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Cellular volume homeostasis

Kevin Strange
Departments of Anesthesiology, Molecular Physiology and Biophysics, and Pharmacology,
Vanderbilt University Medical Center, Nashville, Tennessee 37232

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Strange, Kevin. Cellular volume homeostasis. Adv Physiol Educ 28: 155–159, 2004; doi:10.1152/advan.00034.2004.—All cells face constant challenges to their volume either through changes in intracellular solute content or extracellular osmotic pressure. Cells respond to volume perturbations by activating membrane transport and/or metabolic processes that result in net solute loss or gain and return of cell volume to its normal resting state. This paper provides a brief overview of fundamental concepts of osmotic water flow across cell membranes, mechanisms of cell volume perturbation, the role of inorganic ions and organic osmolytes in cell volume regulation and the signaling mechanisms that regulate the activity of cell volume-sensitive transport and metabolic pathways.

osmosis; cell volume regulation; organic osmolytes; membrane transport; signal transduction

MAINTENANCE of a constant volume in the face of extracellular and intracellular osmotic perturbations is a critical problem faced by all cells. Most cells respond to swelling or shrinkage by activating specific membrane transport and/or metabolic processes that serve to return cell volume to its normal resting state. These processes are essential for normal cell function and survival. This paper provides an overview of the cellular and molecular events underlying cell volume homeostasis.

Osmosis

The bulk movement of water across a semipermeable membrane is termed osmosis. An ideal semipermeable membrane is one that is permeable only to water. If such as membrane separates solutions with different solute concentrations, say, 0.1 M NaCl on one side and 1 M NaCl on the other, water will move from the dilute into the concentrated NaCl solution (Fig. 1). Water flow will continue until the NaCl concentrations in both solutions are equalized. The driving force for water flow is the concentration gradient for water. The concentration of water is higher in the 0.1 M NaCl solution compared with the 1 M NaCl solution.

Osmotic water flow across the membrane can be prevented by applying an opposing hydrostatic force (Fig. 1). The pressure required to stop water flow is termed the osmotic pressure. The mathematical expression that defines osmotic pressure was derived by van’t Hoff and is

\[ \Delta \pi = RT \Delta C_i \]  

where \( \Delta \pi \) is osmotic pressure difference, \( R \) is the gas constant, \( T \) is the absolute temperature, and \( \Delta C_i \) is the difference in solute concentration across the membrane.

Osmotic pressure is dependent on the total concentration of dissolved solute particles. The terms osmolality and osmolarity indicate the total number of particles present in a kilogram of solvent and a liter of solution, respectively. One osmole is 1 mol of particle, which are 6.02 \( \times \) 10\(^{23} \) individual particles. Osmolality and osmolarity are used interchangeably when referring to the relatively dilute solutions of the body.

The above discussion of osmosis is based on the simplifying concept that water flow is occurring across a membrane permeable only to water. Real membranes are not quite so tidy. All membranes have finite solute permeabilities. While many biologically relevant solutes have permeabilities substantially lower than water and behave as though they were effectively impermeable, some solutes have permeabilities approaching that of water. These high-permeability solutes diffuse across the membrane down their concentration gradient. As they do so, the osmotic pressure driving water flow is reduced. If the movement of solute is fast enough, the concentrations of the solute on the two sides of the membrane can become equalized before significant osmotic water flow occurs.

To account for the nonideal behavior of membranes, Staverman derived the term reflection coefficient for solute \( i \), \( \sigma_i \), as

\[ \sigma_i = \frac{\Delta \pi_{obs}}{\Delta \pi_{th}} \]  

where \( \Delta \pi_{obs} \) is the observed osmotic pressure and \( \Delta \pi_{th} \) is the theoretical osmotic pressure obtained from Eq. 1. The reflection coefficient is a dimensionless term that ranges from 1 for a solute that behaves as though it were effectively impermeant (i.e., the solute is “reflected” by the membrane), to 0 for a solute whose permeability is similar to that of water. The effective osmotic pressure, \( \Delta \pi_{eff} \), across a membrane generated by solute \( i \) is therefore

\[ \Delta \pi_{eff} = \sigma_i RT \Delta C_i \]  

The flow of water \( J_v \) across a membrane is defined as

\[ J_v = L_p (\sigma_i \Delta \pi_{th} - \Delta P) \]  

where \( L_p \) is the hydraulic conductivity coefficient of the membrane and \( \Delta P \) the hydrostatic pressure difference across the membrane. The hydraulic conductivity coefficient is a measure of the water permeability of the membrane. Cell membranes do not generate and maintain significant hydrostatic pressure gradients. Thus, when considering water flow into and out of

Address for reprint requests and other correspondence: K. Strange, Anesthesiology Research Division, Vanderbilt Univ. Medical Center, T-4202 Medical Center North, Nashville, TN 37232-2520 (E-mail: kevin.strange @vanderbilt.edu).

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animal cells, the ΔP term in Eq. 4 can be ignored. However, in organisms with relatively rigid cell walls, such as bacteria, plants, and yeast, significant hydrostatic pressure gradients can be generated and play important roles in driving water flow.

Water flow across most biological membranes occurs by simple diffusion of water molecules through the lipid bilayer. However, some cells possess specialized proteins that form transmembrane water-selective pores termed aquaporins (1, 28). Aquaporins dramatically increase the water permeability of cell membranes.

Fundamentals of Cell Volume Regulation

Water is effectively in thermodynamic equilibrium across the plasma membrane. In other words, the osmotic concentration of cytoplasmic (c) and extracellular (e) fluids are equal under steady-state conditions. Changes in intracellular or extracellular solute content generate a transmembrane osmotic gradient (Δπ). Because cell membranes are freely permeable to water, any such gradient results in the immediate flow of water into or out of the cell until equilibrium is again achieved. Since animal cell membranes are unable to generate or sustain significant hydrostatic pressure gradients, water flow causes cell swelling or shrinkage.

Cell volume changes are usually grouped into two broad categories, anisosmotic and isosmotic. Anisosmotic volume changes are induced by alterations in extracellular osmolality. Under normal physiological conditions, most mammalian cells, with a few noteworthy exceptions (e.g., cells in the renal medulla and gastrointestinal tract), are protected from anisosmotic swelling or shrinkage. Under steady-state conditions, intracellular solute levels are held constant by a precise balance between solute influx and efflux across the plasma membrane, and by the metabolic production and removal of osmotically active substances. A variety of physiological and pathophysiological conditions, however, can disrupt this balance (17, 18). For example, the cell swelling that occurs in the brain after a stroke or head trauma is an example of isosmotic volume increase and is due to intracellular accumulation of NaCl and other solutes.

Experimental Measurement of Cell Volume

A variety of methods are available for measuring absolute and relative cell volume changes under experimental conditions including electronic sizing by Coulter-type impedance techniques, light scattering, ion-sensitive microelectrode methods that measure the concentration of intra- or extracellular impermeant molecules, and radioactive tracer methods. Microscopy methods are widely used for measuring cell volume changes. Single cells can be imaged by bright field microscopy and volume changes quantify by three-dimensional reconstruction. If cell shape is spherical, relative volume changes can be quantified simply by measuring cell diameter. Fluorescence microscopy methods can be used to measure changes in the intensity of fluorescent probes trapped within the cell. As long as the probes are not sensitive to other cellular parameters such as pH, Ca²⁺ levels, etc., changes in fluorescence intensity reliably track swelling- and shrinkage-induced volume perturbations.

Regulation of Cell Volume

Cells respond to volume perturbations by activating volume regulatory mechanisms. The processes by which swollen and shrunken cells return to normal volume are collectively termed regulatory volume decrease and regulatory volume increase, respectively (Fig. 2). Cell volume can only be regulated by the gain or loss of osmotically active solutes, primarily inorganic ions such as Na⁺, K⁺, Cl⁻, or small organic molecules termed organic osmolytes.

Volume regulatory electrolyte loss and gain are mediated exclusively by membrane transport processes (12, 13, 20). In most animal cells, regulatory volume decrease occurs through...
loss of KCl via activation of separate K\(^+\) and Cl\(^-\) channels or by activation of the K-Cl cotransporter. Regulatory volume increase occurs by uptake of both KCl and NaCl. Accumulation of these salts is brought about by activation of Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3\)\(^-\) exchangers or the Na-K-2Cl cotransporter. Figure 3 illustrates the ion transport systems commonly involved in cell volume regulation. Activation of these transport pathways is rapid and occurs within seconds to minutes after volume perturbation. Rapid stimulation of electrolyte transport is possible because the channels and transporters by which it is mediated reside continuously in the plasma membrane or are stored in submembrane cytoplasmic vesicles. Certain volume-sensitive ion transport systems play multiple roles, participating in volume regulation as well as transepithelial salt and water movement, and intracellular pH control.

Organic osmolytes are found in high (tens to hundreds of millimolar) concentrations in the cytosol of all organisms from bacteria to humans (3, 4, 12, 31). These solutes play key roles in cell volume homeostasis and may also function as general cytoprotectants. In animal cells, organic osmolytes are grouped into three distinct classes: 1) polyols (e.g., sorbitol and myo-inositol), 2) amino acids and their derivatives (e.g., taurine, alanine, and proline), and 3) methylamines (e.g., betaine, glycerophosphorylcholine) (Fig. 4).

Organic osmolytes are “compatible” or “nonperturbing” solutes (3, 4, 31). They have unique biophysical and biochemical properties that allow cells to accumulate them to high levels or to withstand large shifts in their concentration without deleterious effects on cellular structure and function. In contrast, so-called “perturbing” solutes, such as electrolytes or urea can harm cells or disrupt metabolic processes when they are present at high concentrations or when large shifts in their concentrations occur. For example, elevated electrolyte levels and intracellular ionic strength can denature or precipitate cell macromolecules. Even smaller changes in cellular inorganic ion levels can alter resting membrane potential, the rates of enzymatically catalyzed reactions and membrane solute transport that is coupled to ion gradients.

Accumulation of organic osmolytes is mediated either by energy-dependent transport from the external medium or by changes in the rates of osmolyte synthesis and degradation (Fig. 5) (3, 4, 12, 31). Volume regulatory organic osmolyte accumulation is typically a slow process relative to electrolyte uptake and requires many hours after initial activation to reach completion. This slow time course is observed because activation of organic osmolyte accumulation pathways usually requires transcription and translation of genes coding for organic osmolyte transporters and synthesis enzymes.

Loss of organic osmolytes from cells is elicited by swelling and occurs in two distinct steps. First, swelling induces a very rapid (i.e., seconds) increase in passive organic osmolyte efflux (Fig. 5) (9–11). Downregulation of organic osmolyte synthesis
and uptake mechanisms also contributes to the loss of these solutes from the cell. Overall, this process is slow. Cell swelling inhibits transcription of the genes coding for organic osmolyte transporters and synthesis enzymes (3, 31). As transcription decreases, mRNA levels drop and the number of functional proteins declines over a period of many hours to days.

**How Do Cells Sense Their Size?**

Volume sensing mechanisms appear to be extremely sensitive. For example, studies by Lohr and Grantham (14) on the renal proximal tubule have demonstrated that cells can sense and respond to volume changes of <3%. However, our understanding of the mechanisms by which cells sense volume perturbations and transduce those changes into regulatory responses is rudimentary.

Several possible volume signals have been postulated including swelling- and shrinkage-induced changes in membrane tension, cytoskeletal architecture, cellular ion concentrations and the concentration of cytoplasmic macromolecules (12, 20, 21). All of these hypotheses have their strengths and weaknesses. At present, it appears that no one signaling mechanism can account for the volume sensitivity of the various genes and membrane transport pathways that are activated or inactivated in response to cell volume perturbations. To further complicate the picture, recent evidence suggests that cells can detect more than simple swelling or shrinkage. Cells most likely possess a wide array of volume detector and effector mechanisms that respond selectively to both the magnitude and nature of the volume perturbation (7, 16, 24). Such functionally distinct sensor and effector pathways may afford the cell simultaneous control over a variety of parameters (e.g., intracellular pH and ionic composition) in addition to volume.

Molecular insight into the signals that initiate volume regulatory responses has been gained from studies on bacteria and kidney cells. The large conductance mechanosensitive channel, mechanosensitive channel large conductance (MscL), in *Escherichia coli* mediates osmolyte efflux in response to hypotonic shock. MscL has been purified, reconstituted and characterized structurally using X-ray crystallography (2, 25, 26). The purified channel protein reconstituted into planar lipid bilayers is activated by membrane stretch demonstrating that the channel protein itself senses directly membrane tension transmitted through the bilayer (2, 25, 26).

The mechanisms by which hypertonic stress induces increased expression of genes encoding organic osmolyte transporters and enzymes involved in their synthesis have been studied extensively in the kidney. The major organic osmolytes present in the hypertonic medulla of the mammalian kidney include sorbitol, betaine and myo-inositol. Sorbitol is accumulated by increased synthesis mediated by the enzyme aldose reductase (AR). Betaine and myo-inositol accumulation is mediated by transport from the extracellular space via the NaCl-betaine (BGT1) and the Na-myo-inositol (SMIT) co-transporters (3).

Early studies by Burg and co-workers (3) suggested that increases in intracellular ion strength brought about by hypertonic shrinkage and subsequent regulatory volume increase triggered increased gene transcription. For example, Uchida et al. (27) observed a linear relationship between AR activity and total intracellular Na\(^+\) and K\(^+\) levels.

The promoter regions of AR, SMIT, and BGT1 contain regulatory domains termed toxicity-responsive enhancers (TonE) that modulates gene expression in response to hypertonic stress (3). Kwon and co-workers (19) cloned the transcription factor TonEBP (TonE binding protein) that binds to this regulatory domain and initiates gene transcription. TonEBP is a member of the Rel family of transcription factors. In response to hypertonic shock, TonEBP translocates from the cytoplasm into the nucleus where it binds to genes containing TonE sites (29, 30). Nuclear translocation and TonEBP binding may be mediated by increases in cytoplasmic ion strength.

As with the initial volume signal sensed by the cell, there is little clear-cut understanding of the signaling mechanisms by which cell volume changes are transduced into regulatory responses. Numerous signal transduction pathways have been implicated in the control of volume regulatory transport pathways including changes in intracellular Ca\(^{2+}\) concentration, GTPase activity, serine/threonine and tyrosine phosphorylation/dephosphorylation, and eicosanoid levels (12).

Perhaps the most extensively studied and best understood volume regulatory signaling mechanisms are the phosphorylation/dephosphorylation reactions that regulate swelling- and shrinkage-induced activation of the K-Cl and Na-K-2Cl co-transporters. Swelling-induced activation and shrinkage-induced inactivation of the K-Cl cotransporter are mediated by serine/threonine dephosphorylation and phosphorylation, respectively. The converse is true for the Na-K-2Cl cotransporter; shrinkage-induced activation is mediated by phosphorylation and swelling-induced inactivation is brought by dephosphorylation. Pharmacological studies suggest that type 1 protein phosphatase mediates protein dephosphorylation (8, 12, 15, 21).
Detailed transport studies suggest that both transporters are regulated by a common kinase whose activity is modulated by cell volume changes (8, 12, 15, 21). The identity of this putative common volume-sensitive kinase is not firmly established. However, Delpire and co-workers (22) demonstrated recently that the STE20-related kinase PASK (Proline-Ala-

nine-rich STE20-related Kinase) interacts with the NH2-termini of both the K-CI and Na-K-2CI cotransporters. PASK is a member of a large kinase superfamily that is divided into p21-activated kinase (PAK) and germlinal center kinase (GCK) subfamilies (5). Members of this superfamily regulate numerous fundamental physiological processes including apoptosis, cellular stress responses, morphogenesis, cytoskeletal architecture, cell cycle, and oocyte meiotic maturation (5).

STE20 was identified originally in yeast, where it functions as a mitogen-activated protein kinase kinase kinase kinase (MAP4K) that is activated in response to hypertonic stress and regulates accumulation of the organic osmolyte glyceroen (23). Dowd and Forbush (6) have shown that PASK plays a role in activation of Na-K-2Cl cotransporter NKCC1 in response to hypertonic shrinkage. The regulatory role of PASK is most likely mediated through direct phosphorylation of the cotransporter (6). The studies in yeast and mammalian cells suggest that STE20-related kinases may be important components of the signal transduction pathways that control cell volume regulatory mechanisms.

In conclusion, the ability to tightly control solute and water balance during osmotic challenge is an essential prerequisite for cellular life. Cellular osmotic homeostasis is maintained by the regulated accumulation and loss of inorganic ions and small organic solutes termed organic osmolytes. Organic osmolytes are “compatible” or “nonperturbing” solutes and are typically found in concentrations of tens to hundreds of millimoles in the cytosol of all organisms from bacteria to humans. The effector mechanisms responsible for osmoregulatory solute accumulation and loss in animal cells are generally well understood. However, major gaps exist in our understanding of the signals and signaling pathways by which animal cells detect volume perturbations and activate volume regulatory mechanisms. Elucidation of volume sensing mechanisms and signaling pathways represents the most pressing and significant challenge in the field and is essential for understanding fully cell volume control and related cellular processes.

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