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PICK1 interacts with α7 neuronal nicotinic acetylcholine receptors and controls their clustering

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Central to synaptic function are protein scaffolds associated with neurotransmitter receptors. α7 neuronal nicotinic acetylcholine receptors (nAChRs) modulate network activity, neuronal survival and cognitive processes in the CNS, but protein scaffolds that interact with these receptors are unknown. Here we show that the PDZ-domain containing protein PICK1 binds to α7 nAChRs and plays a role in their clustering. PICK1 interacted with the α7 cytoplasmic loop in yeast in a PDZ-dependent way, and the interaction was confirmed in recombinant pull-down experiments and by co-precipitation of native proteins. Some α7 and PICK1 clusters were adjacent at the surface of SH-SY5Y cells and GABAergic interneurons in hippocampal cultures. Expression of PICK1 caused decreased α7 clustering on the surface of the interneurons in a PDZ-dependent way. These data show that PICK1 negatively regulates surface clustering of α7 nAChRs on hippocampal interneurons, which may be important in inhibitory functions of α7 in the hippocampus.

Keywords: Nicotinic receptor; α7 nAChR; PICK1; Clustering; Hippocampus; GABAergic interneuron

Introduction

Molecular scaffolds organize synaptic structures and downstream signaling processes. Among nAChRs, members of the PSD95 family interact with α3 and β4 subunits in the peripheral nervous system (Conroy et al., 2003; Parker et al., 2004), but no intracellular proteins regulating clustering of nAChRs have been identified in the central nervous system (CNS), yet. α7 nAChRs are prominent nAChRs and constitute α-bungarotoxin-(α-BT)-binding sites widely expressed throughout the CNS (Jones et al., 1999). They are important in learning, attention, nicotine addiction, and involved in neurodegenerative diseases and schizophrenia (Jones et al., 1999; Martin et al., 2004; O'Neill et al., 2002). α7 nAChRs are highly permeable for calcium (Seguela et al., 1993), present at synaptic and extrasynaptic sites (Fabian-Fine et al., 2001; Kawai et al., 2002; Levy and Aoki, 2002; Shoop et al., 1999) and have numerous functions in cell survival and synaptic plasticity (Dajas-Bailador and Wonnacott, 2004), implying specific interaction with appropriate signaling and scaffolding molecules (Berg and Conroy, 2002; Huh and Fuhrer, 2002). Src-family kinases (SFKs) have recently been found to associate with α7 nAChRs, causing α7 phosphorylation and decreased receptor activity (Charpantier et al., 2005). Unlike in the case of the neuromuscular AChR, however (Sadasivam et al., 2005; Willmann et al., 2006), SFKs do not seem to control clustering of α7 nAChRs (Wiesner and Fuhrer, 2006).

In the hippocampus, which receives rich cholinergic innervation from the septal complex, α7 nAChRs are highly expressed in GABAergic interneurons where they form postsynaptic clusters (Kawai et al., 2002), mediate cholinergic synaptic input (Alkondon et al., 1998; Frazier et al., 1998) and regulate inhibition within the hippocampal network (Alkondon et al., 1997; Jones and Yakel, 1997). Activation of these α7 receptors blocks concurrent STP and LTP induction in pyramidal cells (Ji et al., 2001). Inhibition of pyramidal neurons by postsynaptic α7 nAChRs on interneurons also underlies hippocampal auditory gating, suggesting that α7 might play a role in the pathogenesis of schizophrenia (Martin et al., 2004; Ripoll et al., 2004). Neuregulin, neurotrophins and NMDA receptor activity increase interneuronal α7 nAChR levels or clustering in hippocampus (Kawai et al., 2002; Liu et al., 2001) whereas raft-like lipid microdomains are important in α7 clustering in neurons of the ciliary ganglion (Bruses et al., 2001) — but in all these cases the intracellular proteins mediating or modulating α7 clustering remain unknown.

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Here we identify PICK1 as a first scaffolding protein that interacts with α7 nAChRs. PICK1 was originally isolated as a binding protein of protein kinase C (PKCα) (Staudinger et al., 1995), and PICK1 is important in synaptic targeting and clustering of other neurotransmitter receptors. Presynaptically, PICK1 binds to the C-terminus of mGluR7a and causes receptor clustering and phosphorylation by PKC (Boudin et al., 2000; Dev et al., 2000). Postsynaptically, PICK1 binds to and clusters kainate receptors through its PDZ domain (Hirbec et al., 2003). GluR2-containing AMPA receptors are clustered by PICK1 in heterologous cells (Xia et al., 1999). Furthermore, in neurons PICK1 influences glutamate receptor transport processes suggesting a role of PICK1 in the release of AMPA receptors from synaptic anchors and in receptor transport from the synaptic membrane towards endocytotic pathways (Perez et al., 2001; Steinberg et al., 2006; Terashima et al., 2004).

We find that the α7–PICK1 interaction involves the PDZ domain of PICK1 and a segment of the α7 intracellular loop. Interaction is shown in the yeast two-hybrid system and is confirmed in precipitation assays using recombinant and native proteins. Interestingly, PICK1 negatively regulates clustering of α7 receptors in hippocampal GABAergic interneurons, suggesting that PICK1 may play a specific role in α7-mediated inhibition of the hippocampal network.

Results

Identification of PICK1 as an α7 interaction partner using the yeast two-hybrid system

To search for intracellular molecules that interact with α7 nAChRs, we used the cytoplasmic loop of α7 as bait to screen a rat brain cDNA library using the yeast two-hybrid (YTH) technique (Fields and Song, 1989) (bait 1, aa 332–467, Fig. 1A). This loop is situated between transmembrane domains 3 and 4 and comprises most of the cytoplasmic portion of the α7 receptor. Positive candidates were verified by cotransformation of bait and prey clones into yeast and repeated lift filter assays. We classified binding results as positive or negative (+ or −; see Fig. 1A), in accordance to Staudinger et al. (1997), Xia et al. (1999) and Boudin et al. (2000). Among others, we identified two clones that encode full-length PICK1 (aa 1–417, Fig. 1A), showing that the α7 loop interacts with PICK1 in yeast.

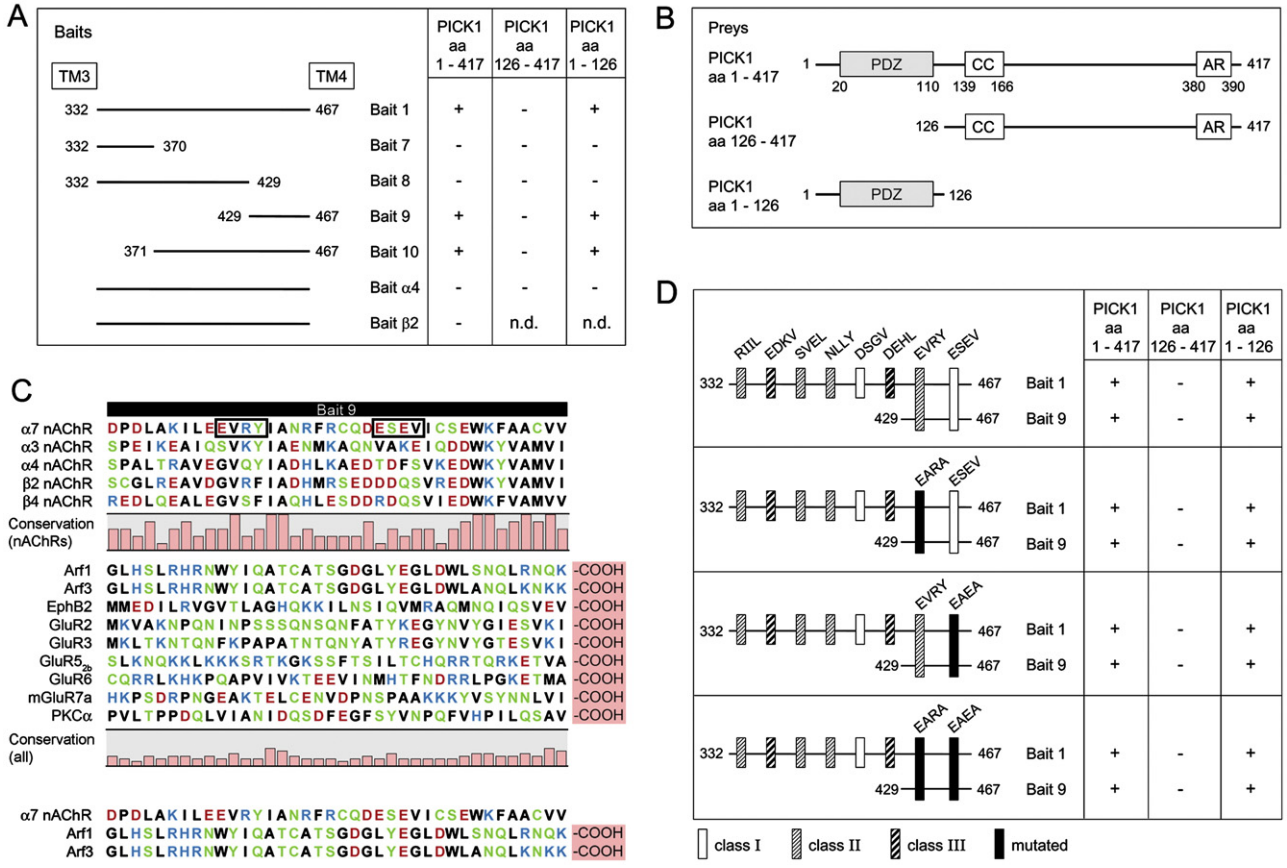

Fig. 1. Interaction between α7 nAChR and PICK1 in yeast. (A) Yeast strain AH109 was cotransformed with plasmids encoding the GAL4 DNA-binding domain fused to different sequences of the cytoplasmic loop of rat α7 nAChR (or rat α4 or β2 nAChR, as indicated) and the GAL4 activation domain fused to different PICK1 sequences. Protein–protein interaction was assayed by growing the yeast on selective medium and by galactosidase assays. The specificity of this interaction was tested using control plasmids; + indicates interaction, − no interaction. n.d., not done. (B) PICK1 prey constructs used. CC, coiled coil domain; AR, acidic region. (C) Sequence alignment of the bait 9 region of α7 with other nAChR subunits and with the C-terminus of other proteins known to bind PICK1. A separate alignment of α7 with Arf1 and Arf3 is shown at the bottom. Note the two putative PDZ-binding motifs, EVRY and ESEV. (D) Mutation of the putative PDZ-binding motifs (EVRY and ESEV) in α7 nAChR bait 1 and bait 9. The interaction with PICK1 prey vectors was not affected. Polarity colors mark the residues according to the polarity of amino acids (www.clcbio.com).