Proteins involved in the synaptic organization of AMPA (α-amino-3-hydroxy-5-methylisoxazolepropionate) receptors

J. Henley

MRC Centre for Synaptic Plasticity, Anatomy Department, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, U.K.

Abstract

As well mediating most synaptic transmission in the mammalian central nervous system, α-amino-3-hydroxy-5-methylisoxazolepropionate (AMPA) receptors are involved in the processes of synapse formation, stabilization and plasticity. Thus the mechanisms that control the developmental and activity-dependent changes in the functional synaptic expression of AMPA receptors are of fundamental importance. In the last few years dramatic advances have been achieved towards elucidating some of the molecular events involved, and a previously unsuspected complexity of proteins that selectively interact directly or indirectly with individual AMPA receptor subunits have been identified. Here I present an overview of some of the main interacting proteins, and describe what we know about how these may fit into cellular pathways that control AMPA receptor targeting, trafficking and functional expression.

Introduction

α-Amino-3-hydroxy-5-methylisoxazolepropionate (AMPA) receptors mediate most synaptic transmission in the mammalian central nervous system, they play a fundamental role in synapse stabilization and plasticity, and their prolonged activation is potently neurotoxic. The developmental and activity-dependent changes in the functional synaptic expression of these receptors are therefore tightly regulated by the neuron. Because of their crucial importance for proper synaptic function, the molecular and cellular mechanisms that control the postsynaptic insertion and arrangement of AMPA receptors have been the subject of intense investigation. In the last few years there has been significant progress towards elucidating some of the processes involved.

Much of the new information has come from assays designed to identify proteins that interact with AMPA receptor subunits, in combination with confocal microscopy and electrophysiological techniques [1]. The success of several groups using these combined approaches has led to the discovery of a hitherto unsuspected complexity of proteins that can selectively interact with and regulate individual AMPA receptor subunits. These newly discovered interacting proteins have been implicated in, among other things, the regulation of AMPA receptor post-translational modification, targeting and trafficking, surface expression and anchoring.

Based on the information currently available, it appears that the intracellular C-terminal domain of the GluR2 subunit (ct-GluR2) has most interacting partners, at least as far as can be detected using the yeast two-hybrid assay [1]. Two distinct interaction domains on ct-GluR2 have been characterized: an N-ethylmaleimide-sensitive fusion factor (NSF)-binding site between residues 844 and 853, and an extreme C-terminal PDZ-binding motif (ct-GluR2/3 PDZ). The PDZ-binding motif has been shown to interact with three different PDZ-domain-containing proteins: glutamate receptor interacting protein (GRIP) [2] and AMPA receptor-binding protein (ABP) (one splice form is also known as GRIP2; [3]), which are closely related, and protein interacting with C-kinase 1 (PICK1) [4,5].

Recent work from our laboratories in the MRC Centre for Synaptic Plasticity here at Bristol and others around the world has suggested that AMPA receptors containing the subunit GluR2 undergo rapid recycling between their functional location in the postsynaptic membrane and a non-functional intradendritic pool. In this presentation I shall outline the situation with two of the best characterized proteins that interact with AMPA receptors, namely NSF and PICK1.

Key words: GluR subunits, NSF, PDZ domains, PICK1, protein kinase C.

Abbreviations used: AMPA, α-amino-3-hydroxy-5-methylisoxazolepropionate; ABP, AMPA receptor-binding protein; ct-GluR2, intracellular C-terminal domain of the GluR2 subunit; GRIP glutamate receptor interacting protein; LTD, long-term depression; NSF, N-ethylmaleimide-sensitive fusion factor; PICK protein interacting with C-kinase; PKC, protein kinase C.

*e-mail j.m.henley@bris.ac.uk

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NSF

NSF is a multihomomeric ATPase which plays an essential role in the membrane fusion processes underlying protein traffic through the Golgi apparatus and the vesicular release of neurotransmitters at the presynaptic membrane [6]. NSF is highly expressed in the central nervous system, and is most abundant in the hippocampus [7,8]. Furthermore, despite its known presynaptic role, transient cerebral ischaemia has been reported to cause an accumulation of NSF in the postsynaptic density [9]. It has also been reported recently that induction of long-term potentiation is blocked by N-ethylmaleimide, a potent inhibitor of NSF [10].

Thus, even before a direct interaction between NSF and GluR2 was demonstrated, there were data available to suggest that postsynaptic NSF could play a role in activity-dependent and pathological changes in synaptic function.

Several years ago we were the first to report that NSF binds to a seemingly unique recognition site located at the C-terminus of the GluR2 subunit of AMPA receptors [11]. Subsequently we [12-14], and others [15,16], demonstrated that the NSF interaction with GluR2 regulated the surface expression of AMPA receptors in hippocampal neurons. Blockade of the interaction between NSF and GluR2 by infusion of inhibitor peptides from a patch pipette into the postsynaptic neurons in hippocampal slices resulted in a rapid and substantial decrease in evoked AMPA-receptor-mediated excitatory postsynaptic currents. These data are consistent with the existence of a pool of AMPA receptors dependent on the NSF-GluR2 interaction for functional surface expression, and with the view that LTD expression involves the removal of these receptors from synapses [14].

Recent work [17] by Scott Ralph and James Uney at Bristol has shown that expression of the inhibitory peptide from a newly developed neuronal-specific adenoviral system resulted in significant neuroprotection to primary CA1–CA3 hippocampal neurons following stimulation with the agonist and potent neurotoxin kainate. This protection was accompanied by a reduction in Ca$^{2+}$ influx, and was also effective following glucose deprivation or exposure to ischaemic buffer, suggesting that GluR2-containing AMPA receptors may play a direct role in mediating post-ischaemic neurotoxicity [17].

PICK1

PICK1 is a PDZ-domain-containing protein that has been shown to interact with the GluR2 and GluR3 subunits of AMPA receptors in the yeast two-hybrid assay [4,5]. PICK1 is a 46.5 kDa protein that was originally isolated as interacting with the catalytic region of protein kinase Cz (PKCz) [18], and is also an efficient substrate for PKC phosphorylation. It does not interact with PKCβ or PKCε [18]. Although the functional consequences of the GluR2–NSF interaction have received considerable attention, only recently has progress been made in understanding the functional roles of the PDZ interactions [19–22].

PDZ domains are protein interaction modules that bind to specific target sequences, and have been shown to play an important role in a wide range of protein–protein interactions. PDZ domains comprise three repeats of ~90 amino acids, and the name derives from three proteins first shown to possess this motif, namely PSD-95 [23], its homologue Drosophila disc-large tumour suppressor gene (Dlp-A) product [24] and ZO-1, a tight junction protein [25]. PDZ domain motifs have so far been shown to be present either singly or as repeats in over 100 otherwise unrelated proteins. Some members of the family of proteins...
containing PDZ domains bind to specific amino acid residues at the C-termini of a variety of membrane proteins present at synapses. The current view is that the PDZ proteins act as adaptors to attach specific membrane proteins to defined scaffolding and/or trafficking proteins. In this way PDZ proteins may be intimately involved in the spatial organization of synaptic proteins, and also possibly in arranging the components of the intracellular signalling apparatus.

In addition to its interaction with GluR2/3, we [26], and others [27], have reported the interaction of PICK with the metabotropic glutamate receptor mGluR7a. PICK1 is also an efficient substrate for PKC phosphorylation. Other groups have described interactions between PICK1 and ephrin ligands and receptors [28,29] and the cytoplasmic protein ARF6 (ADP-ribosylation factor 6) [30]. Therefore it is likely that PICK1 might serve as an adaptor to link the target transmembrane proteins to cytoplasmic proteins such as ADP-ribosylation factors and PKC.

All of the transmembrane proteins that have been reported to interact with PICK1 also appear to interact with GRIP. GRIP is a 130 kDa protein that contains seven PDZ domains, of which domains 4 and 5 mediate binding to the extreme C-terminal ESVK1 motif of GluR2 and GluR3 [2]. Interestingly, although PICK1 and GRIP bind to the same proteins, there appears to be some specificity in their interactions. For example, the Eph-receptor family of receptor tyrosine kinases and their membrane-bound ligands, the ephrins, bind to both PICK1 and GRIP [28,29]. However, Eph-receptors bind PDZ domains 6 and 7 of GRIP (as opposed to domains 4 and 5 for GluR2/3). Furthermore, PKC phosphorylation of Ser-880 in the C-terminus of GluR2 differentially regulates the binding of the PDZ-domain-containing proteins GRIP/ABP and PICK1, and peptides corresponding to the GluR2 C-terminal PDZ-binding motif in which Ser-880 is phosphorylated or dephosphorylated can distinguish between GRIP/ABP and PICK1. The dephosphorylated form of the peptide blocked binding of GRIP/ABP and PICK1, whereas the phosphorylated form of the peptide selectively blocked PICK1, but not GRIP [31]. Both peptides attenuated cerebellar LTD, suggesting that expression of cerebellar LTD requires PKC-regulated interactions between the C-terminus of GluR2/3 and PDZ-domain-containing proteins [31].

In recent studies from John Isaac’s laboratory, it has been shown that ct-GluR2/3 PDZ interactions are important for regulating the function of synaptic AMPA receptors in hippocampal CA1 neurons [32]. Infusion of a peptide corresponding to the last 11 amino acids of ct-GluR2/3 (pep2-SVKI) into CA1 neurons caused a potentiation of basal synaptic transmission in approximately one third of cells. In addition, it blocked LTD in all cells tested. An inactive control peptide with a single amino-acid substitution in the PDZ-binding motif, pep2-SVKE, had no effect on transmission. Another peptide, pep2-EVK1, which blocked the PICK1 but not GRIP/ABP interaction, was also without effect. Application of the PKC inhibitor bisindolylmaleimide prevented both the potentiation in basal transmission and the blockade of LTD caused by pep2-SVKI. Prior induction of LTD resulted in pep2-SVKI causing a potentiation of excitatory postsynaptic currents in the majority of cells. Furthermore, de-depression (i.e. re-potentiation of a pathway that had previously received LTD), was selectively blocked by PKC inhibitors. These data suggest that the ct-GluR2/3 PDZ interactions regulate synaptic transmission at CA1 synapses in an activity- and PKC-dependent manner.

The interactions of proteins with ct-GluR2 are likely to have multiple roles, and are much more dynamic in regulating synaptic transmission than was originally thought. They provide an extra level of complexity for interactions between the protein scaffold and the signal transduction pathways present at the synapse. It is this continual association and dissociation of interacting proteins, both constitutive and activity-dependent, that is important for regulating the number of functional AMPA receptors at hippocampal synapses during basal transmission and synaptic plasticity.

References
The ubiquitin–proteasome pathway regulates lysosomal degradation of the growth hormone receptor and its ligand

P. van Kerkhof and G. J. Strous
Department of Cell Biology, University Medical Center Utrecht and Institute of Biomembranes, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

Abstract
The growth hormone (GH) receptor (GHR) is a mammalian plasma membrane protein whose internalization is mediated by the ubiquitin–proteasome pathway. GH internalization and degradation are inhibited when cells are treated with proteasome inhibitors. Here we show that a GHR truncated at residue 369 can enter the cells in the presence of a proteasome inhibitor, but that the subsequent lysosomal degradation of GH is blocked. Lysosomal inhibitors prolong the half-life of both receptor and ligand. Experiments with antibodies against different receptor tail sections show that degradation of the GHR cytosolic domain precedes degradation of the extracellular GH-binding domain. A possible role for the ubiquitin–proteasome pathway in the degradation of the receptor and ligand is discussed.

Introduction
Degradation of cytokine receptors generally occurs in lysosomes as the final step in signal down-regulation. Preceding events take place at the cell surface, i.e. dimerization of two receptors by a hormone followed by internalization of the complex, and in the endosome, i.e. either sorting to the lysosome or recycling back to the plasma membrane. The latter possibility is unlikely and generally unwanted, because the timing and intensity of cytokine receptor signalling must be

Key words: degradation, EGF receptor, endocytosis, lysosome, ubiquitination.
Abbreviations used: EGF, epidermal growth factor; GH, growth hormone; GHR, growth hormone receptor; UbE, ubiquitin-dependent endocytosis.

To whom correspondence should be addressed (e-mail strous@med.uu.nl).

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