Cell Volume: A Second Message in Regulation of Cellular Function

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To survive, cells must avoid excessive alterations of cell volume. Thus cells have acquired a variety of strategies to main constancy of their volume, including the modification of ion flux across cell membranes and metabolic pathways. Hormones exploit these mechanisms for regulation of cellular function.

To fulfill their metabolic functions, cells accumulate a number of osmotically active substances such as amino acids and metabolites of glycolysis. The cellular osmolarity thus created must be counterbalanced by electrolytes (12).

That is, cells extrude Na^+ in exchange for K^+, accomplished by the Na^+-K^+-ATPase. K^+ thus accumulated tends to exit the cell through K^+ channels, leaving a cell-negative potential across the cell membrane. This cell membrane potential drives anions such as Cl^- into the extracellular space. As a result, Cl^- is some 80 mM lower within compared with outside the cell, thus counterbalancing the accumulation of osmotically active organic substances.

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Maintenance of the electrolyte distribution across the cell membrane requires expenditure of energy in the form of ATP. Thus any energy depletion will eventually result in a decrease of cellular K^+ and cell membrane potential and an increase of intracellular Cl^- and cell swelling. The constancy of cell volume is further compromised by increasing extracellular K^+ concentration, which leads to depolarization of the cell membrane. Obviously, any alteration of extracellular osmolarity causes an osmotic imbalance across the cell membrane. Constancy of cell volume is further challenged by ion transport across the cell membrane, which occurs during ion channel activation. The expectation is that activation of Na^+ channels will cause cells to swell, whereas the activation of K^+ and/or Cl^- channels will cause them to shrink.

Moreover, cellular osmolarity is increased by concentrative uptake of substrates such as amino acids or glucose and by the breakdown of proteins, glycogen, or triglycerides, which are osmotically less active than the sum of their constituent parts (7, 8, 12). Conversely, release of substrates and formation of proteins, glycogen, or triglycerides decrease cellular osmolarity.

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Volume regulatory ion fluxes

To cope with the challenges listed above, the cell utilizes a number of volume regulatory mechanisms (12). The most efficient means of regulating cell volume is by ion transport across the cell membrane.

Upon swelling, most cells release K^+ and Cl^- through the activation of K^+ channels and/or anion channels. Anion channels allow the passage not only of Cl^- but also of other anions such as HCO_3^- and even negatively charged amino acids. Less frequently, cells release ions through KCl symport, parallel K^+/H^+ exchange and Cl^-/HCO_3^- exchange (leading to KCl loss), as well as Na^+/Ca^{2+} exchange in parallel with Ca^{2+}-ATPase (leading to loss of Na^+).

After cell shrinkage, the cells accumulate ions through Na^+-K^+-$2Cl^-$ cotransport and parallel operation of Na^+/H^+ exchange and Cl^-/HCO_3^- exchange (leading to gain of NaCl). Activation of Na^+-K^+-ATPase leads to replacement of accumulated Na^+ with K^+. Ion loss through ion channels is minimized by inhibition of K^+ and/or Cl^- channels.

Metabolic pathways sensitive to cell volume

Cell swelling inhibits the breakdown of proteins and glycogen and therefore the production of monomers, which are osmotically more active. Protein and glycogen synthesis is stimulated (7, 9, 13). Thus amino acids and hexosephos-

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phates are converted into the osmotically less active macromolecules.

Cell shrinkage, on the other hand, stimulates proteolysis and glycogenolysis. Furthermore, cell shrinkage stimulates the formation and cellular accumulation of so-called osmolytes, including polyols such as sorbitol, glycerol, and inositol; the amino acid taurine; and methylamines, such as glycerophosphorylcholine. These substances are metabolically largely inert and are thought to serve mainly to create intracellular osmolarity (6). However, the influence of cell volume is not restricted to the metabolism of macromolecules and osmolytes.

Cell swelling inhibits glycolysis and stimulates flux through the pentose phosphate pathway. The latter probably enhances the availability of NADPH and thus protects the cell from oxidative stress (7). Glutathione formation and efflux into blood are enhanced (7). Further metabolic functions stimulated by cell swelling include glycine oxidation, glutamine breakdown, formation of NH_4^+ and urea from amino acids, ketoisocaproate oxidation, and lipogenesis from glucose (7, 9).

The mRNA for phospho*enol*pyruvate carboxykinase, a key enzyme for gluconeogenesis, is decreased by cell swelling (7). Cell volume modifies the cytoskeleton, whereby cell swelling stabilizes the microtubule network, stimulates actin polymerization, and increases mRNA for β-actin and tubulin (7). Most of these metabolic functions are influenced in the opposite direction by cell shrinkage.

Hormones modify cell volume and thus metabolism

The metabolic pattern stimulated by cell swelling can be triggered by hormones that increase cell volume. In hepatocytes, insulin stimulates Na^+-K^+-$2Cl^-$ cotransport and Na^+/H^- exchange, thus leading to accumulation of NaCl and KCl and subsequent cell swelling (1, 7). It is exclusively the influence of the hormone on cell volume that mediates its antiproteolytic action in the liver. That is, the antiproteolytic action of insulin is abolished in the presence of inhibitors of Na^+-K^+-$2Cl^-$ cotransport and Na^+/H^+ exchange and under conditions in which cell swelling is reversed by the increase of extracellular osmolarity (7).

Glucagon, on the other hand, stimulates ion channels and thus shrinks the cells to trigger proteolysis. The same effect is exerted by adenosine 3',5'-cyclic monophosphate (7). Another mediator shown to swell hepatocytes is phenylephrine, whereas ATP and adenosine have been shown to shrink hepatocytes (7). Peroxides similarly activate ion channels and shrink the cells (7).

pH in acidic cellular compartments: a third messenger

The link between cell volume and metabolism has been elusive until very recently, when it was observed that cell volume modifies pH in acidic cellular compartments (15). As revealed by acridine orange and fluorescein isothiocyanate-dextran fluorescence studies, cell swelling leads to alkalinization of acidic cellular compartments, whereas cell shrinkage enhances the acidity in these compartments (Fig. 1). In hepatocytes, this effect probably accounts for or contributes to the antiproteolytic action of cell volume, since the lysosomal proteases are known to have their pH optima in the acidic range and alkalinization of the lysosomes is well known to inhibit hepatic proteolysis.

The alkalinization of acidic cellular compartments in hepatocytes occurs not only if cell swelling is imposed by a decrease of extracellular osmolarity but also when cell swelling is caused by inhibition of K^+ channels and by concentrative uptake of amino acids. Because the effect of cell volume on proteolysis (7), bile acid excretion (7), and pH in hepatocellular acidic cellular compartments (4) is partially inhibited by colchicine and colcemid, the signal from cell volume to the acidic cellular compartments likely involves microtubules (4).

It appears that the influence of cell volume on the pH of acidic cellular compartments is not confined to lysosomes in hepatocytes but involves a number of distinct compartments in various cells, such as pancreatic β-cells, glial cells, neurons, smooth muscle cells, cardiac myocytes, proximal renal tubules, Madin-Darby canine kidney cells, pulmonary alveolar cells, lymphocytes, and fibroblasts (Völkl and Busch, unpublished observations). Accordingly, the functions of these compartments may be modified by alterations of cell volume.

In pancreatic β-cells, for instance, acidic proteases within the acidic secretory granules cleave proinsulin to yield insulin, a function probably compromised by cell swelling and fostered by cell shrinkage. Furthermore, the release of insulin may be modified by the luminal pH of the secretory granules and thus be sensitive to alterations of cell volume.

In neurons, metabolism, uptake, and release of neurotransmitters may be modified by the luminal pH of synaptic vesicles. For instance, the uptake of neurotransmitters, such as catecholamines, glutamate, γ-aminobutyric acid

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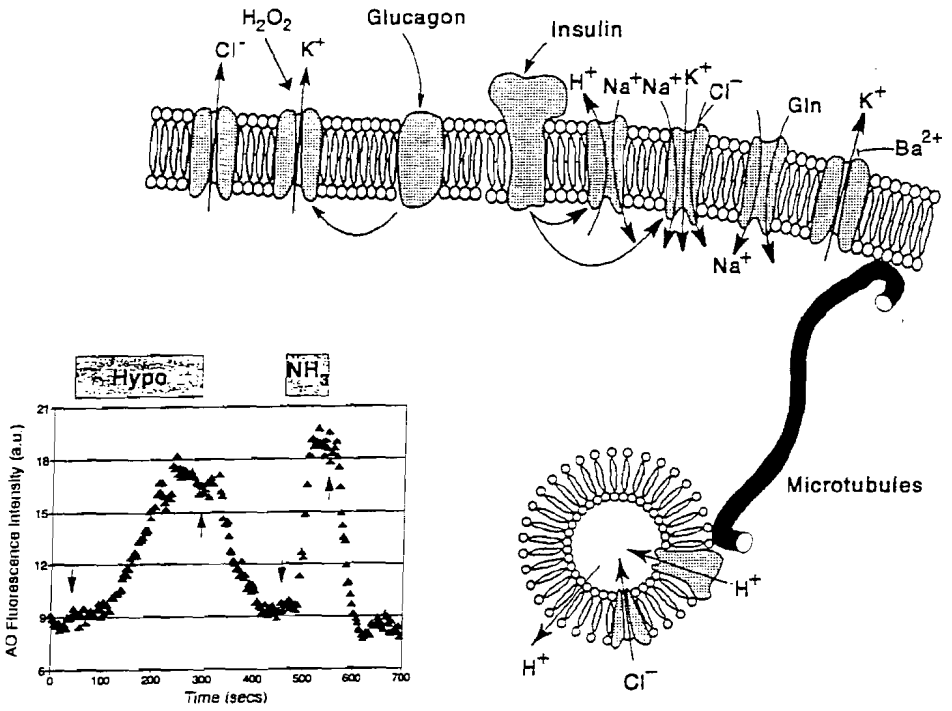

FIGURE 1. *Tentative model illustrating effect of insulin, Ba^{2-}, and glutamine as well as glucagon and peroxides on lysosomal pH in hepatocytes. Insulin, Ba^{2-}, and glutamine swell cells, whereas glucagon and peroxides shrink cells. Via cell volume, maneuvers influence luminal pH of acidic cellular vesicles, an effect mediated by microtubules. Inset: effect of osmotic cell swelling (hypo = 25% reduction of osmolarity) compared with ammonia (NH_3 = addition of 2 mmol NH_4Cl) on acridine orange (AO) fluorescence, which increases on alkalinization of acidic cellular compartments.*

(GABA), and acetylcholine, into small synaptic vesicles is driven by a proton gradient between the cytosol and the acid lumen (5). In a wide variety of cells, trafficking of vesicles and/or receptors may also be modified by luminal pH.

In tracheal epithelium, sulfation and sialation of membrane proteins have been shown to be sensitive to luminal pH of acidic cellular compartments. Thus it has been argued that defective acidification of these compartments in cystic fibrosis leads to altered sulfation and sialation and thus to enhanced adhesion of *Pseudomonas aeruginosa* (3). Whether cell volume modifies the pH in these compartments has not been tested thus far.

It must be pointed out, however, that not all effects of cell volume changes are secondary to altered pH in acidic cellular compartments. Other proposed mechanisms include macromolecular crowding (14) as well as intracellular Ca^{2-} and eicosanoid formation (12).

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Cell volume participates in the regulation of cellular function other than metabolism

As pointed out above, the studies on volume sensitivity of pH in acidic cellular compartments suggest a role for cell volume in the regulation of hormone and transmitter release. Furthermore, the activation of ion channels may influence cell membrane potential and thus voltage-sensitive Ca^{2+} channels. In pancreatic β-cells, cell swelling leads to transient hyperpolarization (by activation of K^- channels) followed by depolarization (possibly due to activation of anion channels) (S. H. Britsch, G. Drews, P. Krippeit-Drews, and F. Lang, unpublished observations). The depolarization leads to activation of voltage-sensitive Ca^{2-} channels, Ca^{2+} entry, and transient stimulation of insulin release.

A similar sequence of events, i.e., anion channel activation, depolarization, and activation of voltage-sensitive Ca^{2+} channels, leads to contraction of vascular smooth muscle cells upon cell swelling (J. H. Dietlefsen, H. Heinke, and F. Lang, unpublished observations). In neurons, volume regulatory mechanisms may similarly modify cell membrane potential and excitability. In sympathetic neurons, for example, activation of Cl^- channels leads to sustained depolarization of the cell membrane. The cellular ion loss leads to cell shrinkage, which in turn activates Na^+-K^+-$2Cl^-$ cotransport. The ion uptake through this carrier maintains not only cell volume but also cellular Cl^- activity. If the carrier is inhibited by furosemide, cellular Cl^-

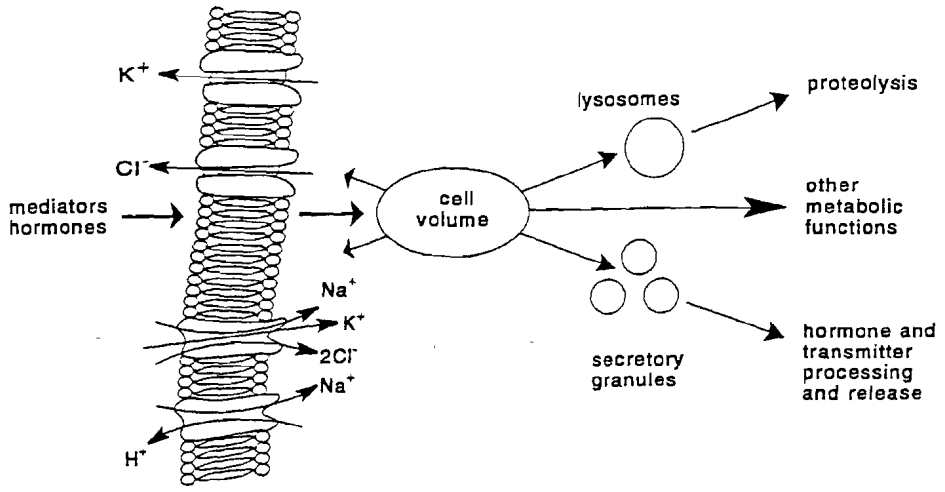

FIGURE 2. Cell volume as an element in signal transduction of hormones and mediators.

declines to equilibrium and the GABA-induced depolarization is only transient (2).

In epithelial cells, volume regulatory activation of ion channels modifies the electrical driving force and thus transepithelial transport (12). Furthermore, cell volume may modify the incorporation and/or activation of transporters, as has been suggested for bile acid transport in the liver (7).

Cell volume regulatory mechanisms may further be involved in complex functions of the cell such as cell proliferation or apoptosis. A variety of growth promoters stimulate Na^+/H^+ exchange, one of the two ion transporters serving to increase cell volume during cell shrinkage (12). Furthermore, in certain cells, growth promoters have been shown to activate the Na^+-K^+-$2Cl^-$ cotransporter, the other carrier increasing cell volume (12). Accordingly, the expression of *ras* oncogene has been demonstrated to shift the set point for cell volume regulation toward greater cell volume (12) and to increase the pH in lysosomes (10).

Apoptosis, defined as programmed cell death, is accompanied by marked cell shrinkage (11). The role of cell volume in the complex machinery to stimulate cell proliferation or apoptosis is ill defined, but the metabolic sequelae of alterations of cell volume may contribute to the adjustment of cellular function to produce proliferation or apoptosis.

Conclusions and synopsis

Alterations of cell volume markedly influence a variety of metabolic functions, epithelial transport, hormone and transmitter release, and possibly complex functions such as cell proliferation or apoptosis (Fig. 2). They do so, in part, by changing the pH within acidic cellular compartments. Hormones such as insulin, glucagon, and growth factors influence cellular functions by stimulating volume regulatory ion flux across the cell membrane. Thus it is appropriate to consider cell volume as a second messenger in the regulation of cellular function.

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