The activity of epithelial sodium channel is negatively regulated by H-Ras *via* an ERK1/2 dependent pathway

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The amiloride-sensitive epithelial Na^+ channel (ENaC) plays a critical role in regulating Na^+ and fluid homeostasis, blood pressure and the lung fluid clearance. ENaC is expressed in a variety of tissues including the renal collecting duct, the distal colon, the lungs and the ducts of exocrine glands. Activity of ENaC in these tissues is regulated by an array of physiological factors including hormones, nucleotides, and cytosolic ion concentrations. Some of these regulators of ENaC are known to exert their effect on the channel by mechanisms that involve GTPase proteins.

The small GTPases of the Ras family, are guanine nucleotide binding proteins that work as molecular switches that relay of signals from cell-surface receptors to intracellular effectors responsible for regulating cell proliferation, differentiation and apoptosis. Various downstream effectors of the three main Ras isoforms *i.e.*, H-Ras, N-Ras and K-Ras are believed to be responsible for their distinct physiological roles.

It has been reported that K-Ras upregulates activity of ENaC in a Na^+ absorptive epithelium *via* a PI3 kinase-dependent mechanism. Using modified Ussing chamber, however, we found that over-expression of constitutively active mutant of H-Ras strongly inhibited the amiloride-sensitive equivalent short-circuit current in mouse collecting duct (M1) cells and Fisher rat thyroid (FRT) cells expressing exogenous ENaC. Conversely, over-expression of a dominant negative mutant of H-Ras or knockdown of endogenous H-Ras expression by gene silencing techniques increased activity of ENaC. We found that expression of constitutively active mutant of H-Ras increased ERK1/2 phosphorylation in FRT cells, and inhibitory effect of H-Ras on ENaC was abolished by an ERK1/2 inhibitor, PD98059. Together, these findings suggest that H-Ras mediates its inhibitory effect on activity ENaC by a different mechanism to that of K-Ras and that the cellular mechanism underlying the effect of H-Ras on ENaC regulation involves activation of ERK1/2.