REVIEW

Swelling-activated ion channels: functional regulation in cell-swelling, proliferation and apoptosis

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Abstract

Cell volume regulation is one of the most fundamental homeostatic mechanisms and essential for normal cellular function. At the same time, however, many physiological mechanisms are associated with regulatory changes in cell size meaning that the set point for cell volume regulation is under physiological control. Thus, cell volume is under a tight and dynamic control and abnormal cell volume regulation will ultimately lead to severe cellular dysfunction, including alterations in cell proliferation and cell death. This review describes the different swelling-activated ion channels that participate as key players in the maintenance of normal steady-state cell volume, with particular emphasis on the intracellular signalling pathways responsible for their regulation during hypotonic stress, cell proliferation and apoptosis.
Keywords apoptosis, cation channels, cell volume regulation, chloride channels, cytoskeleton, proliferation, reactive oxygen species, regulatory volume decrease.

Outline

This mini-review addresses the relevant topics on the regulation of cell-swelling activated ion channels covering volume-regulated anion and cation channels: function and regulation, activation and regulation of volume-sensitive potassium channels and upstream volume signals and sensor.

The regulatory volume decrease

As most animal cell membranes are significantly more permeable to water than ions, a decrease in the extracellular osmolarity or an increase in the intracellular amount of osmolytes will lead to fast cell swelling. Such changes in cell volume can be observed under physiological and under pathophysiological conditions. Under physiological conditions most mammalian cells, with the exception of gastrointestinal and lung epithelial cells, do rarely see a decrease in extracellular osmolarity (see e.g. Hoffmann & Dunham 1995 for examples) and cell volume changes are most often caused by changes in the amount of intracellular solutes. In fact, several cellular physiological functions elicit cell volume perturbations caused by changes in intracellular osmolytes, including secretion of fluid and electrolytes (Greger *et al.* 1999, Hoffmann *et al.* 2002), Na^+-dependent sugar and amino acid uptake, stimulation of Na/H exchange or Na–K–2Cl cotransport with insulin, growth factors and mitogenic factors or finally entry from G1 to S phase (see e.g. Hoffmann & Ussing 1992, Hoffmann & Dunham 1995). Cell swelling under pathophysiological conditions can, however, well be due to a decreased plasma osmolarity (hyponatremia), as well as to an increase in intracellular osmolarity, as observed in metabolic disturbances, cellular acidosis and hypoxia (Nilius *et al.* 1996, 1999, Strange *et al.* 1996, Okada 1997b).

In recent years, it has been realized that regulation of, on the one hand, cell growth and proliferation, and on

the other, demise of cells through apoptosis is closely associated with regulatory changes in cell size (Lang *et al.* 1998). Proliferation is frequently associated with cell swelling, and is stimulated by osmotic swelling and inhibited by osmotic shrinkage, respectively (Burg 2002). Conversely, cell shrinkage is an early event in apoptosis (Okada *et al.* 2001), and osmotic shrinkage *per se* can elicit apoptosis (Bortner & Cidlowski 1996, Friis *et al.* 2005). Little is known regarding the sequence of events leading from cell volume changes to cell cycle progression or apoptosis, although some signalling components have been described in recent years (Lang *et al.* 2002, Friis *et al.* 2005). Evidence linking cell volume to control of cell proliferation and apoptosis opens up for a whole new framework in which to understand the mechanisms controlling cell growth and cell death. It is, therefore, imperative at this time that specialists in the fields of cell volume control and the control of cell growth/cell death utilize each others knowledge to further the understanding of these important processes. In the present review, the current knowledge about the control of cell volume after cell swelling will be discussed.

Increase in cell volume evokes a series of signalling events resulting in activation of volume regulatory mechanisms and a regulatory volume decrease response (RVD) dependent of an efflux of K^+, Cl^- and organic osmolytes followed by water (Hoffmann & Dunham 1995). Below, we will briefly describe the major effectors in volume regulation which are the volume sensitive channels as well as the signal transduction pathways involved in RVD. Electroneutral KCl release mainly via KCC1 which is an important feature during RVD in many systems (see e.g. Hoffmann & Mills 1999) is not dealt with here, neither is the coupled K^+/H^+ exchanger and Cl^-/HCO_3^- exchanger which is best described in the *Amphiuma* red blood cell (Cala *et al.* 1986). Upstream to the signal transduction pathways is the elusive volume sensing machinery, which will not be discussed in any detail in the present review. Actually, it is still not clear whether the signal sensed is changes in cell volume as such or whether the cell rather detects changes in intracellular ionic strength, changes in macromolecular crowding (Minton 1994, Burg 2000) or a combination of all these signals (see e.g. Wehner *et al.* 2003). It is thus important to realize that cell volume regulation must also be considered within the context of intracellular water homeostasis.

The few early events activated by cell swelling that will be dealt with below include activation of small GTP-binding proteins (G proteins) of the Rho family and reorganization of the cortical cytoskeleton. For more general reviews on the role of cytoskeleton in cell volume regulation (see e.g. Hoffmann & Mills 1999, Pedersen *et al.* 2001).

Volume-regulated anion channels: function and regulation

Increases in K^+ conductance during RVD will, due to the outwardly directed K^+ gradient increase K^+ efflux and hyperpolarize the plasma membrane. This hyperpolarization favours conductive Cl^- efflux even if the basal Cl^- conductance is unchanged. Nevertheless, Cl^- channels activated by cell swelling were found to be crucial elements in the RVD process. This is caused by the fact that Cl^- transport in most cells is completely dominated by an exchange diffusion process with the conductive Cl^- permeability being extremely low in isotonic steady-state cells. In Ehrlich ascites tumour cells (EATC), it was found that (Hoffmann *et al.* 1975, 1979) <5% of the Cl^- flux was a conductive leak flux, and thus, the Cl^- conductance was found to be much lower than the K^+ conductance (Hoffmann *et al.* 1979). Simultaneously with that observation, it was demonstrated that there is actually an increase in Cl^- flux after cell swelling in EATC which reflects an increase in the Cl^- conductance (Hoffmann 1978, 1982). This was the first evidence of swelling activated Cl^- channels which were soon after also suggested in lymphocytes (Grinstein *et al.* 1982) and later found in all cells investigated. These channels have been termed volume-regulated anion channels (VRAC), volume-sensitive organic osmolyte and anion channel (VSOAC) or volume-sensitive outwardly rectifying anion channel (VSOR) (Strange & Jackson 1995, Nilius *et al.* 1996, 1997, Okada 1997a, Nilius & Droogmans 2001). The biophysical characteristics of the Cl^- current activated by cell swelling are well described and relatively constant in different cell types. The current exhibits a moderate outwardly rectifying behaviour and a depolarization-dependent inactivation, the degree of which varies between cell types (see e.g. Jackson & Strange 1995, Lepple-Wienhues *et al.* 1998, Pedersen *et al.* 1998) and has the typical anion permeability sequence selectivity $SCN^- > I^- > NO_3^- > Br^- > Cl^- > F^-$ > gluconate that corresponds to Eisenman's sequence I (see Nilius & Droogmans 2001). VRAC is blocked by NPPB, DIDS, A9C, niflumic acid, the oestrogen receptor antagonists tamoxifen and clomiphen and several other substances. The pharmacology of VRAC is excellently described in Nilius *et al.* (1997), Jentsch *et al.* (2002), de Tassigny *et al.* (2003). There is a lack of blockers of VRAC with high affinity and selectivity. Nonetheless, DCPIB, an indanone compound, was found to be one of the most selective inhibitor so far (Decher *et al.* 2001), but recently a new inhibitor of the family of acidic di-aryl-ureas, NS3728, has been shown to inhibit VRAC in HEK-293 cells and in EATC with an IC_{50} around 0.4 μM (Helix *et al.* 2003, T.K. Klausen, A. Bergdahl, C. Hougaard, P. Christoffersen, S.F. Pedersen

& E.K. Hoffmann, 2006, unpublished data). VRAC activity is dependent on cytosolic ATP, but not its hydrolysis, suggesting a non-hydrolytic binding of ATP to the channel (Oike *et al.* 1994, Jackson *et al.* 1996, Meyer & Korbmacher 1996), yet the ATP dependence varies with the rate of cell swelling (Bond *et al.* 1999).

A number of other functions other than cell volume regulation have been attributed to the volume regulated anion channels. They may modulate cardiac electrical activity by modifying the repolarization of the action potential and in ciliary epithelial cells VRAC has been suggested to be relevant for aqueous humour secretion (Hume *et al.*, 2000). Evidence has been put forward linking VRAC activation and cell cycle progression (Lang *et al.* 2000b, Nilius 2001, Wondergem *et al.* 2001, Varela *et al.* 2004a). Inhibition of VRAC abrogates cell proliferation in many cell types (Voets *et al.* 1995, Shen *et al.* 2000, Wondergem *et al.* 2001, Xiao *et al.* 2002, Jiang *et al.* 2004, Rouzaire-Dubois *et al.* 2004); however, the actual role of anion channels in control of the cell cycle is still unclear and many of the results are contradictory (Shen *et al.* 2000, Chen *et al.* 2002). In Ehrlich Letree ascites cells, we find that VRAC expression decreases in G1 and increases in the early S phase, the isotonic steady state Cl^- current follows the picture for the VRAC expression being low in G1 and rising in the S phase. It is proposed that in Ehrlich cells, the entrance into the S phase requires an increase in steady-state chloride conductance and/or an increased potential for volume regulation (Kjær-Klausen *et al.* 2006a).

Recent studies have unravelled a pivotal role of VRAC in apoptosis. Apoptosis requires cells to shrink under normotonic conditions, a process known as apoptotic volume decrease (AVD), and activation of AVD was found to be linked to VRAC activation, thus blockade of this channel was shown to prevent AVD (Lang *et al.* 2000a, Okada & Maeno 2001, Okada *et al.* 2001).

Volume-regulated anion channels can be activated under isoosmotic conditions and independently from changes in cell volume. Isoosmotic activation can be achieved, for instance, by reducing intracellular ionic strength or by intracellular dialysis with the non-hydrolysable GTP analogue GTPγS. However, the signalling cascades and mechanisms involved in VRAC activation are poorly understood and several intracellular signals have been investigated, including intracellular Ca^{2+}, ATP and cytoskeleton proteins (for recent review see Nilius & Droogmans 2003). It should be noted, however, that VRAC can be activated under strong Ca^{2+}_i buffering conditions, and that these currents are distinct from those activated by an increase in $[Ca^{2+}]_i$. This was first shown by Szücs *et al.* (1996) in endothelial cells suggesting that a 'permissive' $[Ca^{2+}]_i$ is necessary to activate VRAC, and later by several other laboratories (see e.g. Pedersen *et al.* 1998).

Changes in membrane cholesterol content have been found to modulate the function of a variety of membrane transporters and channels including VRAC (Levitan *et al.* 2000, Romanenko *et al.* 2004). Cholesterol is a major component of lipid rafts and of caveolins (Brown & London 2000, Simons & Vaz 2004) and cholesterol depletion has been shown to disrupt the structure of both, rafts (Brown & London 2000) and caveolae (Westermann *et al.* 2005). Moreover, cholesterol modulates *membrane deformation energy* (Nielsen *et al.* 1998). Membrane deformation energy seems to be important for channel opening (Lundbaek *et al.* 2004), and it was previously demonstrated in EAT cells that an increase in the membrane content of polyunsaturated fatty acids increased the swelling activated Cl^- permeability and the rate of RVD (Lauritzen *et al.* 1993). Studies in bovine aortic endothelial (BAE) cells (Levitan *et al.* 2000, Romanenko *et al.* 2004) and in EATC (T.K. Klausen, C. Hougaard, E.K. Hoffmann, & S.F. Pedersen 2006, unpublished data) have shown that VRAC activity under modest osmotic gradients is increased by cholesterol depletion but the relationship between VRAC and membrane cholesterol content/microdomain structure is still incompletely understood. That VRAC potentiation by cholesterol depletion should be a result of caveolae disruption seems in contrast to the finding that hypotonically induced activation of VRAC is significantly impaired in caveolin-1-deficient Caco-2 cells, yet transient over-expression of this protein restores VRAC activity (Trouet *et al.* 1999).

Isovolumetric activation of VRAC has been consistently reported by several laboratories, raising a debate on the relation between VRAC activation and cell volume. It has been proposed that a decrease in intracellular ionic strength rather than an increase in cell volume operates as the initial trigger for VRAC activation during anisotonic cell swelling (Nilius *et al.* 1998, Voets *et al.* 1999). The observation that transient activation of VRAC can be achieved by intracellular application of GTPγS without any change in cell volume suggests a relevant modulatory role for GTP binding proteins (Doroshenko 1991, Nilius *et al.* 1994, Mitchell *et al.* 1997). Exposure of cells to toxins that specifically inactivate Rho GTPases significantly reduces VRAC activation by cell swelling or by intracellular GTP (Nilius *et al.* 1999). In NIH3T3 cells, it was demonstrated that the magnitude of VRAC was larger in RhoAV14 than in wild type cells after a 7.5% reduction in extracellular osmolarity, but similar in both strains after a 30% reduction in extracellular osmolarity. VRAC was inhibited by the Rho kinase inhibitor Y-27632 and strongly potentiated by the myosin light

chain kinase inhibitors ML-7 and AV25. It was suggested that RhoA, although not the volume sensor *per se*, is an important upstream modulator (Pedersen *et al.* 2002). Interestingly, it has been demonstrated that in CPAE cells the Rho dependent pathway does not play a causal role in swelling-induced activation of VRAC. These authors show that activation of the Rho pathway does not trigger hypotonicity-induced VRAC activation yet a fully functional Rho pathway is required for achieving VRAC activation, suggesting a permissive effect of this pathway (Carton *et al.* 2002).

Volume-regulated anion channels are also modulated by protein phosphorylation. Although the phosphorylation state of tyrosine residues and the activity of VRAC may indicate that regulatory processes are tissue or species specific, the general observation that several inhibitors of protein tyrosine kinases (PTKs), such as genistein, tyrphostin B46 and tyrphostin A25 prevent VRAC activation and that tyrosine phosphatases (PTPs) blockers Na_3VO_4 and dephosphatin increases VRAC activation, indicate that a sustained VRAC activation critically depends on a precise phosphorylation/dephosphorylation balance (Tilly *et al.* 1993, Sorota 1995, Voets *et al.* 1998, Nilius *et al.* 2000, Du *et al.* 2004, Varela *et al.* 2004b). Even though the tyrosine phosphorylation targets remain largely unknown, it is apparent that these signals are located downstream from the initial trigger(s) that targets VRAC activation. Evidence has been put forward that implicate a member of the Src-family of PTKs because it has been observed that in lck-deficient lymphocytes transfection of p56lck restores osmotic activation of VRAC (Lepple-Wienhues *et al.* 1998). As mentioned above, VRAC was inhibited by a Rho kinase inhibitor and strongly potentiated by myosin light chain kinase inhibitors, thus these two kinases are somehow also involved in the activation of VRAC.

As mentioned above, VRAC seems to be involved in cell proliferation and apoptosis. Although there is ample evidence on the role of radical oxygen species (ROS) in cell proliferation and apoptosis, it was only recently shown that cells subjected to a hypotonic challenge responded by increasing intracellular ROS (Lambert 2003, Varela *et al.* 2004a), prompted several groups to examine a possible role of ROS in VRAC activation. Varela *et al.* (2004a) demonstrated that H_2O_2 elicits VRAC activation under isovolumetric conditions. Using a p47 dominant negative construct, these authors demonstrated that the NADPH oxidase complex played a critical role in the H_2O_2 generation cascade leading to VRAC activation. As shown in Figure 1, H_2O_2 enhances hypotonically induced VRAC currents. They also showed that in isotonic conditions exposure of HeLa cells to the epidermal growth factor (EGF), which is known to induce the generation of H_2O_2, triggered the activation of VRAC. There is profuse evidence indicating that H_2O_2 influences the function of various proteins, including protein tyrosine phosphatases, protein kinases and transcription factors (for reviews see Finkel 2000, Rhee *et al.* 2000, 2005). Stimulation of mitochondrion-mediated apoptotic pathways causes VRAC activation and thus, AVD in HeLa cells (Shimizu *et al.* 2004). These authors also demonstrated that ROS acted upstream to VRAC channel activation in staurosporine-triggered apoptosis. They also found that apoptosis induced by Fas ligand and tumour necrosis factor (TNFα) activated VRAC independently of ROS signalling. ROS involved in VRAC activation was also established in rabbit ventricular myocytes via β1 inte-

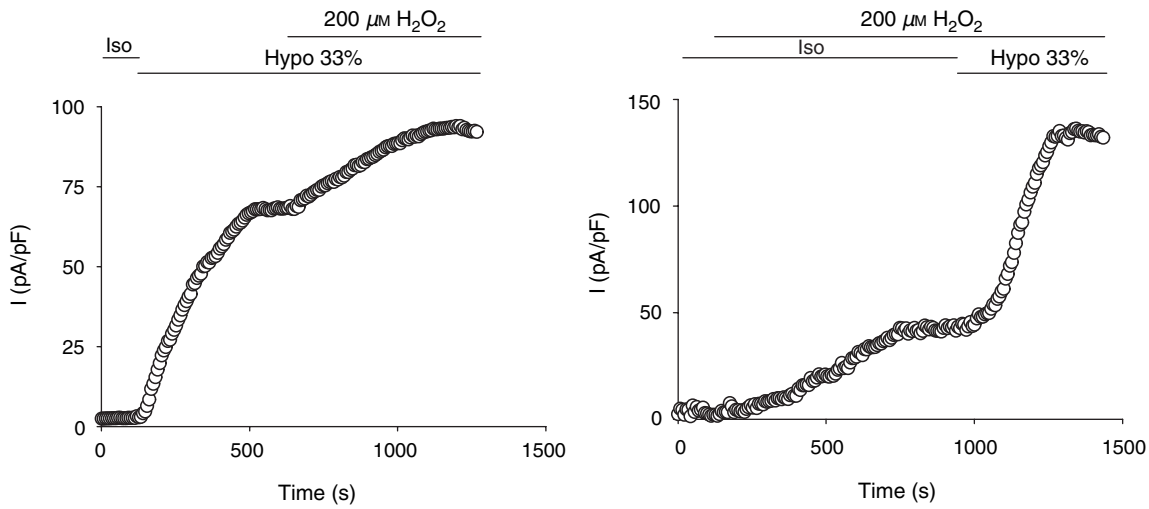

Figure 1 Time course of Cl^- current density in HTC cells stimulated by 33% hypotonicity (left panel) or 200 μM H_2O_2 in isotonicity (right panel) measured at 80 mV at an interval of 7 s. After achieving steady state, 200 μM H_2O_2 was added to the hypotonic solution (left panel) or isotonic solution was changed to hypotonic solution in the presence of 200 μM H_2O_2 (right panel).

grin and angiotensin II and downstream production of superoxide anions by the sarcolemmal NADPH oxidase (Browe & Baumgarten 2004). This response was blocked by diphenyleneiodonium (DPI), a blocker of the NADPH oxidase complex and in addition, the response to stretch was attenuated by catalase, a H_2O_2 scavenger enzyme.

These exciting new data on how VRAC is activated and modulated add a further intriguing feature to a channel that is already known as a very complex permeation pathway, yet still unidentified at the molecular level.

The swelling-activated taurine pathway

The sulphonic amino acid taurine (2-aminoethanesulphonic acid) is the main organic osmolyte of many mammalian cells and is actively accumulated in various tissues to concentrations up to 65 mM. In EATC, it was shown that the cells can maintain a cellular to extracellular taurine concentration ratio of 600 as a functional steady state between the active transport, the passive release and the biosynthesis from cystein (Hoffmann & Lambert 1983). The permeability to taurine is increased after cell swelling and taurine is released from swollen cells together with K^+ and Cl^-, thus playing an important role in the RVD process as it was first shown for mammalian cells in EATC (Hoffmann & Hendil 1976, Hoffmann & Lambert 1983) and later in a great variety of cells (see Wehner *et al.* 2003). Since the molecular identity of the membrane permeability pathway used by taurine to leave the cells is not known, the possibility of a common pathway for taurine and Cl^- translocation has been raised. Evidence favouring a common pathway for organic osmolytes and Cl^- relies on a similar pharmacological profile of the two effluxes and on measurements of single-channel and macroscopic currents mediated by taurine at an alkaline intracellular pH in MDCK kidney cells (Banderali & Roy 1992) and glioma C6 cell (Jackson & Strange 1993), and on the observation that the size of the pore of VRAC is sufficiently large to permeate amino acids (Strange *et al.* 1996, Nilius & Droogmans 2003). Nevertheless, several other laboratories have reported results that cannot be reconciled with a common pathway (Hoffmann & Hendil 1976, Lambert & Hoffmann 1994, Shennan *et al.* 1994, Sánchez-Olea *et al.* 1995, Stutzin *et al.* 1999, Hoffmann 2000, Lambert 2004). In addition to the contradictory reports about the taurine permeability pathway activated under hypoosmotic stress, the transduction signalling elements leading to its activation remain poorly defined (Mongin *et al.* 1999, Mongin & Orlov 2001). Several signalling cascades that transduce cell volume changes into activation of osmolyte pathways or modulate this response have been explored, including intracellular calcium (Falktoft & Lambert 2004), ROS (Lambert 2003, Ortenblad *et al.* 2003) protein kinase C, protein kinase A, and lipid messengers (reviewed in Lang *et al.* 1998), as well as tyrosine kinases (Pasantes-Morales *et al.* 2002, Pasantes-Morales & Franco 2002, Pedersen *et al.* 2002) and Rho proteins (Pedersen *et al.* 2002).

Swelling-activated cation channels: function and regulation

Swelling activated K^+ channels

Activation of a K^+ leak pathway by cell swelling was first demonstrated in lymphocytes (Roti Roti & Rothstein 1973) and in EATC (Hendil & Hoffmann 1974). In contrast to the swelling activated Cl^- channel which is quite ambiguous, the increase in K permeability after cell swelling has been found to be associated with various swelling-activated K^+ channels in different cell types. These channels include: (i) stretch-activated K^+ channels in various cells (Sackin 1989, Filipovic & Sackin 1992, Vanoye & Reuss 1999, Dubinsky *et al.* 2000); (ii) voltage-dependent K^+ channels such as Kv1.3 or Kv1.5 in lymphocytes (Cahalan & Lewis 1988, Deutsch & Chen 1993, Felipe *et al.* 1993, Lewis & Cahalan 1995) and Kv1.5 in murine spermatozoa (Barfield *et al.* 2005); (iii) Ca^{2+}-activated channels of small conductance (SK) in human liver cells (Roman *et al.* 2002), of intermediate conductance (IK) in osteosarcoma cells (Yamaguchi *et al.* 1989), EATC (Christensen & Hoffmann 1992), human T lymphocytes (Khanna *et al.* 1999) and intestine 407 cells (Wang *et al.* 2003) and of large conductance (BK) in human bronchial epithelial cell line (Fernández-Fernández *et al.* 2002), rabbit kidney proximal tubule cells (Dubé *et al.* 1990), hepatocytes (Pon & Hill 1997) and ostoblasts (Weskamp *et al.* 2000); and (iv) KCNQ1/KCNE3 heterotetrameric channels in rat hepatocytes. However, experiments with cloned BK have shown that this channel is not regulated by cell volume changes *per se* and thus, it can not be excluded that substantial changes in cell volume may well induce changes in intracellular Ca^{2+} which subsequently activate BK channels. Finally, members of the family of potassium channels known to have two pore regions and four membrane-spanning segments (2P-4TM) which are thought to be responsible for the leak or background conductances in many cells, have now been shown to be responsible for volume regulation in EATC (Niemeyer *et al.* 2000), in kidney cells (Barriere *et al.* 2003) and in murine spermatozoa (Barfield *et al.* 2005). The most likely candidate in EATC is the TWIK-related, acid sensitive potassium

channel (TASK-2), as Western and Northern blot analyses and reverse transcriptase-polymerase chain reaction (RT-PCR) assays showed the presence of TASK-2 in Ehrlich cells and mTASK-2-expressing HEK-293 cells exhibited a mTASK-2 K^+ current which was osmosensitive, being increased by hypotonic cell swelling, decreased by cell shrinkage and inhibited by chlofilium (Niemeyer *et al.* 2001a,b), a pattern compatible with $I_{K,vol}$ in intact Ehrlich cells (see below). Two other two-pore domain K^+ channels, TREK-1 and TRAAK were shown to be stretch-activated (Patel *et al.* 1998, Maingret *et al.* 1999). At variance with $I_{K,vol}$ in EATC, which is inhibited by AA (Niemeyer *et al.* 2000), TRAAK is stimulated by arachidonic acid (AA) (Fink *et al.* 1998).

Many cloned K^+ channels expressed in *Xenopus* oocytes or HEK 293 cells have also been found to be sensitive to cell volume changes. In addition to the TASK-2 channel discussed above (Niemeyer *et al.* 2001a,b) this is true for SK and IK channels (Grunnet *et al.* 2002, Jørgensen *et al.* 2003), for KCNQ1 (Grunnet *et al.* 2003) and KCNQ4 (Hougaard *et al.* 2004) as well as for HCN2 channels (Calloe *et al.* 2005). It should be mentioned that for the cloned K^+ channels the effect of small cell volume changes (2–8%) were investigated and thus, those experiments are not necessarily comparable to 'classic' RVD experiment in which large changes in cell volume are forced. Even though cloned K^+ channels expressed in *Xenopus* oocytes or HEK 293 cells are found to be sensitive to cell volume changes it is always a question whether any of these channels represent the dominating swelling activated K^+ current ($I_{K,vol}$) seen in a cell types in which they are found to be expressed. As an example, cloned hIK channels expressed in *Xenopus* oocytes are as mentioned volume sensitive and the first swelling-activated K^+ channel demonstrated in EATC was an inwardly rectifying Ca^{2+}-dependent IK channel (Christensen & Hoffmann 1992) with similarities to the cloned hIK1 channel (Ishii *et al.* 1997, Jensen *et al.* 1998). Nevertheless, this IK channel contributes only insignificantly to $I_{K,vol}$ in EATC cells at 37° since the magnitude of $I_{K,vol}$ is unaffected by ChTX, another inhibitor of hIK1 (Riquelme *et al.* 1998), and $I_{K,vol}$ in EATC is activated also under strong $[Ca^{2+}]_i$ buffered conditions (Hougaard *et al.* 2000).

The swelling activated TASK channel in EATC

A detailed description of $I_{K,vol}$ has been performed in Ehrlich cells through whole cell patch clamp studies. Its characteristics are: (i) it is insensitive to a number of known K^+ channel inhibitors such as clotrimazole, ChTX, apamin, kaliotoxin, margatoxin and TEA (Jørgensen *et al.* 1997, Riquelme *et al.* 1998), but

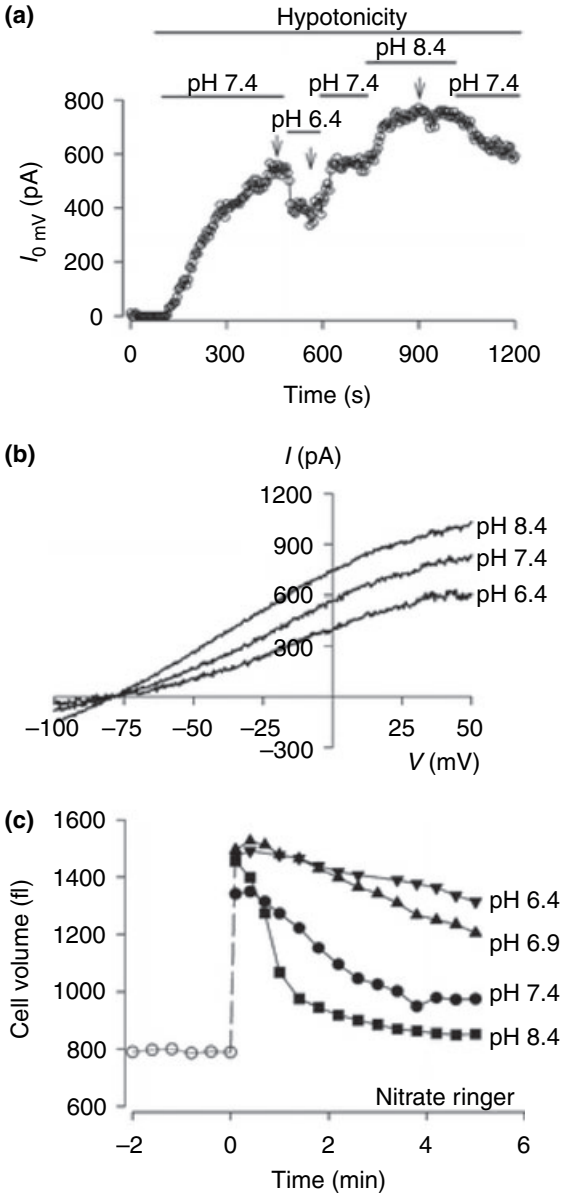

Figure 2 The swelling activated K^+ current ($I_{K,vol}$) and the regulatory volume decrease (RVD) at various external pH values. (a) Activation of $I_{K,vol}$ by hypotonic cell swelling, pH_o was changed as indicated by the bars, pH_i was 7.4. (b) *I/V* relations of $I_{K,vol}$ measured at the time-points depicted in (a). The figure represents seven independent experiments. (c) Cell volume was measured as a function of time by electronic cell sizing using a Coulter Counter. The cells were pre-incubated for 30 min in isotonic Cl^--free $NaNO_3$-containing medium to exchange all Cl^- for NO_3^- in order to avoid any contribution from the KCl cotransporter to RVD. At time zero the cells were diluted into hypotonic $NaNO_3$ medium, pH 6.4, 6.9, 7.4 or 8.4 as indicated. Open circles indicate cell volume under isotonic conditions. The figure represents four independent sets of experiments (results from Hougaard *et al.* 2001).

efficiently blocked by clofilium (Niemeyer *et al.* 2000); (ii) it is independent of intracellular Ca^{2+} and it shows Goldmann–Hodgkin–Katz (GHK) behaviour, suggesting lack of intrinsic voltage dependence (Niemeyer *et al.* 2000). The permselectivity sequence is $K^+ > Rb^+ >> Cs^+$, NH_4^1, Na^+, Li^+ is consistent with that reported for mTASK-2 (Riquelme *et al.* 1998, Niemeyer *et al.* 2000, 2001a); (iii) the maximal current is as shown in Figure 2a,b strongly dependent upon extracellular pH, being enhanced by alkalinization and inhibited by acidification (Hougaard *et al.* 2001) in agreement with the observation shown in Figure 2c that the RVD response of EATC is strongly accelerated at pH_o 8.2 and inhibited at pH_o 6.6 (Kramhøft *et al.* 1986, Hougaard *et al.* 2001). Using noise analysis, Hougaard *et al.* (2001) found that the K^+ channel activity (NP_{open}) obtained after cell swelling increased significantly with increasing pH_o and decreased significantly with decreasing pH_o, similar to what has been reported for TBAK1/TASK1 (Kim *et al.* 1999), TASK2 (Reyes *et al.* 1998) and TASK3 (Kim *et al.* 2000) expressed in COS-7 cells. All the above functional characteristics of $I_{K,vol}$ suggests a TASK channel to be the K^+ conductance activated by swelling of EATC (Niemeyer *et al.* 2001b). TASK-2, which is found to be highly expressed in EATC (Niemeyer *et al.* 2001b), seems to be the most obvious candidate since PCR assays did not detect TASK-1 or -3 transcripts in EATC. The possibility that a different channel is responsible for the pH-dependent $I_{K,vol}$ in EATC cannot, however, be dismissed. The channel responsible, in that case, would have to exhibit very similar functional characteristics to TASK-2.

Activation and regulation of $I_{K,vol}$

Eicosanoids and swelling activated K^+ channels

Eicosanoids seem to play a role in regulation of the channels involved in the loss of KCl during RVD (see Hoffmann 2000). 12-(*S*)-hydroxyeicosatetraenoic acid [12(*S*)-HETE] is a hydroxy derivative of AA made by 12-lipoxygenase. It is one of the most abundant eicosanoids in human bronchi. In human platelets, the 12-HPETE product, hepoxylin A activates $I_{K,vol}$ (Margalit & Livne 1991, Margalit *et al.* 1993), hepoxylin A, however, had no effect in EATC (Hoffmann 2000). In EATC, on the other hand, it was demonstrated that the synthesis of cysteinyl leukotrienes is stimulated, while prostaglandin E_2 synthesis is reduced, during RVD (Lambert *et al.* 1987). The role of prostaglandins was thought to be principally directed towards the control of Na^+ permeability since (i) PGE_2 significantly inhibits RVD following hypotonic swelling in Na^+-containing medium but not in Na^+-free media; (ii) addition of PGE_2 to EATC results in a strong depolarization (Lambert *et al.* 1987). Addition of the cysteinyl leukotriene leukotriene D_4 (LTD_4) accelerated RVD and since the K^+ permeability is rate limiting for RVD this was taken to indicate that LTD_4 stimulated the K permeability. The cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) are metabolites of AA. They are formed via the 5-lipoxygenase pathway by addition of cysteine derivatives to LTA_4. The conversion of LTA_4 to LTC_4 by the enzyme LTC_4 synthase limits the rate of cysteinyl leukotriene formation (Fig. 4). The cysteinyl leukotrienes are potent lipid mediators in inflammation. LTD_4 was first found to be involved in the RVD response in EATC (Lambert *et al.* 1987) and later also in rat colonic enterocytes (Diener & Scharrer 1993, 1997), flounder erythrocytes (Thoroed & Fugelli 1994) mud puppy red blood cells (Light *et al.* 1997) and mouse intact distal colonic crypts. In EATC, LTD_4 activates $I_{K,vol}$ independent of an increase in intracellular Ca^{2+} thus in Ca^{2+}-free media, 3 nM LTD_4 accelerates the RVD response in the absence of an increase in $[Ca^{2+}]_i$ (Jørgensen *et al.* 1997) and addition of LTD_4 to EATC results in a ChTX-sensitive Ca^{2+}-activated K^+ efflux as well as a K^+ efflux which, similar to $I_{K,vol}$, is ChTX-insensitive, with the ChTX-insensitive K^+ efflux being dominating after addition of low concentrations of LTD_4 (Hoffmann 1999). This was confirmed in whole cell patch clamp experiments where addition 5 nM LTD_4 activates a K^+ current with similar conductance (5 pS as estimated from non-stationary noise analysis (Jørgensen *et al.* 2000), IV relation (GHK behaviour) and pharmacological profile (blocked by 100 μM clofilium and insensitive to ChTX) as $I_{K,vol}$ (Hougaard *et al.* 2000). LTD_4 binds to CysLT receptors and the murine $CysLT_1$ (Mollerup *et al.* 2001) have been cloned. Binding of LTD_4 to the receptor results in Ca^{2+} release and Ca^{2+} influx in most cell types studied (Bouchelouche *et al.* 1990, Grönroos *et al.* 1995, 1996, Thodeti *et al.* 2001) included EATC cells (Jørgensen *et al.* 1996, Pedersen *et al.* 1997). The accelerating effect of LTD_4 on the RVD response can result from this increase in $[Ca^{2+}]_i$ and activation of Ca^{2+} activated channels, but as discussed above in EATC LTD_4 activates $I_{K,vol}$ independent of an increase in intracellular Ca^{2+} thus there is another pathway apart from stimulation with LTD_4 leading to the activation of $I_{K,vol}$. In this respect, it is interesting that it has been shown that LTD_4 activates distinct G-proteins in intestinal epithelial cells to regulate stress fibre formation (pertussis-toxin insensitive G proteins) and to generate intracellular Ca^{2+} mobilization (pertussis toxin sensitive G proteins) (Nielsen *et al.* 2005). It is thus likely that the stimulation of the TASK channel in EATC goes via the pertussis–toxin insensitive G proteins. Native $I_{K,vol}$ in the Ehrlich cell has been shown to be under the control of one or more G-proteins (Niemeyer *et al.* 2002). In NIH3T3 cells, it

is shown that the swelling-activated K^+ efflux is strongly potentiated in cells expressing constitutively active Rho (Pedersen *et al.* 2002).

Swelling-activated non selective cation channels and their regulation

In several cell systems including EATC (Christensen & Hoffmann 1992) hypotonic cell swelling leads to activation of non-selective channels which do not discriminate among small monovalent cations and have a sizeable permeability to Ca^{2+} and Ba^{2+} (see e.g. Wehner *et al.* 2003 for references). Molecular candidates for these channels are TRPV4 or VROAC (see e.g. Pedersen *et al.* 2005). TRPV4 is a non-selective cation channel that is modestly permeable for Ca^{2+}. It is thermosensitive but can also be activated by numerous other stimuli including cell swelling and shear stress (see Nilius *et al.* 2004). The mechanism whereby swelling activates TRPV4 is not yet fully solved. One possibility is that hypotonic activation of TRPV4 is downstream to swelling induced phospholipase A_2 (PLA_2) mediated arachidonic acid (AA) release and its subsequent metabolism to one of the eicosanoid products (see below). A similar mechanism was previously demonstrated for the swelling activated K^+ channel in EATC (Pedersen *et al.* 2000) and human neuroblastoma cells (Basavappa *et al.* 1998). In support of such a mechanism for activation of TRPV4 are the following results: (i) AA causes an increase in $[Ca^{2+}]_i$ and activates typical whole cell currents in TRPV4-expressing cells (Watanabe *et al.* 2003); (ii) AA is not able to activate TRPV4 in cell free patches, indicating that cellular metabolism of AA is necessary for channel activation; (iii) ETYA, a non-specific blocker of both lipoxygenases (LOX), cyclo-oxygenase (COX), and cytochrome *P*-450 epoxygenase inhibits the activation of TRPV4 by arachidonic acid; (iv) more specific blockers of COX (indomethacin) and LOX (nordihydroguaiaretic acid), respectively, had no effect; (v) an inhibitor the *P*-450 epoxygenase [17-octadecynoic acid (17-ODYA)] abolished the AA activation of TRPV4 (Watanabe *et al.* 2003) and finally; and (vi) 5′,6′-epoxyeicosatrienoic acid (EET) activates TRPV4. It was thus concluded that the epoxygenase pathway is involved in TRPV4 activation (see Nilius *et al.* 2004). It is possible that the cytoskeleton is also involved. Thus, the NH_2-terminal intracellular domain of TRPV4 contains three or more ankyrin repeat domains that may anchor the channel to the cytoskeleton and form a mechanical link for gating. It is found that TRPV4 activation by swelling is delayed if these ankyrin repeats are lacking (Liedtke *et al.* 2000, Nilius *et al.* 2004). Activation by cell swelling and AA requires cytochrome P450 epoxygenase activity to convert AA to epoxyeicosatrienoic acids such as 5,6-EET, 8,9-EET, which both act as direct TRPV4 agonists. Vriens *et al.* (2005) evaluated the role of TRPV4 and its modulation by the CYP pathway in vascular endothelial cells using mouse aortic endothelial cells (MAECs) isolated from wild-type and $TRPV4^{-/-}$ mice. They demonstrated that all TRPV4-activating stimuli including cell swelling induced robust Ca^{2+} responses in wild-type MAECs but not in MAECs isolated from $TRPV4^{-/-}$ mice. Several other TRP channels (TRPC1, TRPC6, TRPV2, TRPM2, TRPM4 and TRPP2) have been shown or suggested to be activated by cell swelling/modulated by cell volume perturbations (for example see Muraki *et al.* 2003, Kraft & Harteneck 2005); however, in most cases, their direct roles in cell volume regulation remain to be determined (see Pedersen *et al.* 2005).

Upstream volume signals and sensor

The role of PLA_2

Although the eicosanoids responsible for activation of the swelling activated channels varies, a common theme seems to be that cell swelling activates a PLA_2 which releases arachidonic acid from the membrane phospholipids. This was first demonstrated in EAT cells (Thoroed *et al.* 1997) and in IMCD cells (Tinel *et al.* 1997). In EATC, hypoosmotic cell swelling was found to result in an immediate increase (3.3-fold) in the rate of AA release apparently resulting from activation of the 85 kDa Ca^{2+}-dependent $cPLA_2$ (Fig. 3). A similar conclusion was drawn from inhibitor studies in human neuroblastoma cells (Basavappa *et al.* 1998). In EATC, it was demonstrated that AA is predominantly released from the nuclear fraction during cell swelling. Using specific antibodies to $cPLA_2\alpha$ and $cPLA_2\gamma$, respectively, it was shown by laser-scanning microscopy that $cPLA_2\alpha$ but not $cPLA_2\gamma$ translocated to the nucleus (probably the inner nuclear envelope) (Pedersen *et al.* 2000) where the enzyme is simultaneously phosphorylated and activated. Tyrosine kinase inhibitors significantly inhibits the RVD response in EATC (Hoffmann 2000) and it was suggested that tyrosin kinases are involved in the initial activation of $cPLA_2\alpha$ (Hoffmann 2000). The mechanisms involved in cell swelling-induced translocation of $cPLA_2\alpha$ to the nucleus in EATC are unknown. Stimulation of $cPLA_2\alpha$ by an increase in $[Ca^{2+}]_i$ does actually result in such a translocation of the enzyme (Glover *et al.* 1995, Schievella *et al.* 1995, Sierra-Honigmann *et al.* 1996, Pedersen *et al.* 2000) but since swelling of EATC does not result in any increase in $[Ca^2]_i$ the swelling induced translocation of $cPLA_2\alpha$ to the nucleus cannot be such a Ca^{2+} effect. Another possibility is the involvement of the cytoskeleton. Apparently, both catalytic activity and translocation of $cPLA\alpha$ is dependent of the F-actin cytoskeleton (Cybulsky *et al.* 2004), thus depolymeriza-

tion of F-actin by cytochalasin D or latrunculin B reduced AA release, as well as the increase in $cPLA_2$ activity. The same was true after stabilization of F-actin by jasplakinolide supporting the conclusion that $cPLA_2$ activation is dependent, in part, on actin remodelling. In many cell types including EATC, cell swelling is associated with a disorganization of F-actin and in a decrease in the quantitative content of F-actin (see Pedersen *et al.* 2001). In EATC, it was shown using a quantitative F-actin assay and confocal laser scanning microscopy of rhodamine–phalloidin labelled cells that hypotonic cell swelling reduced the cortical F-actin within the first min after osmotic challenge and was associated with a significant decrease in the cellular content of F-actin (Pedersen *et al.* 1999). It is an interesting possibility that this initial remodelling during cell swelling could be somehow involved in the translocation of $cPLA_2$, a possibility that will be investigated in future studies. Of special interest is an intermediate filament called vimentin that forms a cytoskeletal network around the perinuclear area which seems to be a functional adapter for $cPLA_2$ targeting $cPLA_2$ to the perinuclear membrane compartment. It has been suggested that vimentin would act as a scaffold protein that assists the appropriate interaction of $cPLA_2$ with perinuclear phospholipid membranes (Murakami *et al.* 2000). Interestingly, $cPLA_2$ is not the only PLA_2 isoform involved in swelling. In NIH3T3 cells, both $iPLA_2$ and $sPLA_2$ appear to be involved in volume-sensitive AA release as well as in regulation of the swelling-activated taurine pathway, the latter apparently dependent on the oxygenation of AA via the 5-LOX pathway (S.F. Pedersen, K. Poulsen & H. Lambert, 2006, unpublished data).

As seen from Figure 4, the AA released by PLA_2 serves as a substrate for (i) the 5-LOX which catalyses the oxygenation of AA to 5-HPETE, which is converted to leukotrienes, of which LTD_4 is shown to activate volume-sensitive TASK channels (see above); (ii) the *P*-450 epoxygenase which synthesises EET which is shown to activate TRPV4 (see above); (iii) the 12-lipoxygenase which catalyses the hydroxylation of arachidonic acid to 12(*S*)-HETE the product of which is hepoxylin which in human platelets activates $I_{K,vol}$ (see above); and (iv) the cyclooxygenase pathway which converts AA to prostaglandins of which PGE_2 increases Na^+ permeability in EATC (see above).

Cytoskeleton and the swelling-activated channels

Several ion channels are linked to the cytoskeleton and regulation of channel activity by changes in the structure of the cytoskeleton has been shown for some channels (see Cantiello & Prat 1996, Cantiello 1997, Pedersen *et al.* 1999, 2001, Wehner *et al.* 2003). Since hypotonic cell swelling as described above cause alteration of the cytoskeletal organization (see Pedersen *et al.* 2001) this may in turn modulate the channels and could play a role in the swelling activation of channels. In *Xenopus* oocytes expressing HCN2 channels and KCNQ1 channels it was shown that the response to volume changes was abolished when the actin cytoskeleton was depolymerised by CD (Grunnet *et al.* 2003,

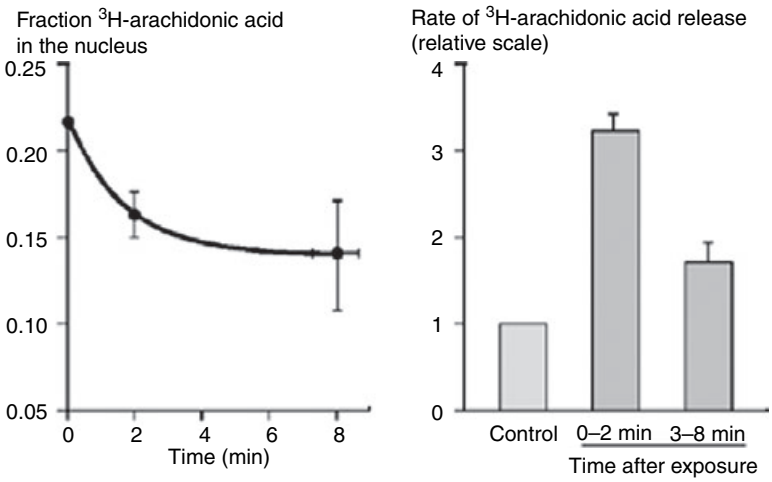

Figure 3 Cell swelling-induced $^{[3H]}$AA release from EATC. The cells were loaded with $^{[3H]}$AA for 2 h, washed and subsequently exposed to an isoosmotic or a hypoosmotic (150 mOsm) solution. *Left panel*: the fraction of $^{[3H]}$AA in the nuclear fraction was estimated as ^{3}H radioactivity in the nuclear fraction divided by that in the nuclear plus cytosolic fraction. *Right panel*: rate of $^{[3H]}$AA release from EATC in an isotonic solution (control) or in a hypoosmotic solution as a function of time. The medium contained 0.5% BSA in order to scavenge the released $^{[3H]}$AA. The results are mean ± SEM of three and four independent set of experiments in left and right and frame, respectively. Reproduced from Hoffmann (2000).

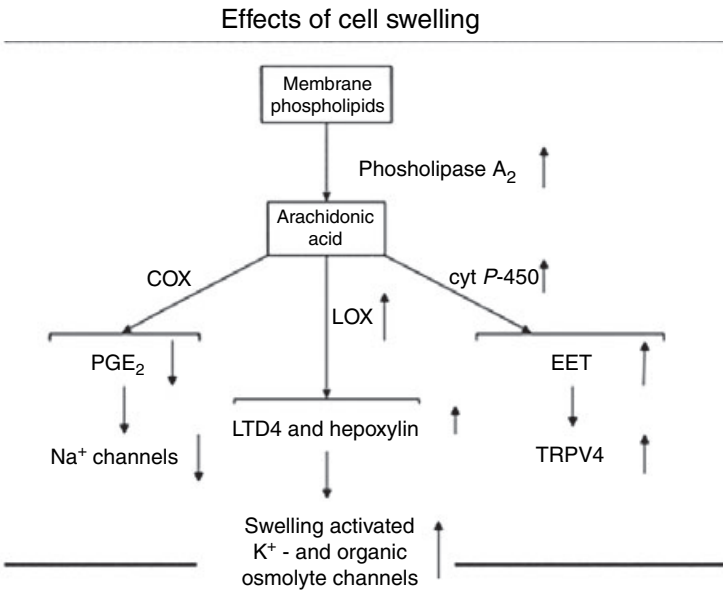

Figure 4 The arachidonic acid cascade and the eicosanoids that are found to regulate volume sensitive channels. Three different pathways are shown: (i) The cyclooxygenase (COX) pathway, forming prostaglandins among which is prostaglandin E_2 ($PDGE_2$), thromboxanes and prostacyclins; (ii) the lipoxygenase (LOX) pathway, forming leukotrienes among which is leukotriene D_4 (LTD_4), lipoxins, hepoxilins among which is hepoxylin A_3 and hydro(pero)xy fatty acids; and (iii) the P-450 (cyt P-450) pathway, forming hydroxylated fatty acids and epoxy derivatives among which is EET. See text for details.

Calloe *et al.* 2005) and the same was found for hIK channels expressed in HEK cells in which cell swelling cause a rapid reversible decrease in net cellular F-actin content (Jørgensen *et al.* 2003). Whether the interaction between the cytoskeleton and the channel is a direct or indirect one is not known but the results by Grunnet *et al.* (2003) suggest that KCNQ1 channels expressed in *Xenopus* oocytes are regulated by cell swelling through interactions between cytoskeleton and structural elements in the N-terminus of the channel protein. Identification of this eventual N- terminal structure has not yet been performed.

Concluding remarks

As highlighted in this mini-review, and perhaps not surprisingly, cell volume regulation is an extremely complex process. A plethora of different signalling pathways and effector molecules are involved, many of them yet unidentified at the molecular level. Abnormal cell volume regulation leads to very distinct endpoints with often significant variations in the pathophysiological consequences for an organism. Exciting new signalling elements are beginning to be recognized as significant players conferring to the field a re-expansion and several existing views on cell volume regulation are being revisited. Exemplary, in this respect, is the unresolved debate on how the different signalling pathways responsible for cell volume regulation interact to respond to the diversity of stimuli capable to alter cell volume. Changes in cell volume, the activity of ion channels and associated ion fluxes are now attributed an important role in events associated with cell proliferation and cell death. Furthermore, reactive oxygen species are now increasingly implicated as important modulators or effectors of cell volume regulation in physiological and pathophysiological conditions.

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References

Banderali, U. & Roy, G. 1992. Anion channels for amino acids in MDCK cells. *Am J Physiol* **263**, C1200–C1207.

Barfield, J.P., Yeung, C.H. & Cooper, T.G. 2005. The effects of putative K^+ channel blockers on volume regulation of murine spermatozoa. *Biol Reprod* **72**, 1275–1281.

Barriere, H., Belfodil, R., Rubera, I. *et al.* 2003. Role of TASK2 potassium channels regarding volume regulation in primary cultures of mouse proximal tubules. *J Gen Physiol* **122**, 177–190.

Basavappa, S., Pedersen, S.F., Jørgensen, N.K., Ellory, J.C. & Hoffmann, E.K. 1998. Swelling-induced arachidonic acid release via the 85-kDa $cPLA_2$ in human neuroblastoma cells. *J Neurophysiol* **79**, 1441–1449.

Bond, T., Basavappa, S., Christensen, M. & Strange, K. 1999. ATP dependence of the $I_{Cl,swell}$ channel varies with rate of cell swelling. Evidence for two modes of channel activation. *J Gen Physiol* **113**, 441–456.

Bortner, C.D. & Cidlowski, J.A. 1996. Absence of volume regulatory mechanisms contributes to the rapid activation

of apoptosis in thymocytes. *Am J Physiol* **271**, C950–C961.

Bouchelouche, P.N., Ahnfelt-Ronne, I. & Thomsen, M.K. 1990. LTD_4 increases cytosolic free calcium and inositol phosphates in human neutrophils: inhibition by the novel LTD_4 receptor antagonist, SR2640, and possible relation to modulation of chemotaxis. *Agents Actions* **29**, 299–307.

Browe, D.M. & Baumgarten, C.M. 2004. Angiotensin II (AT1) receptors and NADPH oxidase regulate Cl^- current elicited by β1 integrin stretch in rabbit ventricular myocytes. *J Gen Physiol* **124**, 273–287.

Brown, D.A. & London, E. 2000. Structure and function of sphingolipid- and cholesterol-rich membrane rafts. *J Biol Chem* **275**, 17221–17224.

Burg, M.B. 2000. Macromolecular crowding as a cell volume sensor. *Cell Physiol Biochem* **10**, 251–256.

Burg, M.B. 2002. Response of renal inner medullary epithelial cells to osmotic stress. *Comp Biochem Physiol A Mol Integr Physiol* **133**, 661–666.

Cahalan, M.D. & Lewis, R.S. 1988. Role of potassium and chloride channels in volume regulation by T-lymphocytes. In: R.B. Gunn & J.C. Parker (eds) *Cell Physiology of Blood*, pp. 281–301. Rockefeller University Press, New York.

Cala, P.M., Mandel, L.J. & Murphy, E. 1986. Volume regulation by *Amphiuma* red blood cells: cytosolic free Ca^{2+} and alkali metal–H^+ exchange. *Am J Physiol* **250**, C423–C429.

Calloe, K., Elmedyb, P., Olesen, S.P., Jørgensen, N.K. & Grunnet, M. 2005. Hypoosmotic cell swelling as a novel mechanism for modulation of cloned HCN2 channels. *Biophys J* **89**, 2159–2169.

Cantiello, H.F. 1997. Role of actin filament organization in cell volume and ion channel regulation. *J Exp Zool* **279**, 425–435.

Cantiello, H.F. & Prat, A.G. 1996Role of actin filament organization in ion channel activity and cell volume regulation. In: W.J. Nelson (ed.) *Membrane Protein – Cytoskeleton Interactions*, Academic Press, San Diego, CA.

Carton, I., Trouet, D., Hermans, D. *et al.* 2002. RhoA exerts a permissive effect on volume-regulated anion channels in vascular endothelial cells. *Am J Physiol* **283**, C115–C125.

Chen, L.X., Wang, L.W., Zhu, L.Y., *et al.* 2002. Cell cycle-dependent expression of volume-activated chloride currents in nasopharyngeal carcinoma cells. *Am J Physiol* **283**, C1313–C1323.

Christensen, O. & Hoffmann, E.K. 1992. Cell swelling activates K^+ and Cl^- channels as well as nonselective, stretch-activated cation channels in Ehrlich ascites tumor cells. *J Membr Biol* **129**, 13–36.

Cybulsky, A.V., Takano, T., Papillon, J., Khadir, A., Bijian, K. & Le Berre, L. 2004. The actin cytoskeleton facilitates complement-mediated activation of cytosolic phospholipase A_2. *Am J Physiol* **286**, F466–F476.

Decher, N., Lang, H.J., Nilius, B., Bruggemann, A., Busch, A.E. & Steinmeyer, K. 2001. DCPIB is a novel selective blocker of $I_{Cl,swell}$ and prevents swelling-induced shortening of guinea-pig atrial action potential duration. *Br J Pharmacol* **134**, 1467–1479.

Deutsch, C. & Chen, L.Q. 1993. Heterologous expression of specific K^+ channels in T lymphocytes: functional consequences for volume regulation. *Proc Natl Acad Sci USA* **90**, 10036–10040.

Diener, M. & Scharrer, E. 1993. The leukotriene D_4 receptor blocker, SK&F 104353, inhibits volume regulation in isolated crypts from the rat distal colon. *Eur J Pharm* **238**, 217–222.

Diener, M. & Scharrer, E. 1997. Effects of short-chain fatty acids on cell volume regulation and chloride transport in the rat distal colon. *Comp Biochem Physiol A Physiol* **118**, 375–379.

Doroshenko, P. 1991. Second messengers mediating activation of chloride current by intracellular GTPγS in bovine chromaffin cells. *J Physiol* **436**, 725–738.

Du, X.L., Gao, Z., Lau, C.P. *et al.* 2004. Differential effects of tyrosine kinase inhibitors on volume-sensitive chloride current in human atrial myocytes: evidence for dual regulation by Src and EGFR kinases. *J Gen Physiol* **123**, 427–439.

Dubé, L., Parent, L. & Sauvé, R. 1990. Hypotonic shock activates a maxi K^+ channel in primary cultured proximal tubule cells. *Am J Physiol* **259**, F348–F356.

Dubinsky, W.P., Mayorga-Wark, O. & Schultz, S.G. 2000. Potassium channels in basolateral membrane vesicles from *Necturus* enterocytes: stretch and ATP sensitivity. *Am J Physiol* **279**, C634–C638.

Falktoft, B. & Lambert, I.H. 2004. Ca^{2+}-mediated potentiation of the swelling-induced taurine efflux from HeLa cells: on the role of calmodulin and novel protein kinase C isoforms. *J Membr Biol* **201**, 59–75.

Felipe, A., Snyders, D.J., Deal, K.K. & Tamkun, M.M. 1993. Influence of cloned voltage-gated K^+ channel expression on alanine transport, Rb^+ uptake, and cell volume. *Am J Physiol* **265**, C1230–C1238.

Fernández-Fernández, J.M., Nobles, M., Currid, A., Vazquez, E. & Valverde, M.A. 2002. Maxi K^+ channel mediates regulatory volume decrease response in a human bronchial epithelial cell line. *Am J Physiol* **283**, C1705–C1714.

Filipovic, D. & Sackin, H. 1992. Stretch- and volume-activated channels in isolated proximal tubule cells. *Am J Physiol* **262**, F857–F870.

Fink, M., Lesage, F., Duprat, F., *et al.* 1998. A neuronal two P domain K^+ channel stimulated by arachidonic acid and polyunsaturated fatty acids. *EMBO J* **17**, 3297–3308.

Finkel, T. 2000. Redox-dependent signal transduction. *FEBS Lett* **476**, 52–54.

Friis, M.B., Friborg, C.R., Schneider, L. *et al.* 2005. Cell shrinkage as a signal to apoptosis in NIH 3T3 fibroblasts. *J Physiol* **567**, 427–443.

Glover, S., de Carvalho, M.S., Bayburt, T. *et al.* 1995. Translocation of the 85-kDa phospholipase A_2 from cytosol to the nuclear envelope in rat basophilic leukemia cells stimulated with calcium ionophore or IgE/antigen. *J Biol Chem* **270**, 15359–15367.

Greger, R., Heitzmann, D., Hug, M.J., Hoffmann, E.K. & Bleich, M. 1999. The $Na^+2Cl^-K^+$ cotransporter in the rectal gland of *Squalus acanthias* is activated by cell shrinkage. *Pflügers Arch* **438**, 165–176.

Grinstein, S., Clarke, C.A. & Rothstein, A. 1982. Increased anion permeability during volume regulation in human

lymphocytes. *Philos Trans R Soc Lond B Biol Sci* **299**, 509–518.

Grönroos, E., Schippert, A., Engstrom, M. & Sjölander, A. 1995. The regulation of leukotriene D_4-induced calcium influx in human epithelial cells involves protein tyrosine phosphorylation. *Cell Calcium* **17**, 177–186.

Grönroos, E., Andersson, T., Schippert, A., Zheng, L. & Sjölander, A. 1996. Leukotriene D_4-induced mobilization of intracellular Ca^{2+} in epithelial cells is critically dependent on activation of the small GTP-binding protein Rho. *Biochem J* **316**, 239–245.

Grunnet, M., MacAulay, N., Jørgensen, N.K., Jensen, S., Olesen, S.P. & Klaerke, D.A. 2002. Regulation of cloned, Ca^{2+}-activated K^+ channels by cell volume changes. *Pflügers Arch* **444**, 167–177.

Grunnet, M., Jespersen, T., MacAulay, N. *et al.* 2003. KCNQ1 channels sense small changes in cell volume. *J Physiol* **549**, 419–427.

Helix, N., Strobaek, D., Dahl, B.H. & Christophersen, P. 2003. Inhibition of the endogenous volume-regulated anion channel (VRAC) in HEK293 cells by acidic di-aryl-ureas. *J Memb Biol* **196**, 83–94.

Hendil, K.B. & Hoffmann, E.K. 1974. Cell volume regulation in Ehrlich ascites tumor cells. *J Cell Physiol* **84**, 115–125.

Hoffmann, E.K. 1978. Regulation of cell volume by selective changes in the leak permeabilities of Ehrlich ascites tumor cells. In: C.B. Jørgensen & K. Strange (eds) *Alfred Benzon Symposium IX. Osmotic and Volume Regulation*, pp. 397–417. Munksgaard, Copenhagen.

Hoffmann, E.K. 1982. Anion exchange and anion-cation co-transport systems in mammalian cells. *Philos Trans R Soc Lond B Biol Sci* **299**, 519–535.

Hoffmann, E.K. 1999. LTD_4 activates charybdotoxin-sensitive and -insensitive K^+ channels channels in Ehrlich ascites tumor cells. *Pflügers Arch* **438**, 263–268.

Hoffmann, E.K. 2000. Intracellular signalling involved in volume regulatory decrease. *Cell Physiol Biochem* **10**, 273–288.

Hoffmann, E.K. & Dunham, P.B. 1995. Membrane mechanisms and intracellular signalling in cell volume regulation. *Int Rev Cytol* **161**, 173–262.

Hoffmann, E.K. & Hendil, K.B. 1976. The role of amino acids and taurine in isoosmotic intracellular regulation in Ehrlich ascites mouse tumour cells. *J Comp Physiol* **108**, 279–286.

Hoffmann, E.K. & Lambert, I.H. 1983. Amino acid transport and cell volume regulation in Ehrlich ascites tumour cells. *J Physiol* **338**, 613–625.

Hoffmann, E.K. & Mills, J.W. 1999Membrane events involved in volume regulation. In: D.W. Deamer, A. Kleinzeller & D.M. Fambrough (eds) *Current Topics In Membranes*, pp. 123–195. Academic Press, San Diego, CA.

Hoffmann, E.K. & Ussing, H.H. 1992. Membrane mechanisms in volume regulation in vertebrate cells and epithelia. In: G.H. Giebisch, J.A. Schafer, H.H. Ussing & P. Kristensen (eds) *Membrane Transport in Biology*, Vol. 5, pp. 317–399. Springer Verlag, Berlin.

Hoffmann, E.K., Simonsen, L.O. & Sjöholm, C. 1975. *Chloride Fluxes and Membrane Potential in Ehrlich Ascites Tumor Cells*. 5th International Biophysical Congress, Copenhagen.

Hoffmann, E.K., Simonsen, L.O. & Sjöholm, C. 1979. Membrane potential, chloride exchange, and chloride conductance in Ehrlich mouse ascites tumour cells. *J Physiol* **296**, 61–84.

Hoffmann, E.K., Hoffmann, E., Lang, F. & Zadunaisky, J.A. 2002. Control of Cl^- transport in the operculum epithelium of *Fundulus heteroclitus*: long- and short-term salinity adaptation. *Biochim Biophys Acta* **1566**, 129–139.

Hougaard, C., Niemeyer, M.I., Hoffmann, E.K. & Sepúlveda, F.V. 2000. K^+ currents activated by leukotriene D_4 or osmotic swelling in Ehrlich ascites tumour cells. *Pflügers Arch* **440**, 283–294.

Hougaard, C., Jørgensen, F. & Hoffmann, E.K. 2001. Modulation of the volume-sensitive K^+ current in Ehrlich ascites tumour cells by pH. *Pflügers Arch* **442**, 622–633.

Hougaard, C., Klaerke, D.A., Hoffmann, E.K., Olesen, S.P. & Jørgensen, N.K. 2004. Modulation of KCNQ4 channel activity by changes in cell volume. *Biochim Biophys Acta* **1660**, 1–6.

Hume, J.R., Duan, D., Collier, M.L., Yamazaki, J. & Horowitz, B. 2000. Anion transport in heart. *Physiol Rev.* **80**, 31–81.

Ishii, T.M., Silvia, C., Hirschberg, B., Bond, C.T., Adelman, J.P. & Maylie, J. 1997. A human intermediate conductance calcium-activated potassium channel. *Proc Natl Acad Sci USA* **94**, 11651–11656.

Jørgensen, N.K., Lambert, I.H. & Hoffmann, E.K. 1996. Role of LTD_4 in the regulatory volume decrease response in Ehrlich ascites tumor cells. *J Membr Biol* **151**, 159–173.

Jørgensen, N.K., Christensen, S., Harbak, H. *et al.* 1997. On the role of calcium in the regulatory volume decrease (RVD) response in Ehrlich mouse ascites tumor cells. *J Membr Biol* **157**, 281–299.

Jørgensen, F., Hougaard, C., Niemeyer, M.I., Stutzin, A., Hoffmann, E.K. & Sepúlveda, F.V. 2000. Noise analysis of the K^+ current activated by leukotriene D_4 in Ehrlich ascites tumour cells. *J Physiol* **526P**, 116P.

Jørgensen, N.K., Pedersen, S.F., Rasmussen, H.B., Grunnet, M., Klaerke, D.A. & Olesen, S.P. 2003. Cell swelling activates cloned Ca^{2+}-activated K^+ channels: a role for the F-actin cytoskeleton. *Biochim Biophys Acta* **1615**, 115–125.

Jackson, P.S. & Strange, K. 1993. Volume-sensitive anion channels mediate swelling-activated inositol and taurine efflux. *Am J Physiol* **265**, C1489–C1500.

Jackson, P.S. & Strange, K. 1995. Characterization of the voltage-dependent properties of a volume- sensitive anion conductance. *J Gen Physiol* **105**, 661–676.

Jackson, P.S., Churchwell, K., Ballatori, N., Boyer, J.L. & Strange, K. 1996. Swelling-activated anion conductance in skate hepatocytes: regulation by cell Cl^- and ATP. *Am J Physiol* **270**, C57–C66.

Jensen, B.S., Strøbæk, D., Christophersen, P. *et al.* 1998. Characterization of the cloned human intermediate-conductance Ca^{2+}-activated K^+ channel. *Am J Physiol* **275**, C848–C856.

Jentsch, T.J., Stein, V., Weinreich, F. & Zdebik, A.A. 2002. Molecular structure and physiological function of chloride channels. *Physiol Rev* **82**, 503–568.

Jiang, B.H., Hattori, N., Liu, B., Nakayama, Y., Kitagawa, K. & Inagaki, C. 2004. Suppression of cell proliferation with induction of p21 by Cl^- channel blockers in human leukemic cells. *Eur J Pharmacol* **488**, 27–34.

Khanna, R., Chang, M.C., Joiner, W.J., Kaczmarek, L.K. & Schlichter, L.C. 1999. hSK4/hIK1, a calmodulin-binding KCa^{2+} channel in human T lymphocytes. Roles in proliferation and volume regulation. *J Biol Chem* **274**, 14838–14849.

Kim, Y., Bang, H. & Kim, D. 1999. TBAK-1 and TASK-1, two-pore K^+ channel subunits: kinetic properties and expression in rat heart. *Am J Physiol* **277**, H1669–H1678.

Kim, Y., Bang, H. & Kim, D. 2000. TASK-3, a new member of the tandem pore K^+ channel family. *J Biol Chem* **275**, 9340–9347.

Kraft, R. & Harteneck, C. 2005. The mammalian melastatin-related transient receptor potential cation channels: an overview. *Pflügers Arch* **451**, 204–211.

Kramhøft, B., Lambert, I.H., Hoffmann, E.K. & Jørgensen, F. 1986. Activation of Cl^- dependent K^+ transport in Ehrlich ascites tumor cells. *Am J Physiol* **251**, C369–C379.

Lambert, I.H. 2003. Reactive oxygen species regulate swelling-induced taurine efflux in NIH3T3 mouse fibroblasts. *J Membr Biol* **192**, 19–32.

Lambert, I.H. 2004. Regulation of the cellular content of the organic osmolyte taurine in mammalian cells. *Neurochem Res* **29**, 27–63.

Lambert, I.H. & Hoffmann, E.K. 1994. Cell swelling activates separate taurine and chloride channels in Ehrlich mouse ascites tumor cells. *J Membr Biol* **142**, 289–298.

Lambert, I.H., Hoffmann, E.K. & Christensen, P. 1987. Role of prostaglandins and leukotrienes in volume regulation by Ehrlich ascites tumor cells. *J Membr Biol* **98**, 247–256.

Lang, F., Busch, G.L., Ritter, M. *et al.* 1998. Functional significance of cell volume regulatory mechanisms. *Physiol Rev* **78**, 247–306.

Lang, F., Madlung, J., Bock, J. *et al.* 2000a. Inhibition of Jurkat-T-lymphocyte Na^+/H^+-exchanger by CD95(Fas/Apo-1)-receptor stimulation. *Pflügers Arch* **440**, 902–907.

Lang, F., Ritter, M., Gamper, N. *et al.* 2000b. Cell volume in the regulation of cell proliferation and apoptotic cell death. *Cell Physiol Biochem* **10**, 417–428.

Lang, K.S., Fillon, S., Schneider, D., Rammensee, H.G. & Lang, F. 2002. Stimulation of TNF alpha expression by hyperosmotic stress. *Pflügers Arch* **443**, 798–803.

Lauritzen, L., Hoffmann, E.K., Hansen, H.S. & Jensen, B. 1993. Dietary (n-3) and (n-6) fatty acids are equipotent in stimulating volume regulation in Ehrlich ascites tumor cells. *Am J Physiol* **264**, C109–C117.

Lepple-Wienhues, A., Szabó, I., Laun, T., Kaba, N.K., Gulbins, E. & Lang, F. 1998. The tyrosine kinase $p56^{lck}$ mediates activation of swelling-induced chloride channels in lymphocytes. *J Cell Biol* **141**, 281–286.

Levitan, I., Christian, A.E., Tulenko, T.N. & Rothblat, G.H. 2000. Membrane cholesterol content modulates activation of volume-regulated anion current in bovine endothelial cells. *J Gen Physiol* **115**, 405–416.

Lewis, R.S. & Cahalan, M.D. 1995. Potassium and calcium channels in lymphocytes. *Annu Rev Immunol* **13**, 623–653.

Liedtke, W., Choe, Y., Marti-Renom, M.A. *et al.* 2000. Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell* **103**, 525–535.

Light, D.B., Mertins, T.M., Belongia, J.A. & Witt, C.A. 1997. 5-Lipoxygenase metabolites of arachidonic acid regulate volume decrease by mudpuppy red blood cells. *J Membr Biol* **158**, 229–239.

Lundbaek, J.A., Birn, P., Hansen, A.J. *et al.* 2004. Regulation of sodium channel function by bilayer elasticity: the importance of hydrophobic coupling. Effects of micelle-forming amphiphiles and cholesterol. *J Gen Physiol* **123**, 599–621.

Maingret, F., Fosset, M., Lesage, F., Lazdunski, M. & Honore, E. 1999. TRAAK is a mammalian neuronal mechano-gated K^+ channel. *J Biol Chem* **274**, 1381–1387.

Margalit, A. & Livne, A.A. 1991. Lipoxygenase product controls the regulatory volume decrease of human platelets. *Platelets* **2**, 207–214.

Margalit, A., Livne, A.A., Funder, J. & Granot, Y. 1993. Initiation of RVD response in human platelets: mechanical-biochemical transduction involves pertussis-toxin-sensitive G protein and phospholipase A_2. *J Membr Biol* **136**, 303–311.

Meyer, K. & Korbmacher, C. 1996. Cell swelling activates ATP-dependent voltage-gated chloride channels in M-1 mouse cortical collecting duct cells. *J Gen Physiol* **108**, 177–193.

Minton, A.P. 1994 Influence of macromolecular crowding on intracellular association reactions: possible role in volume regulation. In: K. Strange (ed.) *Cellular and Molecular Physiology of Cell Volume Regulation*, pp. 181–190. CRC Press, Boca Raton, USA.

Mitchell, C.H., Zhang, J.J., Wang, L. & Jacob, T.J. 1997. Volume-sensitive chloride current in pigmented ciliary epithelial cells: role of phospholipases. *Am J Physiol* **272**, C212–C222.

Mollerup, J., Jørgensen, S.T., Hougaard, C. & Hoffmann, E.K. 2001. Identification of a murine cysteinyl leukotriene receptor by expression in *Xenopus laevis* oocytes. *Biochim Biophys Acta* **1517**, 455–459.

Mongin, A.A. & Orlov, S.N. 2001. Mechanisms of cell volume regulation and possible nature of the cell volume sensor. *Pathophysiology* **8**, 77–88.

Mongin, A.A., Reddi, J.M., Charniga, C. & Kimelberg, H.K. 1999. $^{[3H]}$taurine and D-$^{[3H]}$aspartate release from astrocyte cultures are differently regulated by tyrosine kinases. *Am J Physiol* **276**, C1226–C1230.

Murakami, M., Nakatani, Y., Kuwata, H. & Kudo, I. 2000. Cellular components that functionally interact with signaling phospholipase A_2S. *Biochim Biophys Acta* **1488**, 159–166.

Muraki, K., Iwata, Y., Katanosaka, Y., Ito, T., Ohya, S., Shigekawa, M. & Imaizumi, Y. 2003. TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. *Circ Res* **93**, 829–838.

Nielsen, C., Goulian, M. & Andersern, O.S. 1998. Energetics of inclusion-induced bilayer deformations. *Biophys J.* **74**, 1966–1983.

Nielsen, C.K., Massoumi, R., Sonnerlind, M. & Sjolander, A. 2005. Leukotriene D_4 activates distinct G-proteins in intestinal epithelial cells to regulate stress fibre formation and to generate intracellular Ca^{2+} mobilisation and ERK1/2 activation. *Exp Cell Res* **302**, 31–39.

Niemeyer, M.I., Hougaard, C., Hoffmann, E.K., Jørgensen, F., Stutzin, A. & Sepúlveda, F.V. 2000. Characterisation of a cell swelling-activated K^+-selective conductance of Ehrlich mouse ascites tumour cells. *J Physiol* **524**, 757–767.

Niemeyer, M.I., Cid, L.P., Barros, L.F. & Sepúlveda, F.V. 2001a. Modulation of the two-pore domain acid-sensitive K^+ channel TASK-2 (KCNK5) by changes in cell volume. *J Biol Chem* **276**, 43166–43174.

Niemeyer, M.I., Cid, L.P. & Sepúlveda, F.V. 2001b. K^+ conductance activated during regulatory volume decrease. The channels in Ehrlich cells and their possible molecular counterpart. *Comp Biochem Physiol A Mol Integr Physiol* **130**, 565–575.

Niemeyer, M.I., Stutzin, A. & Sepúlveda, F.V. 2002. A voltage-independent K^+ conductance activated by cell swelling in Ehrlich cells is modulated by a G-protein-mediated process. *Biochim Biophys Acta* **1562**, 1–5.

Nilius, B. 2001. Chloride channels go cell cycling. *J Physiol* **532**, 581.

Nilius, B. & Droogmans, G. 2001. Ion channels and their functional role in vascular endothelium. *Physiol Rev* **81**, 1415–1459.

Nilius, B. & Droogmans, G. 2003. Amazing chloride channels: an overview. *Acta Physiol Scand* **177**, 119–147.

Nilius, B., Oike, M., Zahradnik, I. & Droogmans, G. 1994. Activation of a Cl^- current by hypotonic volume increase in human endothelial cells. *J Gen Physiol* **103**, 787–805.

Nilius, B., Eggermont, J., Voets, T. & Droogmans, G. 1996. Volume-activated Cl^- channels. *Gen Pharmacol* **27**, 1131–1140.

Nilius, B., Eggermont, J., Voets, T., Buyse, G., Manolopoulos, V. & Droogmans, G. 1997. Properties of volume-regulated anion channels in mammalian cells. *Prog Biophys Mol Biol* **68**, 69–119.

Nilius, B., Prenen, J., Voets, T., Eggermont, J. & Droogmans, G. 1998. Activation of volume-regulated chloride currents by reduction of intracellular ionic strength in bovine endothelial cells. *J Physiol* **506**, 353–361.

Nilius, B., Voets, T., Prenen, J. *et al.* 1999. Role of Rho and Rho kinase in the activation of volume-regulated anion channels in bovine endothelial cells. *J Physiol* **516**, 67–74.

Nilius, B., Prenen, J., Walsh, M.P. *et al.* 2000. Myosin light chain phosphorylation-dependent modulation of volume-regulated anion channels in macrovascular endothelium. *FEBS Lett* **466**, 346–350.

Nilius, B., Vriens, J., Prenen, J., Droogmans, G. & Voets, T. 2004. TRPV4 calcium entry channel: a paradigm for gating diversity. *Am J Physiol* **286**, C195–C205.

Oike, M., Droogmans, G. & Nilius, B. 1994. The volume-activated chloride current in human endothelial cells depends on intracellular ATP. *Pflügers Arch* **427**, 184–186.

Okada, Y. 1997a. Volume expansion-sensing outward-rectifier Cl^- channel: fresh start to the molecular identity and volume sensor. *Am J Physiol* **273**, C755–C789.

Okada, Y. 1997b. Volume expansion-sensing outward-rectifier Cl^- channel: fresh start to the molecular identity and volume sensor. *Am J Physiol* **273**, C755–C789.

Okada, Y. & Maeno, E. 2001. Apoptosis, cell volume regulation and volume-regulatory chloride channels. *Comp Biochem Physiol A Mol Integr Physiol* **130**, 377–383.

Okada, Y., Maeno, E., Shimizu, T., Dezaki, K., Wang, J. & Morishima, S. 2001. Receptor-mediated control of regulatory volume decrease (RVD) and apoptotic volume decrease (AVD). *J Physiol* **532**, 3–16.

Ortenblad, N., Young, J.F., Oksbjerg, N., Nielsen, J.H. & Lambert, I.H. 2003. Reactive oxygen species are important mediators of taurine release from skeletal muscle cells. *Am J Physiol* **284**, C1362–C1373.

Pasantes-Morales, H. & Franco, R. 2002. Influence of protein tyrosine kinases on cell volume change-induced taurine release. *Cerebellum* **1**, 103–109.

Pasantes-Morales, H., Franco, R., Ochoa, L. & Ordaz, B. 2002. Osmosensitive release of neurotransmitter amino acids: relevance and mechanisms. *Neurochem Res* **27**, 59–65.

Patel, A.J., Honoré, E., Maingret, F. *et al.* 1998. A mammalian two pore domain mechano-gated S-like K^+ channel. *EMBO J* **17**, 4283–4290.

Pedersen, S., Hoffmann, E.K., Hougaard, C., Jørgensen, N.K., Wybrandt, G.B. & Lambert, I.H. 1997. Leukotriene D_4-induced Ca^{2+} mobilization in Ehrlich ascites tumor cells. *J Membr Biol* **155**, 61–73.

Pedersen, S.F., Prenen, J., Droogmans, G., Hoffmann, E.K. & Nilius, B. 1998. Separate swelling- and Ca^{2+}-activated anion currents in Ehrlich ascites tumor cells. *J Membr Biol* **163**, 97–110.

Pedersen, S.F., Mills, J.W. & Hoffmann, E.K. 1999. Role of the F-actin cytoskeleton in the RVD and RVI processes in Ehrlich ascites tumor cells. *Exp Cell Res* **252**, 63–74.

Pedersen, S., Lambert, I.H., Thoroed, S.M. & Hoffmann, E.K. 2000. Hypotonic cell swelling induces translocation of the α isoform of cytosolic phospholipase A_2 but not the γ isoform in Ehrlich ascites tumor cells. *Eur J Biochem* **267**, 5531–5539.

Pedersen, S.F., Hoffmann, E.K. & Mills, J.W. 2001. The cytoskeleton and cell volume regulation. *Comp Biochem Physiol A Mol Integr Physiol* **130**, 385–399.

Pedersen, S.F., Beisner, K.H., Hougaard, C., Willumsen, B.M., Lambert, I.H. & Hoffmann, E.K. 2002. Rho family GTP binding proteins are involved in the regulatory volume decrease process in NIH3T3 mouse fibroblasts. *J Physiol* **541**, 779–796.

Pedersen, S.F., Owsianik, G. & Nilius, B. 2005. TRP channels: an overview. *Cell Calcium* **38**, 233–252.

Pon, D.C. & Hill, C.E. 1997. Existence, properties, and functional expression of "Maxi-K$^+$"-type, Ca^{2+}-activated K^+ channels in short-term cultured hepatocytes. *J Cell Physiol* **171**, 87–94.

Reyes, R., Duprat, F., Lesage, F. *et al.* 1998. Cloning and expression of a novel pH-sensitive two pore domain K^+

channel from human kidney. *J Biol Chem* **273**, 30863–30869.

Rhee, S.G., Bae, Y.S., Lee, S.R. & Kwon, J. 2000. Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. *Sci STKE* **2000**, E1.

Rhee, S.G., Kang, S.W., Jeong, W., Chang, T.S., Yang, K.S. & Woo, H.A. 2005. Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. *Curr Opin Cell Biol* **17**, 183–189.

Riquelme, G., Sepúlveda, F.V., Jørgensen, F., Pedersen, S. & Hoffmann, E.K. 1998. Swelling-activated potassium currents of Ehrlich ascites tumour cells. *Biochim Biophys Acta* **1371**, 101–106.

Roman, R., Feranchak, A.P., Troetsch, M. *et al.* 2002. Molecular characterization of volume-sensitive $SKCa^{2+}$ channels in human liver cell lines. *Am J Physiol* **282**, G116–G122.

Romanenko, V.G., Rothblat, G.H. & Levitan, I. 2004. Sensitivity of volume-regulated anion current to cholesterol structural analogues. *J Gen Physiol* **123**, 77–87.

Roti Roti, L.W. & Rothstein, A. 1973. Adaptation of mouse leukemic cells (L5178Y) to anisotonic media. II. Cell growth. *Exp Cell Res* **79**, 311–317.

Rouzaire-Dubois, B., Malo, M., Milandri, J.B. & Dubois, J.M. 2004. Cell size-proliferation relationship in rat glioma cells. *Glia* **45**, 249–257.

Sackin, H. 1989. A stretch-activated K^+ channel sensitive to cell volume. *Proc Natl Acad Sci U S A* **86**, 1731–1735.

Sánchez-Olea, R., Fuller, C., Benos, D. & Pasantes-Morales, H. 1995. Volume-associated osmolyte fluxes in cell lines with or without the anion exchanger. *Am J Physiol* **269**, C1280–C1286.

Schievella, A.R., Regier, M.K., Smith, W.L. & Lin, L.L. 1995. Calcium-mediated translocation of cytosolic phospholipase A_2 to the nuclear envelope and endoplasmic reticulum. *J Biol Chem* **270**, 30749–30754.

Shen, M.R., Droogmans, G., Eggermont, J., Voets, T., Ellory, J.C. & Nilius, B. 2000. Differential expression of volume-regulated anion channels during cell cycle progression of human cervical cancer cells. *J Physiol* **529**, 385–394.

Shennan, D.B., McNeillie, S.A. & Curran, D.E. 1994. The effect of a hyposmotic shock on amino acid efflux from lactating rat mammary tissue: stimulation of taurine and glycine efflux via a pathway distinct from anion exchange and volume-activated anion channels. *Exp Physiol* **79**, 797–808.

Shimizu, T., Numata, T. & Okada, Y. 2004. A role of reactive oxygen species in apoptotic activation of volume-sensitive Cl^- channel. *Proc Natl Acad Sci U S A* **101**, 6770–6773.

Sierra-Honigmann, M.R., Bradley, J.R. & Pober, J.S. 1996. "Cytosolic" phospholipase A_2 is in the nucleus of subconfluent endothelial cells but confined to the cytoplasm of confluent endothelial cells and redistributes to the nuclear envelope and cell junctions upon histamine stimulation. *Lab Invest* **74**, 684–695.

Simons, K. & Vaz, W.L. 2004. Model systems, lipid rafts, and cell membranes. *Annu Rev Biophys Biomol Struct* **33**, 269–295.

Sorota, S. 1995. Tyrosine protein kinase inhibitors prevent activation of cardiac swelling-induced chloride current. *Pflügers Arch* **431**, 178–185.

Strange, K. & Jackson, P.S. 1995. Swelling-activated organic osmolyte efflux: a new role for anion channels. *Kidney Int* **48**, 994–1003.

Strange, K., Emma, F. & Jackson, P.S. 1996. Cellular and molecular physiology of volume-sensitive anion channels. *Am J Physiol* **270**, C711–C730.

Stutzin, A., Torres, R., Oporto, M. *et al.* 1999. Separate taurine and chloride efflux pathways activated during regulatory volume decrease. *Am J Physiol* **277**, C392–C402.

Szücs, G., Heinke, S., Droogmans, G. & Nilius, B. 1996. Activation of the volume-sensitive chloride current in vascular endothelial cells requires a permissive intracellular Ca^{2+} concentration. *Pflügers Arch* **431**, 467–469.

de Tassigny, A.D., Souktani, R., Ghaleb, B., Henry, P. & Berdeaux, A. 2003. Structure and pharmacology of swelling-sensitive chloride channels, $I_{Cl,swell}$. *Fundam Clin Pharmacol* **17**, 539–553.

Thodeti, C.K., Nielsen, C.K., Paruchuri, S., Larsson, C. & Sjölander, A. 2001. The epsilon isoform of protein kinase C is involved in regulation of the LTD_4-induced calcium signal in human intestinal epithelial cells. *Exp Cell Res* **262**, 95–103.

Thoroed, S.M. & Fugelli, K. 1994. The Na^+-independent taurine influx in flounder erythrocytes and its association with the volume regulatory taurine efflux. *J Exp Biol* **186**, 245–268.

Thoroed, S.M., Lauritzen, L., Lambert, I.H., Hansen, H.S. & Hoffmann, E.K. 1997. Cell swelling activates phospholipase A_2 in Ehrlich ascites tumor cells. *J Membr Biol* **160**, 47–58.

Tilly, B.C., van den Berghe, N., Tertoolen, L.G., Edixhoven, M.J. & de Jonge, H.R. 1993. Protein tyrosine phosphorylation is involved in osmoregulation of ionic conductances. *J Biol Chem* **268**, 19919–19922.

Tinel, H., Wehner, F. & Kinne, R.K. 1997. Arachidonic acid as a second messenger for hypotonicity-induced calcium transients in rat IMCD cells. *Pflügers Arch* **433**, 245–253.

Trouet, D., Nilius, B., Jacobs, A., Remacle, C., Droogmans, G. & Eggermont, J. 1999. Caveolin-1 modulates the activity of the volume-regulated chloride channel. *J Physiol* **520**, 113–119.

Vanoye, C.G. & Reuss, L. 1999. Stretch-activated single K^+ channels account for whole-cell currents elicited by swelling. *Proc Natl Acad Sci U S A* **96**, 6511–6516.

Varela, D., Simon, F., Riveros, A., Jørgensen, F. & Stutzin, A. 2004a. NAD(P)H oxidase-derived H_2O_2 signals chloride channel activation in cell volume regulation and cell proliferation. *J Biol Chem* **279**, 13301–13304.

Varela, D., Simon, F., Riveros, A., Jørgensen, F. & Stutzin, A. 2004b. The volume-activated chloride current depends on phospholipase C activation and intracellular calcium mobilization. In: P.K. Lauf & N.C. Adragna (eds) *Cell Volume and Signaling*, pp. 141–146. Springer + Business, New York.

Voets, T., Szücs, G., Droogmans, G. & Nilius, B. 1995. Blockers of volume-activated Cl^- currents inhibit endothelial cell proliferation. *Pflügers Arch* **431**, 132–134.

Voets, T., Manolopoulos, V., Eggermont, J., Ellory, C., Droogmans, G. & Nilius, B. 1998. Regulation of a swelling-activated chloride current in bovine endothelium by protein tyrosine phosphorylation and G proteins. *J Physiol* **506**, 341–352.

Voets, T., Droogmans, G., Raskin, G., Eggermont, J. & Nilius, B. 1999. Reduced intracellular ionic strength as the initial trigger for activation of endothelial volume-regulated anion channels. *Proc Natl Acad Sci U S A* **96**, 5298–5303.

Vriens, J., Owsianik, G., Fisslthaler, B. *et al.* 2005. Modulation of the Ca^{2+} permeable cation channel TRPV4 by cytochrome P450 epoxygenases in vascular endothelium. *Circ Res* **97**, 908–915.

Wang, J., Morishima, S. & Okada, Y. 2003. IK channels are involved in the regulatory volume decrease in human epithelial cells. *Am J Physiol* **284**, C77–C84.

Watanabe, H., Vriens, J., Prenen, J., Droogmans, G., Voets, T. & Nilius, B. 2003. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* **424**, 434–438.

Wehner, F., Olsen, H., Tinel, H., Kinne-Saffran, E. & Kinne, R.K.H. 2003. Cell volume regulation: osmolytes, osmolyte transport, and signal transduction. *Rev Physiol, Biochem Pharmacol* **148**, 1–80.

Weskamp, M., Seidl, W. & Grissmer, S. 2000. Characterization of the increase in $[Ca^{2+}]_i$ during hypotonic shock and the involvement of Ca^{2+}-activated K^+ channels in the regulatory volume decrease in human osteoblast-like cells. *J Membr Biol* **178**, 11–20.

Westermann, M., Steiniger, F. & Richter, W. 2005. Belt-like localisation of caveolin in deep caveolae and its re-distribution after cholesterol depletion. *Histochem Cell Biol* **123**, 613–620.

Wondergem, N., Gong, W., Monen, S.H. *et al.* 2001. Blocking swelling-activated chloride current inhibits mouse liver cell proliferation. *J Physiol* **532**, 661–672.

Xiao, G.N., Guan, Y.Y. & He, H. 2002. Effects of Cl^- channel blockers on endothelin-1-induced proliferation of rat vascular smooth muscle cells. *Life Sci* **70**, 2233–2241.

Yamaguchi, D.T., Green, J., Kleeman, C.R. & Muallem, S. 1989. Characterization of volume-sensitive, calcium-permeating pathways in the osteosarcoma cell line UMR-106-01. *J Biol Chem* **264**, 4383–4390.