Supplementary information

P_f of a confluent epithelial monolayer. The cell monolayer grown on a Transwell-polycarbonate membrane was vertically mounted in a Teflon chamber that thereby was divided into two compartments. The hypertonic DMEM solutions in both compartments were stirred continuously to guarantee an invariant size of the unstirred layers (USL) in the immediate epithelial vicinity. The USL thickness, δ, is defined in terms of the K^+ concentration gradient at the membrane water interface:

$$\frac{|C_s - C_b|}{\delta} = \frac{fC}{fx}\Big|_x$$

(1)

where x is the distance from the membrane. C_b and C_s denote the K^+ concentrations in the bulk and at the interface, respectively. Osmotic water flux was induced by addition of 0.4 M D-sorbitol to the basolateral compartment. The resulting K^+ dilution in the hypertonic compartment was used to derive P_f. Therefore, K^+ concentration was measured (i) in the steady-state as a function of the distance to the surface of the epithelial cells and (ii) after stimulation with forskolin as a function of time at a distance of 20 μm away from the epithelium ($C_{20}(t)$).

Steady state P_f: C(x) measured within the USL ($-\delta < x < \delta$), can be used to derive the linear velocity of the osmotic volume flow, v (Pohl et al., 1997):

$$C(x) = C_s e^{\frac{-vx}{D} + \frac{ax^3}{3D}}$$

(2)

where D and a are the K^+ diffusion coefficient and the stirring parameter, respectively. v allows calculation of P_f:

$$P_f(t) = \frac{v}{V_W C_{osm}}$$

(3)

where V_w is the partial molar volume of water, C_{osm} the osmolyte concentration, corrected (i) for dilution by water flux and (ii) by the osmotic coefficient (here 1.09).

P_f after stimulation with forskolin: Because in a well stirred system, δ does not depend on v, C_s was expressed from a combination of the Eqs. 1 and 2. Subsequently, Eq. 2 was used to derive $v(t)$ as a function of $C_{20}(t)$, where $vx/D >> ax^3/3D$. For the hypertonic USL we got:

$$P_f(t) = \frac{D[C_{20}(t) - C_b]}{C_{osm}V_W[C_{20}(t)\delta - C_b x]}$$

(4)

The K^+ sensitive microelectrodes were made of glass capillaries, the tips (1 - 2 μm in diameter) of which where filled with cocktail B of K^+ Ionophore I (Fluka). Movement of the electrodes relative to the epithelial monolayer was realized by a hydraulic stepdrive (Narishige, Japan) with a velocity of 3 – 5 μm/s.

Flash photolysis of BCMCM-caged 8-Br-cAMP. The [6,7-bis(carboxymethoxy)coumarin-4-yl]methyl (BCMCM) ester of 8-Br-cAMP (BCMCM-caged 8-Br-cAMP) was synthesized in analogy to BCMCM-caged cAMP (Hagen et al., 2001). Flash photolysis of BCMCM-caged 8-Br-cAMP was induced by a Xenon flash-lamp (Till Photonics, Martinsried, Germany) coupled to a Zeiss Axiovert TV135 microscope (Fluar 40x, 1.3, oil immersion objective, Carl Zeiss, Jena, Germany). The release of 8-Br-cAMP was observed by monitoring the fluorescence emitted from the liberated photoproduct 6,7-bis(carboxymethoxy)-4-(hydroxymethyl)coumarin (BCMCM-OH), using excitation at 347 nm (polychromatic illumination system, Till Photonics). The fluorescence immediately before and after the flash (10 ms excitations) was collected through a dichroic mirror FT 395, a 400 nm long pass filter (both Carl Zeiss), detected by a photodiode and acquired simultaneously with C_m, G_m and G_s data by the EPC-9. Fluorescence images of the cells were captured as described (Lorenz et al., 1998).

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