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Contextual utilization of enzymes in discrete AKAP79/150 signalling complexes

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Abstract

Cellular function involves the concerted action of signal transduction enzymes. Restriction of enzyme location contributes to the fidelity of each cellular response. A kinase-anchoring proteins (AKAPs) target the cAMP-dependent protein kinase and other signalling enzymes to defined subcellular locations. We have developed a new strategy that combines RNA interference of the endogenous protein and replacement with AKAP79/150 forms unable to anchor selected binding partners. Using this approach we show that AKAP79/150 coordinates different enzyme combinations to modulate the activity of two distinct neuronal ion channels: AMPA-type glutamate receptors and M-type potassium channels. Utilization of distinct enzyme combinations in this manner provides a means to expand the repertoire of cellular events that the same AKAP modulates.
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Many A kinase-anchoring proteins (AKAPs) are multivalent scaffold proteins (Wong and Scott, 2004). Accumulating evidence shows that AKAPs restrict the subcelluar localization of enzymes with broad substrate specificities, thereby contributing to the fidelity of each response. However, it is not clear whether the whole set of enzymes in the complex is required as a functional unit or only a portion of the complex is sufficient for mediating a certain pathway. To address this question, we developed an RNA interference (RNAi)/rescue approach (Hoshi, et al., 2005). Plasmid-based RNAi of AKAP150 was performed in cultured rat hippocampal neurons. The gene silencing of AKAP150 was confirmed by immunohistochemistry. The neurons in which the endogenous AKAP150 gene was silenced were followed by electroporation of plasmid and were rescued with various fragments from the human orthologue, AKAP79 (Fig. 1A). When protein kinase A (PKA) is inhibited by protein kinase inhibitor (PKI), gene silencing of AKAP150 abolished glutamate-dependent attenuation of AMPA currents when compared to controls, whereas ectopic expression of AKAP79 rescued the phenomena (Fig. 1B). Functional rescue of AMPA channel regulation was obtained with AKAP79 complexes lacking PKA or lacking protein kinase C (PKC), whereas reconstruction with AKAP79 forms unable to bind protein phosphatase 2B (PP2B) were ineffective (Fig. 1B). These results suggest anchored PP2B is essential for the downregulation of GluR1. Further, our study showed that neurons replaced with AKAP79 lacking PKA showed accelerated downregulation, which indicated PKA is required for the maintenance of GluR1 during repetitive glutamate application.

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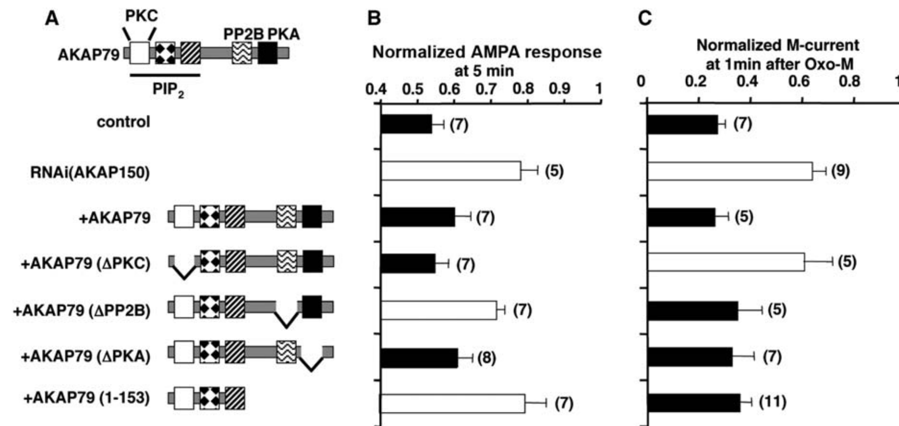

Fig. 1. Functional dissection of the AKAP79 complex for AMPA channel and M-channel. (A) Schematic diagram of AKAP79 deletion mutants lacking enzyme-binding sites for protein kinases A (PKA), protein kinase C (PKC), or protein phosphatase 2B (PP2B) and phosphatidylinositol 4,5-bisphosphate (PIP_2). (B) Amalgamated data depicting the level of normalized AMPA current from each experimental group taken 5 min after the application of glutamate (100 μM). White columns indicate significantly different from control and black columns indicate significantly different from RNAi(AKAP150). (C) Amalgamated data depicting the level of normalized M current from each experimental group taken 1 min after the application of oxotremorine-M (10 μM). Error bars indicate SEM.

Our RNAi/rescue approach was applied to examine a second AKAP79/150-mediated event, the suppression of the M-type potassium current. Muscarinic agonists inhibit this channel via a Gq-coupled pathway that evokes phosphoinositide turnover. Although a role for PKC in this process has been elusive, recent pharmacological studies imply that an anchored pool of this kinase facilitates this suppression of M current in superior cervical ganglion (SCG) neurons (Hoshi et al., 2003). Accordingly, gene silencing of AKAP150 in cultured rat SCG neurons reduced suppression of M currents in response to the muscarinic agonist, oxotremorine-M (Oxo-M) (Fig. 1C) when compared to controls. Ectopic expression of AKAP79 rescued the phenomenon (Fig. 1C). Only the PKC-anchoring-deficient form of AKAP79 failed to restore the M current inhibition. In complementary experiments, muscarinic inhibition of M current was restored upon expression of AKAP79 1-153, a fragment that encompasses the phosphoinositide-binding sites and PKC-anchoring region.

These studies imply that AKAP150 facilitates muscarinic suppression of M current by bringing PKC and the m1 muscarinic receptor in close proximity to the channel. These components and this configuration are distinct from the anchored signalling complex that functions to downregulate AMPA channels. This evidence suggests that AKAPs can mediate distinct pathways using different configuration.

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