CELL VOLUME REGULATION AND AQUAPORINS.

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INTRODUCTION.

In the recent years, the importance of the volume of a given cell has been accepted not only in defining its intracellular osmolality and its shape, but also in defining other cellular functions, such as transepithelial transport, cell migration, cell growth, cell death and the regulation of intracellular metabolism (35). Since most cells have to perform these physiological functions under a variable osmotic stress, cell volume must be carefully regulated. Based on the origin of the disturbance, cell volume changes are frequently classified into two categories: **anisosmotic** (alterations in extracellular solute concentration) and **isosmotic** (alterations in intracellular solute concentration) volume changes. Because of the relatively high permeability of the plasma membrane for water, any such gradient results in the immediate flow of water into or out of the cell causing cell swelling or shrinkage. To regulate cell volume, cells use channels and transport systems to flux osmolytes across the plasma membrane, followed by the obligatory movement of water. The current review reflects these developments and focuses on the contributions of aquaporins water channels in regulatory volume processes in a variety of cells.

CELL VOLUME REGULATION IN ANISOSMOTIC MEDIUM.

Alterations in extracellular osmolality induce anisosmotic volume changes. Under normal physiological conditions, most mammalian cells (with a few exceptions like renal and gastrointestinal tract cells), are protected from anisosmotic volume changes by the precise regulation of plasma osmolality. However, plasma osmolality can be disrupted by a variety of disease states and their treatments (31). For instance, hypo and hypernatremia are common electrolyte disorders that require careful management.

If cells are exposed to hypotonic extracellular fluid, they first respond by swelling and second by initiating mechanisms that allow them to recover their original volume (22, 24, 26). This complex mechanism called regulatory volume decrease (RVD), depends on the release of inorganic ions, including K^+, Na^+, Cl^-, and HCO_3^- and organic osmolytes (e.g. taurine, betaine) that reverse the osmotic gradient and direction of water flow (Figure 1 A) (22, 24, 31). Essentially all cells respond to hypotonic swelling with an RVD, but the response is usually incomplete probably due to the fact that a new steady-state volume was achieved (26).

Under exposure to hyperosmotic conditions, cells immediately shrink as the result of an efflux of intracellular water brought about by the higher extracellular osmolarity. In some cell types, cell shrinkage triggers a cellular response known as regulatory volume increase (RVI) by activating the inward transport of organic and inorganic osmolytes, followed by the obligatory movement of water (22, 26, 31) (Figure 1 B). Cell volume regula-

tion after cell shrinkage involves accumulation of ions through activation of $Na^+K^+2Cl^-$ co-transport, Na^+/H^+ exchange in parallel to Cl^-/HCO_3^- exchange, NaCl cotransport or Na^+ channels. In addition, chronic adaptation to hyperosmolarity involves accumulation of organic osmolytes within the cell by stimulating the uptake, enhancing the formation or decreasing the degradation (22). While essentially all cells are capable of RVD, RVI mechanisms have only been reported in some cell types like epithelial cells, lymphocytes and skeletal muscle.
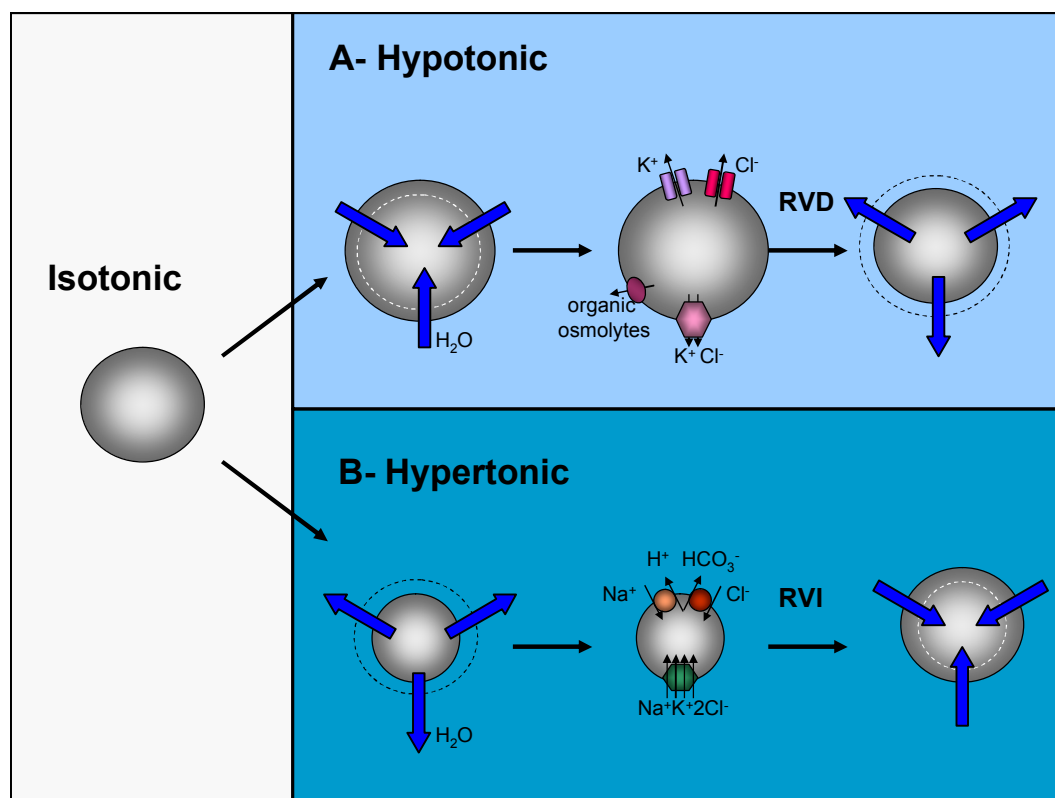

Figure 1: Schematic illustration of the principal mechanisms involved in cell volume regulation after anisotonic shocks. A: Under hypotonic medium cells first swell and then activate a regulatory volume decrease response (RVD). B: Under hypertonic medium cells first shrink and then activate a regulatory volume increase response (RVI).

CELL VOLUME REGULATION IN ISOSMOTIC MEDIUM.

Alterations in intracellular solute content induce isosmotic volume changes. Upon steady-state conditions, intracellular solute levels are kept constant by a precise balance between solute influx and efflux across the plasma membrane, and by the metabolic production and removal of osmolytes (31). However, several cellular physiological functions elicit cell volume perturbations caused by changes in intracellular osmolytes. Under isosmotic conditions cell volume can be altered by hormones, substrates, second messengers and oxidative stress (12). For instance a decrease in cell volume may occur under secretion of fluid and electrolytes, and an increase in cell volume takes place when nutrients enter the cell. To maintain cell volume, cells respond activating the same mechanisms (RVD and RVI) usually involved in anisosmotic regulation (Figure 2 A and C).

In addition, cell swelling due to isoosmotic osmolyte uptake could be a mechanism that contributes to cell cycle progression (12). Interestingly, the initial processes of apop-

tosis and necrosis are associated with persistent shrinkage (apoptotic volume decrease: AVD) and with persistent swelling (necrotic volume increase: NVI) respectively (Figure 2 B and D) (24). It has become evident that impairments of cell volume regulation are closely associated with apoptotic or necrotic cell death. AVD proceeds presumably by activation of K^+ and Cl^- efflux and it was suggested that the impairment of RVI mechanisms may be a general feature of apoptosis (Figure 2 B) (24). Contrary, NVI is initiated by uptake of osmolytes, such as Na^+, Cl^- and lactate and persistence of NVI is caused by dysfunction of RVD due to impairment of volume-sensitive Cl^- channels under conditions of ATP deficiency or lactacidosis (Figure 2 D) (24, 25).
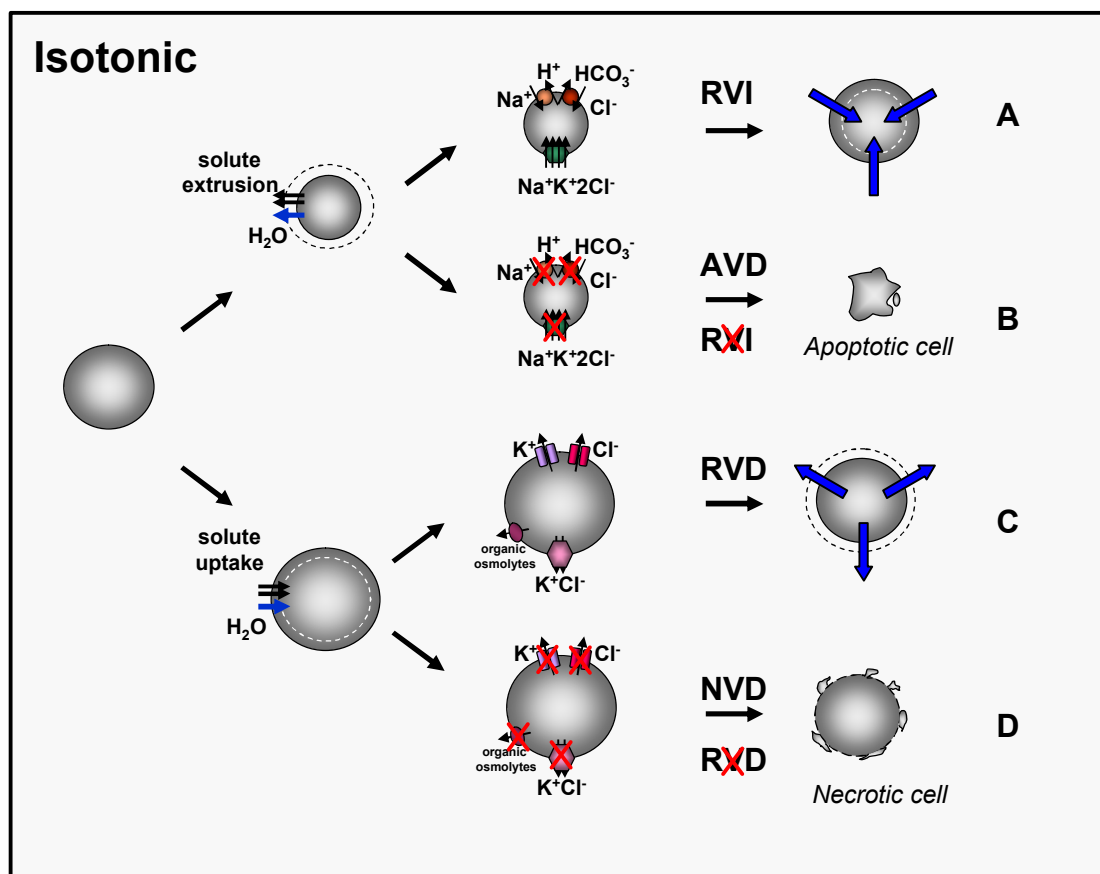

Figure 2: *Cell volume regulation in isosmotic medium. A: After solute extrusion cell volume decrease and RVI mechanisms are activated to restore cell volume. B: Impaired of RVI could lead to apoptotic volume decrease (AVD). C: After solute uptake cell volume increase and RVD mechanisms are activated to restore cell volume. D: Impaired RVD could lead to necrotic volume decrease (NVD).*

Finally, cell volume under isosmotic conditions can also be disrupted in a variety of pathophysiological disorders such as in metabolic disturbances, cellular acidosis, hypoxia, ischemia, stroke (22, 31, 33).

AQUAPORINS AND CELL VOLUME REGULATION.

As it has been above described a number of ion channels and transporters were identified as the pathways for volume-regulatory ionic flux (22, 25), however, the pathway

for volume-regulatory water flow across the plasma membrane has not been methodically investigated (19).

Over the past decade, significant advances have been made in understanding how water moves into and out of cells (2, 9, 27, 34). Today the accepted water pathway for transcellular movements is the lipid bilayer itself and specific water channels called aquaporins (AQPs) (1, 2, 34). Alternatively, solute-water cotransport, a controversial mechanism conceptually different from those previously mentioned, has been proposed (36).

The AQPs are a family of small membrane transport proteins that assemble in membranes as tetramers and act primarily as water-selective pores, facilitating osmotically driven water transport across cell plasma membranes (1, 2, 34). These selective channels are present in all forms of life, including mammals, amphibia, insects, plants, protozoa and bacteria. In humans, 13 different AQPs (AQP0–AQP12) have been characterized. New approaches like transfection, knockdown and knockout of specific water channels provide evidence for a role of AQPs in cell volume regulation.

AQP5 was the first water channel described to have a role in cell volume regulation (20). In this work the authors informed that parotid and sublingual acinar cells from AQP5 deficient mice have decreased membrane water permeability and inhibited RVD (20). These results are in line with the faster swelling and more complete RVD recently shown by Hansen et al., in a rat submandibular acinar cell line in which AQP5 levels have been increased (15). Furthermore, it has been demonstrated that deletion of **AQP1** in mice corneal endothelium reduces osmotic water permeability and impaired the extent of RVD after a hyposmotic challenge (21). Regarding the involvement of the **AQP2** water channel in volume regulation, we have reported that in cortical collecting duct cells transfected with AQP2, hypotonic shock induces RVD more rapidly than in wild type cells not expressing AQP2 (11). Our data also suggest that the rapid activation of RVD mechanisms in cells expressing AQP2 would be linked to cystic fibrosis transmembrane conductance regulator (CFTR) and to barium-sensitive potassium channels. Finally, downregulation of **AQP3** expression by antisense treatment was found to suppress the RVD activity of Intestine 407 cells, indicating a major role of AQP3 in the RVD process (19). In some others non epithelial cells, a role in cell volume regulation for **AQP4, AQP8, AQP9** and AQPs of protozoa has been suggested (4, 5, 7, 8). Altogether these studies demonstrate that AQP water channels are essential for cell volume regulation in epithelial and non epithelial cells, however, the mechanisms involved are still not clear.

WHY AQPS MIGHT BE IMPORTANT IN CELL VOLUME REGULATION?

Figure 3 shows a schematic representation of the time evolution of the relative cell volume under a hyposmotic shock of cells expressing or not AQPs. When the osmotic gradient is applied, changes in cell volume can be divided in two phases: **1-** cell volume increase due to passive water transport drives by the external osmotic shock and **2-** cell volume decrease due to isosmotic water movement drives by solute efflux. The presence of an AQP, as a water path, is critical for the fast rate of cell swelling. If AQPs are not present in the cell membrane osmotic swelling (phase 1) also occurs, even to the same extent, however, the rate is markedly reduced. After the increase in cell volume a "rapid activation" of RVD mechanisms takes place only in the presence of AQPs (11). In the absence of AQPs the subsequent RVD (phase 2) is sometimes totally inhibited and others reduced or later activated (11, 15, 19, 20, 21, 23). Although the expression of AQP clearly favors RVD, an important question remains to be answered: are AQPs important because they are the main route for water transport once the RVD response was elicited and/or because they play

some role in the activation/regulation of the RVD mechanisms? It is plausible that AQPs play some role other than the aquapore water pathway since water permeability can be largely provided by lipid bilayers. In this line AQPs have been proposed as sensors of mechanostress or of turgor pressure difference in the plasma membrane (11, 16, 21). Moreover, it was also suggested that AQPs may contribute to the set point for resting cell volume (30).
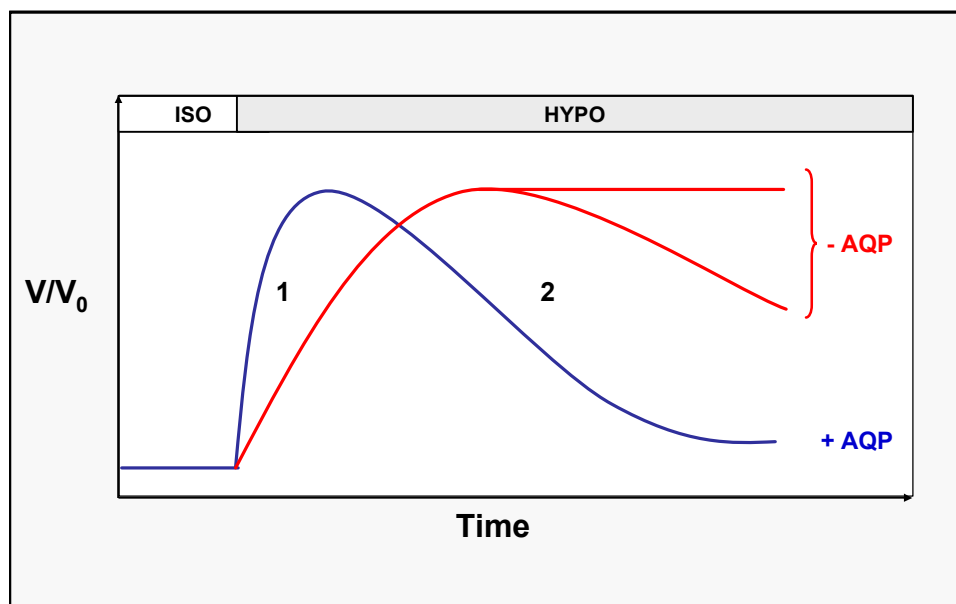

Figure 3: Schematic representation of the time evolution of the relative single cell volume (V/V$_0$) under a hyposmotic shock in cells expressing (+AQP) or not (-AQP) aquaporins. A biphasic response can be observed: 1- phase where passive water transport drives the evolution of cell volume and 2- phase where RVD drives the evolution of cell volume.

AQUAPORINS AND OSMOSENSING.

Cell volume regulation operates through a chain of events that essentially consists of a "sensor or sensing mechanism" to detect changes in cell volume and a "signaling cascade" to amplify the sensing signal and orient it to activate pathways for osmolyte fluxes (28, 31). Although a large amount of experimental results have been accumulated, the nature of the volume sensor and the signaling pathways are yet to be fully understood. Transmembrane molecules such as integrins, receptors of growth factor with intrinsic tyrosine kinase activity and the transient receptor potential channels (TRP) are all candidates proposed to play a role in the volume sensing mechanisms (28, 33). In recent years, it is becoming increasingly apparent that Ca^{2+} influx via TRP channels plays a crucial role in the response to mechanical and osmotic perturbations in a wide range of cell types (29). Some possible mechanisms of mechano or osmosensing by TRP channels are: changes in the tension and/or curvature of the lipid bilayer, changes in the cortical cytoskeleton, and signaling events such as lipid metabolism and protein phosphorylation/dephosphorylation. Among the mammalian TRP channels, a role in volume regulation has been documented most thoroughly for TRPV4 (transient receptor potential vanalloid 4). Importantly, activation of TRPV4 by cell swelling appears to be modulated by protein–protein interactions (29). In salivary gland epithelial cells TRPV4 activation by hypotonicity appears to depend

on the interaction with its binding partner aquaporin 5 (AQP5) rather than on cell swelling directly (23). The authors demonstrated in acinar cells that AQP5 is required for the activation of TRPV4 which increases intracellular Ca^{2+}, and that both proteins are assembled in a signaling complex that control RVD. In line with this we have recently found, in renal cells, that the presence of AQP2 facilitates a Ca^{2+} entry path, necessary for the activation of rapid RVD mechanisms (13). In a recent study, Taguchi et al. found, in the epithelial layer of the human endolymphatic sac, a similar distribution pattern of AQP2, V2-receptor, and TRPV4 to the one observed in the kidney and they proposed that AQP2 and TRPV4 channels are expected to be closely interconnected (32). Notably, in astroglia the same plasma membrane domains that were identified as strongly TRPV4 immunopositive are also enriched in the water channel AQP4 (3). Other members of the TRP family could also interact with AQPs since it has been recently reported that AQP2 physically associates with TRPC3 in cells of the rat renal collecting duct (14). Future work investigating functional and/or physical interactions between AQPs with TRPs will contribute to understand osmosense and osmoregulatory cell physiology.

Recent evidence raises the intriguing possibility that changes in cell volume act as signals for basic cell functions such as migration, proliferation, and apoptosis. One common point of all these processes is that to take place they require transient changes in cell volume as the result of ion movement. However, the mechanisms involved are not yet completely understood. It is interesting to note that AQPs have also been implicated in all these processes (34). AQP1, AQP3 and AQP4 have been shown to increase cell migration in renal, epidermal and astroglial cells. AQP3 was also shown to facilitate cell proliferation in different cell types (34). Additionally, AQP5 plays a role in promoting cell proliferation in human chronic myelogenous leukemia (6). Jablonski et al. reported in thymocytes and granulosa cells that AQP1 directly affects the rate of apoptotic progression (18). Specifically, the authors demonstrated that inhibition of AQP1 reduces the AVD and the downstream apoptotic cascades while AQP1 over-expression increases the rate of apoptosis Furthermore, the same group reported that, in hepatocytes, AQP8 and AQP9 expression also contribute to apoptosis (17). We have recently proposed that under apoptotic stimulation AQP2 would act as sensor leading to a coordinated activation of specific ionic channels for K^+ and Cl^- efflux resulting in both more rapid AVD and more rapid achievement of adequate levels of ions necessary to activate the enzymatic apoptotic cascade (10). All these studies provide new insights into the physiological roles of AQPs. However, it appears that AQPs expression is not an absolute requirement for migration, proliferation or apoptosis (10, 18, 34). Future studies are necessary to understand the roles of AQPs in the modulation of these processes.

CONCLUDING REMARKS.

Cells adapt to volume changes by a complex and dynamic process resulting from the concerted action of volume sensing mechanisms and intricate signaling chains. It is becoming evident that AQPs would be a critical factor in promoting a regulatory volume response, however, the mechanisms involved are not yet fully understood. The straightforward explanation would be that AQPs increase cell volume regulation because they provide an additional water path to the lipid bilayer. In anisotonic conditions it is clear the contribution of the AQPs as water paths since they significantly increase the osmotic permeability, causing an extremely rapid swollen or shrinkage. Nevertheless, the role of AQPs as simple water paths in the subsequent cell volume recovery, where water transport is isosmotically driven by solute fluxes, is not so evident. Several lines of evidence suggest that

AQPs might act someway like activators/regulators of the channels/transporters implicated in the cell volume regulation. The activation/regulation of the effectors could be due to the fact that immediate changes of cell volume elicit rapid alterations in their microenvironment (membrane tension, cytoskeletal architecture, ions strength, etc) or to specific interactions between AQPs and proteins involved in the sensing/signaling cascade. The recent finding that AQPs interact with some members of TRPs family opens a possibility to explain a role of AQPs in volume sensing. Elucidation of volume sensing mechanisms and signaling pathways represent the most important challenge in the field of cell volume regulation.

ACKNOWLEDGMENTS.

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