Regulation of epithelial Na^+ channels

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Body sodium and fluid homeostasis is largely determined by the activity of Na^+ transport proteins that are expressed in the kidney and the GI tract. Among these, the epithelial Na^+ channels (ENaC) play an important role in Na^+ transport by the distal kidney and the distal colon. This is evident from the observations that gain of function mutations of ENaC, as occur in Liddle's syndrome, cause hyperabsorption of Na^+ in the distal collecting duct of the kidney, leading to salt-sensitive hypertension, whereas loss of function mutations, as occur in pseudohypoaldosteronism type I, cause hypotension.

It is well established that the activity of ENaC is tightly regulated. The most important regulators of ENaC are aldosterone and arginine vasopressin which increase activity of the channel during extracellular fluid volume depletion. ENaC activity is also regulated by the concentration of Na^+ in the luminal fluid facing the apical membrane. This regulation is mediated by cytosolic Na^+ concentration which inactivates the channels by a mechanism involving the G protein, G_o, and an ubiquitin-protein ligase, either Nedd4 or Nedd4-2, which ubiquitinates the channels and triggers their endocytosis (Dinudom *et al.*, 1998).

Recent studies have suggested that, in addition to its genomic effects, aldosterone may activate ENaC via a mechanism that involves the serum- and glucocorticoid-stimulated kinase, Sgk. This kinase is believed to phosphorylate Nedd4-2 so as to prevent it binding the channels. Contrary to this belief, we have found in whole-cell patch-clamp studies on mouse mandibular duct cells that inclusion of recombinant, constitutively-active Sgk in the pipette solution does not prevent inactivation of ENaC by increased intracellular Na^+. We found instead that ENaC activity is increased by another protein kinase, the G-protein coupled receptor kinase, Grk2. Our experiments in salivary duct cells further showed that Grk2 phosphorylates the β subunit of ENaC and that this phosphorylation prevents Na^+ feedback inhibition of the channel by preventing the binding of Nedd4/Nedd4-2 to channel (Dinudom *et al.*, 2004)). We then investigated the regulation of ENaC by Grk2 in Fisher Rat Thyroid (FRT) cells, a model epithelium. We found that expression of Grk2 in FRT cells expressing ENaC caused a two-fold increase in the activity of ENaC compared to FRT cells in which ENaC alone is expressed. Conversely transfection of siRNA directed against Grk2 into FRT cells expressing ENaC inhibited ENaC activity. Interestingly, the mechanism by which Grk2 regulates ENaC in FRT cells differed from the mechanism in salivary duct cells. We found in FRT cells that a kinase-dead mutant of Grk2 activated ENaC in a same manner as wild-type Grk2, and that activation of the channels by Grk2 was due to binding of α-subunits of the Gq,11 family of G proteins by the Regulatory G-protein Signalling (RGS) domain of Grk2. The exact identity of the G protein that inhibits ENaC in FRT cells, and the mechanism by which it does so, are currently being investigated.

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