Caveolae and cell swelling. Focus on “Stimulation by caveolin-1 of the hypotonicity-induced release of taurine and ATP at basolateral, but not apical, membrane of Caco-2 cells”

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IN MAMMALIAN CELLS under steady-state conditions, intracellular and extracellular osmolarity are equal and water exists at equilibrium across the plasma membrane. Perturbation of the osmotic equilibrium, through physiological processes such as active solute uptake by hepatocytes, or pathological conditions such as elevated plasma osmolarity due to diabetes, results in a transmembrane flow of water to restore this equilibrium and thus cell swelling or shrinkage. Cell swelling in response to a hypotonic stimulus triggers a regulatory volume decrease (RVD). RVD is predominantly mediated by the release of K\(^+\), Cl\(^-\), and other organic osmolytes from the cell and the compensatory flow of water (reviewed in Refs. 4 and 9). A key component of RVD is the release of Cl\(^-\) through the volume-regulated anion channel (VRAC). However, VRAC activity is not restricted to a hypotonic response, but is also involved in setting membrane potential, mechanotransduction, cell proliferation, cell cycle regulation, apoptosis, and angiogenesis (4, 9).

Astonishingly, for a cellular activity with so many important functional roles, the molecular identity of VRAC is not yet known. Similarly, it is unclear whether VRAC activity constitutes a single molecular entity. However, VRAC has been extensively characterized at both the pharmacological and electrophysiological levels (4, 9). Many compounds inhibit VRAC activity, including tamoxifen and 5-nitro-2-(3-phenylpropylamino)benzoic acid. In addition to the release of Cl\(^-\) ions, VRAC can also release other anions, organic osmolytes such as the amino acids glycine, glutamate, aspartate, and taurine, lactate, bicarbonate, and ATP (for recent reviews, see Ref. 9 and references therein). While the function of ATP release is unknown, it has been suggested to be autocrine or paracrine.

Although the molecular nature of the VRAC remains obscure, several components of the cellular machinery involved in cytoskeletal control, membrane trafficking, and signaling have been implicated in the control of VRAC. These include members of the caveolin family of integral membrane proteins (17), Rho GTPases (11), annexin II (10), and the Src family of nonreceptor tyrosine kinases (15). The identification of these proteins is beginning to shed light on the molecular mechanisms underlying VRAC regulation.

Caveolin is one of the main components of caveolae, flask-shaped invaginations of the cell surface (12). Expression of caveolin is sufficient to drive the formation of caveolae in cell lines normally lacking caveolin, such as human colon cancer cells (Caco-2) (20). Caveolae are a structurally identifiable type of lipid raft, microdomains of the cell membrane enriched in cholesterol, sphingolipids, and specific subsets of proteins. Caveolae have been implicated in a variety of cell functions, including signal transduction, endocytosis, membrane trafficking, lipid homeostasis, and Ca\(^{2+}\) signaling (comprehensively reviewed in Ref. 3), and are thought to act as a platform for the formation of protein complexes. However, caveolae are not present in all cells, and their abundance varies considerably between cell types. An early indication for the involvement of caveolin-1 in the regulation of VRAC was suggested by the observation that the current density of VRAC correlated with caveolin-1 expression (17).

Previous studies (17) have shown that hypotonic stimulation of caveolin-1-negative cells, such as Caco-2 cells, only triggers a low swelling-induced Cl\(^-\) current (\(I_{\text{Cl,swell}}\)), which can be rescued by transient expression of caveolin-1. Furthermore, expression of a dominant-negative caveolin mutant can suppress \(I_{\text{Cl,swell}}\) (16). In previous studies (20), caveolin-1 expression in polarized Caco-2 cells induced the formation of caveolae at the basolateral, but not the apical, surface. In the article in focus, Ulrich et al. (Ref. 18a, see p. C1287 of this issue) established stable Caco-2 cell lines expressing caveolin-1 and showed that these cells have a robust \(I_{\text{Cl,swell}}\) in response to a hypotonic stimulus. After these cells were allowed to form a polarized epithelium, the authors demonstrated that caveolin expression facilitates taurine efflux from the basolateral surface in response to hypotonicity, consistent with the reported formation of caveolae, although this was not sensitive to tamoxifen inhibition. In contrast, hypotonic stimulation of taurine release from the apical surface was gradual and not statistically different between wild-type and caveolin-1-expressing cells, but was sensitive to tamoxifen inhibition. Similarly, caveolin-1 expression facilitated the release of ATP from the basolateral surface but had no effect on ATP release from the apical surface. These results indicate that reconstitution of the VRAC response is directly related to the presence of caveolae in the membrane. However, it also suggests that distinct mechanisms operate at the basolateral vs. the apical surface.

In the absence of a molecular identity for VRAC, it is difficult to determine the role of caveolin in its regulation. However, it is possible to speculate about the nature of caveolin involvement in VRAC function. Caveolin could directly modulate the functional properties of the channel by direct binding. Alternatively, caveolin could play a role in delivering VRAC, or component(s) thereof, to the cell surface. In this case, caveolin would not be required to remain in contact with VRAC after delivery to the plasma membrane. Caveolin has been implicated in the delivery of proteins, such as the transient receptor potential (canonical) channel-1 (1), the angiotensin receptor (21), and dysferlin (5), to the cell surface. However, in the latter two cases, there is only limited colocalization of caveolin and the transported protein once the cell
surface is reached. However, one caveat to this model is the observation that in polarized epithelial cells, such as Madin-Darby canine kidney cells that express endogenous caveolin-1, caveolin-1 is localized to both the apical and basolateral surfaces but caveolae are formed only at the basolateral membrane (8). A detailed analysis of the trafficking of caveolin-1 in Caco-2 cells is needed to determine whether a proportion of this protein reaches the apical membrane without causing caveolae formation.

Alternatively, caveolae could be required for the functional assembly of VRAC, or for the formation of multimeric protein platforms that facilitate VRAC activation or function. Many proteins shown to be involved in the regulation of VRAC have also been localized to caveolae or have been shown to interact with caveolin. For instance, activation of VRAC requires tyrosine phosphorylation (19), and while the target of phosphorylation is unknown, there is evidence that the tyrosine kinase involved might be a member of the Src family of nonreceptor tyrosine kinases (15). Numerous studies have linked caveolin-1 and Src (see Ref. 3 and references therein). Mutation of c-Src to generate a diacylated form, which is restricted to caveolae (14), results in an inhibition of VRAC (15). Therefore, it is possible that Src activation forms a part of the signaling pathway regulating VRAC. There is also evidence that functional Rho GTPases and Rho kinase pathways are necessary for VRAC activity. Rho is a monomeric GTPase involved in organization of the actin cytoskeleton, the formation of focal adhesions, endo- or exocytosis, and the formation of actin stress fibers (6). Stretch-induced activation of RhoA requires its localization to, and subsequent dissociation from, caveolae (7). Inhibition of Rho and Rho kinase results in impaired cell swelling induced by hypotonicity (11), although the effect of Rho is suggested to be permissive rather than directly activating (2). Finally, annexin II, which localizes to caveolae (13) and can form a complex with caveolin-1 (18), has also been implicated in the activation of VRAC (10).

It is difficult to distinguish cell components involved in sensing changes in tonicity or swelling, from signaling pathways and direct activation of VRAC. The clear involvement of caveolae in the response to changes in cell shape adds another dimension to the multitude of roles for these fascinating organelles. Until the components of VRAC have been identified at the molecular level, fully understanding its regulation and formation is difficult. The eventual molecular identification of VRAC components promises to be a major leap forward in our appreciation of this fundamental physiological activity.

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GRANTS

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REFERENCES


