

Amiloride-sensitive epithelial Na⁺ channels reconstituted into planar lipid bilayer membranes

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High resistance epithelia actively transport sodium from the luminal side to the blood. Aldosterone and vasopressin stimulate this sodium transport system; the diuretic drug amiloride inhibits it in a reversible fashion. The first step in the transepithelial transport of Na⁺ is the facilitated diffusion of Na⁺ across the apical membrane via Na⁺-specific, amiloride-sensitive channels. We report here the first direct measurements of single, amiloride-sensitive Na⁺ channel activity. The channel was isolated after incorporation of purified apical membrane vesicles from A6 cells into planar lipid bilayers. The channel had the following characteristics: single-channel conductance ranged from 4 to 80 pS at 200 mM NaCl; it was perfectly cation-selective; amiloride reduced the open-state conductance in a dose-dependent fashion when present in the cis compartment, and induced flickering when present in the trans chamber; channel conductance and gating were voltage-independent; and the Na⁺/K⁺ sel