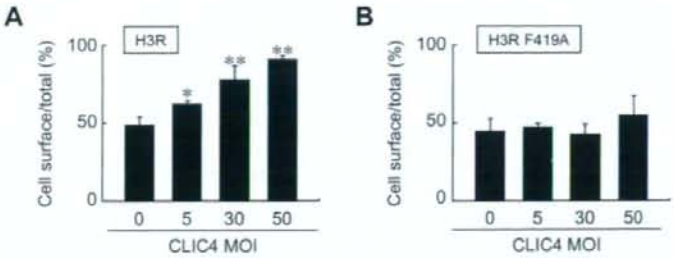

Fig. 4. Effects of CLIC4 on H3R cell surface expression in CHO cells. Cell surface H3R was measured by cell-based ELISA. (A) CHO cells were coinfected with rAd-H3R and rAd-CLIC4, and cell surface and total cell H3R were determined. The percentage of cell surface H3R/total cell H3R are shown. $^{*}p < 0.05$, $^{**}p < 0.01$ versus cells infected only with rAd-H3R. (B) CHO cells were infected with rAd-H3RF419A and rAd-CLIC4. Data are expressed as means ± SE of three independent experiments.

of α_{2B}-AR and AT1R receptors with their phenylalanine residues replaced with alanines, the F419A mutant form of H3R is itself capable of exiting ER to be expressed on cell surface. This observation suggests that, for H3R to be trafficked to cell surface, some other protein is also operating during its transit from ER to cell surface that is insensitive to the F419 mutation.

Because the ligand binding affinity of GPCR would be defined generally by its three dimensional conformation and its state of association with heterotrimeric G proteins [5], CLIC4 might influence assembly of G protein signaling complex with H3R. Molecular mechanisms of G protein assembly with GPCR, and subcellular locations of the assembly sites along the trafficking route to the cell surface are poorly understood. Thus, it would be an interesting possibility that CLIC4 is a chaperon component of a GPCR trafficking machinery that operates at some location along the exocytic pathway where heterotrimeric G protein assembly takes place. It is also important to know if this unpredicted function of CLIC4 is shared among other members of CLIC protein family, and if any anion channel activity of the protein is essential to this function.

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