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ELEVATION OF INTRACELLULAR Ca^{2+} INHIBITS EPITHELIAL SODIUM CHANNELS BY DECREASING MEMBRANE PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE

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The epithelial sodium channel (ENaC) in distal nephron plays an important role in regulating sodium balance to control systemic blood pressure and consequently affect cardiovascular function. Using patch-clamp techniques, we found that application of ionomycin (a Ca^{2+} ionophore) to A6 cells reduced open probability of ENaC from 0.56 ± 0.17 to 0.09 ± 0.05 (initial inhibition; $p < 0.01$) and 0.07 ± 0.06 (sustained inhibition; $p < 0.01$) in 5 cell-attached patches. GF 109203X, a protein kinase C (PKC) inhibitor, did not affect the initial inhibition, but abolished the sustained inhibition. In contrast, U-73122, a phospholipase C (PLC) inhibitor, but not U-73343 (an inactive analog of U-73122), abolished the initial inhibition and partially reduced the sustained inhibition. Stimulation of PKC with phorbol 12-myristate 13 acetate (PMA) inhibited ENaC with a 3-min delay in control cells, but not in the cells pre-treated with U-73122. Using confocal microscopy, we found that ionomycin induced a significant decrease in membrane phosphatidylinositol-4,5-bisphosphate (PIP_2), monitored by a GFP-tagged pleckstrin homology domain of PLC-d1. Like ENaC activity, the sustained decrease in membrane PIP_2 was abolished by inhibition of PKC, while the initial decrease in membrane PIP_2 induced by ionomycin was abolished by inhibition of PLC. Our results suggest that the secondary messenger Ca^{2+} alters ENaC activity through both PLC- and PKC-dependent decreases in membrane PIP_2 .