PIP_2 mediates sodium feedback inhibition in the epithelial sodium channel

C.R. Campbell, A. Dinudom and D.I. Cook, Discipline of Physiology, University of Sydney, NSW 2006, Australia.

Epithelial Na^+ channels (ENaC) are important for regulation of Na^+ and fluid homeostasis and blood pressure. It has been previously reported that phosphatidylinsositol 4,5-bisphosphate (PIP_2) upregulates ENaC activity (Yue *et al.*, 2002) and that PIP_2 may mediate the regulation of ENaC by purinergic stimulation (Kunzelmann *et al.*, 2005). Details of the mechanism by which PIP_2 acts on ENaC are, however, currently unknown. In this project we used the whole-cell patch-clamp technique to measure ENaC activity in isolated mouse mandibular duct cells (Dinudom *et al.*, 1998) in order to investigate the pathway through which PIP_2 acts to increase ENaC activity. We found that the activity of ENaC in the duct cells is inhibited by inclusion in the pipette solution of a specific antibody directed against PIP_2. Inclusion in the pipette solution of peptides corresponding to the N-termini of β- or γ-ENaC significantly decreased ENaC activity. These peptides contain consensus PIP_2 binding motifs. We found that inclusion of 25 μM PIP_2 in the pipette solution completely overcame the inhibitory effect of increased cytosolic Na^+ on ENaC. Interestingly, PIP_2 did not overcome the inhibitory effect of inclusion of recombinant Nedd4-2 protein in the pipette solution. Taken together, these data suggest that PIP_2 is essential for maintaining ENaC activity in salivary duct cells and that it exerts its effect on ENaC by blocking the Na^+ feedback pathway, possibly at a step upstream from Nedd4-2. The mechanism by which PIP_2 inhibits Na^+ feedback inhibition of ENaC is currently under investigation.

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