Published in final edited form as:
Circ Res. 2009 January 30; 104(2): e22–e23. doi:10.1161/CIRCRESAHA.108.191338.

Harmony and discord in endothelial calcium entry

David J Beech
Institute of Membrane & Systems Biology, Faculty of Biological Sciences, and Multidisciplinary Cardiovascular Research Centre, University of Leeds, Leeds, LS2 9JT, UK.

This letter addresses the recent report from Abdullaev et al[1]. In 2006, the seminal work of Feske et al[2] identified membrane proteins which were named Orais, after the Greek keepers of Heaven's gate: Harmony (Orai1), Justice (Orai2) and Peace (Orai3). The proteins emerged through a study of severe combined immune deficiency (SCID), which is caused by a defect in Ca^{2+}-entry of T-lymphocytes. A mutation in Orai1 gene underlies the defect.

There has been particular interest in Orais because of their relationship to the widely-occurring store-operated Ca^{2+}-entry (SOCE) phenomena, which reflect Ca^{2+}-permeable plasma membrane mechanisms that respond to the depletion status of endoplasmic reticular Ca^{2+} stores. In lymphocytes the store-operated mechanism is commonly observed as a Ca^{2+}-selective and inwardly rectifying current, I-CRAC. The mechanism is defective in SCID and rescued by expression of wild-type Orai1[2]. In predicted structure Orais belong to the tetraspanin protein family[3] rather than resembling known ion channels but mutations in Orais modify ion-selectivity, suggesting Orais generate ion permeation[4].

Endothelial I-CRAC-like phenomenon

Abdullaev et al's search for I-CRAC in cultured endothelial cells[1] adds to an expanding view that the importance of Orai1 and I-CRAC is not restricted to the immune system. A striking feature of I-CRAC is its small amplitude; well below the size of most other whole-cell currents and close to the resolving power of whole-cell patch-clamp. Abdullaev et al[1] are to be commended for persisting when the current turned out to be at least 5 times smaller than the current of immune cells. Unfortunately, the current could only be convincingly shown in the absence of divalent cations, leaving open the possibility that Ca^{2+} shuts it down and, strictly, not enabling it to be described as I-CRAC (i.e. a Ca^{2+} current). Nevertheless, the current was lost when Orai1 was knocked-down by RNAi. An ionic current was also observed in the presence of a high concentration of extracellular Ca^{2+}, but barely above background noise, making it difficult to define. Therefore, Abdullaev et al[1] observed an I-CRAC-like phenomenon linked to Orai1 but its existence in the presence of Ca^{2+} is unsure.

Although an I-CRAC-like phenomenon was observed using a common I-CRAC protocol[1], there is little direct evidence it depended on store-depletion. All recordings of a clear I-CRAC-like signal used a patch pipette containing a high concentration of the Ca^{2+} chelator,

Correspondence to: Prof David J Beech, Faculty of Biological Sciences, Garstang Building, Mount Preston Street, University of Leeds, Leeds, LS2 9JT, England, UK; d.j.beech@leeds.ac.uk; Tel +44 (0) 113 34 34323.

Europe PMC Funders Author Manuscripts

BAPTA. While BAPTA depletes intracellular stores, it also lowers the global cytosolic Ca^{2+} concentration. Therefore, observation of current with BAPTA in the pipette is not proof that the current arose because of store-depletion. Therefore, Abdullaev et al[1] also used another method for store-depletion (application of the SERCA inhibitor, thapsigargin). However, the observed current had unexpected characteristics: It developed abruptly and then declined (unlike I-CRAC of immune cells), the current-voltage relationship was not like that of I-CRAC, and gadolinium-sensitivity was not demonstrated because the current declined before gadolinium was applied. Ca^{2+}- activated current could have been a confounding factor because the Ca^{2+}-free BAPTA available for buffering may have been relatively little, especially given the dialysis time required for such large cells.

TRPC discord

Abdullaev et al[1] addressed the contribution of canonical transient receptor potential (TRPC) proteins because previous studies of endothelial SOCE all suggested TRPCs contribute the underlying ion permeation pathway[5-9]. In studies of HUVECs (the same cell type as used by Abdullaev et al[1]) store-depletion evoked non-selective cationic current that was abolished by dominant-negative TRPC3 construct. In other studies of HUVECs (and other endothelial cells), store-depletion evoked a similar non-selective cationic current that was enhanced by over-expressing TRPC1 and suppressed by antisense DNA or antibody targeting TRPC1. In other endothelial cells, a mutant form of TRPC4 inhibited Ca^{2+} current observed during store-depletion. Current resembling I-CRAC was observed in endothelial cells from wild-type but not TRPC4 knockout mice.

The TRPC hypothesis has attractions because TRPCs are *bona fide* ion pore-forming proteins with Ca^{2+} permeability. However, TRPCs do not have the Ca^{2+}-selectivity and inward rectification of I-CRAC and are activated by a multitude of factors[10], such that association with or dependence on store-depletion could be a relatively minor aspect of their biology. Nevertheless, independent investigators have published data suggesting TRPCs are involved in endothelial SOCE as well as associated I-CRAC-like or non-selective cationic currents. Resolutions to the apparent discord may come through answers to very specific questions, such as: (1) Do TRPCs activate when physiological concentrations of physiological agonists evoke store-depletion in physiological conditions? Probably yes. (2) Are TRPCs activated by only some experimental protocols designed to evoke store-depletion? Probably yes. (3) When the only event is store-depletion, do TRPCs respond? Although many investigators are keen to answer this question, it is currently impossible to be sure that store-depletion is the only event. (4) Do protocols isolating I-CRAC shut down other mechanisms? Almost certainly yes.

Abdullaev et al exclude TRPCs based on RNAi experiments aimed at knocking down expression of TRPC1 or TRPC4[1], two of the six human TRPCs. Demonstration of effective knock-down is important in such a situation. Unfortunately, the size of the protein suggested to be TRPC1 is 20 % larger than TRPC1's predicted mass, over-expressed TRPC1 clone, or native TRPC1 validated in studies of TRPC1−/− mice. While glycosylation of TRPC1 is suggested, supporting data are not provided and the only potential N-linked glycosylation site is weak. While the band intensity was reduced by TRPC1 siRNA, other bands labeled

by the antibody were also decreased, as was the amount of ß-actin. Although TRPC1 RNAi affected cell proliferation, the western blotting fails to confer confidence that the effect resulted from knock-down of TRPC1. The TRPC4 data, however, appear convincing.

What are we to conclude when previous studies provide data suggesting TRPCs contribute to SOCE of endothelial cells[5-9], where as Abdullaev et al's data indicate no role[1]? Abdullaev et al[1] suggest that most previous studies relied on antibodies that yielded non-specific effects, but the antibody data comprise only a small component of the endothelial cell evidence. Alternatively, could the details of the experimental protocol be crucial in determining whether an investigator observes a TRPC contribution? This would not be surprising but, if so, where does the difference lie and does it matter for understanding the biology?

Because I-CRAC is so small, particular conditions are needed to remove other, larger, unwanted currents. Might these conditions compromise the function of TRPCs? The Ca^{2+} concentration may be critical. Store-depletion can lead to a rise in cytoplasmic Ca^{2+}, stimulating Ca^{2+}-activated ion channels. However, this is not what is usually meant by store-operated channels, rather that the channels sense only the depletion status of the stores. To exclude changes in Ca^{2+} the experimenter must tightly buffer cytosolic Ca^{2+}. "Ca^{2+} add-back" experiments using a Ca^{2+} indicator dye do not meet this condition. Commonly I-CRAC is studied with inclusion of a high concentration of BAPTA in the patch pipette, which helps to deplete the stores and buffer Ca^{2+}, but also reduces cytosolic Ca^{2+} to sub-physiological levels. Less commonly, I-CRAC is evoked by a SERCA inhibitor during buffering of cytosolic Ca^{2+} at the physiological concentration. Almost always, two non-physiological conditions are employed in the extracellular medium: a high concentration of Ca^{2+} (10-20 mM) or a DVF (divalent cation free) solution. Such manoeuvres aid observation of I-CRAC but may divert experimenters from TRPCs, which have complex Ca^{2+} dependencies and regulation.

An additional factor to consider is IP_3. Notably, it was included in the patch pipette for the TRPC4−/−[6] but not Abdullaev et al[1] studies. TRPC channels couple to IP_3 receptors and so involvement of TRPCs in SOCE may conceivably depend on a permissive concentration of IP_3. However, non-selective cationic store-operated current has been observed in endothelial cells without IP_3 in the patch pipette[5,8,9] and activation of TRPCs by other stimuli often does not depend on including IP_3 in the patch pipette. If permissive IP_3 is nevertheless required (or just important), we know that it would usually be present when agonists cause Ca^{2+}-release. In contrast, physiological concentrations of agonists may evoke Ca^{2+}-release without there being appreciable store-depletion. It is unclear how often cells experience store-depletion of the type evoked by thapsigargin or intracellular BAPTA.

Potential for harmony

If Orai1 does indeed confer a widespread I-CRAC, Abdullaev et al's data show us that the current may often be so small in physiological conditions that it is undetectable.

Nevertheless, the Ca^{2+}-entry may be sufficient to alter behaviour of other ion channels, especially if they are physically coupled to Orai. Such close relationships between ion

channels are precedented, for example between voltage-gated Ca^{2+} channels and Ca^{2+}-activated K^+ channels, and different ligand-gated ion channels. Similarly, Orais and TRPCs may form complex molecular arrangements or webs. There is biochemical evidence for Orais interacting with TRPCs, either directly or via the auxiliary STIM1 protein and knock-down of Orai1 can abolish current carried by over-expressed TRPC1[11-13]. Perhaps Orais and TRPCs are separable by experimental conditions but often partners in physiology. There is much work to do and Abdullaev et al's study makes an important contribution to the campaign to solve these complex processes.

Acknowledgments

Research in our laboratory is funded by the British Heart Foundation and the Wellcome Trust.

References

1. Abdullaev IF, Bisaillon JM, Potier M, Gonzalez JC, Motiani RK, Trebak M. Stim1 and Orai1 Mediate CRAC Currents and Store-Operated Calcium Entry Important for Endothelial Cell Proliferation. Circ Res. Oct 9.2008
2. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature. 2006; 441:179–185. [PubMed: 16582901]
3. Wissenbach U, Philipp SE, Gross SA, Cavalié A, Flockerzi V. Primary structure, chromosomal localization and expression in immune cells of the murine ORAI and STIM genes. Cell Calcium. 2007; 42:439–446. [PubMed: 17659338]
4. Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. Nature. 2006; 443:226–229. [PubMed: 16921385]
5. Groschner K, Hingel S, Lintschinger B, Balzer M, Romanin C, Zhu X, Schreibmayer W. Trp proteins form store-operated cation channels in human vascular endothelial cells. FEBS Lett. 1998; 437:101–106. [PubMed: 9804180]
6. Freichel M, Suh SH, Pfeifer A, Schweig U, Trost C, Weissgerber P, Biel M, Philipp S, Freise D, Droogmans G, Hofmann F, Flockerzi V, Nilius B. Lack of an endothelial store-operated Ca2+ current impairs agonist-dependent vasorelaxation in TRP4−/− mice. Nat Cell Biol. 2001; 3:121–127. [PubMed: 11175743]
7. Paria BC, Vogel SM, Ahmmed GU, Alamgir S, Shroff J, Malik AB, Tiruppathi C. Tumor necrosis factor-alpha-induced TRPC1 expression amplifies store-operated Ca2+ influx and endothelial permeability. Am J Physiol Lung Cell Mol Physiol. 2004; 287:L1303–1313. [PubMed: 15347566]
8. Ahmmed GU, Mehta D, Vogel S, Holinstat M, Paria BC, Tiruppathi C, Malik AB. Protein kinase Calpha phosphorylates the TRPC1 channel and regulates store-operated Ca2+ entry in endothelial cells. J Biol Chem. 2004; 279:20941–20949. [PubMed: 15016832]
9. Wu S, Cioffi EA, Alvarez D, Sayner SL, Chen H, Cioffi DL, King J, Creighton JR, Townsley M, Goodman SR, Stevens T. Essential role of a Ca2+-selective, store-operated current (ISOC) in endothelial cell permeability: determinants of the vascular leak site. Circ Res. 2005; 96(8):856–863. [PubMed: 15790951]
10. Abramowitz J, Birnbaumer L. Physiology and pathophysiology of canonical transient receptor potential channels. Faseb J. Oct 21.2008
11. Liao Y, Erxleben C, Yildirim E, Abramowitz J, Armstrong DL, Birnbaumer L. Orai proteins interact with TRPC channels and confer responsiveness to store depletion. Proc Natl Acad Sci U S A. 2007; 104:4682–4687. [PubMed: 17360584]
12. Cheng KT, Liu X, Ong HL, Ambudkar IS. Functional requirement for Orai1 in store-operated TRPC1-STIM1 channels. J Biol Chem. 2008; 283:12935–12940. [PubMed: 18326500]

13. Zeng W, Yuan JP, Kim MS, Choi YJ, Huang GN, Worley PF, Muallem S. STIM1 Gates TRPC Channels, but Not Orai1, by Electrostatic Interaction. Mol Cell. 2008; 32:439–448. [PubMed: 18995841]

Europe PMC Funders Author Manuscripts