma membrane,

Key words: glutamate receptor, kainate receptor-interacting protein, membrane trafficking, splice variant.

Abbreviations used: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; COPI, coatomer protein 1; EPSC, excitatory postsynaptic current; ER, endoplasmic reticulum; GRIP, glutamate receptor-interacting protein; PKC, protein kinase C; PSD-95, postsynaptic density 95; RXR, retinoid X receptor; SAP, synapse-associated protein.

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which strongly depend on the alternative splicing at their C-terminus [7,8]. In analogy with KA2, GluR5c splice variant is essentially retained in the ER due to the presence of an RXR (retinoid X receptor) retention motif, similar to the one described for NMDA receptors [9] and potassium channels [10]. GluR7b also contains an RXR motif, but it does not act as an ER retention signal and may play a role during receptor biogenesis and assembly [11].

GluR6a and GluR7a are expressed at high levels in the plasma membrane and can promote surface expression of other subunits that contain ER retention motifs [7,11,12] due to the existence in their C-terminal of a forward trafficking motif. This stretch of positively charged amino acids (CQRRLKHK) is crucial for ER exit but, if mutated, the receptors can nevertheless reach the plasma membrane if associated with other receptors containing the intact sequence. The molecular mechanisms by which the CQRRLKHK motif acts as a forward trafficking signal are not known. The other subunit splice variants (GluR5a, GluR5b, GluR6b and GluR7b) are present at low levels at the plasma membrane as homomers. Other positively charged residues that are conserved between GluR5b and GluR5c are also important for their exit from the ER [8]. Oligomerization of kainate receptor subunits plays a major role in their surface expression, as demonstrated, for instance, in the case of GluR6a–GluR6b heteromers, which form native kainate receptors [13].

In conclusion, cis-acting regulatory elements encoded within the C-terminal domain of kainate receptor subunits affect their trafficking and surface expression. These processes are not regulated by PDZ-binding domains of kainate receptors [5,7,12], in contrast with NMDA receptor subunits.

Recent studies have demonstrated that the N-terminal domain of kainate receptors also plays an important role in their trafficking and surface expression [14–16]. Formation of an intact glutamate-binding site was suggested to act as a quality control checkpoint for the forward trafficking of intracellular homomeric and heteromeric kainate receptors [15,16]. When a key ligand-binding amino acid in the second extracellular domain of KA2 (Thr675) is mutated, KA2 can assemble with GluR6 but the receptor is degraded instead of being targeted to the plasma membrane. Mutation of the corresponding amino acid in GluR6 yields immaturely glycosylated receptors that are retained in the ER. Non-functional GluR6 receptors are thus retained intracellularly, suggesting that glutamate binding and associated conformational changes are prerequisites for the forward trafficking of intracellular kainate receptors following multimeric assembly.

Two cysteine residues located in the C-terminal domain of GluR6 can be palmitoylated [17]. Palmitoylation could affect channel function indirectly by regulating phosphorylation of kainate receptors. GluR6-containing receptors are subject to rapid endocytosis followed by sorting to either recycling or degradation pathways, depending on the endocytic stimulus. In cultured hippocampal neurons, whereas exogenous kainate causes a PKC (protein kinase C)-dependent internalization of kainate receptors targeted to lysosomes for degradation, NMDA triggers a Ca2+-, PKA- (cAMP-dependent protein kinase) and PKC-dependent endocytosis of kainate receptors to early endosomes for recycling to the plasma membrane [18].

Identification of kainate receptor-interacting proteins

Many kainate receptor-interacting proteins have been identified in recent studies. Some of these proteins have been implicated in trafficking, synaptic localization and modulation of the properties of kainate receptors. Kainate receptors contain a PDZ-binding motif at their C-terminus that can bind to prototypic PDZ domain-containing proteins such as PSD-95 (postsynaptic density 95), SAP97 (synapse-associated protein 97) and SAP102. In heterologous systems, binding of GluR6a to PSD-95 causes the clustering of kainate receptors [19–21]. Interestingly, there is some specificity in the association of kainate receptors with this family of proteins that may dictate the subcellular localization of the receptors; both PSD-95 and SAP102 can associate with KA2 and GluR6, but the presynaptically localized SAP97 was found to be specific for this class of receptors. These proteins differentially regulate the function and synaptic stability of kainate and AMPA receptors. However, PSD-95, PICK1 and GRIP do not seem to play any role in the exit from the ER of either GluR5 or GluR6 [7,8].

Kainate receptors can also bind to β2-catenin and proteins of the cadherin–catenin complex, through an indirect interaction with the extreme C-terminus of GluR6 [21]. Although the two splice variants GluR6a and GluR6b do not significantly differ in their functional properties, they can co-assemble into the same heteromeric complex in native and recombinant receptors, bringing into close proximity different sets of interacting cytosolic proteins [13]. The set of proteins identified by a proteomic approach to interact with GluR6a–GluR6b includes dynamin-1, NSF protein (N-ethylmaleimide-sensitive fusion protein), dynamin and 14-3-3 proteins that are possibly involved in the assembly and trafficking of membrane receptors, and also spectrin and profilin II that may participate in cytoskeletal organization. GluR6b also interacts with a group of proteins including calcineurin, calmodulin, VILIP-1 (visinin-like protein 1) and neurocalcin-δ that are involved in the regulation of receptors and ion channels by Ca2+.

Given the apparent complexity of the processes of kainate receptor trafficking and polarized targeting in neurons, it is likely that they are governed by intricate protein interactions with as yet unknown partners.

Role of kainate receptor-interacting proteins in the regulation of trafficking

An early checkpoint for biosynthesis of functional kainate receptors is the interaction between KA2, COPI and 14.3.3ζ.
Regulation of kainate receptor function by interacting proteins

Little is known about the role of interacting proteins in the regulation of the functional properties of kainate receptors. It was proposed that interaction of GluR6a with cytoplasmic proteins such as PSD-95 [19] might change the functional properties of kainate receptors. However, re-evaluation of these effects has shown only very minor regulation of desensitization by these proteins [28].

The functional properties of recombinant kainate receptors can be modified by direct PKA phosphorylation of the C-terminal domain [29–31]. Influx of Ca\(^{2+}\) through NMDA receptor channels down-regulates native kainate receptors in a rapid and reversible manner through activation of calcineurin [32]. This regulation requires the heteromeric assembly of GluR6a and GluR6b and the association of calcineurin with GluR6b in the kainate receptor complex [13].

In perirhinal cortex neurons, activation of PKC by stimulation of mGluR5 enhances kainate receptor-mediated Ca\(^{2+}\) responses and kainate receptor-mediated EPSCs (excitatory postsynaptic currents) [33,34]. In these neurons, a train of 200 stimuli at 5 Hz results in long-term decrease in kainate receptor-mediated EPSCs via a mechanism involving mGluR5, PKC and PICK1 PDZ-domain interactions [34]. How exactly the interaction of kainate receptors with these PDZ-domain proteins affects synaptic function or synaptic localization is still under investigation.

Perspectives

Our understanding of the mechanisms by which kainate receptor trafficking and function are regulated during synapse formation and synaptic plasticity lags far behind similar studies for AMPA and NMDA receptors. The role of numerous identified interacting proteins is yet to be uncovered. In particular, 14–3–3 proteins play an essential role in controlling the function and localization of ion channels. Similarly, binding of 14–3–3 to the C-terminal domain of GluR6a or KA2 might be necessary for the efficient trafficking of kainate receptors containing these subunits. Profilin and spectrin are involved in the organization of the cytoskeleton. Finally, GluR6b binds proteins involved in the calcium regulation of ion channels and receptors. These interactions are probably necessary for fine regulation of native kainate receptor function.

The proper targeting and function of kainate receptors within the brain is likely to depend not only on molecular determinants and cytoplasmic protein interactions, but also on a variety of cellular cues. As an example, in CA3 pyramidal cells, not only are kainate receptors localized in the dendrites, but also this localization is restricted to the stratum lucidum at the zone of contact with mossy fibres, suggesting the existence of trans-synaptic signals that stabilize synaptic kainate receptors. Hence, a crucial point in the future will be to evaluate properly the trafficking of kainate receptors in vivo, in the context of complex synaptic circuits. The combination of imaging techniques in vivo with the
production of transgenic mice and viral-expressing vectors certainly opens very promising avenues in this regard.

References


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