

P002 Phosphodiesterase-4 influences the PKA phosphorylation status and membrane translocation of G-protein receptor kinase 2 (GRK2) in HEK293 β 2 cells and cardiac myocytes.

George S. Baillie.

Molecular Pharmacology Group, Division of Biochemistry & Molecular Biology, IBL, University of Glasgow, University Avenue, GLASGOW, G12 8QQ, UK

Membrane-recruitment of G-protein receptor kinase-2 (GRK2) provides a fundamental step in the desensitization process controlling G-protein coupled receptors such as the β_2 -adrenoceptor (β_2 AR). Here we show that challenge of HEK293 β 2 cells with isoprenaline causes GRK2 to become phosphorylated by PKA. This action is facilitated when phosphodiesterase-4 activity is inactivated, either chemically with rolipram or by siRNA-mediated knockdown of PDE4B and PDE4D. PDE4 inhibition by rolipram facilitates isoprenaline-induced membrane translocation of GRK2, phosphorylation of the β_2 AR by GRK2 and membrane translocation of β arrestin. PDE4 inhibition also enhances the ability of isoprenaline to trigger PKA phosphorylation of GRK2 in cardiac myocytes. In the absence of isoprenaline, rolipram-induced inhibition of PDE4 activity acts to stimulate PKA phosphorylation of GRK2, with consequential effects on GRK2 membrane recruitment and GRK2-mediated phosphorylation of the β_2 AR. We propose that a key role for PDE4 enzymes is (i) to gate the action of PKA on GRK2, influencing the rate of GRK2 phosphorylation of the β_2 AR and consequential recruitment of β arrestin subsequent to β -adrenoceptor agonist challenge and (ii) to protect GRK2 from inappropriate phosphorylation by PKA and membrane recruitment in unstimulated cells in response to basal levels of cAMP production.