Neuronal calcium sensors and synaptic plasticity

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Abstract

Calcium entry plays a major role in the induction of several forms of synaptic plasticity in different areas of the central nervous system. The spatiotemporal aspects of these calcium signals can determine the type of synaptic plasticity induced, e.g. LTP (long-term potentiation) or LTD (long-term depression). A vast amount of research has been conducted to identify the molecular and cellular signalling pathways underlying LTP and LTD, but many components remain to be identified. Calcium sensor proteins are thought to play an essential role in regulating the initial part of synaptic plasticity signalling pathways. However, there is still a significant gap in knowledge, and it is only recently that evidence for the importance of members of the NCS (neuronal calcium sensor) protein family has started to emerge. The present minireview aims to bring together evidence supporting a role for NCS proteins in plasticity, focusing on emerging roles of NCS-1 and hippocalcin.

Overview of NCSs (neuronal calcium sensors)

Calcium-binding proteins are generally divided into two classes, depending on affinity for calcium, binding off-rates and functionality: (i) calcium buffers and (ii) calcium sensors. CaM (calmodulin) is probably the best studied calcium sensor to date, and its role in synaptic plasticity is well established. Members of the NCS protein family have also been extensively studied, but many details regarding their specific roles in synaptic plasticity remain to be elucidated. NCS proteins share little (<20%) homology with CaM. They are characterized by four EF-hand calcium-binding motifs, of which only three are functional calcium-binding sites. The NCS proteins are classified into five different subgroups (A–E) based on their appearance in evolution and their sequence similarity (Figure 2A). All of the mammalian NCS proteins from subgroups A–D and one member from subgroup E [KChIP1 (K, channel-interacting protein 1)] contain an N-terminal myristoyl group [1], which enables the NCS protein to associate with the membrane. This process is termed a 'calcium myristoyl switch' [5] and is a prominent mechanism when considering micro- (or nano-) domains of calcium. NCS proteins are known to interact with several molecules, but we are still a long way from identifying all of their targets. We are, however, beginning to unravel the complex interplay between the emerging signalling components and the ability of NCS proteins to sense and respond to calcium. The ability of NCS proteins to interact with different target molecules together with their differing abilities for sensing calcium clearly makes them versatile components of the calcium signalling tool kit. In the present paper, we focus on the emerging roles of NCS-1 and hippocalcin in synaptic plasticity; for a more extensive overview of NCS proteins, readers are referred to more general reviews [2,3].

Myristoylation and the myristoyl switch

Myristoylation is a post-translational modification that adds a covalently linked short-chain fatty acid to a glycine residue at position 2 of the protein [4]. This lipid modification enables NCS proteins to interact directly with cellular membranes. Many members of the NCS protein family are permanently membrane-associated because their myristoyl groups are exposed when the protein is either in its calcium-free or calcium-bound form. Other members of the NCS family, however, associate with membranes only when they are in their calcium-bound form. In such cases, binding of calcium to the functional EF hands of the NCS protein induces a conformational modification that results in exposure of the myristoyl group, thus allowing it to associate with the membrane. This process is termed a 'calcium myristoyl switch' [5] and is a prominent mechanism for recovery and class B NCS proteins, of which hippocalcin is a member. O’Callaghan et al. [6] experimentally verified

Key words: hippocalcin, hippocampus, neuronal calcium sensor (NCS), peripheral cortex, synaptic plasticity.

Abbreviations used: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; AP2, adaptor protein 2; BAR, Bin/amphiphysin/Rvs; CaM, calmodulin; EGFP, enhanced green fluorescent protein; EPSC, excitatory postsynaptic current; GSK, glutathione transferase; LTD, long-term depression; LTP, long-term potentiation; mGluR, metabotropic glutamate receptor; NCS, neuronal calcium sensor; NMDAR, N-methyl-D-aspartate receptor; P531, protein that interacts with protein C-kinase 1; RNAi, RNA interference.

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that hippocalcin undergoes a calcium myristoyl switch in living cells [HeLa cells transfected with EYFP (enhanced yellow fluorescent protein)–hippocalcin], responding to free calcium in the 200–800 nM range. The same group later showed that different NCS proteins can be targeted to different membrane compartments within the cell and that the essential targeting information is contained in the N-terminal myristoylation sequence, comprising 11–14 amino acids [7]. Interestingly, although NCS-1 is always associated with the membrane, in contrast with hippocalcin, which undergoes translocation after calcium binding, both are localized to the plasma membrane and trans-Golgi network. The N-terminal myristoylation motifs for NCS-1 and hippocalcin are very similar (nine out of the 14 amino acids are identical) and have been shown to be sufficient for effective membrane targeting [7]. Moreover, the myristoyl motif of hippocalcin shows a high-affinity interaction with PtdIns(4,5)P$_2$, the most abundant phosphoinositide in cellular membranes, probably via interaction of its basic residues with lipid head groups [7].

**Hippocalcin and synaptic plasticity**

Hippocalcin, a 22 kDa protein comprising 193 amino acids (Figure 1A), is highly expressed in the principal cells of the hippocampus and moderately expressed in neurons of the cortex, cerebellum and striatum [8]. Hippocampal neurons overexpressing YFP (yellow fluorescent protein)-tagged hippocalcin have recently been used to show that transient site specific translocation of hippocalcin to the plasma membrane can occur under resting conditions and after stimulation.
that causes calcium entry through voltage-operated calcium channels [9]. These reversible increases in fluorescence were observed in spatially restricted areas (1–5 μm) of dendritic trees and axons. Translocation of hippocalcin to the plasma membrane decreased when the extracellular calcium concentration was reduced and was blocked when glutamate was mutated to glutamine, to prevent calcium binding, in EF hands 2 and 3 [9]. Hippocalcin has been shown to play important roles in modulating spatial and associative memory [10] and gating calcium activation of the slow after-hyperpolarization [11].

Of particular interest to the field of synaptic plasticity, hippocalcin plays an important role, acting as a molecular link between calcium entry through NMDARs (N-methyl-D-aspartate receptors) and the subsequent endocytosis of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors [12]. Specifically, hippocalcin has been shown to interact with the β2-adaptin subunit of the AP2 (adaptor protein 2) adaptor complex, which interacts with the GluA2/3 subunit of AMPA receptors in a calcium-dependent manner (Figure 1B). The N-terminal region of hippocalcin (comprising amino acids 2–72; Figure 1A) lacks functional calcium-binding domains and is essential for interaction with the β2-adaptin subunit. A fragment of hippocalcin derived from the N-terminal region (residues 2–72) acts as a dominant negative and inhibits the interaction of full-length hippocalcin with GluA2/3 subunits (Figure 1B). Infusion of this peptide (Hip2-72) into CA1 hippocampal neurons blocks LTD (long-term depression) induction (Figure 1C), but has no effect on basal transmission [12]. Cellular localization of hippocalcin has been shown to be calcium-dependent, with increased levels of AP2 and hippocalcin detected in postsynaptic density fractions from rat brains in the presence of calcium [12]. The detection of AP2, but not hippocalcin, in clathrin-coated vesicles purified from rat brain, fits the idea that hippocalcin can interact with AP2–GluA2/3 only transiently and at the plasma membrane. Following this model, hippocalcin recruits the β2 subunit of AP2 and translocates it to the plasma membrane once calcium entry has activated its myristoyl switch. Once at the plasma membrane, the hippocalcin–AP2 complex interacts with AMPA receptor subunits and, after dissociation of
hippocalcin, AP2 initiates clathrin-dependent endocytosis. We have recently obtained data from organotypic hippocampal brain slices transfected biolistically with hippocalcin RNAi (RNA interference) (Figure 2B) that confirm a critical role for hippocalcin in NMDAR-dependent LTD (Figure 2D).

NCS-1 and synaptic plasticity

Many of the initial studies regarding NCS-1 were performed in lower organisms [13,14], but NCS-1 is also highly expressed in mammals in all brain regions [8] as well as in non-neuronal cell types. NCS-1 interacts with a plethora of target molecules in the central nervous system and has a half-maximal affinity for calcium lower than 1 μM. NCS-1 interacts with most of its target molecules (e.g. the D2 receptor) in a calcium-dependent manner, but some interactions are calcium-independent (e.g. with the InsP3 receptor). Some of the proteins with which NCS-1 interacts have also been shown to bind to CaM, but the fact that these calcium sensors have differing affinities for calcium probably means that they regulate any shared target molecules in different ways [3].

NCS-1 has been shown to act as a calcium sensor in short-term plasticity in rat hippocampal cell cultures, switching paired pulse depression to paired pulse facilitation without altering basal transmission or initial transmitter release probability, probably by recruiting 'dormant' vesicles [15]. More recently, it has been shown that different calcium signalling pathways are involved in two independent forms of synaptic plasticity in the perirhinal cortex: NMDAR-dependent and mGluR (metabotropic glutamate receptor)-dependent LTDs [16] (Figure 3). Both forms of plasticity require postsynaptic calcium entry and release of calcium from intracellular stores, but the two forms of LTD have a different sensitivity to calcium (as shown by changing the BAPTA [1,2-bis(o-aminophenoxy)ethane-2,2,2′,2′-tetra-acetic acid] concentration in the patch pipette). In contrast with NMDAR-LTD, mGluR-LTD requires PKC (protein kinase C) activation, InsP3-mediated calcium release and a role for NCS-1 as a calcium sensor but not CaM [16]. The essential role of NCS-1 in mGluR-LTD has been shown using a dominant-negative mutant of NCS-1 (Figure 3A) and NCS-1 RNAi (Figure 3E). None of these treatments affect NMDAR-LTD (Figure 3D), which highlights the specificity of the interaction. In terms of the molecular mechanism, NCS-1 binds to PICK1 (protein that interacts with protein C-kinase 1) via its BAR (Bin/amphiphysin/Rvs) domain in a calcium-dependent manner, and this association is enhanced after stimulation of mGluRs, but not NMDARs [16]. Moreover, blocking this interaction with an inhibitory peptide [GST (glutathione transferase)–BAR] prevents the induction of mGluR-LTD (Figure 3G), but not NMDAR-LTD (Figure 3F).

Concluding remarks

Calcium sensors are exciting molecules to study in relation to synaptic plasticity because they interact with different target molecules and respond to different types of calcium signals in the central nervous system. Many NCS proteins undergo a conformational change after calcium binding, which allows them to translocate to, and associate with, cellular membranes. NCS proteins have been implicated in various diseases, including schizophrenia, bipolar disorder [17], Alzheimer’s disease and cancer [18]. Many target
molecules for NCS proteins remain to be identified, and we are still far from elucidating the intricate details of the roles that NCS proteins play in signalling pathways underlying synaptic plasticity. Further investigation into the role of calcium sensors in the central nervous system will shed new light on how they contribute to both the normal and abnormal functioning of neurons.

References

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