International Journal of Biochemistry and Cell Biology 111 (2019) 37–41

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Molecules in focus

Calcium-activated chloride channels: Potential targets for antinociceptive therapy

Isabella Salzer*, Stefan Boehm

Division of Neurophysiology and Neuropharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Waehringer Strasse 13a, 1090, Vienna, Austria

ARTICLE INFO

Keywords:
Ca-activated chloride channels
CaCC
TMEM16
Nociception
Therapeutic target

ABSTRACT

The molecular identity of calcium-activated chloride channels (CaCCs) was clarified only some ten years ago when it was linked to the family of "transmembrane proteins of unknown function 16″ (TMEM16). Since then, numerous studies have been conducted both to define their role in physiology and identify their biophysical functions. For the latter, the ultrastructural description of mouse TMEM16 A was a breakthrough. CaCCs were functionally described in a number of different tissues including first-order sensory neurons. The activating rise in intracellular calcium concentration can be caused by an influx of calcium through other calcium permeable ion channels. Calcium release from intracellular stores, mediated by G-protein coupled receptors, also leads to CaCC activation. Prominent inflammatory mediators like bradykinin or serotonin stimulate CaCCs via such a mechanism. The (patho) physiological function of these ion channels renders them promising targets for antinociceptive treatment.

1. A brief history of calcium-activated chloride channels

One of the first descriptions of calcium-activated chloride channels (CaCCs) was as chloride channels endogenously expressed in *Xenopus laevis* oocytes (Miledi, 1982). However, currents through these channels were rather viewed as experimentally disadvantageous as they potentially interfered with currents through recombinant channels. Furthermore, their molecular identity remained elusive (Bader et al., 1982; Miledi, 1982).

A seemingly unconnected event occurred in the early 2000s, when a protein began gaining interest in the field of cancer research. This protein was termed "DOG1″ (discovered on GIST 1; GIST: gastrointestinal stroma tumor) and was found to be expressed on GIST. It was suggested to use DOG1 as a biomarker for GIST and other cancers, where its expression profile can be used to predict cancer progression and outcome (Oh and Jung, 2016).

Finally in 2008, the molecular identity of CaCCs was clarified: members A and B of family 16 of "transmembrane proteins with unknown function" (TMEM16 A and B) were found to be anion channels activated by a rise in intracellular calcium (Caputo et al., 2008; Schroeder et al., 2008; West et al., 2004). It also became clear that the cancer cell marker DOG1 and TMEM16 A were identical (Oh and Jung, 2016). Meanwhile, CaCCs were found to play important roles in a variety of physiological functions, among them neuronal excitability (Duran and Hartzell, 2011).

2. The surprising structure of TMEM16A

As indicated previously, the structure of DOG1/TMEM16 A was originally thought to consist of eight transmembrane domains lending substance to the name anoctamin currently extended to ten family members as anoctamin 1–10 (Falzone et al., 2018; Oh and Jung, 2016). More recently, an X-ray structure of nhTMEM16, a fungal phospholipid scramblase paralog (Brunner et al., 2014), which also acts as a non-selective ion channel (Lee et al., 2016), and a cryo-EM structure of mouse TMEM16 A, the classic CaCC, were published (Dang et al., 2017; Paulino et al., 2017a, 2017b). Since both structures contain ten membrane-spanning domains, the name anoctamin is currently being disputed.

The aforementioned two cryo-EM structures shed some light on several mysteries: while it was suggested earlier that TMEM16 A forms homodimers (Fallah et al., 2011; Sheridan et al., 2011), it was unclear how these two subunits would interact or form an ion conduction pore. It is now clear that transmembrane domains 3–7 of each subunit define a separate ion conduction, creating a two-pore anion channel upon dimerization (Dang et al., 2017; Paulino et al., 2017a, 2017b). Comparison of the structures of the fungal scramblase nhTMEM16 and the mouse channel equivalent TMEM16 A further revealed a common

* Corresponding author.
E-mail address: isabella.salzer@meduniwien.ac.at (I. Salzer).

https://doi.org/10.1016/j.biocel.2019.04.006
Received 31 August 2018; Received in revised form 13 April 2019; Accepted 17 April 2019
Available online 18 April 2019
1357-2725/

conduction pathway for lipids and ions. In murine TMEM16 A, TM3 to TM7 shield the hydrophilic ion-conduction pathway entirely from the hydrophobic plasma membrane. In the fungal lipid scramblase paralog, that same tunnel is not completely sealed, in that it resembles a slit hose. Hydrophilic heads of phospholipids can, therefore, slide through the hydrophilic pore of the scramblase, while their lipophilic tails remain in the plasma membrane (Falzone et al., 2018). This process was termed "credit-card mechanism" and works only for phospholipids with small head groups. Another mechanism that allows transport of lipids with larger head groups, involves a thinning of the membrane which in turn reduces the energy barrier for transport of hydrophobic residues through lipid environments (Malvezzi et al., 2018).

3. Activation mechanisms of TMEM16A

As the name suggests, CaCCs are gated by rising concentrations of intracellular calcium. In dorsal root ganglion neurons, the change in intracellular calcium concentration can be achieved by activating a G-protein coupled receptor that releases calcium from intracellular stores via the formation of IP_3. Additionally, activation of calcium-permeable ion channels, like TRPV1- (Takayama et al., 2015), voltage-gated calcium channels (Jin et al., 2016) can gate CaCCs (Fig. 1). Additionally, non-selective CNG channels were shown to gate CaCCs in olfactory sensory neurons (Dibattista et al., 2017). Changes in calcium concentrations can be sensed either directly, via a calcium-recognition domain within the protein of interest, or indirectly through interaction with specialized calcium-recognition proteins such as calmodulin (Galietta, 2009). Interestingly, both mechanisms have been previously proposed for the mode of calcium-sensing of CaCCs. The topic remained disputed since CaCCs lack both classical calmodulin binding-sites and calcium-recognition domains and experimental evidence from different groups supported both ideas (Tian et al., 2011; Yu et al., 2012). Recently, the cryo-electron microscopy (EM) structure of a mouse CaCC (mTMEM16 A) revealed that calcium is sensed directly. It involves negatively charged amino acid residues in transmembrane domains 6–8 (Fig. 2). Binding of two calcium ions then leads to a major rearrangement of the transmembrane domain 6, which finally gates the anion channel (Dang et al., 2017; Paulino et al., 2017a).

In addition to calcium-sensitivity, CaCCs were described as being voltage dependent (Duran and Hartzell, 2011), but not to the same extent as classical voltage-gated ion channels: i.e. a mere change of membrane voltage within physiological limits in absence of an increase of intracellular calcium is not able to gate CaCCs. However, it was shown that CaCCs do gate at very high, supraphysiological, voltages in absence of Ca^{2+} (Xiao et al., 2011). Instead, the voltage-dependence of CaCCs shifts to a physiological range in the presence of different calcium concentrations (Ma et al., 2017). The absence of classic voltage-gating can be explained by an absence of a canonical voltage-sensor from the structure of CaCCs (Duran and Hartzell, 2011). Contrarily, the calcium binding site, described above, projects into a part of TM6 to TM8, which are situated in the range of the inner leaflet of the plasma membrane. Hence, binding of positively charged calcium ions at this position may easily interfere with the movement of TM 6 (Fig. 2) once

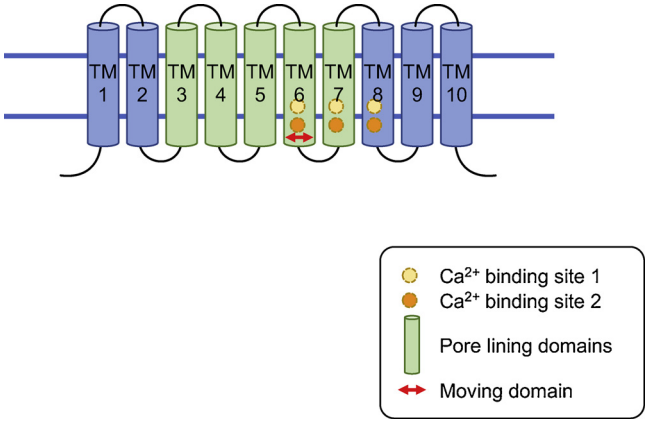

Fig. 2. Simplified topology of a TMEM16 A subunit.
Functional calcium-activated chloride channels (CaCC) are composed of two TMEM16 A subunits. Transmembrane domains TM3 to TM7 (green barrels) of each subunit contribute to a separate ion conduction pore, forming a two-barreled ion channel. Two calcium ions first bind to negatively charged amino acid residues on TM7 and TM8 (the location of each of the two Ca^{2+} binding sites is roughly indicated by dark and bright yellow circles on the three participating transmembrane domains). Subsequently, TM6 moves (red arrow) towards the calcium-bound transmembrane domains and opens the ion conduction pathway. The calcium binding-site is located at a part of the transmembrane domains that resides within the inner leaflet of the plasma membrane. Upon binding of positive charges (calcium ions), the membrane potential is also thought to interfere with TM6 movement, explaining the voltage-dependent nature of CaCC currents. Information for this figure was taken from (Dang et al., 2017; Falzone et al., 2018; Paulino et al., 2017a).

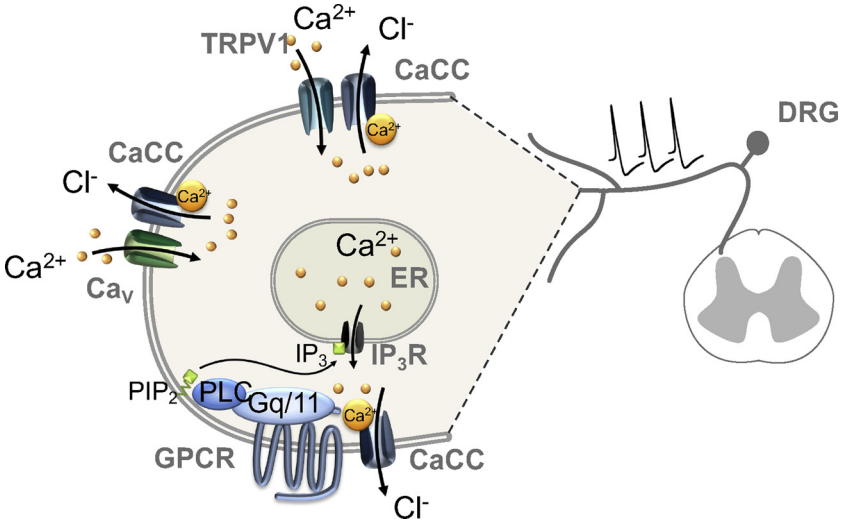

Fig. 1. Calcium sources for channel activation.
Calcium required for gating of calcium activated chloride channels (CaCC) in dorsal root ganglion (DRG) neurons can be provided by different sources: activation of G-protein coupled receptors (GPCR) coupled to $G_{q/11}$ G-proteins triggers phospholipase C (PLC) activity. Activated PLC hydrolyzes phosphatitylinositol 4,5 bisphosphate (PIP_2) into diacylglycerol (DAG, not shown) and inositol 1,4,5 trisphosphate (IP_3). IP_3 diffuses to the ER (endoplasmic reticulum) membrane, where it opens calcium-permeable IP_3 receptors (IP_3R). The subsequently released calcium (Ca^{2+}) can bind to and activate CaCCs (bottom). Cytosolic calcium can also rise due to an influx through ion channels from the extracellular space: one possibility is voltage-gated calcium channels (Ca_V). An increase in membrane potential leads to gating of these channels. Subsequently, calcium ions directly enter the cell and can then activate CaCCs (left). Another type of ion channel, that allows calcium influx is transient receptor potential vanilloid family 1 (TRPV1). These channels can be activated by noxious heat, voltage or presence of ligands, such as capsaicin. TRPV1 channels are highly permeable for calcium, which can bind to CaCCs and activate them (top). In all three cases, the source of calcium needs to be in close proximity to CaCCs to be able to activate these channels. Information for this figure was taken from (Jin et al., 2013).

the membrane potential changes (Ma et al., 2017).

Additionally, CaCCs have been proposed as sensors for noxious heat. TRPV1 channels represent canonical heat sensors. However, mice lacking these channels still retain some heat sensitivity (Oh and Jung, 2016). In this context, CaCCs have been proposed as candidates for additional heat sensors. Indeed, heterologously expressed TMEM16 A were found to produce currents at temperatures higher than 44 °C (Cho and Oh, 2013). Moreover, a tissue-specific knock-out of TMEM16 A lowered both thermally and mechanically induced nocifensive behavior (Cho et al., 2012; Lee et al., 2014). Yet, only heterologously expressed CaCCs were clearly shown to produce currents in response to noxious heat (Cho and Oh, 2013). Evidence to further support the hypothesis of heat-evoked currents through endogenously expressed CaCCs in DRG neurons is still lacking.

4. Ion selectivity of calcium-activated chloride channels

Even though the name “calcium-activated chloride channel” suggests chloride as the sole permeant ion (Falzone et al., 2018), it additionally permits a lesser flux for fluoride but also a higher flux for iodide and bromide ions. However, since chloride is physiologically the most abundant anion and plays the predominant functional role for CaCCs, this fact is imprinted on the channel’s name. Yet, it is still debated if CaCCs are truly selective anion channels, since some reports suggest that up to 20% of CaCC currents may be carried by small cations (Oh and Jung, 2016), while others propose high selectivity for anions (Dang et al., 2017; Paulino et al., 2017a). Interestingly, when TMEM16 A was investigated under purified conditions, the anion selectivity was preserved (Terashima et al., 2013). Hence, one may suspect the suggestion of cation flux through CaCCs may have been due to the presence of a contaminating conductance or other technical limitations (Falzone et al., 2018).

The TMEM16 family of plasma membrane proteins consists of ten members: only the first two, named TMEM16 A and TMEM16B, were unequivocally proven to be ion channels. Recently, other members of the TMEM16 family were identified as calcium-dependent phospholipid scramblases (randomly translocating phospholipids between the two layers of the membrane) as well as dual-function channel/scramblase proteins (see above).

5. Calcium-activated chloride channels in sensory neurons

CaCCs were functionally characterized in a variety of neuronal tissues, including dorsal root ganglion (DRG) neurons (Mayer, 1985). These neurons are first-order afferent sensory neurons, which transmit sensory information to the spinal cord. A subset of these neurons specifically encodes noxious stimuli. Noxious information is relayed in the spinal dorsal horn to a second-order neuron, which subsequently transmits the signal to the brain (Basbaum et al., 2009).

An inward current was described in DRG neurons, which either occurred after activation of calcium currents (Mayer, 1985) or in response to bradykinin application (Burgess et al., 1989). This inward current was most likely carried by chloride, a current that requires the presence of intracellular calcium, and which can be blocked by non-selective chloride channel blockers. Therefore the name calcium-activated chloride channels was implemented (Burgess et al., 1989; Mayer, 1985). In current-clamp recordings, activation of CaCCs in DRG neurons depolarize the membrane and trigger action potentials (Mayer, 1985). On the other hand, a general misconception is that activation of a chloride conductance, like CaCCs or $GABA_A$ receptors, leads to a decrease (and not an increase) in neuronal firing and a hyperpolarization. This difference can be easily explained: opening of a channel brings the membrane potential to the Nernst potential of the permeating ion. The equilibrium- or Nernst potential of a specific ion is the voltage where the concentration gradient is cancelled out by the electrical gradient that is formed by an imbalance of positive and negative charges. The equilibrium potential is dependent on intracellular and extracellular concentrations of that specific ion (Nernst, 1888). In case of chloride, the intracellular concentration varies considerably between, for example, central neurons and peripheral neurons. Mature central neurons display very low intracellular concentrations of chloride (Kahle et al., 2008), whereas some peripheral neurons maintain a higher chloride concentration (Liu et al., 2010). Accordingly, the intracellular chloride concentration in DRG neurons was determined to range around 30 mM (Kaneko et al., 2002) and that of sympathetic neurons even amounts to 70 mM (Woodward et al., 1969). However, these different cytosolic concentrations can be created by expressing different chloride transport proteins (Kahle et al., 2008). Hence, activation of a chloride conductance in mature central neurons leads to a hyperpolarization and a reduction of action potential frequency, whereas activation of the same conductance in a peripheral neuron leads to depolarization and an increase of action potential frequency (Liu et al., 2010).

CaCCs are expressed on small diameter nociceptive DRG neurons (Cho et al., 2012). There, they are activated in the presence of inflammatory mediators, such as bradykinin (Liu et al., 2010), serotonin (Salzer et al., 2016), or proteases (Jin et al., 2013; Liu et al., 2010). In these cases, the activation of CaCCs is indirect as it involves G_q-coupled receptors (Jin et al., 2016). In animal experiments, injection of bradykinin increases nocifensive behavior (Liu et al., 2010), which is absent in mice lacking TMEM16 A. Hence, the contribution of these ion channels to the formation of bradykinin-induced inflammatory hyperalgesia is undisputed (Lee et al., 2014). Serotonin induces acute inflammatory pain in rats, which involves G_q-coupled 5-HT_2 receptors (Nakajima et al., 2009). Hence, it is reasonable to assume that indirect activation of CaCCs by 5-HT_2 receptors contributes to the formation of serotonin-mediated inflammatory nocifensive behavior. Additionally, stimulation of protease activated receptors (PAR) induces chronic pain states in rats and the associated nocifensive behavior (Tillu et al., 2015). Again, one might speculate that indirect activation of CaCCs by G_q-coupled PAR-2 receptors is involved in this process.

Bradykinin, serotonin, and proteases, amongst others, are released locally in response to tissue damage (Basbaum et al., 2009) and are part of the so-called “inflammatory soup” (Basbaum et al., 2009; Kessler et al., 1992). This induced inflammation leads to an amplification of pain sensation and subsequent prevention of potentially harmful behavior. On a molecular level, indirect activation of CaCCs by such inflammatory mediators leads to a depolarization of the respective nociceptive neuron. As a result, neurons fire more action potentials in response to an otherwise unaltered stimulus (Salzer et al., 2017), which is then sensed as increased pain (Basbaum et al., 2009). Hence, CaCCs appear crucially involved in the formation of chronic and inflammatory pain.

In addition, CaCCs were implied in sensing noxious heat (Cho and Oh, 2013). Additional heat sensors are necessary, since knock-out of the canonical heat sensor TRPV1 leaves animals with some residual heat sensitivity (Palkar et al., 2015). However, additional evidence needs to be gathered to corroborate the role of CaCCs as sensors for noxious heat (see above).

As mentioned before, Ca^{2+} influx through TRPV1 channels provides a Ca^{2+} source for CaCC gating (Jin et al., 2016). This is very relevant since both channels are expressed on the same set of nociceptive DRG neuron (Oh and Jung, 2016). Interestingly, inhibition of TMEM16 A reduces capsaicin-evoked nocifensive behavior in mice. This suggests that the direct interaction of TRPV1 receptors and CaCCs is an important pain-enhancing mechanism (Takayama et al., 2015).

6. Future perspective

The prominent expression pattern of CaCCs in the nociceptive pathway (Oh and Jung, 2016) makes it tempting to speculate about CaCCs as potential drug targets in antinociceptive therapy. Indeed,

Inhibitors of CaCCs were tested for their actions in animal models of acute (Deba and Bessac, 2015) as well as inflammatory nociception (García et al., 2014). In both models, CaCC inhibitors were found to reduce nocifensive behavior. These promising results revealed that CaCC inhibitors could potentially be used as treatment of both acute as well as inflammatory pain.

Potential drug candidates should first be tested for their selectivity on TMEM16 A or B. New, (proposed) selective inhibitors of CaCCs, especially TMEM16 A, include substances like $T16A_{inh}$-A01, $CaCC_{inh}$-A01 and MONNA. However, all three drugs were found to act rather unspecifically, at least in vascular tissue (Boedtkjer et al., 2015). It is unlikely that the situation should be different in neurons of the pain pathway. Another candidate is Ani9, a more potent, small-molecule inhibitor, that was found to be selective for TMEM16 A over TMEM16B (Seo et al., 2016). It still needs to be clarified if this drug is also more selective over other targets.

A further potential problem is the tissue distribution of CaCCs and their variety of (patho)physiological functions. CaCCs were found in a number of cell types, including vascular smooth muscle cells, where they control vascular tone. CaCCs are also present in a number of different epithelia, where they regulate chloride secretion. Moreover, in tumor cells, CaCCs are involved in proliferation and tumorigenesis (Oh and Jung, 2016). Hence, when looking for a new analgesic, drug effects in these tissues may cause serious side effects. Nevertheless, new treatment options are needed, especially for chronic and inflammatory pain, and CaCCs represent promising targets.

Acknowledgements

I.S. is a member of the doctoral program CCHD co-funded by the Austrian Science Fund (W1205) and the Medical University of Vienna. S. Boehm receives support from the interuniversity cluster project “Novel scaffolds for improved antiepileptic drugs” financed by the University of Vienna and the Medical University of Vienna.

References

Bader, C.R., Bertrand, D., Schwartz, E.A., 1982. Voltage-activated and calcium-activated currents studied in solitary rod inner segments from the salamander retina. J. Physiol. 331, 253–284. https://doi.org/10.1038/nature13984.

Basbaum, A.I., Bautista, D.M., Scherrer, G., Julius, D., 2009. Cellular and molecular mechanisms of pain. Cell 139, 267–284. https://doi.org/10.1016/j.cell.2009.09.028.

Boedtkjer, D.M.B., Kim, S., Jensen, A.B., Matchkov, V.M., Andersson, K.E., 2015. New selective inhibitors of calcium-activated chloride channels - T16A(inh) -A01, CaCC(inh) -A01 and MONNA - what do they inhibit? Br. J. Pharmacol. 172, 4158–4172. https://doi.org/10.1111/bph.13201.

Brunner, J.D., Lim, N.K., Schenck, S., Duerst, A., Dutzler, R., 2014. X-ray structure of a calcium-activated TMEM16 lipid scramblase. Nature 516, 207–212. https://doi.org/10.1038/nature13984.

Burgess, G.M., Mullaney, I., McNeill, M., Dunn, P.M., Rang, H.P., 1989. Second messengers involved in the mechanism of action of bradykinin in sensory neurons in culture. J. Neurosci. 9, 3314–3325.

Caputo, A., Caci, E., Ferrera, L., Pedemonte, N., Barsanti, C., Sondo, E., Pfeffer, U., Ravazzolo, R., Zegarra-Moran, O., Galietta, L.J.V., 2008. TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. Science 322, 590–594. https://doi.org/10.1126/science.1163518.

Cho, H., Oh, U., 2013. Anoctamin 1 mediates thermal pain as a heat sensor. Curr. Neuropharmacol. 11, 641–651. https://doi.org/10.2174/1570159X113119990038.

Cho, H., Yang, Y.D., Lee, J., Lee, B., Kim, T., Jang, Y., Back, S.K., Na, H.S., Harfe, B.D., Wang, F., Raouf, R., Wood, J.N., Oh, U., 2012. The calcium-activated chloride channel anoctamin 1 acts as a heat sensor in nociceptive neurons. Nat. Neurosci. 15, 1015–1021. https://doi.org/10.1038/nn.3111.

Dang, S., Feng, S., Tien, J., Peters, C.J., Bulkley, D., Lolicato, M., Zhao, J., Zuberbühler, K., Ye, W., Qi, L., Chen, T., Craik, C.S., Jan, Y.N., Minor, D.L., Cheng, Y., Jan, L.Y., 2017. Cryo-EM structures of the TMEM16A calcium-activated chloride channel. Nature 552, 426–429. https://doi.org/10.1038/nature25024.

Deba, F., Bessac, B.F., 2015. Anoctamin-1 Cl(-) channels in nociception: activation by an N-aroylaminothiazole and capsaicin and inhibition by T16A[inh]-A01. Mol. Pain 11, 55. https://doi.org/10.1186/s12990-015-0061-y.

Dibattista, M., Pifferi, S., Boccaccio, A., Menini, A., Reisert, J., 2017. The long tale of the calcium activated Cl- channels in olfactory transduction. Channels (Austin) 11, 399–414. https://doi.org/10.1080/19336950.2017.1307489.

Duran, C., Hartzell, H.C., 2011. Physiological roles and diseases of Tmem16/Anoctamin proteins: are they all chloride channels? Acta Pharmacol. Sin. 32, 685–692. https://doi.org/10.1038/aps.2011.48.

Fallah, G., Römer, T., Detro-Dassen, S., Braam, U., Markwardt, F., Schmalzing, G., 2011. TMEM16A(a)/anoctamin-1 shares a homodimeric architecture with CLC chloride channels. Mol. Cell Proteomics 10, M110.004697. https://doi.org/10.1074/mcp.M110.004697.

Falzone, M.E., Malvezzi, M., Lee, B.-C., Accardi, A., 2018. Known structures and unknown mechanisms of TMEM16 scramblases and channels. J. Gen. Physiol. 150, 933–947. https://doi.org/10.1085/jgp.201711957.

Galietta, L.J.V., 2009. The TMEM16 protein family: a new class of chloride channels? Biophys. J. 97, 3047–3053. https://doi.org/10.1016/j.bpj.2009.09.024.

García, G., Martínez-Rojas, V.A., Rocha-González, H.I., Granados-Soto, V., Murbartián, J., 2014. Evidence for the participation of Ca(2+)-activated chloride channels in formalin-induced acute and chronic nociception. Brain Res. 1579, 35–44. https://doi.org/10.1016/j.brainres.2014.07.011.

Jin, X., Shah, S., Liu, Y., Zhang, H., Lees, M., Fu, Z., Lippiat, J.D., Beech, D.J., Sivaprasadarao, A., Baldwin, S.A., Zhang, H., Gamper, N., 2013. Activation of the Cl- channel ANO1 by localized calcium signals in nociceptive sensory neurons requires coupling with the IP3 receptor. Sci. Signal. 6, ra73. https://doi.org/10.1126/scisignal.2004184.

Jin, X., Shah, S., Du, X., Zhang, H., Gamper, N., 2016. Activation of Ca(2+) -activated Cl (-) channel ANO1 by localized Ca(2+) signals. J. Physiol. 594, 19–30. https://doi.org/10.1113/jphysiol.2014.275107.

Kahle, K.T., Staley, K.J., Nahed, B.V., Gamba, G., Hebert, S.C., Lifton, R.P., Mount, D.B., 2008. Roles of the cation-chloride cotransporters in neurological disease. Nat. Clin. Pract. Neurol. 4, 490–503. https://doi.org/10.1038/ncpneuro0883.

Kaneko, H., Putzier, I., Frings, S., Gensch, T., 2002. Determination of intracellular chloride concentration in dorsal root ganglion neurons by fluorescence lifetime imaging. Curr. Top. Membr. 167–189. https://doi.org/10.1016/S1063-5823(02)53033-7.

Kessler, W., Kirchhoff, C., Reeh, P.W., Handwerker, H.O., 1992. Excitation of cutaneous afferent nerve endings in vitro by a combination of inflammatory mediators and conditioning effect of substance P. Exp. Brain Res. 91, 467–476. https://doi.org/10.1007/BF00227842.

Lee, B., Cho, H., Jung, J., Yang, Y.D., Yang, D.-J., Oh, U., 2014. Anoctamin 1 contributes to inflammatory and nerve-injury induced hypersensitivity. Mol. Pain 10, 5. https://doi.org/10.1186/1744-8069-10-5.

Lee, B.-C., Menon, A.K., Accardi, A., 2016. The nhTMEM16 scramblase is also a non-selective ion channel. Biophys. J. 111, 1919–1924. https://doi.org/10.1016/j.bpj.2016.09.032.

Liu, B., Linley, J.E., Du, X., Zhang, X., Ooi, L., Zhang, H., Gamper, N., 2010. The acute nociceptive signals induced by bradykinin in rat sensory neurons are mediated by inhibition of M-type K+ channels and activation of Ca2+-activated Cl- channels. J. Clin. Invest. 120, 1240–1252. https://doi.org/10.1172/JCI41084.

Ma, K., Wang, H., Yu, J., Wei, M., Xiao, Q., 2017. New insights on the regulation of Ca2+-Activated chloride channel TMEM16A. J. Cell. Physiol. 232, 707–716. https://doi.org/10.1002/jcp.25621.

Malvezzi, M., Andra, K.K., Pandey, K., Lee, B.-C., Falzone, M.E., Brown, A., Iqbal, R., Menon, A.K., Accardi, A., 2018. Out-of-the-groove transport of lipids by TMEM16 and GPCR scramblases. Proc. Natl. Acad. Sci. U. S. A. 115, E7033–E7042. https://doi.org/10.1073/pnas.1806721115.

Mayer, M.L., 1985. A calcium-activated chloride current generates the after-depolarization of rat sensory neurones in culture. J. Physiol. 364, 217–239.

Miledi, R., 1982. A calcium-dependent transient outward current in Xenopus laevis oocytes. Proc. R. Soc. London. Ser. B Biol. Sci. 215, 491–497. https://doi.org/10.1098/rspb.1982.0056.

Nakajima, K., Obata, H., Ito, N., Goto, F., Saito, S., 2009. The nociceptive mechanism of 5-hydroxytryptamine released into the peripheral tissue in acute inflammatory pain in rats. Eur. J. Pain 13, 441–447. https://doi.org/10.1016/j.ejpain.2008.06.007.

Nernst, W., 1888. Zur Kinetik der in Lösung befindlichen körper. Erste abhandlung. Theorie der Diffusion. Z. Phys. Chem 2.

Oh, U., Jung, J., 2016. Cellular functions of TMEM16/anoctamin. Pflugers Arch. 468, 443–453. https://doi.org/10.1007/s00424-016-1790-0.

Palkar, R., Lippoldt, E.K., McKemy, D.D., 2015. The molecular and cellular basis of thermosensation in mammals. Curr. Opin. Neurobiol. 34, 14–19. https://doi.org/10.1016/j.conb.2015.01.010.

Paulino, C., Kalienkova, V., Lam, A.K.M., Neldner, Y., Dutzler, R., 2017a. Activation mechanism of the calcium-activated chloride channel TMEM16A revealed by cryo-EM. Nature 552, 421–425. https://doi.org/10.1038/nature24652.

Paulino, C., Neldner, Y., Lam, A.K.M., Kalienkova, V., Brunner, J.D., Schenck, S., Dutzler, R., 2017b. Structural basis for anion conduction in the calcium-activated chloride channel TMEM16A. Elife 6, 1–23. https://doi.org/10.7554/eLife.26232.

Salzer, I., Gantumur, E., Yousuf, A., Boehm, S., 2016. Control of sensory neuron excitability by serotonin involves 5HT2C receptors and Ca(2+)-activated chloride channels. Neuropharmacology 110, 277–286. https://doi.org/10.1016/j.neuropharm.2016.08.006.

Salzer, I., Schicker, K., Boehm, S., 2017. Ca2+ activated Cl- channels as targets for analgesics. Oncotarget 8, 45038–45039. https://doi.org/10.18632/oncotarget.18354.

Schroeder, B.C., Cheng, T., Jan, Y.N., Jan, L.Y., 2008. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. Cell 134, 1019–1029. https://doi.org/10.1016/j.cell.2008.09.003.

Seo, Y., Lee, H.K., Park, J., Jeon, D.-K., Jo, S., Jo, M., Namkung, W., 2016. Ani9, a novel potent small-molecule ANO1 inhibitor with negligible effect on ANO2. PLoS One 11, e0155771. https://doi.org/10.1371/journal.pone.0155771.

Sheridan, J.T., Worthington, E.N., Yu, K., Gabriel, S.E., Hartzell, H.C., Tarran, R., 2011. Characterization of the oligomeric structure of the Ca(2+)-activated Cl- channel Ano1/TMEM16A. J. Biol. Chem. 286, 1381–1388. https://doi.org/10.1074/jbc.

M110.174847.
Takayama, Y., Uta, D., Furue, H., Tominaga, M., 2015. Pain-enhancing mechanism through interaction between TRPV1 and anoctamin 1 in sensory neurons. Proc. Natl. Acad. Sci. U. S. A. 112, 5213–5218. https://doi.org/10.1073/pnas.1421507112.
Terashima, H., Picollo, A., Accardi, A., 2013. Purified TMEM16A is sufficient to form Ca2+-activated Cl- channels. Proc. Natl. Acad. Sci. U. S. A. 110, 19354–19359. https://doi.org/10.1073/pnas.1312014110.
Tian, Y., Kongsuphol, P., Hug, M., Ousingsawat, J., Witzgall, R., Schreiber, R., Kunzelmann, K., 2011. Calmodulin-dependent activation of the epithelial calcium-dependent chloride channel TMEM16A. FASEB J. 25, 1058–1068. https://doi.org/10.1096/fj.10-166884.
Tillu, D.V., Hassler, S.N., Burgos-Vega, C.C., Quinn, T.L., Sorge, R.E., Dussor, G., Boitano, S., Vagner, J., Price, T.J., 2015. Protease-activated receptor 2 activation is sufficient to induce the transition to a chronic pain state. Pain 156, 859–867. https://doi.org/10.1097/j.pain.0000000000000125.
West, R.B., Corless, C.L., Chen, X., Rubin, B.P., Subramanian, S., Montgomery, K., Zhu, S., Ball, C.A., Nielsen, T.O., Patel, R., Goldblum, J.R., Brown, P.O., Heinrich, M.C., van de Rijn, M., 2004. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. Am. J. Pathol. 165, 107–113. https://doi.org/10.1016/S0002-9440(10)63279-8.
Woodward, J.K., Bianchi, C.P., Erulkar, S.D., 1969. Electrolyte distribution in rabbit superior cervical ganglion. J. Neurochem. 16, 289–299.
Xiao, Q., Yu, K., Perez-Cornejo, P., Cui, Y., Arreola, J., Hartzell, H.C., 2011. Voltage- and calcium-dependent gating of TMEM16A/Ano1 chloride channels are physically coupled by the first intracellular loop. Proc. Natl. Acad. Sci. U. S. A. 108, 8891–8896. https://doi.org/10.1073/pnas.1102147108.
Yu, K., Duran, C., Qu, Z., Cui, Y.-Y., Hartzell, H.C., 2012. Explaining calcium-dependent gating of anoctamin-1 chloride channels requires a revised topology. Circ. Res. 110, 990–999. https://doi.org/10.1161/CIRCRESAHA.112.264440.