Biochem. Soc. Symp. **74**, 37–45
(Printed in Great Britain)

4

Inositol lipids and TRPC channel activation

James W. Putney, Jr[1]

National Institute of Environmental Health Sciences, NIH, Department of Health and Human Services, Research Triangle Park, NC 27709 U.S.A.

Abstract

The original hypothesis put forth by Bob Michell in his seminal 1975 review held that inositol lipid breakdown was involved in the activation of plasma membrane calcium channels or 'gates'. Subsequently, it was demonstrated that while the interposition of inositol lipid breakdown upstream of calcium signalling was correct, it was predominantly the release of Ca^{2+} that was activated, through the formation of Ins(1,4,5)P_3. Ca^{2+} entry across the plasma membrane involved a secondary mechanism signalled in an unknown manner by depletion of intracellular Ca^{2+} stores. In recent years, however, additional non-store-operated mechanisms for Ca^{2+} entry have emerged. In many instances, these pathways involve homologues of the *Drosophila trp* (transient receptor potential) gene. In mammalian systems there are seven members of the TRP superfamily, designated TRPC1–TRPC7, which appear to be reasonably close structural and functional homologues of *Drosophila* TRP. Although these channels can sometimes function as store-operated channels, in the majority of instances they function as channels more directly linked to phospholipase C activity. Three members of this family, TRPC3, 6 and 7, are activated by the phosphoinositide breakdown product, diacylglycerol. Two others, TRPC4 and 5, are also activated as a consequence of phospholipase C activity, although the precise substrate or product molecules involved are still unclear. Thus the TRPCs represent a family of ion channels that are directly activated by inositol lipid breakdown, confirming Bob Michell's original prediction 30 years ago.

Introduction

In 1975, Michell postulated that degradation of membrane inositol lipids represented a signalling mechanism that was upstream of the generation of Ca^{2+} signals [1]. Subsequently, he more specifically suggested that plasma membrane

[1]email putney@niehs.nih.gov

inositol lipids were involved in regulating the influx of Ca^{2+} across the plasma membrane, through hypothetical channels or Ca^{2+} 'gates' [2]. It is an understatement to say that this idea was not universally accepted (e.g. [3]). With the discovery of the Ca^{2+}-mobilizing function of the PLC (phospholipase C) product, Ins(1,4,5)P_3 [4,5], attention turned to regulation of release of Ca^{2+} from intracellular stores, rather than influx of Ca^{2+}. Subsequently, the idea developed that in the PLC signalling pathway, Ca^{2+} entry was generally signalled by a mechanism that was downstream of intracellular Ca^{2+} store depletion, a mechanism known as capacitative calcium entry or store-operated calcium entry [6–8]. In the search for the channels that mediate capacitative calcium entry, recent attention has focused on a family of channels that are homologues of a *Drosophila* photoreceptor channel protein, named for the mutant phenotype, TRP (transient receptor potential). Most of the members of the mammalian TRP superfamily fall within three major subgroups: TRPCs (canonical TRPs), TRPVs (vanilloid-related TRPs) and TRPMs (melastatin-related TRPs). A number of other family members comprise small subgroups, most representing genes discovered by their association with specific genetic diseases (for a review of the larger TRP superfamily see [9–11]). However, in terms of channels involved in store-operated Ca^{2+} entry, most attention has focused on the TRPC subfamily, because they are structurally and functionally most similar to the parental *Drosophila* TRP, which has been known for some time to be activated downstream of PLC.

The channel proteins encoded by the TRPC genes, like the parental *Drosophila* TRP protein, span the membrane six times [12], and contain a short hydrophobic sequence believed to be involved in forming the pore of the channel [13]. By analogy with other channels, it is believed that a functional TRPC channel will be formed by the coming together of four TRPC proteins [13]; thus channels could be formed as homotetramers, if all four TRPCs are the same, or heterotetramers, if more than one kind of TRPC is involved. There appear to be restrictions as to which TRPCs can come together to form channels, although there is not complete agreement on what these restrictions are [13,14]. Thus Hofmann et al. [13] found that when TRPC channels were exogenously expressed in HEK (human embryonic kidney)-293 cells, the only permitted combinations were TRPC3, 6 and 7, or TRPC1, 4 and 5. However, a number of reports have found that TRPC1 and 3 can associate, with both exogenous and endogenous expression [14–16]. Unlike previous studies of ion channel function, investigators attempted to understand the function of mammalian TRPC channels beginning only with knowledge of their coding sequences. This may underlie the unusual degree of inconsistency in the literature regarding activation mechanisms of TRPC channels. In this review, I will discuss ideas about mechanisms of TRPC activation, emphasizing the role of the PLC/inositol lipid pathway, and offer an hypothesis to reconcile some of the conflicting findings in the literature.

TRPCs: mechanisms of regulation *in transfecto*

The TRPCs are so named [17] because this family is most closely related to the original *Drosophila* channel, TRP (i.e. they are the 'canonical' TRPs). Because

Drosophila TRP was clearly activated downstream of PLC and Ins(1,4,5)P_3 generation in photoreceptor cells, Hardie and Minke [18] suggested that TRP might be the long-sought store-operated Ca^{2+} channel. The store-operated or capacitative calcium entry channels are activated by a process triggered by the depletion of intracellular endoplasmic reticulum Ca^{2+} stores [8,19]. Recent findings indicate that the sensor of intracellular Ca^{2+} store depletion may be the Ca^{2+}-binding membrane protein, STIM1 (stromal interaction molecule 1) [20,21]. Ironically, it is now very clear that store-depletion is not the mechanism by which *Drosophila* TRP is activated in its native environment in photoreceptor cells [22]. The significance of this basic finding to the larger body of work on mammalian TRPCs perhaps should not be overlooked. Yet, it is clear from PLC-deficient mutants that *Drosophila* TRP is activated in some manner downstream of PLC. It is most likely, although not proven, that, in flies, a lipid mediator derived from PLC products activates TRP, perhaps the immediate breakdown product, DAG (diacylglycerol), and it is also regulated in complex ways by the substrate of PLC, PtdIns(4,5)P_2 [22].

For the seven mammalian TRPCs, there is considerable evidence that these channels are also activated in some manner downstream of PLC. Although initial studies suggested that TRPC1 and 3 were activated by store depletion [23], it was subsequently shown that this resulted from the constitutive activity of the channels [24] (however, see [25]). One report suggested that Ins(1,4,5)P_3 and the Ins(1,4,5)P_3 receptor were involved in activation of TRPC3 [26], but other laboratories failed to reproduce these results [27,28]. Likewise, the original studies on TRPC4 and TRPC5 presented evidence for activation by store depletion [29,30], but others failed to reproduce these findings [31,32] (but see [33]). For the TRPC3/6/7 subfamily, the vast majority of published results suggest that DAG, produced upon PLC activation, is the signal activating these channels (see references in [34,35]). For TRPC4 and 5, the case is not so clear. These channels are highly sensitive to inhibition by PKC (protein kinase C), making it difficult to obtain evidence that DAG can activate them. Indeed, while TRPC3/6/7 channels can be activated by exogenous DAG [usually OAG (oleyl acetyl glycerol)], OAG inhibits the activation of TRPC4 or 5 and this inhibition is blocked by inhibitors of PKC [36]. Thus if DAG is involved in the activation of TRPC4 or 5, there must be considerable compartmentalization of the signalling pathway to prevent concomitant inhibition by PKC. An interesting possibility is that TRPC4 and 5 may be tonically inhibited by PtdIns(4,5)P_2, such that PLC-mediated degradation of PtdIns(4,5)P_2 relieves this inhibition, resulting in channel activation. Normally, PtdIns(4,5)P_2 is thought to provide a positive regulation of TRPs [37–39]; however, for TRPV1, PtdIns(4,5)P_2 appears to regulate negatively, although this is not thought to constitute a primary mechanism for channel activation [40].

As mentioned above, TRPC1 is often considered together with TRPC4 and 5, as it is somewhat similar in sequence and is known to associate with TRPC4 and 5 to form heteromultimeric channels [13,14]. The actions of TRPC1 when expressed on its own are controversial; some laboratories demonstrate activated ion channel behaviour following expression of TRPC1 [23,41–44], whereas others find that the channel does not traffic to the plasma membrane correctly

unless co-expressed with TRPC4 or 5 [13]. Interestingly, and importantly for arguments to be advanced below, Strübing et al. [45] found that co-expression of TRPC1 and TRPC5 produced functional heteromultimers with different electrophysiological properties from homotetramers of TRPC5.

A second mechanism that is important for the function of TRPC channels involves regulation of their trafficking to the plasma membrane [46–48]. The published reports on this topic are not entirely consistent with one another. However, it is clear from the study of Bezzerides et al. [46] that growth factors [such as EGF (epidermal growth factor)] activate the insertion of TRPC5 channels into the plasma membrane. Once the channels reach the membrane, subsequent manoeuvres that activate the channels result in larger currents. Reports from other laboratories indicate that for other TRPCs, the translocation event leads to increased current [47,48]. In either case, either constitutive or stimulated currents would be increased simply by increasing the number of channels, and would not necessarily involve any further increase in the open probability of each channel. The mechanism by which TRPC5 secretion is signalled does not seem to involve activation of PLC, rather it involves a pathway requiring phosphoinositide 3-kinase, Rac1 and phosphatidylinositol 4-kinase [46]. Finally, it is significant that only TRPC5 homotetrameric channels were regulated in this manner. Heterotetramers containing TRPC5 and TRPC1 did not translocate to the plasma membrane in response to EGF. Note that regulated trafficking is emerging as a general mechanism of regulation of membrane signalling proteins [49], and may apply to members of the broader TRP superfamily; there is at least one example of a TRPV channel that appears to be regulated in this way [50].

The third major mechanism which has been described for TRPC channel activation is the store-depletion or capacitative calcium entry mechanism. Thus a number of studies have reported activation of TRPC channels by store depletion (see references cited in [34,51]), and knockout or knockdown of TRPCs often reduces store-operated calcium entry (see references cited in [34,51]). Yet, in many instances these channels clearly are not store-operated (see references cited in [34,51]). It is becoming increasingly clear that the basis for the different behaviours is expression conditions. Thus in DT40 B-lymphocytes, TRPC3 formed a store-operated channel at low levels of expression and a DAG-activated channel at higher levels of expression [52]. The loss of store-operated behaviour at higher expression levels may result from inappropriate stoichiometry among members of a signalling complex [51], as has been argued previously for scaffolding proteins [53,54]. Significantly, the pharmacology of the channels differed in these two modes, the store-operated TRPC3 channels being much more sensitive to block by Gd^{3+} than the non-store-operated ones [55]. Both TRPC7 [56] and TRPC5 [33] are store-operated when stably expressed in HEK-293 cells; in this case, the store-operated channels are either capable of activation by alternative, non-store-operated mechanisms, or they co-exist with channels that are activated by the non-store-operated mechanisms. For TRPC7, it was demonstrated that this store-operated behaviour is only seen with stable transfection. Transient transfection of HEK-293 cells with TRPC7 results in channels that can only be activated by receptor activation or OAG, not by store depletion [56].

Thus the available data demonstrate that when ectopically expressed, TRPCs can be activated by one of three mechanisms: store-depletion, PLC (or its products) or channel translocation to the plasma membrane. The determinant of which mode of activation occurs appears to be the environment in which the subunit finds itself, including perhaps the nature of other subunits that compose the channel pore. This principle is most clearly illustrated by the case of translocation of TRPC5, which only occurs with TRPC5 homotetramers, not with TRPC5/1 heterotetramers [46]. It is also a logical interpretation of the observed difference in Gd^{3+} sensitivity of TRPC3 channels in store-operated as compared to PLC-activated mode [55]. The question then arises: do these different modes of regulation occur with TRPC subunits when expressed in their native environments in untransfected cells?

Mechanisms of TRPC activation *in situ*

The answer to the aforeposed question seems to be clearly, yes. There is now considerable evidence for each of these modes of TRPC function in native, untransfected cells. In the case of store-operated channels, I have already mentioned the considerable number of examples of disrupted behaviour of native store-operated channels by knock-down of TRPCs. However, a sticking point has been the failure of ectopically expressed TRPCs (or as yet any expressed ion channel) to faithfully reproduce the electrophysiological characteristics of I_{crac} (first described in detail in [57,58], and for example see also [59]). However, it is clear that there are a variety of different kinds of store-operated channels [60–67], some of which have properties reminiscent of TRPCs. Thus it is realistic to expect that TRPCs may contribute to the composition of store-operated channels in such instances. It is not out of the question to consider that TRPCs might play a role in I_{crac} channels, given the possibility of heteromultimers with drastically altered electrophysiological properties. Two reports have provided evidence for a role for TRPC1 [68] and TRPC3 [69] in I_{crac}.

There are a number of examples of native channels that appear to be activated by DAGs under physiological conditions [70–77]. In a particularly thorough study, Inoue et al. [73] investigated the role of TRPC6 in α-adrenergic-activated cation channels. The authors examined the electrophysiological and pharmacological characteristics of the endogenous cation entry controlled by α1-adrenoceptors in rabbit portal vein smooth muscle cells and found this cation entry to be reminiscent of that of the TRPC6 current when transiently expressed in HEK-293 cells. Both the endogenous current, and the current due to expressed TRPC6 could be activated by OAG in a PKC-independent fashion. Furthermore, TRPC6 mRNA and protein expression was demonstrated in the smooth muscle cells, and knockdown of TRPC6 with antisense RNA in smooth muscle cells resulted in almost complete abrogation of the α1-adrenergic response. Similarly, Jung et al. [74] showed that vasopressin activates a non-CCE (capacitative Ca^{2+} entry), non-selective cation current in the smooth muscle cell line, A7r5, resembling that observed with expression of TRPC6 in HEK-293 cells. The DAG analogue, OAG, activated the endogenous, agonist-sensitive

current in A7r5 cells and those cells were shown to express mRNA for TRPC1 and TRPC6 but no other TRPC proteins. The authors then concluded that TRPC6 is likely to be a component of the endogenous agonist-activated channel in the A7r5 cell line. Lievremont et al. [75] found that DAG could activate Ca^{2+} entry and a non-selective cation current, but only in the presence of inhibitors of PKC. This requirement for inhibitors of PKC is interesting because it distinguishes endogenously expressed channels from those overexpressed in transfection experiments, probably because in the latter case the channels are present in large numbers and are not completely inhibited by PKC. These same authors used targeted homologous recombination to disrupt the coding sequence of the TRPC7 gene. This resulted in complete loss of the agonist- and DAG-activated non-selective cation current. Transfection of human TRPC7 into the TRPC7-knockout cells restored the DAG-activated current. Thus TRPC7 appears to constitute a major component of the non-store-operated Ca^{2+} entry pathway in B-lymphocytes.

There are far fewer examples of stimulated translocation of native TRPC channels, probably due to the rather recent discovery of this mode of activation. However, Bezzerides et al. [46] reported that in cultured hippocampal neurons,

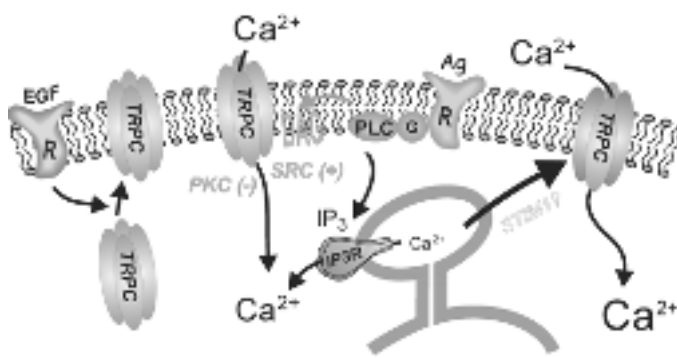

Figure 1 Activation mechanisms for calcium-permeable TRPC cation channels. TRPC channels can be activated in any of three distinct ways. From left: channels sequestered in a vesicular compartment can be translocated to the plasma membrane in response to growth factor (e.g. EGF) where the expression of their constitutive activity will contribute to membrane signalling and electrical properties. TRPC channels can be activated by DAG, or possibly as a result of loss of PtdIns(4,5)P_2 (not shown) following agonist (Ag) activation of PLC by a G-protein-coupled (G) pathway. In some instances, this activation mode requires the tyrosine kinase, Src [79], and is negatively regulated by PKC [28,36,80–82]. Finally, the activation of PLC leads to the production of Ins(1,4,5)P_3 (IP_3) which activates the Ins(1,4,5)P_3 receptor (IP_3R) causing release of Ca^{2+} from a critical component of the endoplasmic reticulum. This can in turn activate TRPC channels through the capacitative or store-operated pathway, possibly involving the Ca^{2+} sensor protein, STIM1. There is evidence that in the latter case, the subunit composition of the store-operated channels may differ from that of the PLC-regulated channels.

native TRPC5 channels were localized to active growth cones (see also [78]). Surface expression of TRPC5 was increased by several different growth factors. TRPC1 was expressed in the cell soma and processes, and not in growth cones, consistent with the observation that only TRPC5 homotetramers undergo regulation through translocation.

Summary

As predicted by Bob Michell over thirty years ago [1], turnover of plasma membrane inositol lipids results in regulation of a variety of calcium-permeable ion channels. Among the channels regulated through PLC activity, the TRPC channels appear to be directly regulated by the products of inositol lipid breakdown. Alternatively, these channels can be regulated by other mechanisms downstream of PLC, including by the depletion of Ca^{2+} stores in the case of the store-operated pathway. Finally, the channels are regulated through receptor-mediated activation of their trafficking to and insertion into the plasma membrane [34] (See Figure 1). It is becoming increasingly clear that this multiplicity of regulatory mechanisms does not simply reflect (at least in all cases) aberrant behaviour due to over expression, but rather is indicative of true diversity of channel function *in vivo*. At least one of the factors that determines the function and regulation of TRPC channels appears to be the subunit composition of the assembled tetrameric channel. Additional factors may include partners in a signalling complex, such as regulatory subunits or scaffolding structures. The propensity of TRPC channels to interact with the Ca^{2+} sensor, STIM1 [20,21], may be important for their regulation by Ca^{2+} store depletion. As argued previously [51], the ability of cells to utilize TRPCs in diverse ways may have significance beyond the ion channel field; such a multiplicity offers a means by which the complex mammalian organism can be assembled from what has turned out to be a surprisingly limited genome.

I gratefully acknowledge ideas and criticisms from Mohamed Trebak, Dave Armstrong and Steve Shears. This research was supported by funds from the Intramural Program of NIH (National Institutes of Health) and NIEHS (National Institute of Environmental Health Sciences).

References

1. Michell, R.H. (1975) Biochim. Biophys. Acta **415**, 81–147
2. Michell, R.H., Jafferji, S. and Jones, L.M. (1977) Adv. Exp. Biol. Med. **83**, 447–464
3. Hawthorne, J.N. (1982) Nature **295**, 281–282
4. Berridge, M.J. (1983) Biochem. J. **212**, 849–858
5. Streb, H., Irvine, R.F., Berridge, M.J. and Schulz, I. (1983) Nature **306**, 67–68
6. Berridge, M.J. (1995) Biochem. J. **312**, 1–11
7. Putney, Jr, J.W. (1986) Cell Calcium **7**, 1–12
8. Putney, Jr, J.W. (1997) Capacitative Calcium Entry, Landes Biomedical Publishing, Austin, TX
9. Montell, C. (2005) Sci STKE **2005**, re3
10. Nilius, B. and Voets, T. (2005) Pfleugers Arch. **451**, 1–10

11. Vriens, J., Owsianik, G., Voets, T., Droogmans, G. and Nilius, B. (2004) Pfleugers Arch. **449**, 213–226
12. Vannier, B., Zhu, X., Brown, D. and Birnbaumer, L. (1998) J. Biol. Chem. **273**, 8675–8679
13. Hofmann, T., Schaefer, M., Schultz, G. and Gudermann, T. (2002) Proc. Nat. Acad. Sci. U.S.A. **99**, 7461–7466
14. Strubing, C., Krapivinsky, G., Krapivinsky, L. and Clapham, D.E. (2003) J. Biol. Chem. **278**, 39014–39019
15. Lintschinger, B., Balzer-Geldsetzer, M., Baskaran, T., Graier, W.F., Romanin, C., Zhu, M.X. and Groschner, K. (2000) J. Biol. Chem. **275**, 27799–27805
16. Xu, X.-Z.S., Li, H.-S., Guggino, W.B. and Montell, C. (1997) Cell **89**, 1155–1164
17. Montell, C., Birnbaumer, L., Flockerzi, V., Bindels, R.J., Bruford, E.A., Caterina, M.J., Clapham, D.E., Harteneck, C., Heller, S., Julius, D. et al. (2002) Mol. Cell **9**, 229–231
18. Hardie, R.C. and Minke, B. (1993) Trends Neurosci. **16**, 371–376
19. Parekh, A.B. and Penner, R. (1997) Physiol. Rev. **77**, 901–930
20. Liou, J., Kim, M.L., Heo, W.D., Jones, J.T., Myers, J.W., Ferrell, Jr, J.E. and Meyer, T. (2005) Curr. Biol. **15**, 1235–1241
21. Roos, J., DiGregorio, P.J., Yeromin, A.V., Ohlsen, K., Lioudyno, M., Zhang, S., Safrina, O., Kozak, J.A., Wagner, S.L., Cahalan, M.D., Velicelebi, G. and Stauderman, K.A. (2005) J. Cell Biol. **169**, 435–445
22. Hardie, R.C. (2003) Ann. Rev. Physiol. **65**, 735–759
23. Zhu, X., Jiang, M., Peyton, M., Boulay, G., Hurst, R., Stefani, E. and Birnbaumer, L. (1996) Cell **85**, 661–671
24. Zhu, X., Jiang, M. and Birnbaumer, L. (1998) J. Biol. Chem. **273**, 133–142
25. Yildirim, E., Kawasaki, B.T. and Birnbaumer, L. (2005) Proc. Natl. Acad. Sci. U.S.A **102**, 3307–3311
26. Kiselyov, K., Xu, X., Mozhayeva, G., Kuo, T., Pessah, I., Mignery, G., Zhu, X., Birnbaumer, L. and Muallem, S. (1998) Nature **396**, 478–482
27. Hofmann, T., Obukhov, A.G., Schaefer, M., Harteneck, C., Gudermann, T. and Schultz, G. (1999) Nature **397**, 259–262
28. Trebak, M., Bird, G.S., McKay, R.R., Birnbaumer, L. and Putney, Jr, J.W. (2003) J. Biol. Chem. **278**, 16244–16252
29. Philipp, S., Cavalié, A., Freichel, M., Wissenbach, U., Zimmer, S., Trost, C., Marguart, A., Murakami, M. and Flockerzi, V. (1996) EMBO J. **15**, 6166–6171
30. Philipp, S., Hambrecht, J., Braslavski, L., Schroth, G., Freichel, M., Murakami, M., Cavalié, A. and Flockerzi, V. (1998) EMBO J. **17**, 4274–4282
31. McKay, R.R., Szmeczek-Seay, C.L., Lièvremont, J.-P., Bird, G.S., Zitt, C., Jüngling, E., Lückhoff, A. and Putney, Jr, J.W. (2000) Biochem. J. **351**, 735–746
32. Schaefer, M., Plant, T.D., Obukhov, A.G., Hofmann, T., Gudermann, T. and Schultz, G. (2000) J. Biol. Chem. **275**, 17517–17526
33. Zeng, F., Xu, S.Z., Jackson, P.K., McHugh, D., Kumar, B., Fountain, S.J. and Beech, D.J. (2004) J. Physiol. **559**, 739–750
34. Putney, Jr, J.W. (2005) Pfleugers Arch. **451**, 29–34
35. Trebak, M., Vazquez, G., Bird, G.S. and Putney, Jr, J.W. (2003) Cell Calcium **33**, 451–461
36. Venkatachalam, K., Zheng, F. and Gill, D.L. (2003) J. Biol. Chem. **278**, 29031–29040
37. Liu, B. and Qin, F. (2005) J. Neurosci. **25**, 1674–1681
38. Rohacs, T., Lopes, C.M., Michailidis, I. and Logothetis, D.E. (2005) Nat. Neurosci. **8**, 626–634
39. Runnels, L.W., Yue, L. and Clapham, D.E. (2002) Nat. Cell Biol. **4**, 329–336
40. Liu, B., Zhang, C. and Qin, F. (2005) J. Neurosci. **25**, 4835–4843
41. Chen, J. and Barritt, G.J. (2003) Biochem. J. **373**, 327–336
42. Liu, X., Wang, W., Singh, B.B., Lockwich, T., Jadlowiec, J., O'Connell, B., Wellner, R., Zhu, M.X. and Ambudkar, I.S. (2000) J. Biol. Chem. **275**, 3403–3411
43. Sinkins, W.G., Estacion, M. and Schilling, W.P. (1998) Biochem. J. **331**, 331–339
44. Zitt, C., Zobel, A., Obukhov, A.G., Harteneck, C., Kalkbrenner, F., Lückhoff, A. and Schultz, G. (1996) Neuron **16**, 1189–1196
45. Strübing, C., Krapivinsky, G., Krapivinsky, L. and Clapham, D.E. (2001) Neuron **29**, 645–655
46. Bezzerides, V.J., Ramsey, I.S., Kotecha, S., Greka, A. and Clapham, D.E. (2004) Nat. Cell Biol. **6**, 709–720

47. Cayouette, S., Lussier, M.P., Mathieu, E.L., Bousquet, S.M. and Boulay, G. (2004) J. Biol. Chem. **279**, 7241–7246
48. Singh, B.B., Lockwich, T.P., Bandyopadhyay, B.C., Liu, X., Bollimuntha, S., Brazer, S.C., Combs, C., Das, S., Leenders, A.G., Sheng, Z.H. et al. (2004) Mol. Cell **15**, 635–646
49. Royle, S.J. and Murrell-Lagnado, R.D. (2003) BioEssays **25**, 39–46
50. Kanzaki, M., Zhang, Y.-Q., Mashima, H., Li, L., Shibata, H. and Kojima, I. (1999) Nat. Cell Biol. **1**, 165–170
51. Putney, Jr, J.W. (2004) Trends Cell Biol. **14**, 282–286
52. Vazquez, G., Wedel, B.J., Trebak, M., Bird,G.S. and Putney, Jr, J.W. (2003) J. Biol. Chem. **278**, 21649–21654
53. Burack, W.R. and Shaw, A.S. (2000) Curr. Opin. Cell Biol. **12**, 211–216
54. Levchenko, A., Bruck, J. and Sternberg, P.W. (2000) Proc. Nat. Acad. Sci. U.S.A. **97**, 5818–5823
55. Trebak, M., Bird, G.S., McKay, R.R. and Putney, Jr, J.W. (2002) J. Biol. Chem. **277**, 21617–21623
56. Lievremont, J.P., Bird, G.S. and Putney, Jr, J.W. (2004) Am. J. Physiol. Cell Physiol. **287**, C1709–C1716
57. Hoth, M. and Penner, R. (1992) Nature **355**, 353–355
58. Hoth, M. and Penner, R. (1993) J. Physiol. **465**, 359–386
59. Voets, T., Prenen, J., Fleig, A., Vennekens, R., Watanabe, H., Hoenderop, J.G.J., Bindels, R.J.M., Droogmans, G., Penner, R. and Nilius,B. (2001) J. Biol. Chem. **276**, 47767–47770
60. Albert, A.P. and Large, W.A. (2002) J. Physiol. **538**, 717–728
61. Gusev, K., Glouchankova, L., Zubov, A., Kaznacheyeva, E., Wang, Z., Bezprozvanny, I. and Mozhayeva, G.N. (2003) J. Gen. Physiol **122**, 81–94
62. Krause, E., Pfeiffer, F., Schmid, A. and Schulz, I. (1996) J. Biol. Chem. **271**, 32523–32528
63. Liu, X., Groschner, K. and Ambudkar, I.S. (2004) J. Membr. Biol. **200**, 93–104
64. McDaniel, S., Platoshyn, O., Wang, J., Yu, Y., Sweeney, M., Krick, S., Rubin, L.J. and Yuan, J.X.J. (2001) Am. J. Physiol. **280**, L870–L880
65. Trepakova, E.S., Gericke, M., Hirakawa, Y., Weisbrod, R.M., Cohen, R.A. and Bolotina, V.M. (2001) J. Biol. Chem. **276**, 7782–7790
66. Wayman, C.P., Wallace, P., Gibson, A. and McFadzean, I. (1999) Eur. J. Pharmacol. **376**, 325–329
67. Zhang, H., Inazu, M., Weir, B., Buchanan, M. and Daniel, E. (1994) Eur. J. Pharmacol. **251**, 119–125
68. Mori, Y., Wakamori, M., Miyakawa, T., Hermosura, M., Hara, Y., Nishida, M., Hirose, K., Mizushima, A., Kurosaki, M., Mori, E., et al. (2002) J. Exp. Med. **195**, 673–681
69. Philipp, S., Strauss, B., Hirnet, D., Wissenbach, U., Mery, L., Flockerzi, V. and Hoth, M. (2003) J. Biol. Chem. **278**, 26629–26638
70. Albert, A.P. and Large, W.A. (2003) J. Physiol **552**, 789–795
71. Gamberucci, A., Giurisato, E., Pizzo, P., Tassi, M., Giunti, R., McIntosh, D.P. and Benedetti, A. (2002) Biochem. J. **364**, 245–254
72. Hassock, S.R., Zhu, M.X., Trost, C., Flockerzi, V. and Authi, K.S. (2002) Blood **100**, 2801–2811
73. Inoue, R., Okada, T., Onoue, H., Hara, Y., Shimizu, S., Naitoh, S., Ito, Y. and Mori, Y. (2001) Circ. Res. **88**, 325–332
74. Jung, S., Strotmann, R., Schultz, G. and Plant, T.D. (2002) Am. J. Physiol. **282**, C347–C359
75. Lievremont, J.P., Numaga, T., Vazquez, G., Lemonnier, L., Hara, Y., Mori, E., Trebak, M., Moss, S.E., Bird, G.S., Mori, Y. and Putney, Jr, J.W. (2005) J. Biol. Chem. **280**, 35346–35351
76. Tesfai, Y., Brereton, H.M. and Barritt, G.J. (2001) Biochem. J. **358**, 717–726
77. Thebault, S., Zholos, A., Enfissi, A., Slomianny, C., Dewailly, E., Roudbaraki, M., Parys, J. and Prevarskaya, N. (2005) J. Cell Physiol. **204**, 320–328
78. Greka, A., Navarro, B., Oancea, E., Duggan, A. and Clapham, D.E. (2003) Nat. Neurosci. **6**, 837–845
79. Vazquez, G., Wedel, B.J., Kawasaki, B.T., Bird, G.S. and Putney, Jr, J.W. (2004) J. Biol. Chem. **279**, 40521–40528
80. Ahmmed, G.U., Mehta, D., Vogel, S., Holinstat, M., Paria, B.C., Tiruppathi, C. and Malik, A.B. (2004) J. Biol.Chem. **279**, 20941–20949
81. Okada, T., Inoue, R., Yamazaki, K., Maeda, A., Kurosaki, T., Yamakuni, T., Tanaka, I., Shimizu, S., Ikenaka, K., Imoto, K. and Mori, Y. (1999) J. Biol. Chem. **274**, 27359–27370
82. Trebak, M., Hempel, N., Wedel, B.J., Smyth, J.T., Bird, G.S. and Putney, Jr, J.W. (2005) Mol. Pharmacol. **67**, 558–563