Review

Mechanisms and Significance of Cell Volume Regulation

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Survival of human and animal cells requires avoidance of excessive alterations of cell volume. The osmolarity amassed by cellular accumulation of organic substances must be compensated by lowering cytosolic ion concentrations. The Na^+/K^+ ATPase extrudes Na^+ in exchange for K^+, which can permeate the cell membrane through K^+ channels. K^+ exit generates a cell-negative potential difference across the cell membrane, driving the exit of anions such as Cl^-. The low cytosolic Cl^- concentrations counterbalance the excess cellular osmolarity by organic substances. Cell volume regulation following cell swelling involves releasing ions through activation of K^+ channels and/or anion channels, KCl-cotransport, or parallel activation of K^+/H^+ exchange and Cl^-/HCO_3^- exchange. Cell volume regulation following cell shrinkage involves accumulation of ions through activation of Na^+,K^+,$2Cl^-$ cotransport, Na^+/H^+ exchange in parallel to Cl^-/HCO_3^- exchange, or Na^+ channels. The Na^+ taken up is extruded by the Na^+/K^+ ATPase in exchange for K^+. Shrunken cells further accumulate organic osmolytes such as sorbitol and glycerophosphorylcholine, and monomeric amino acids by altered metabolism and myoinositol (inositol), betaine, taurine, and amino acids by Na^+ coupled transport. They release osmolytes during cell swelling. Challenges of cell volume homeostasis include transport, hormones, transmitters, and drugs. Moreover, alterations of cell volume participate in the machinery regulating cell proliferation and apoptotic cell death. Deranged cell volume regulation significantly contributes to the pathophysiology of several disorders such as liver insufficiency, diabetic ketoacidosis, hypercatabolism, fibrosing disease, sickle cell anemia, and infection.

Key teaching points:

- A diverse array of regulatory mechanisms adjust cell volume to functional demands.
- Cell volume and cell volume-sensitive cellular functions participate in a wide variety of physiological and pathophysiological mechanisms
- Cell hydration is an important determinant of cell performance.
- The physiological and pathophysiological role of cell volume regulation in integrated function is frequently unrecognized or poorly understood.
- Further research is necessary to define the role of cell volume in health and disease.

INTRODUCTION

Upon ingestion of fluids, water distributes between intra- and extracellular compartments. The entry of water into cells leads to cell swelling. Conversely, loss of cellular water upon dehydration leads to cell shrinkage.

The maintenance of adequate cell volume is, however, one of the most obvious prerequisites for cell survival [1]. Excessive alterations of cell volume interfere with the integrity of cell membrane and cytoskeletal architecture. Moreover, the state of hydration has a profound influence on cytosolic proteins. Proteins and protein-bound water occupy a large fraction of the intracellular space (macromolecular crowding), leaving little space for unbound water [2]. Loss or gain of even a small

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percentage of cellular water thus exerts a profound effect on protein function and cellular performance.

Due to the presence of water channels, water easily permeates the plasma membrane of most cells [3,4]. The movement of water is driven by osmotic pressure gradients [1]. In mammalian cells, hydrostatic gradients across cell membranes remain negligibly low. To avoid swelling or shrinkage, cells have to accomplish osmotic equilibrium across the cell membrane. At an intracellular osmolarity exceeding extracellular osmolarity, water enters the cell following its osmotic gradient and the cell swells. Conversely, at an extracellular osmolarity exceeding intracellular osmolarity, water exits and the cell shrinks.

A wide variety of factors modify intra- or extracellular osmolarity and thus challenge the osmotic equilibrium across the cell membrane [1]. Volume regulation may be required even in a perfectly isotonic environment. Cells employ an array of mechanisms to maintain cell volume constancy, including altered transport across the cell membrane and metabolism. Hormones and mediators may modify the activity of these cell volume regulatory mechanisms and thus influence cell volume sensitive functions. Accordingly, cell volume regulatory mechanisms participate in the signaling of those hormones and mediators [5].

Following untoward cell swelling, volume regulatory mechanisms decrease intracellular osmolarity and cell volume thus accomplishing regulatory cell volume decrease (RVD). Following untoward cell shrinkage, cell volume regulatory mechanisms increase intracellular osmolarity and cell volume, thus accomplishing regulatory cell volume increase (RVI) [6].

The most rapid and efficient cell volume regulatory mechanisms are ion transporters in the cell membrane [7]. Following cell swelling, they mediate cellular ion release and upon cell shrinkage, they allow cellular ion accumulation. The use of ions in cell volume regulation is limited, however, as high inorganic ion concentrations interfere with the stability of proteins and altered ion gradients across the cell membrane interfere with the function of gradient driven transporters [8]. Thus, cells additionally utilize organic osmolytes for osmoregulation. Moreover, cells adapt a variety of metabolic functions and thus modify the cellular generation or disposal of osmotically active organic substances [8]. Organic osmolytes are particularly important in the grossly hypertonic environment of kidney medulla [9].

In this brief overview, cell volume regulatory mechanisms and factors challenging cell volume constancy will be described. Moreover, examples will be provided on the interplay of cell volume regulatory mechanisms, cell hydration, and cell function in disease. It should be pointed out that virtually all mechanisms described below have been shown to operative in humans and are thus pertinent to human physiology.

MAINTENANCE OF CELL VOLUME IN ISOTONIC MEDIUM

Even in a isotonic environment, the intracellular concentration of inorganic ions needs to be lower than the extracellular ion concentration to counterbalance the cellular accumulation of organic substances [1,10]. The cells extrude Na^+ in exchange for K^+ by the Na^+/K^+ ATPase pump. The cell membrane is less permeable to Na^+ than to K^+. The chemical K^+ gradient drives K^+ exit through K^+ channels. The movement of K^+ generates a cell-negative potential difference across the cell membrane that then drives Cl^- into the extracellular space. At a cell membrane potential of -18 mV and an extracellular Cl^- concentration of 110 mmol/l, intracellular Cl^- concentration is in electrochemical equilibrium at 55 mmol/l. Thus, at this membrane potential the uneven Cl^- distribution would allow the excess accumulation of some 55 mmol/l organic substances. In most cells, the potential difference across the cell membrane is more negative than -18 mV and intracellular Cl^- even lower than 55 mmol/l. The operation of Na^+/K^+ ATPase, and thus the establishment of the ionic gradients, requires energy expenditure.

Energy depletion impairs the function of the Na^+/K^+ ATPase, dissipates the Na^+ and K^+ gradients, depolarizes the cell membrane, and leads to cellular accumulation of Cl^- and thus cell swelling [1]. During ischemia, the swelling is compounded by an increase of extracellular K^+ concentration, which further dissipates the K^+ gradient. Moreover, excessive formation and reduced clearance of lactate leads to cellular acidosis, which enhances Na^+/H^+ exchange activity and thus increases cellular Na^+ accumulation and cell swelling. In the brain, the depolarization triggers the release of glutamate, which activates nonspecific cation channels and thus induces further cell swelling.

The energy requirements for maintaining ionic gradients and cell volume constancy depend on the rate of Na^+ entry [1]. In theory, in a completely Na^+ impermeable cell, K^+ and Cl^- approach an equilibrium that does not require any energy expenditure to maintain cell volume constancy. In some cells, energy depletion leads to transient cell shrinkage preceding the eventual cell swelling. In those cells, the increase of intracellular Na^+ concentration reverses the driving force for the Na^+/Ca^{2+} exchanger and thus leads to Ca^{2+} entry, activation of Ca^{2+}-sensitive K^+ channels and/or Cl^- channels, KCl exit, and thus cell shrinkage.

REGULATORY CELL VOLUME INCREASE

Exposure of cells to hypertonic medium or cellular loss of osmolytes leads to exit of water according to the osmotic gradient and thus to cell shrinkage. The following RVI (Fig. 1) is accomplished by ion uptake [7]. Cell shrinkage leads to

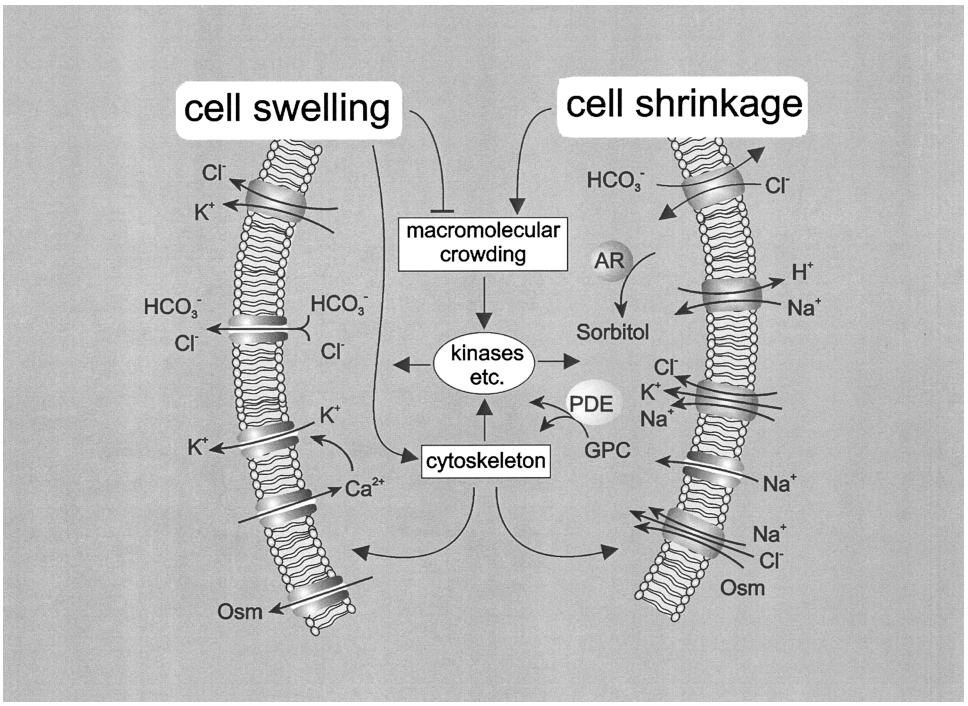

Fig. 1. Cell volume regulatory mechanisms (from Lang, editor, Cell volume regulation, in Contributions to Nephrology Vol. 123).

activation of the Na^+-K^+-$2Cl^-$ cotransporter and/or the combined activation of the Na^+/H^+ exchanger in parallel to the Cl^-/HCO_3^- exchanger [7]. The H^+ and the HCO_3^- extruded by the Na^+/H^+ exchanger and the Cl^-/HCO_3^- exchanger, respectively, are replenished in the cell from CO_2 via H_2CO_3. The net effect of those two carriers is thus NaCl entry. Na^+ accumulated by either Na^+-K^+-$2Cl^-$ cotransport or Na^+/H^+ exchange is extruded by the Na^+/K^+ ATPase in exchange for K^+. Thus, the transporters eventually lead to uptake of KCl. Several Na^+-K^+-$2Cl^-$ cotransporter [11] and Na^+/H^+-exchanger isoforms [7] have been cloned, which do not all serve cell volume regulation. For instance, the Na^+/H^+ exchangers NHE-1, NHE-2 and NHE-4 are activated by, and NHE-3 is inhibited by, cell shrinkage [7].

Shrinkage of some cells leads to activation of Na^+ channels and depolarization, which in turn dissipates the electrical gradient for Cl^- and thus leads to Cl^- entry [12]. Some cells inhibit K^+ channels again leading to depolarization. Some cells inhibit Cl^- channels upon cell shrinkage to avoid cellular Cl^- loss [7].

Cell shrinkage is not only counteracted by cellular accumulation of ions, but also by cellular uptake or generation of organic osmolytes [8,13]. The most important osmolytes are polyols (such as sorbitol and myoinositol), methylamines (such as betaine and glycerophosphorylcholine), amino acids, and the amino acid derivative taurine.

Sorbitol is generated from glucose [8]. The reaction is catalyzed by aldose reductase, which is expressed following osmotic cell shrinkage. The gene expression of the protein takes several hours and the appropriate increase of sorbitol concentration requires hours to days. Glycerophosphorylcholine (GPC) is produced from phosphatidylcholine. The reaction is catalyzed by a phospholipase A_2 distinct from the arachidonyl selective enzyme. GPC is degraded by a phosphodiesterase to glycerol-phosphate and choline. Cell shrinkage inhibits the phosphodiesterase enzyme and leads to cellular accumulation of GPC.

Myoinositol (inositol), betaine, and taurine are accumulated by their respective Na^+ coupled transporters (SMIT, BGT and NCT) [14]. BGT and NCT transport Cl^- and Na^+ as well as their respective organic osmolyte. Moreover, the excess positive charge of these carriers depolarizes the cell membrane and favors Cl^- entry. Accordingly, these transporters accumulate NaCl in parallel to organic osmolytes. Cell shrinkage stimulates the gene expression of these transporters and thus the cellular accumulation of the respective osmolytes. Again, the expression of the transporters is slow and full adaptation requires hours to days. Moreover, the osmolyte uptake depends on the availability of osmolytes in extracellular fluid. Similar to the organic osmolytes, some amino acids are accumulated by cell volume sensitive Na^+ coupled transport, such as the amino acid transport system A [1].

In contrast to inorganic ions, organic osmolytes do not destabilize proteins. Moreover, some osmolytes counteract the destabilizing effects of inorganic ions, some organic ions (spermidine), and urea. For instance, the effects of urea are counteracted by betaine and glycerophosphorylcholine and by myoinositol to a lesser extent. The osmolytes further protect

against the destabilizing effects of heat shock, dessication, and presumably radiation [1].

REGULATORY CELL VOLUME DECREASE

Exposure of cells to hypotonic extracellular fluid or cellular gain of osmolytes leads to water influx, along the osmotic gradient across the cell membrane. Regulatory cell volume decrease (Fig. 1) requires release of cellular ions, by activation of K^+ channels and/or anion channels in most cells [15–18]. Both ion channel types must be operative for KCl exit, as neither K^+ nor anions can exit without the respective counterion. Cell volume regulatory ion channels include the K^+ channels Kv1.3, Kv1.5, and KCNE1/KCNQ1 and the anion channels ClC-2 and ClC-3 [7]. The role of I_{Cln} and P-glycoprotein (MDR) in cell volume regulation has been a matter of controversy [19,20]. In any case, many different ion channels likely participate in cell volume regulation.

Swelling leads to activation of nonspecific cation channels in some cells [7]. The electrochemical gradient favors entry rather then exit of cations through those channels. Thus, permeation of ions through those channels cannot directly serve cell volume regulation. Instead, the channels mediate the entry of Ca^{2+} which in turn activates Ca^{2+}-sensitive K^+ channels and/or Cl^- channels.

Cell volume regulatory decrease could be further accomplished by activation of carriers, such as KCl-cotransport, which allows coupled exit of both ions [21]. Some cells dispose cellular KCl via parallel activation of K^+/H^+ exchange and Cl^-/HCO_3^- exchange. The H^+ and HCO_3^- taken up by those transporters react via H_2CO_3 to CO_2 which easily crosses the cell membrane and is not osmotically relevant. Thus, the tandem serves to release KCl [7].

Cell swelling stimulates the rapid exit of GPC, sorbitol, inositol, betaine, and taurine [12,22]. The mechanisms mediating the release of organic osmolytes are ill-defined and may involve several transporters and/or channels in parallel.

CELL VOLUME-SENSITIVE METABOLIC PATHWAYS

A variety of metabolic pathways are sensitive to cell volume [1]. The effects of cell volume on metabolism result from activation, inhibition, or altered expression of enzymes.

Cell shrinkage stimulates the degradation of proteins to amino acids and of glycogen to glucosephosphate. Cell shrinkage further inhibits protein and glycogen synthesis. The degradation products are osmotically more active than the macromlecules and their breakdown generates cellular osmolarity. Conversely, cell swelling stimulates protein and glycogen synthesis and inhibits proteolysis and glycogenolysis, thus converting the intracellular amino acids and glucose phosphate into the osmotically less active macromolecules [1].

Alterations of cell volume further influence several pathways of glucose and amino acid metabolism [1]. Cell swelling inhibits glycolysis, stimulates flux through the pentose phosphate pathway, favors lipogenesis from glucose, and decreases transcription of phosphoenolpyruvate carboxykinase, a key enzyme for gluconeogenesis. It stimulates glycine and alanine oxidation, glutamine breakdown, as well as formation of NH_4^+ and urea from amino acids. Cell swelling stimulates ketoisocaproate oxidation, acetyl CoA carboxylase, and lipogenesis; inhibits carnitine palmitoyltransferase I; decreases cytosolic ATP and phosphocreatine concentrations; increases respiration; and stimulates RNA and DNA synthesis. All those effects are reversed by cell shrinkage.

Stimulation of flux through the pentose phosphate pathway increases NADPH production and thus enhances glutathione (GSH) formation. Conversely, cell shrinkage decreases NADPH production and GSH formation. As a result, cell swelling increases and cell shrinkage decreases cellular resistance to oxidative stress [1]. At the same time, cell shrinkage decreases the activity of NADPH-oxidase and thus impedes cellular O_2^- formation. Thus a hypertonic environment, as it prevails in kidney medulla, suppresses leukocyte oxidative burst and antibacterial response [1].

CELL VOLUME-SENSITIVE GENES

Expression of a wide variety of genes is sensitive to cell volume [23,24]. Some of those genes serve cell volume regulation. For instance, cell shrinkage stimulates expression of the Na^+-K^+-$2Cl^-$ cotransporter and of the ATPase α1-subunit. It also stimulates expression of enzymes or transporters engaged in cellular formation or accumulation of osmolytes including the aldose reductase and the Na^+-coupled transporters for betaine (BGT), taurine (NCT), myoinositol (SMIT), and amino acids.

Other cell volume-sensitive genes encode elements in the signaling of cell volume regulatory mechanisms. For instance, cell swelling stimulates the expression of the extracellular signal regulated kinases ERK1, ERK2 and the Jun kinase JNK-1 [1], cell shrinkage enhances the expression of the serum and glucocorticoid inducible kinase SGK1 and cycloxygenase-2 [25].

Cell shrinkage stimulates the expression of heat shock proteins, which stabilize proteins. Their expression following cell shrinkage presumably protects against the destabilizing effects of increased cytosolic ion concentrations [1].

A number of cell volume-sensitive genes do not play an obvious role in cell volume regulation [1]. Cell swelling stimulates the expression of β-actin and tubulin, the immediate

early genes c-jun and c-fos, and the enzyme ornithine decarboxylase. Cell shrinkage stimulates the expression of the cytokine TNF-α, the Cl^- channel ClC-K1, P-glycoprotein, the immediate early genes Egr-1 and c-fos, the GTPase inhibitor α1-chimaerin, the CDβ antigen, the enzymes phosphoenolpyruvate carboxykinase (PEPCK), arginine succinate lyase, tyrosine aminotransferase, tyrosine hydroxylase, dopamine β-hydroxylase, matrix metalloproteinase 9 and tissue plasminogen activator, as well as matrix proteins including biglycan and laminin B_2. Cell shrinkage further stimulates expression and release of antidiuretic hormone ADH [1].

The stimulation of transcription is partially mediated by respective promoter region in the cell volume sensitive genes: the genes encoding aldose reductase, BGT, and SGK1 contain osmolarity responsive (ORE), tonicity responsive (TonE), or cell volume responsive (CVE) elements. TonE binds a tonicity responsive element binding protein TonEBP for stimulation of expression [24].

Signaling of Cell Volume Regulation

Little is known about sensors of cell volume or osmolarity. Possibly, cells recognize cellular protein content or macromolecular crowding [2]. The protein density somehow influences a serine/threonine kinase [presumably the WNK kinase with no lysine], which in turn regulates the activity of cell volume regulatory KCl- and Na^+-K^+-$2Cl^-$ cotransport by respective phosphorylation of the transport proteins [26].

Cell swelling may impose stretch on the cytoskeleton and/or cell membrane, which may similarly serve as sensors of cell volume [26]. The sensors trigger a variety of cellular signaling pathways, which may vary considerably between different cells or a given cell in different functional states [26–28].

In many, but not all cells, swelling increases intracellular activity of Ca^{2+}, which enters through Ca^{2+} channels in the plasma membrane and/or is released from intracellular stores following formation of 1,4,5-inositol-trisphosphate. Ca^{2+} activates volume-regulatory K^+ channels and Cl^- channels and influences other cell volume sensitive cellular functions [7,29].

Cell volume affects cytoskeletal architecture and expression of cytoskeletal proteins [30]. Microtubules and actin filaments may participate in cell volume regulation and their disruption may interfere with cell volume regulation.

Alterations of cell volume modify the phosphorylation of a variety of proteins [31,32]. Kinases activated during cell swelling include tyrosine kinases, protein kinase C, adenylate cyclase, MAP kinases, Jun-kinase, and focal adhesion kinase ($p121^{FAK}$). Osmotic cell shrinkage triggers WNK and several MAP (mitogen activated protein) kinase cascades, leading to activation of SAPK, p38 kinase, and myosin light chain kinase (MLCK). The kinases may directly phosphorylate cell volume regulating carriers or the cytoskeleton and they may lead to activation of transcription factors governing expression of cell volume-regulated genes.

In some cells, swelling activates phospholipase A_2 [33,34], which leads to formation of the 15-lipoxygenase product hepoxilin A_3 and the 5-lipoxygenase product leukotriene LTD_4 [33]. The eicosanoids in turn stimulate cell volume-regulatory K^+ and/or Cl^- channels and/or taurine release. Cell swelling inhibits formation of PGE_2 and thus prevents activation of PGE_2-sensitive Na^+ channels [33]. Cell volume signaling also may involve nitric oxide [35,36].

Cell swelling alkalinizes and cell shrinkage acidifies cellular compartments such as endosomes, lysosomes, and secretory granules. The alkalinization of the acidic cellular compartments, which in turn inhibits autophagic proteolysis [37].

CHALLENGE OF CELL VOLUME CONSTANCY BY ALTERATIONS OF EXTRACELLULAR FLUID OSMOLARITY

Most cells in mammals are normally bathed in isotonic extracellular fluid. In the human kidney medulla, however, extracellular osmolarity may approach 1400 mosmol/l [9]. Moreover, the extracellular osmolarity may change within less than one hour from this high value to almost isotonicity, during transition from antidiuresis to diuresis. Thus, renal medullar cells have to cope with rapid changes of extracellular osmolarity. Within less than a minute, blood cells passing through the kidney medulla are exposed to the high medullary osmolarity and return to the isoosmolarity of systemic blood.

Food is usually not isotonic and intestinal cells may be exposed to anisosmotic luminal fluid. Absorption of anisotonic nutrients leads to usually minor alterations of portal blood osmolarity. Hepatocytes are thus exposed to moderate alterations of osmolarity [38]. Upon ingestion of water, for instance, liver cells swell and buffer the alterations of blood osmolarity.

Other tissues are exposed to moderately-altered extracellular osmolarity during hypernatriemic or hyponatriemic conditions. Na^+ salts (mainly NaCl) normally contribute more than 90% to extracellular osmolarity and thus hypernatremia is necessarily paralleled by an increase of extracellular osmolarity. Hyponatremia may be associated with increased, normal, or decreased extracellular osmolarity, depending on the concentration of osmotically active organic substances which may reach excessive concentrations in blood [1].

Hypernatremia may result from excessive oral NaCl intake, renal Na^+ retention, and/or renal or extrarenal loss of water [39]. During hypernatremia, extracellular osmolarity is enhanced. Cells trigger mechanisms of regulatory cell volume increase including the accumulation of osmolytes. When extracellular osmolarity increases slowly, cell volume may remain normal despite enhanced extracellular osmolarity. Rapid correction of chronically-enhanced osmolarity may then result in cell swelling. The brain is particularly vulnerable as cerebral betaine, inositol, and glycerophosphorylcholine may remain

elevated for days following correction of extracellular hypertonicity; also, the rapid correction of hyperosmolarity may lead to brain edema [1].

Hyponatremia may be due to excessive oral water load or impaired renal elimination of water [40]. Moreover, hyponatremia may be due to a Na^+ deficit resulting from renal or extrarenal loss. Hyponatremia is not necessarily associated with hypoosmolarity but may occur in isoosmolar or even hyperosmolar states (as in alcohol poisioning), hyperglycemia of uncontrolled diabetes mellitus, or hypercatabolic states (such as burns, pancreatitis, and crush syndrome). In all those disorders, cell shrinkage may prevail despite hyponatriemia.

At decreased extracellular osmolarity, cells trigger mechanisms of regulatory cell volume decrease including the release of organic osmolytes. Rapid correction of hypoosmolar hyponatremia may lead to untoward cell shrinkage as the cells are unable to rapidly reaccumulate the osmolytes. This may be more harmful than untreated hypoosmolarity [1].

INFLUENCE OF EXTRACELLULAR FLUID COMPOSITION ON CELL VOLUME HOMEOSTASIS

Even at constant extracellular osmolarity, cell volume constancy may be challenged (Fig. 2) by alterations of extracellular fluid composition [1]. An increase of extracellular K^+ concentration decreases the chemical gradient for K^+ ions, impedes K^+ efflux, depolarizes the cell membrane, and leads to Cl^- entry. Cellular KCl accumulation may result in cell swelling or, conversely, low extracellular K^+ may lead to cell shrinkage due to the cellular loss of KCl.

Increase of extracellular HCO_3^- concentration blunts cellular HCO_3^- release through anion channels and Na^+,HCO_3^- cotransport; the decreased efflux of negative charge hyperpolarizes the cell membrane thus decreasing the electrochemical gradient for K^+ efflux. The cellular accumulation of K^+ and HCO_3^- then results in cell swelling.

Alkaline extracellular pH stimulates cellular H^+ elimination through the Na^+/H^+ exchanger, leading to cellular Na^+ accumulation and thus to cell swelling.

During hypercapnea, CO_2 enters cells and dissociates to HCO_3^- and H^+. H^+ is extruded by the Na^+/H^+ exchanger, leading to cellular Na^+ accumulation and cell swelling. In general, intracellular acidification stimulates and intracellular alkalinization inhibits the Na^+/H^+ exchanger, leading to cell swelling or shrinkage, respectively.

Several organic anions including acetate, lactate, propionate, or butyrate enter cells as unionized acids. The intracellular dissociation of the acids then leads to intracellular acidification, enhanced Na^+/H^+ exchange, accumulation of Na^+ and organic anions, and thus cell swelling. Isotonic replacement of Cl^- with impermeant anions may lead to cell shrinkage due to cellular Cl^- loss.

Cell volume is further influenced by extracellular urea concentration [1]. Urea readily passes through cell membranes and does not usually create osmotic gradients across the cell membrane. On the other hand, urea destabilizes proteins and thus

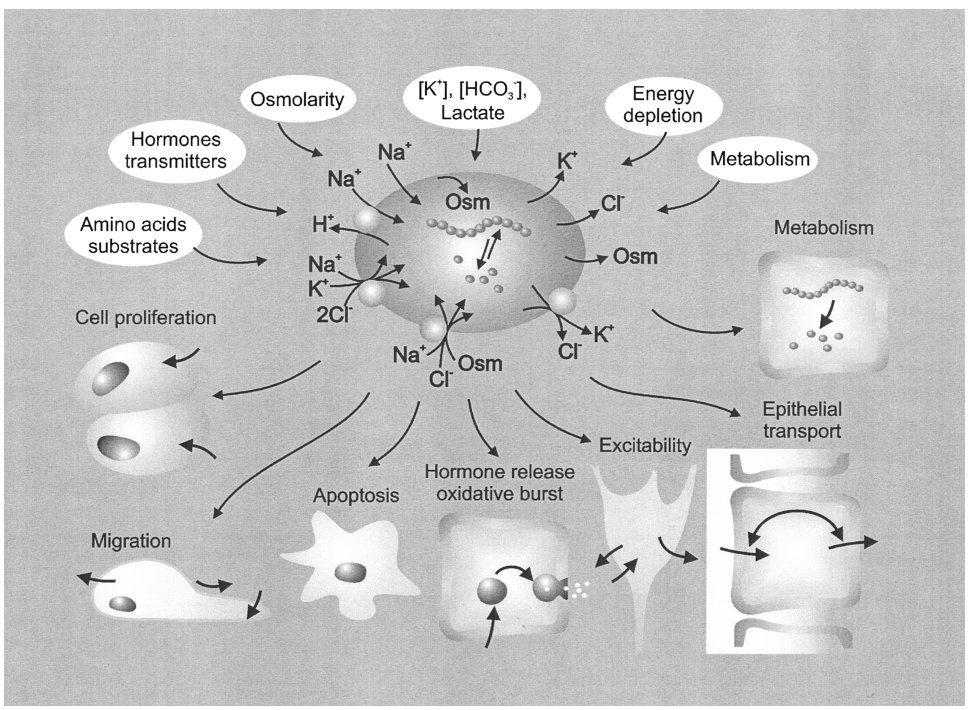

Fig. 2. Functional significance of cell volume.

shifts the cell volume regulatory set point towards a smaller cell volume. Through activation of regulatory mechanisms such as KCl cotransport, urea shrinks cells. Renal insufficiency leads to increase of extracellular urea concentration. The high urea concentrations stimulate the formation of methylamines that counteract urea's perturbing effect. Rapid alterations of urea concentration during dialysis sessions do not allow full adjustment of the osmolyte concentration and thus lead to transient disturbance of the balance between stabilizing osmolytes and destabilizing urea.

TRANSPORT CHALLENGES CELL VOLUME HOMEOSTASIS

The transcellular flux of osmotically active substances during epithelial transport requires the coordination of different transport systems at the apical and basolateral cell membranes (Fig. 2). For instance, Na^+-coupled transport of substrates, such as amino acids or glucose, across the luminal cell membrane of proximal renal tubules or intestine lead to cellular accumulation of Na^+ and substrate [41,42]. In addition, the entry of positive charge depolarizes the cell and impedes the exit of Cl^- and HCO_3^-. The resulting cell swelling is counteracted by activation of cell volume regulatory mechanisms including activation of K^+ channels, which in turn maintain the electrical driving force for Na^+ entry into the cell [41,42]. Na^+ coupled cellular uptake of nutrients challenges similarly cell volume constancy of nonepithelial cells.

Na^+ entry via Na^+ channels of the renal collecting duct and the colon similarly challenges cell volume constancy. Again, activation of K^+ channels serves to maintain cell volume constancy and driving force [1]. In several Cl^- secreting epithelia, activation of Cl^-- and/or K^+ channels decreases intracellular Cl^- activity. The resulting cell shrinkage stimulates Na^+-K^+-$2Cl^-$ cotransport and/or Na^+/H^+ exchanger with Cl^-/HCO_3^- exchanger [1].

EFFECTS OF HORMONES, TRANSMITTERS, AND DRUGS

Several hormones and other mediators alter the volume of their target cells [5]. Insulin swells liver cells by activating both Na^+/H^+ exchange and Na^+-K^+-$2Cl^-$ cotransport; glucagon shrinks hepatocytes, presumably by activation of ion channels [5]. The effect of these hormones on cell volume contributes to their effects on metabolism. For instance, the swelling effect of insulin accounts for its antiproteolytic effect. Conversely, the shrinking effect of glucagon accounts for its proteolytic effect.

Growth factors increase cell volume by stimulating Na^+/H^+ exchange and partially Na^+-K^+-$2Cl^-$ cotransport. The increase of cell volume is a prerequisite for stimulation of cell proliferation [43]. Several excitatory neurotransmitters, such as glutamate, activate Na^+ channels or nonselective cation channels with subsequent Na^+ entry, depolarization, Cl^- entry, and cell swelling. Some inhibitory neurotransmitters, such as GABA, activate K^+ channels and/or anion channels, leading to hyperpolarization, Cl^- exit and thus cell shrinkage [1].

Regulators of epithelial transport may swell or shrink epithelial cells, depending on their effect on their respective ion transport mechanisms. Stimulation of Na^+/H^+ exchange, Na^+-K^+-$2Cl^-$ cotransport, or Na^+ channels lead to cell swelling; stimulation of Cl^-- and/or K^+ channels leads to cell shrinkage [1].

Transforming growth factor beta (TGFβ) stimulates Na^+/H^+ exchanger and Na^+,K^+,$2Cl^-$ cotransport thus leading to cell volume increase. Cell volume increase stimulates protein synthesis and inhibits lysosomal degradation of matrix proteins; this contributes to the enhanced deposition of matrix proteins in conditions with enhanced TGFβ formation, such as fibrosing disease [44].

Cell volume is influenced by a wide variety of drugs and toxins, interfering with cell volume regulatory mechanisms, such as K^+ channels, Na^+-K^+-$2Cl^-$ cotransport and/or Na^+/H^+ exchanger. Their effect on cell volume may contribute to their effect on cellular function [1].

HORMONE RELEASE AND NEUROEXCITABILITY

Cell volume not only participates in the regulation of cell function by hormones, but also regulates hormone release [45]. The release of several hormones is triggered by cell swelling and inhibited by cell shrinkage. The link between cell volume and hormone release is ill-defined but partially involves cell volume sensitive alterations of cytosolic Ca^{2+} activity [45]. Similarly, neuroexcitability critically depends on cell hydration [46]. Accordingly, increased plasma osmolarity decreases, and decreased plasma osmolarity increases, the susceptibility to epileptic seizures [47,48].

IMPACT OF METABOLISM ON CELL VOLUME

Degradation of proteins to amino acids, glycogen to glucose phosphate, or triglycerides to glycerol and fatty acids increases the number of osmotically active particles and thus increases intracellular osmolarity. The degradation of the substrates to CO_2 and H_2O decreases intracellular osmolarity [1].

Glycolysis leads to cellular accumulation of lactate and H^+, subsequent activation of the Na^+/H^+ exchanger, and cell swelling [1]. In addition, metabolic pathways may influence cell volume indirectly through alteration of transport across the cell membrane. In cells expressing ATP-sensitive K^+ channels, a decrease of cellular ATP could activate those channels and thus lead to cell shrinkage. Cellular formation of peroxides may

shrink cells by activation of oxidant sensitive K^+ channels or by inhibition of oxidant sensitive Na^+-K^+-$2Cl^-$ cotransport. On the other hand, oxidation inhibits Kv1.3 K^+ channels and KCNE1/KCNQ1 K^+ channels in a variety of tissues, effects rather increasing cell volume [1].

In liver insufficiency, the impaired formation of urea leads to accumulation of NH_3, which enters the brain, is taken up by glial cells, stimulates cellular formation and accumulation of glutamine, and thus results in glial cell swelling. Glial cells release myoinositol to counteract swelling. Glial cell swelling is apparently a major cause for the development of hepatic encephalopathy [49–51].

Diabetic ketoacidosis leads to cellular accumulation of organic acids and cellular acidity that stimulates Na^+/H^+ exchange activity. Moreover, hyperglycemia stimulates cellular formation and accumulation of sorbitol from glucose, through aldose reductase [52] which results in cell swelling. Hyperglycemia further leads to formation of advanced glycation end products which similarly induce cell swelling. To compensate for cell swelling, the cells release osmolytes such as myoinositol. This cell swelling leads to antiproteolysis, which may add to the excessive disposal of matrix proteins. On the other hand, hyperglycemia is paralleled by hyperosmolarity, which may lead to shrinkage and subsequent activation of Ca^{2+} entry in some cells [53]. At least partly through cell shrinkage, hyperglycemia increases the expression of SGK1, which in turn participates in the stimulation of matrix protein formation and thus diabetic nephropathy [54].

Several hypercatabolic states, such as burns, acute pancreatitis, severe injury, or liver carcinoma are paralleled by a decrease of muscle cell volume correlating with urea excretion, an indicator of protein degradation [55]. The decrease of cell volume may play a causal role for the triggering of hypercatabolism. Accordingly, hypercatabolism can be reversed by glutamine, which enlarges cells via Na^+ coupled cellular uptake.

CELL MIGRATION

Cell volume regulatory mechanisms participate in the locomotion of cells [56]. During cell migration, water enters at the leading edge and exits at the rear end. The water movement is driven by osmotic gradients generated by volume regulatory transport. NaCl enters at the leading edge via Na^+,K^+,$2Cl^-$ cotransport and Na^+/H^+ exchange in parallel to Cl^-/HCO_3^- exchange; ions exit at the rear end via K^+ and Cl^- channels.

CELL PROLIFERATION AND APOPTOTIC CELL DEATH

Cell volume participates in the cellular machinery driving cell proliferation [57–59]. Mitogenic factors stimulate Na^+/H^+ exchange and in some cells Na^+-K^+-$2Cl^-$ cotransport [43]. Activation of those carriers may lead to a shift of the set point for cell volume regulation towards greater volumes. The Na^+/H^+ exchange further leads to cellular alkalinization. As cell shrinkage and cytosolic acidity inhibit cell proliferation, the stimulation of the Na^+/H^+ exchanger is required for the stimulation of cell proliferation in acid and/or hypertonic extracellular environment.

Cell shrinkage is one of the hallmarks of apoptotic cell death [1,60,61]. Cell shrinkage further parallels suicidal erythrocyte death, i.e. eryptosis [62] which is similar to senescence [63,64], and neocytolysis [65] which leads to clearance of circulating erythocytes. Apoptotic cell shrinkage is accomplished by adjustment of the respective cell volume regulatory mechanisms such as activation of Cl^- and/or K^+ channels, stimulation of organic osmolyte release, and inhibition of the Na^+/H^+ exchanger [1,15,60,66–68].

Marked osmotic cell shrinkage triggers apoptotic cell death, which may involve PGE_2-sensitive Ca^{2+}-permeable cation channels [69]. A moderate decrease of cell volume (<30%) leads to a blunting of receptor (CD95-) triggered apoptotic cell death [1]. The latter effect is apparently due to interference with the CD95 signaling, such as cellular O_2^- formation.

SICKLE CELL ANEMIA

In sickle cell disease, a point mutation of hemoglobin (HbS) favors polymerization of desoxyhemoglobin, which dramatically decreases erythrocyte deformability [70]. As a result, blood viscosity is increased, which leads to a severe disturbance of microcirculation. The polymerization of hemoglobin is favored by cell shrinkage following excess extracellular osmolarity, activation of KCl cotransport by urea, or activation of Ca^{2+}-sensitive K^+ channels by increase of intracellular Ca^{2+} activity [70,71]. The high osmolarity and urea concentration in kidney medulla tissue contribute to the particular vulnerability of this tissue to ischemia in sickle cell anemia.

INFECTION

Immune defense mechanisms are dependent on cell volume in several ways [1]. The proliferation, migration, and O_2^- formation of leukocytes, phagocytosis, and matrix deposition are all cell volume-sensitive mechanisms. The impaired immune defense in hypertonic kidney medulla thus renders this tissue especially vulnerable for infections [1]. Moreover, cell volume constancy is challenged by intracellular pathogens. The cell volume-dependent survival of host cells critically determines the course of infectious disease [72]. Apoptotic cell death leads to phagocytosis and degradation not only of host cells but also of the pathogen. Thus, inability of host cells to undergo apoptosis is associated with a particularly severe

course of the disease [73]. To the extent that cell volume-regulatory mechanisms participate in the machinery leading to cell death, they influence the eventual outcome of infections.

CONCLUSIONS

Cells are equipped with a diverse array of cell volume regulatory mechanisms (Fig. 1), which adjust cell volume to functional demands. Those mechanisms are under the control of even more diverse signaling pathways. Many, but by far not all, of those are known at the molecular level. A myriad of extracellular and intracellular mechanisms challenge cell volume constancy. Conversely, cell volume and cell volume-sensitive cellular functions participate in a wide variety of physiological and pathophysiological mechanisms (Fig. 2). While there is little doubt that cell hydration is an important determinant of cell performance, many cell volume sensitive molecular mechanisms have remained elusive and the physiological and pathophysiological role of cell volume regulation in integrated function is frequently unrecognized or poorly understood. Thus, extensive further experimental effort is required to define the role of cell volume in health and disease.

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