Data Supplement

CPT-cGMP Stimulates Human Alveolar Fluid Clearance by Releasing External Na^+ Self-Inhibition of ENaC

Dong-Yun Han[1,2], Hong-Guang Nie[2,3], Xue-Feng Su[1,], Xue-Mei Shi[1], Deepa Bhattarai[1], Meimi Zhao[1], Run-Zhen Zhao[1], Katlin Landers[1], Hua Tang[1], Lin Zhang[4], Hong-Long Ji[1,2]

METHODS and MATERIALS

Method for the two-electrode voltage clamp recording

The use of *Xenopus laevis* and surgical procedure were approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at Tyler. Oocytes were surgically removed from appropriately anesthetized adult female *Xenopus laevis* (Xenopus Express Inc., Brooksville, FL) and cRNAs for full length and mutated ENaC were prepared as described previously (1). Briefly, the ovarian tissue was removed from frogs under anesthesia by ethyl 3-aminobenzoate methanesulfonate salt (Sigma, St. Louis, MO) through a small incision in the lower abdomen. Ovary lobes were removed and digested in OR-2 calcium-free medium (in mM: 82.5 NaCl, 2.5 KCl, 1.0 $MgCl_2$, 1.0 Na_2HPO_4, and 10.0 HEPES, pH 7.5) with the addition of 2 mg/ml collagenase (Roche, Indianapolis, IN). Defolliculated oocytes were cytosolically injected with ENaC cRNAs and incubated in half-strength L-15 medium at 18°C. The two-electrode voltage clamp technique was used to record whole-cell currents 24 or 48 h post injection. Oocytes were impaled with two electrodes filled with 3M KCl, having resistances of 0.5–2 MΩ. A TEV-200 voltage clamp amplifier (Dagan, Minneapolis, MN) was used to clamp oocytes with concomitant recording of currents. Two reference electrodes were connected to the bath. The basic bathing solution was ND96 medium (in mM: 96.0 NaCl, 1.0 $MgCl_2$, 1.8 $CaCl_2$, 2.5 KCl, and 5.0 HEPES, pH 7.5). Whole-cell steady-state currents were recorded as previously reported (2). Experiments were controlled by pCLAMP 10.1 software (Molecular Devices, Sunnyvale, CA), and currents at -40 mV, -100 mV, and +80 mV were continuously monitored with a sampling interval of 10 s. Data were digitized at 1,000 Hz and filtered at 500 Hz.

Whole-cell and single channel patch clamp recordings

When whole-cell mode was established, the bath solution was fast switched to the regular bath solution (in mM: NaCl 145, $MgCl_2$ 2, $CaCl_2$ 1.8, HEPES 10, pH 7.4) from a low Na^+ perfusate (containing 1mM NaCl) to elicit self-inhibition. Pipette solution comprised of (in mM) KCl 145, $MgCl_2$ 2, $CaCl_2$, 0.5, EGTA 4, K_2ATP 2, HEPES 10 (pH 7.4). Solutions supplemented with CPT-cGMP (0.5 mM) were applied following the acquisition of basal self-inhibition traces (3).

Single channel patch clamp assay were carried out as described preciously (4).

Cell cGMP measurements

Lung tissues, H441 cells, and oocytes were exposed CPT-cGMP under the experimental conditions for AFC, patch clamp, and voltage clamp studies. Saline treated samples were controls. Cyclic GMP contents were measured using the cGMP Enzyme Immunoassay kits (Assay Designs, Ins. Ann Arbor, MI) following the user manual.

References:

1. Ji HL, Parker S, Langloh AL, Fuller CM, Benos DJ. Point mutations in the post-M2 region of human alpha-ENaC regulate cation selectivity. *Am J Physiol Cell Physiol* 2001;281(1):C64-74.

2. Ji HL, Su XF, Kedar S, Li J, Barbry P, Smith PR, Matalon S, Benos DJ. Delta-subunit confers novel biophysical features to alpha beta gamma-human epithelial sodium channel (ENaC) via a physical interaction. *J Biol Chem* 2006;281(12):8233-8241.
3. Nie HG, Chen L, Han DY, Li J, Song WF, Wei SP, Fang XH, Gu X, Matalon S, Ji HL. Regulation of epithelial sodium channels by cGMP/pkgii. *J Physiol* 2009;587(Pt 11):2663-2676.
4. Nie HG, Zhang W, Han DY, Li QN, Li J, Zhao RZ, Su XF, Peng JB, Ji HL. 8-pcpt-cGMP stimulates alphabetagamma-ENaC activity in oocytes as an external ligand requiring specific nucleotide moieties. *Am J Physiol Renal Physiol* 2010;298(2):F323-334.