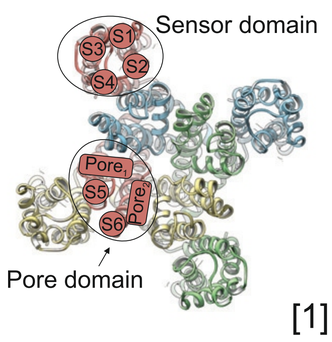
The role of the S1-S4 sensor domain in the activation and modulation of the TRPA1 ion channel

L. Zímová[1], V. Synytsya[1,2], A. Hynková[1,2], V. Zíma[3], I. Barvík[3], V. Vlachová[1]

[1]Institute of Physiology of the Czech Academy of Sciences, Department of Cellular Neurophysiology, Prague, Czech Republic; [2]Faculty of Science, Charles University in Prague, Prague, Czech Republic; [3]Faculty of Mathematics and Physics, Charles University in Prague, Prague, Czech Republic

INTRODUCTION

Transient receptor potential ankyrin 1 (TRPA1) is a polymodal ion channel sensitive to noxious chemical agents. It is mainly expressed in peripheral nociceptor neurons. TRPA1 agonists include a broad class of electrophiles that activate the channel through covalent modification of reactive cysteines [2,3]. When activated, TRPA1 is partially calcium selective and permeating Ca^{2+} ions critically regulate its gating by promoting both potentiation and inactivation [4]. The molecular details behind these processes remain unknown as well as the nature of a weak TRPA1 voltage sensitivity. It has been recently [5] recognized that TRP channels possess an asymmetrically solvated sensor domain, possibly involved in voltage sensing. Whereas the symmetrical hourglass shape of solvation in voltage-gated potassium (Kv) channel-sensor is known [6] to focus the membrane electric field onto the gating charges, allowing efficient transport of charge.



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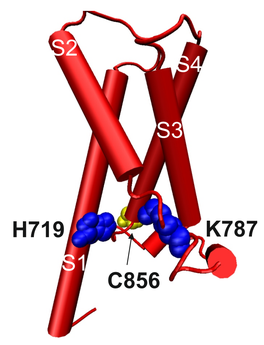
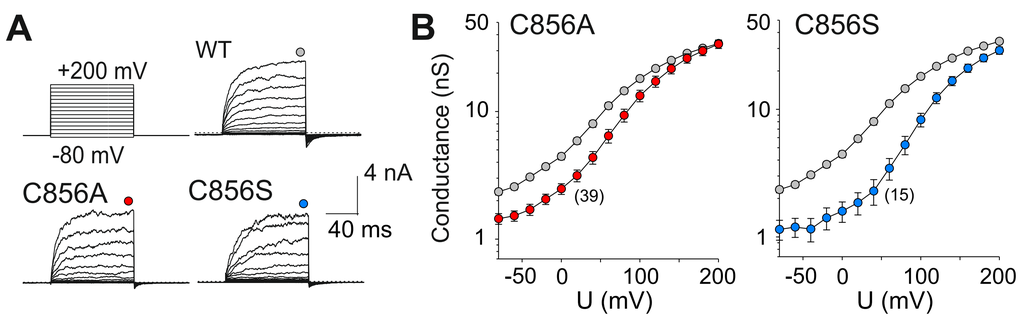


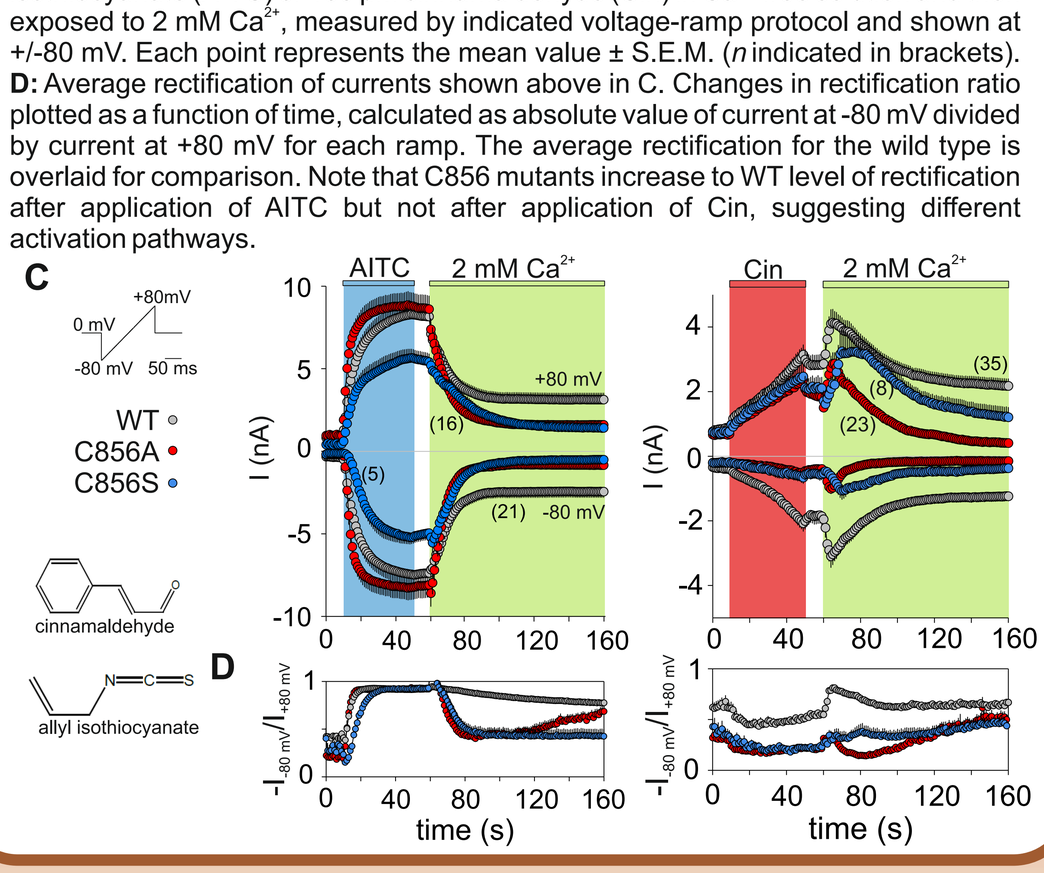
Here, we investigate the involvement of S1-S4 sensor domain in TRPA1 channel gating. We focus on the reactive residues in the lower vestibule which contribute to asymmetrical solvation of the sensor domain.

RESULTS

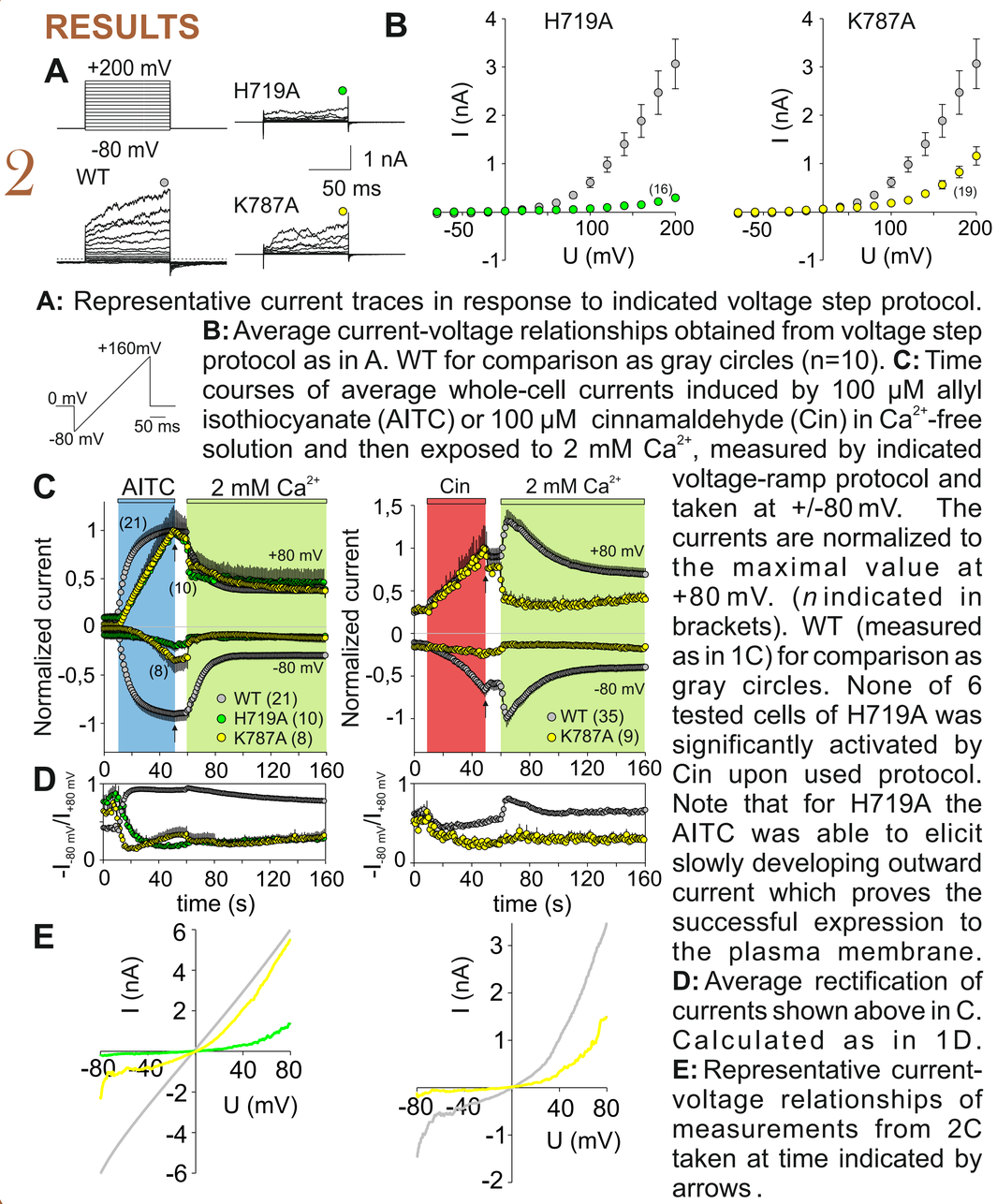
Mutations of C856 dramatically reduce inward current upon activation by week agonist



A: Representative current traces in response to indicated voltage step protocol. **B:** Average conductances obtained from voltage step protocol as in A. Note the rightward shift in half-maximal activation voltage. WT for comparison as gray circles (n=126). **C:** Time courses of average whole-cell currents induced by 100 µM allyl isothiocyanate (AITC) or 100 µM cinnamaldehyde (Cin) in Ca^{2+}-free solution and then exposed to 2 mM Ca^{2+}, measured by indicated voltage-ramp protocol and shown at +/-80 mV. Each point represents the mean value ± S.E.M. (*n* indicated in brackets). **D:** Average rectification of currents shown above in C. Changes in rectification ratio plotted as a function of time, calculated as absolute value of current at -80 mV divided by current at +80 mV for each ramp. The average rectification for the wild type is overlaid for comparison. Note that C856 mutants increase to WT level of rectification after application of AITC but not after application of Cin, suggesting different activation pathways.



RESULTS

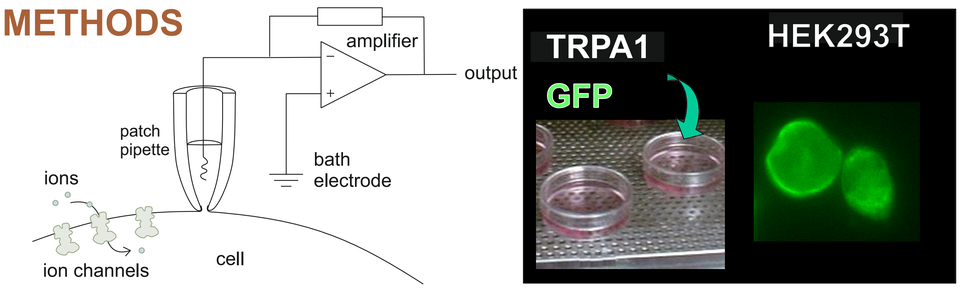


A: Representative current traces in response to indicated voltage step protocol. **B:** Average current-voltage relationships obtained from voltage step protocol as in A. WT for comparison as gray circles (n=10). **C:** Time courses of average whole-cell currents induced by 100 µM allyl isothiocyanate (AITC) or 100 µM cinnamaldehyde (Cin) in Ca^{2+}-free solution and then exposed to 2 mM Ca^{2+}, measured by indicated voltage-ramp protocol and taken at +/-80 mV. The currents are normalized to the maximal value at +80 mV. (*n* indicated in brackets). WT (measured as in 1C) for comparison as gray circles. None of 6 tested cells of H719A was significantly activated by Cin upon used protocol. Note that for H719A the AITC was able to elicit slowly developing outward current which proves the successful expression to the plasma membrane. **D:** Average rectification of currents shown above in C. Calculated as in 1D. **E:** Representative current-voltage relationships of measurements from 2C taken at time indicated by arrows.

CONCLUSION

- Mutations of the reactive residues C856, H719 and K787 located at the base of the S1-S4 sensor domain shift the equilibrium of voltage-dependent gating
- There are different mechanisms involving C856 which couple the AITC or Cin-sensing domains with the voltage-dependent gate opening
- The solvated vestibule of the sensor domain defines voltage- and Ca^{2+}-dependence of TRPA1 channel gating

METHODS



Whole-cell membrane currents were recorded by an Axopatch 200B amplifier. The extracellular bath solutions contained (mM): 150 NaCl and 10 HEPES, with an added 2 HEDTA for the Ca^{2+} - free solution, and 2 $CaCl_2$ for the Ca^{2+}-containing solution; adjusted to pH 7.3 with NaOH, 300 mOsm. The whole-cell pipette solution contained the high-buffer internal solution: 145 mM CsCl, 5 mM EGTA, 3 mM $CaCl_2$, 10 mM HEPES, 2 mM MgATP; pH 7.3 adjusted with CsOH, 320 mOsm [4]. Only one recording was performed on each coverslip.

REFERENCES

[1] Paulsen C. E., et al. Nature (2015) [2] Hinman A., et al. PNAS (2006) [3] Macpherson L.J., et al. Nature (2007) [4] Wang Y. Y., et al. JBC (2008) [5] Palovcak E., JGP (2015) [6] Krepkiy D.M., et al. Nature (2009)

ACKNOWLEDGEMENTS

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