BJ www.biochemj.org

BJ Signal

Biochem. J. (2011) **433**, 197–204 (Printed in Great Britain) doi:10.1042/BJ20101256

online data

SUPPLEMENTARY ONLINE DATA

The C-terminal basic residues contribute to the chemical and voltage-dependent activation of TRPA1

Abdul SAMAD*†[1], Lucie SURA*[1], Jan BENEDIKT*, Rudiger ETTRICH†, Babak MINOFAR†, Jan TEISINGER* and Viktorie VLACHOVA*[2]

*Department of Cellular Neurophysiology, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic, and †Laboratory of Structural Biology, Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, Zamek 136, 373 33 Nove Hrady, Czech Republic

See the pages that follow for Supplementary Figure S1

[1] These authors contributed equally to this work.
[2] To whom correspondence should be addressed (email vlachova@biomed.cas.cz).

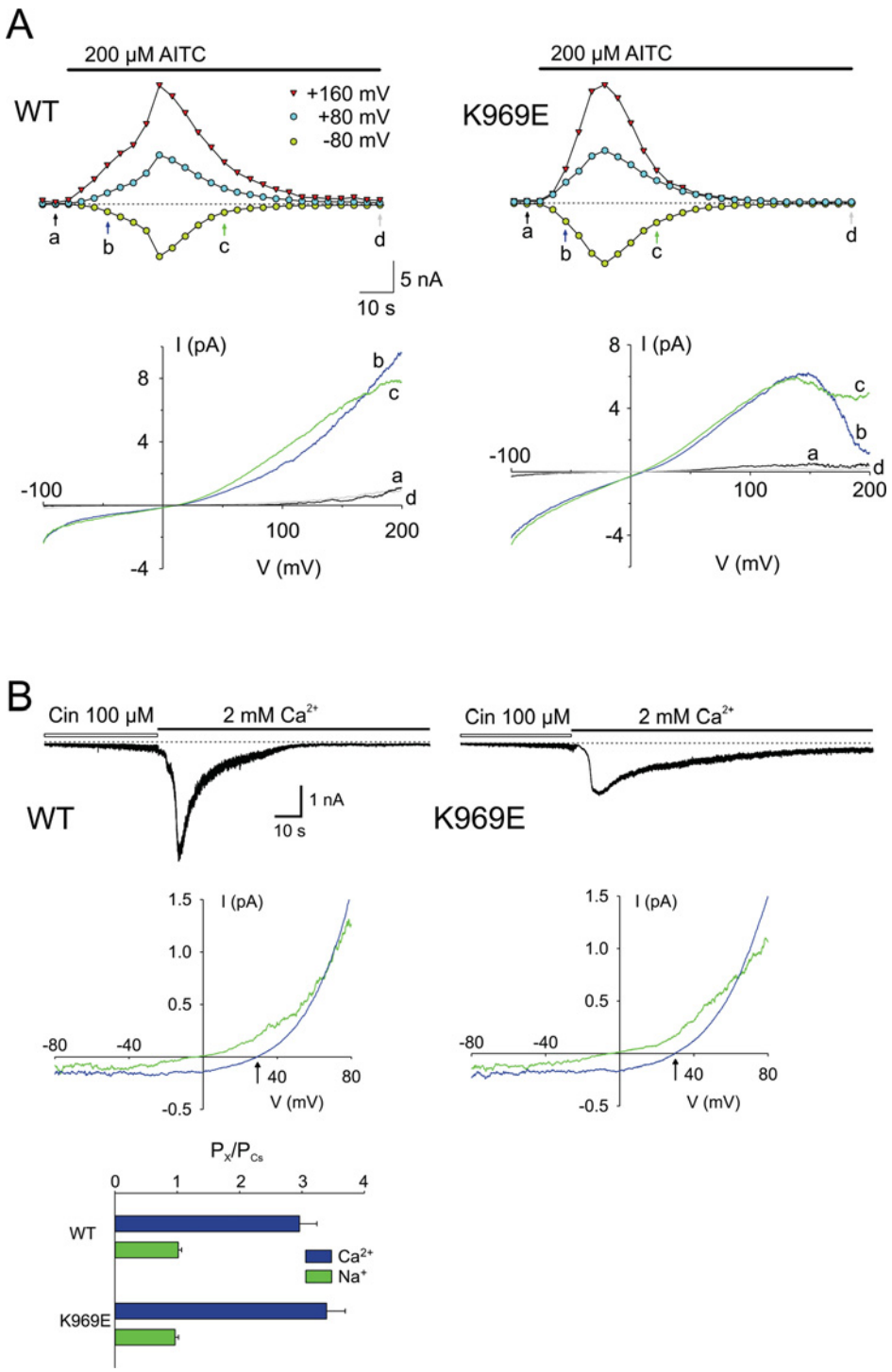

Figure S1 Mutation K969E does not affect the ion permeation properties of TRPA1

(**A**) Effects of the charge-reversing mutation K969E on chemical sensitivity and voltage-dependent properties of the recombinant human TRPA1 channel transiently expressed in HEK-293T cells. The voltage protocol consisted of 600 ms ramps from -100 mV to $+200$ mV applied every 3.8 s, first in extracellular control solution, and then in the maintained presence of 200 μM AITC (bar above the record). Time course of whole-cell membrane currents mediated by wild-type (left) and mutant (right) TRPA1 channel, measured at -80 mV (green circles), $+80$ mV (blue circles) and $+160$ mV (red triangles). Note the changes in the onset of activation in K969E. Voltage ramp traces measured without any stimulation (a), at the onset phase of the AITC response (b), at the inactivation phase (c) and at fully inactivated state of the channels (d). Panels below the time courses are current–voltage relationships of the traces measured at the times indicated by arrows as a, b, c and d above. Currents through K969E decay at more positive membrane potentials (>100 mV). The very close proximity of the first C-terminus helix (comprising Lys^{969}, Arg^{975}, Lys^{988} and Lys^{989}) to the putative inner vestibule of the channel makes it tempting to speculate that this helix can be orientated toward the plane of the membrane and its interface with the cytosol and may constitute a gating ring that transforms AITC-modification energy or depolarization into the mechanical energy necessary to open the pore. (**B**) Ca^{2+} permeability relative to Cs^{+} was measured by using a ramp depolarization (1 V/s) in bi-ionic conditions similar to those described previously [1]. Inward currents were activated by $\sim$40 s exposure to cinnamaldehyde (Cin) and voltage-induced currents were measured first in Ca^{2+}-free bath solution and then in high Ca^{2+} bath solution (128 mM Ca^{2+}). In agreement with published data [2,3], wild-type (WT) TRPA1 channels exhibited relative permeability P_{Ca}/P_{Cs} of 3.0 ± 0.2 ($n = 7$). The K969E mutation yielded channels with unchanged permeability to Ca^{2+} ($P_{Ca}/P_{Cs} = 3.4 \pm 0.3$; $P < 0.05$; $n = 6$), which indicates that this mutation does not affect the ion

permeation process and most likely does not indirectly influence the Ca^{2+}-dependent gating of the channel. The Ca^{2+}-free bath solution contained 150 mM NaCl, 10 mM Hepes and 2 mM EDTA, pH 7.3. For ion-permeability experiments, the high Ca^{2+} bath solution contained 128 mM $CaCl_2$ and 10 mM Hepes (pH 7.3 with NaOH) and the pipette solution contained 5 mM EGTA, 3 mM $CaCl_2$ (100 nM free Ca^{2+}), 145 mM CsCl, 2 mM MgATP and 10 mM Hepes, pH 7.3 with CsOH. Cinnamaldehyde solution was prepared from a 0.1 M stock solution in DMSO so that the final concentration of DMSO in the solutions applied to cells was 0.1 %. The relative permeability P_{Ca}/P_{Cs} was calculated based on the reversal potential (V_{rev}) of the currents measured first in Ca^{2+}-free bath solution and then in the high Ca^{2+} bath solution (with Ca^{2+} as the only permeant ion) according to the rearranged constant-field equation $P_{Ca}/P_{Cs} = ([Cs^+]_i/4 \cdot [Ca^{2+}]_o) \exp(V_{rev}F/RT) [\exp(V_{rev}F/RT)+1]$, where F is Faraday's constant, R is the gas constant and T is temperature [4]. The permeability ratio P_{Na}/P_{Cs} was calculated according to the equation $P_{Na}/P_{Cs} = \exp(V_{rev}F/RT)$. The reversal potential was corrected for the liquid junction potential (in the range 2–5 mV).

REFERENCES

1 Wang, Y. Y., Chang, R. B., Waters, H. N., McKemy, D. D. and Liman, E. R. (2008) The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. J. Biol. Chem. **283**, 32691–32703

2 Ding, S. and Horn, R. (2003) Effect of S6 tail mutations on charge movement in Shaker potassium channels. Biophys. J. **84**, 295–305

3 Benedikt, J., Samad, A., Ettrich, R., Teisinger, J. and Vlachova, V. (2009) Essential role for the putative S6 inner pore region in the activation gating of the human TRPA1 channel. Biochim. Biophys. Acta **1793**, 1279–1288

4 Owsianik, G., Talavera, K., Voets, T. and Nilius, B. (2006) Permeation and selectivity of TRP channels. Annu. Rev. Physiol. **68**, 685–717

Received 10 August 2010/28 September 2010; accepted 14 October 2010
Published as BJ Immediate Publication 14 October 2010, doi:10.1042/BJ20101256