Regulation of the epithelial sodium channel by phosphatidylinositol 4,5-bisphosphate

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Epithelial Na^+ channels (ENaC) control sodium and fluid homeostasis and regulate blood pressure. In addition to being tightly regulated by well-known hormonal and homocellular factors, recent studies in A6 cells indicate that activity of ENaC may also be influenced by phosphatidylinositol 4,5-bisphosphate (PIP_2), a membrane lipid[1,2]. Here we investigate whether PIP_2 is involved in regulation of ENaC activity in mammalian epithelial cells by examining whether inhibition of PIP_2 activity reduces ENaC activity in isolated mouse mandibular duct cells.

Amiloride-sensitive Na^+ conductance was measured using whole-cell patch-clamp analysis in duct cells prepared from mandibular glands of euthanased Quackenbush mice[3]. Whole-cell current was measured three minutes after the whole-cell configuration was obtained. The amiloride-sensitive Na^+ conductance was calculated from the difference between the currents observed with and without 100 Î¼M amiloride.

Under control conditions, the amiloride-sensitive chord conductance of the duct cells was 275.2 ± 51.1 pS (n = 9). In the presence of an antibody directed against PIP_2 (60 nM) in the pipette solution, the amiloride-sensitive conductance was reduced by 38.2% to 170.1 ± 12.5 pS (n = 8). Therefore, PIP_2 activity may contribute to maintenance of ENaC function in duct cells. To confirm this finding, poly-L-lysine (300 Î¼M) was added to the pipette solution to disrupt the electrostatic interaction between PIP_2 and its target molecule. Poly-L-lysine reduced the amiloride-sensitive conductance by 32.8% from 304.0 ± 36.0 pS (n = 10) to 204.4 ± 44.8 pS (n = 7). The detailed mechanism of how PIP_2 influences ENaC activity in duct cells is currently under investigation.

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