Regulation of the epithelial Na^+ channel (ENaC) by G protein-coupled receptor kinase (GRK2)

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The G protein-coupled receptor kinase (GRK2) belongs to a family of proteins that phosphorylates agonist-activated G protein-coupled receptors, leading to G protein-receptor uncoupling and termination of G protein signaling. This kinase has been implicated in the development of essential hypertension. We have made a novel finding that GRK2 regulates activity of the epithelial Na^+ channel (ENaC), a heterotrimeric Na^+ transport protein expressed in the distal nephron, the distal colon and ducts of salivary and sweat glands and which plays an important role in Na^+ homeostasis and the regulation of blood pressure. Inhibition of GRK2 activity in isolated mouse mandibular duct cells attenuated the activity of ENaC (Dinudom *et al.*, 2004), suggesting that basal activity of this kinase is important to maintain an appropriate level of Na^+ absorption in these epithelial cells. In the same tissue, a recombinant GRK2 and a phosphatase inhibitor, okadeic acid, interrupted feedback inhibition on ENaC by cytosolic Na^+. This was due to the inhibitory effect of GRK2 on Nedd4-2, an ubiquitin protein ligase that facilitates endocytosis of ENaC, a key component of the Na^+ feedback mechanism. We found that GRK2 increased serine phosphorylation of the C-terminus of the β-ENaC subunit. Taken together, these finding suggest that GRK2 increases activity of ENaC by phosphorylating the C-terminus of the β-ENaC rendering the channel insensitive to Nedd4-2.

GRK2 contains a regulator of G protein signaling homology (RH) domain, which selectively interacts with α-subunits of Gq/11 that are released during G protein-coupled receptor activation. We found that this RH domain of GRK2 also plays a role in regulating activity of ENaC. In M1 mouse collecting duct cells, expression of a kinase-dead GRK2 mutant increased activity of ENaC (Lee *et al.*, 2011). Conversely, a GRK2 mutant that lacks the C-terminal RH-domain or a GRK2 mutant that cannot interact with Gαq/11, has no effect on the activity of ENaC. These findings suggest that GRK2 upregulates ENaC as a consequence of the RH domain of GRK2 sequestering the α-subunits of Gq/11. This effect of GRK2 is independent of Nedd4-2 and does not involve inhibition of expression of ENaC in the plasma membrane or proteolytic activation of the channel (Lee *et al.*, 2011). The phosphorylation-independent effect that is mediated by the RH domain of GRK2 provides an additional regulatory mechanism for controlling epithelial Na^+ absorption.

Dinudom A, Fotia AB, Lefkowitz RJ, Young JA, Kumar S & Cook DI. (2004) The kinase GRK2 regulates Nedd4/Nedd4-2-dependent control of epithelial Na^+ channels. *Proceedings of the National Academy of Sciences U.S.A.* **101**: 11886-11890.

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