Epithelial Sodium channels are regulated by the tyrosine kinase, c-Abl

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Epithelial Na^+ channels (ENaC) mediate Na^+ absorption in kidney, lung and colon epithelia. The function of ENaC is important in the maintenance of Na^+ and fluid homeostasis, and the regulation of plasma volume and blood pressure. Activity of ENaC is controlled by hormones, such as aldosterone and insulin, and paracrine signalling molecules, such as tumor necrosis factor (TNF) and transforming growth factor-β (TGF-β). Many of these regulators of ENaC activity exert their effects via protein kinases. Abelson kinase, c-Abl, is a 150-KDa non-receptor tyrosine kinase that is stimulated by two regulators of ENaC, TNF and TGF-β. c-Abl contains multiple interactive domains, including Src homology (SH) 3 domain and SH2 domain, a tyrosine kinase catalytic domain, proline-rich motifs, DNA-binding domains and actin-binding domains, which allow transduction of a variety of cellular signals. Although c-Abl is wildly expressed in epithelial cells, the role of this kinase in regulating ENaC is currently unknown.

Using RT-PCR and Western blot analysis, we confirmed endogenous expression of c-Abl kinase in mouse kidney collecting duct (M1) cells, human lung epithelial (H441) cells, and in Fisher rat thyroid (FRT) cells. We found that expression of a constitutively active mutant of c-Abl strongly inhibited the activity of ENaC in both M1 cells, which endogenously express ENaC and FRT cells which express exogenous ENaC. Interestingly, c-Abl cannot downregulate activity of a mutated ENaC in which the c-terminal of the β-subunit of the channel has been truncated, or a mutated ENaC in which a consensus phospholylation site for c-Abl on βENaC, βY618, is mutated to alanine. The amino acid Y618 on βENaC is part of the PY motif that interacts with the ubiqitin protein ligase, Nedd4-2, a known regulator of ENaC which mediates ubiquitin-dependent downregulation of the channel. c-Abl, however, downregulated activity of ENaC in cells in which Nedd4-2 expression was knocked-down by expression of siRNA directed against Nedd4-2.

We conclude that c-Abl kinase mediates its inhibitory effect on the activity of ENaC activity via the PY motif of the β-subunit of ENaC in a Nedd4-2 independent manner.