Feedback Regulation of Epithelial Na+ Channel (ENaC)

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Summary

The epithelial Na+ channel (ENaC), composed of three subunits (α, β, and γ), is expressed in several epithelia and plays a critical role in salt and water balance and in the regulation of blood pressure. The activity of ENaC is tightly regulated not only by various hormones such as aldosterone and vasopressin, but also by intracellular factors such as Na+. A mechanism by which the activity of ENaC is regulated in native Na+ transport epithelia is called the 'feedback inhibition', which is defined as channel downregulation due to the transport of Na+ across the apical membrane and its accumulation intracellularly. However, molecular mechanisms of the feedback inhibition are not yet fully understood. The aim of the present study was to assess the role of C-terminus of γ subunit of rat ENaC (rENaC) in the "feedback inhibition" of ENaC activity by cytosolic Na+ concentration. Using the patch-clamp techniques, we have now examined the effect of deletion of amino acid residues at C-terminus of γ subunit (R576stop: γLiddle) on the feedback inhibition. Under conventional whole-cell patch clamp configuration, $\alpha\beta\gamma$liddle rENaC-expressing MDCK cells exhibited Na+ conductance reversibly inhibited by 10μM amiloride applied extracellularly. Ion selectivity sequence of the Na+ conductance was $Li^+ > Na^+ >> K^+ =$ N-methyl-D-glucamine+ (NMDG+). Using excised inside-out patches, single channel conductance, likely responsible for the macroscopic Na+ channel current, was found to be 9 pS when Li+ was used as a charge carrier. Therefore, these biophysical properties of both macroscopic and microscopic currents of $\alpha\beta\gamma$Liddle rENaC was similar to those of $\alpha\beta\gamma$ENaC expressed in MDCK cells. In inside-out patches obtained from MDCK cells expressing $\alpha\beta\gamma$ENaC, the channel activity (nPo), defined as a product of the number of active channel (n) and open probability (Po), was decreased when cytosolic Na+ concentration was increased from 0 to 25 mM. In $\alpha\beta\gamma$Liddle rENaC-expressing MDCK cells, however, nPo was not affected even when cytosolic Na+ concentration was increased from 0 to 150 mM in inside-out patches. These results provide evidence for the role of C-terminus of γ subunit of rENaC in the "feedback inhibition" by cytosolic Na+.