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Co-expression of a novel ankyrin-containing protein, rSIAP, can modulate gating kinetics of large-conductance calcium-activated potassium channel from rat brain.

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We isolated a novel ankyrin-repeat containing protein, rSIAP (*r*Slo *I*nteracting *A*nkyrin-repeat *P*rotein), as an interacting protein to the cytosolic domain of the alpha-subunit of rat large-conductance Ca^{2+}-activated K^+ channel (rSlo) by yeast two-hybrid screening. Affinity pull-down assay showed the direct and specific interaction between rSIAP and rSlo domain. The channel-binding proteins can be classified into several categories according to their functional effects on the channel proteins, *i.e.* signaling adaptors, scaffolding net, molecular tuners, molecular chaperones, *etc.* To obtain initial clues on its functional roles, we investigated the cellular localization of rSIAP using immunofluorescent staining. The results showed the possible co-localization of rSlo and rSIAP protein near the plasma membrane, when co-expressed in CHO cells. We then investigated the functional effects of rSIAP on the rSlo channel using electrophysiological means. The co-expression of rSIAP accelerated the activation of rSlo channel. These effects were initiated at the micromolar $[Ca^{2+}]_i$ and gradually increased as $[Ca^{2+}]_i$ raised. Interestingly, rSIAP decreased the inactivation kinetics of rSlo channel at micromolar $[Ca^{2+}]_i$, while the rate was accelerated at sub-micromolar $[Ca^{2+}]_i$. These results suggest that rSIAP may modulate the activity of native BK_{Ca} channel by altering its gating kinetics depending on $[Ca^{2+}]_i$. To localize critical regions involved in protein-protein interaction between rSlo and rSIAP, a series of sub-domain constructs were generated. We are currently investigating sub-domain interaction using both of yeast two-hybrid method and *in vitro* binding assay.