Regulation of epithelial sodium channels by $G_{\alpha q}$

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Transepithelial Na^+ absorption mediated by epithelial Na^+ channels (ENaC) is important for Na^+ and fluid balance. The activity of ENaC is regulated by an array of physiological factors, many of which exert their effects on the channel *via* G-protein-coupled receptors. For example, intracellular ions exert their effect on ENaC *via* $G_{\alpha o}$ (Komwatana *et al.*, 1996) and $G_{\alpha i2}$ (Dinudom *et al.*, 1995), whereas the inhibitory effect of purinergic receptor activation on ENaC is mediated *via* an unidentified pertussis toxin-sensitive G-protein (Kunzelmann *et al.*, 2005). The G_q family, G_q, G_{11}, G_{14} and $G_{15/16}$, plays an important role in the regulation of the function of a variety of ion channels and transporters. For instance, G_q modulates L-type and N-type Ca^{2+} channels (Gamper *et al.*, 2004; Lu *et al.*, 2005), TRPC4 (Otsuguro *et al.*, 2008) and TASK-1 and TASK-3 K^+ channels (Chen *et al.*, 2006). So far, the mechanisms and extent to which ENaC is modulated by G_q family G-proteins remain largely unexplored.

To investigate the potential regulation pf ENC by G_q family members, we expressed constitutively active mutants of $G_{\alpha q}$, $G_{\alpha 11}$ and $G_{\alpha 14}$ in Fisher rat thyroid (FRT) cells cotransfected with ENaC and in mouse collecting duct (M1) cells. All α-subunits of G_q family proteins exerted a strong inhibitory effect on the activity of ENaC in both cell types. The effect of $G_{\alpha q}$ on ENaC, however, was not mediated *via* the traditional signaling molecules downstream of GPCR activation, such as PLC, PKC or MAP kinases. We also found that $G_{\alpha q}$ had no effect on the abundance of ENaC at the cell membrane and that its effect on ENaC was independent of Nedd4-2. We further found that the effect of $G_{\alpha q}$ on ENaC was inhibited by Grk2, although the kinase activity of Grk2 was not involved in its inhibition of the $G_{\alpha q}$ effect on ENaC. This effect of Grk2 was totally dependent on the presence of its Regulatory of G-protein Signalling (RGS) domain.

We conclude that the activity of ENaC is regulated by multiple G-protein signalling mechanisms that differentially influence the activity and the membrane expression of the channel. Grk2 acts as a negative regulator of G-protein-dependent regulation of ENaC in two different ways. The kinase activity of Grk2 renders G-protein-mediated regulation of ENaC, such as that of $G_{\alpha o}$, ineffective (Dinudom *et al.*, 2004). Additionally, similar to other RGS-like proteins, the structural domains of Grk2 impinge on the activity of free $G_{\alpha q}$, possibly by accelerating its hydrolysis of GTP, and so render them unable to propagate signals to modulate function of ENaC.

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