PTH receptor-mediated G_s signaling is suppressed by direct binding of the subcortical cytoskeletal protein 4.1G to adenylyl cyclase type 6

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The G protein-coupled receptors (GPCRs) transduce their signaling through the activation of trimeric G proteins, but their associated mechanisms have remained unclear. It has been shown that the proteins that interact with carboxyl (C)-termini of GPCRs regulate the GPCRs-mediated signal transduction by modulating intracellular localization of the receptors. Parathyroid hormone (PTH)/PTH-related protein receptor (PTHR) is a G_s- and G_q-coupled GPCR. We previously showed that the C-terminus of PTHR directly binds to a subcortical cytoskeletal protein 4.1G. Cell surface expression of PTHR and its G_q/$[Ca^{2+}]_i$ signaling were increased by 4.1G, whereas its G_s/adenylyl cyclase (AC)/cyclic AMP (cAMP) signaling was reduced by 4.1G through unknown mechanisms. In the present study, we first found that AC type 6 (AC6) interacted with 4.1G in HEK293 cells and the N-terminus of AC6 (AC6-N) directly and selectively bound to the 4.1/ezrin/radixin/moesin (FERM) domain of 4.1G (4.1G-FERM) *in vitro*. Association of AC6-N with the plasma membrane was disturbed by the knockdown of 4.1G. Next, AC6-N was overexpressed to competitively inhibit the interaction of endogenous AC6 and 4.1G in the cells. Overexpression of AC6-N, as well as 4.1G-knockdown, augmented the cAMP production induced by forskolin, a direct AC activator, and PTH-(1-34). Taken together, our results demonstrate a model in which AC6-N associates with the plasma membrane through binding to 4.1G-FERM, resulting in low AC6 activity. The mechanism is responsible for the attenuation of PTHR-mediated G_s/AC6/cAMP signaling.