

Osmosensory Mechanisms in Cellular and Systemic Volume Regulation

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ABSTRACT

Perturbations of cellular and systemic osmolarity severely challenge the function of all organisms and are consequently regulated very tightly. Here we outline current evidence on how cells sense volume perturbations, with particular focus on mechanisms relevant to the kidneys and to extracellular osmolarity and whole body volume homeostasis. There are a variety of molecular signals that respond to perturbations in cell volume and osmosensors or volume sensors responding to these signals. The early signals of volume perturbation include integrins, the cytoskeleton, receptor tyrosine kinases, and transient receptor potential channels. We also present current evidence on the localization and function of central and peripheral systemic osmosensors and conclude with a brief look at the still limited evidence on pathophysiological conditions associated with deranged sensing of cell volume.

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Osmotic water flux across the plasma membrane, resulting in altered cellular volume and ionic strength, severely affects cell function. Most vertebrates counteract such perturbations by maintaining a remarkably stable osmolarity in the extracellular fluid (ECF; in mammals, close to 300 mOsm) and by possessing a variety of generally indirect mechanisms of volume regulated ion transport that allow individual cells to monitor and recover their volume following osmotic swelling or shrinkage. The purpose of this review is to outline the current evidence on how cells sense volume perturbations, with particular focus on mechanisms relevant to the kidneys and to ECF osmolarity/whole body volume homeostasis. The signaling events downstream from the osmosensor and the volume regulatory ion transport proteins involved in the process of regulatory volume decrease (RVD) and regulatory volume increase (RVI) after osmotic swelling and shrinkage, respectively, have been reviewed in detail elsewhere.^{1–4} We also dis-

cuss a variety of pathophysiological consequences to disturbances in cell-volume sensing.

Two terms are used to describe the osmotic relation between the cell and its surroundings: osmolarity and tonicity. Osmolarity is an absolute term that can be measured typically by freezing-point depression, whereas tonicity is defined by the osmotic gradient across a membrane; by definition, *hypertonic* exposure causes cell shrinkage, and *hypotonic* exposure causes cell swelling. In contrast, exposure of a cell to a fully permeable osmolyte has an osmotic effect, but no tonicity effect, and does not alter cell volume. In this review, we will use the terms osmosensor and osmosensing unless there is direct experimental evidence that the modality sensed is tonicity that perturbs cell volume. Tonicity will be used for describing the experimental perfusion of a cell with an anisotonic solution or for processes in which a direct dependence on cell volume, rather than osmolarity, is established.

BASAL MECHANISMS IN CELLULAR VOLUME SENSING

Arguably the most fundamental issue in osmosensing is precisely what is sensed when a cell is exposed to osmotic stress. Surprisingly, this issue is still not resolved completely. At least three types of osmotic stress signals can be identified in eukaryotic cells: changes in macromolecular crowding, ionic strength, and mechanical or chemical changes in the lipid bilayer or the extracellular matrix (ECM) and the cytoskeleton to which it is tethered.^{1,5–7} Osmosensory systems respond to these signals, resulting in a series of signal transduction events that, in turn, activate volume regulatory, protective, and adaptive events.¹ All three types of osmotic stress signals can be part of the osmosensory machinery in various model systems, and, in some cases, several signals may contribute to a given response, as examples that follow will show.

A poorly understood issue in volume sensing is where, subcellularly, the sensors are located and how that impacts on the signals that can be perceived. As discussed in detail elsewhere,¹ altered curvature and/or composition of the plasma membrane can initiate a number of sig-

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naling events central to the cellular sensing of osmotic stress. However, while altered membrane stretch or curvature is seemingly an attractive mechanism for volume sensing, many cells have such a large membrane reserve that this mechanism is not likely to be globally relevant.^{1,8} On the other hand, proteins located in specific subcellular domains, such as caveolae or lipid rafts, may well experience altered stretch or curvature relevant to osmosensing.^{9–12} Moreover, several volume-sensitive transporters and channels are highly sensitive to membrane curvature and lipid composition.^{7,13–16}

Another subcellular domain of interest in the context of osmosensing is the *primary cilium*, a microtubule-based structure emanating from the mother centriole of most cells in the body controlling essential cellular functions.^{17,18} The primary cilium contains several complexes of transient receptor potential (TRP) channels, with proposed roles in volume sensing and signaling (see *Transient Receptor Potential Channels (TRPs) and Mechanosensitive Channels*). The primary cilium has also been assigned an essential role in renal flow sensing,^{19,20} although this has been partially challenged recently.^{21,22}

Sensors, Transducers, and Effectors?

In fungi, two-component histidine kinase systems initiate the osmosensory re-

sponse.²³ In higher eukaryotes, this system is lacking and a number of others are employed. These are often parsed in terms of the primary volume *sensor*, the signaling events transmitting the signal (the *transducers*), and the mechanisms actually mediating the volume regulatory, protective, and adaptive events in response to osmotic stress (the *effectors*). In many cases, however, it is hard to separate the sensor from the transducer, an example being the cytoskeleton (see below), and some effectors likely directly sense the osmotic or mechanical perturbation. Examples include stretch-activated channels such as two-pore K⁺ channels,^{15,16} as well as, possibly, the transcription factor TonEBP (see below). Figure 1 illustrates some mechanisms through which a membrane protein may be activated by osmotic or mechanical perturbations. Further details are provided below.

The Cytoskeleton in Osmosensing

Rapid, extensive reorganization of the actin cytoskeleton in response to osmotic stress has been demonstrated in a wide variety of cell types.^{24–30} The specific reorganization pattern varies between cell types but typically involves a net increase in F-actin content in the cortical region, loss of stress fibers, and increased length of F-actin protrusions in hypertonically shrunken cells, and the reverse pattern in swollen cells.¹ The mechanisms involved in this reorganization are complex; however, sev-

eral important moieties have been identified, including Rho family G proteins,^{31,32} cofilin,³³ the ezrin/radixin/moesin (ERM) proteins ezrin³⁴ and moesin,³⁵ nonmuscle myosin II,³⁶ cortactin, and the WASP/Arp2/3 system.^{28,37} Recent data also suggests the microtubule-based (MT) cytoskeleton is regulated by osmotic shrinkage.^{1,38,39} In fibroblasts and retinal pigment epithelial cells, we find that, in marked contrast to the actin cytoskeleton, MTs collapse upon hyper-osmotic stress and the MT plus the TIP protein end-binding protein (EB1) are lost from the tips, whereas hypotonic stress is associated with increased MT polymerization and EB1 tip-tracking.³⁹ Is the cytoskeleton osmosensory, then? The sequence of events from osmotic volume perturbations to cytoskeletal reorganization is incompletely elucidated. However, available evidence suggests that, after osmotic shrinkage, very early events include changes in cellular PtdIns(4,5)P₂ content, which can lead to changes in ERM protein activity and, in turn, to altered Rho activity,^{34,40} and which could also, tentatively, be upstream of the shrinkage-induced change in cofilin and WASP/Arp2/3 activity.¹ Volume-dependent integrin activation (see below) could also be upstream of osmotic changes in Rho activity through p190RhoGAP, which is known to couple integrin activation to Rho.⁴¹

The cytoskeleton clearly contributes to the osmosensitivity of some ion transport proteins. An important example is TRPV4-mediated Ca²⁺ influx, which is part of the signaling activated by hypotonic stress in many epithelial cells and is F-actin dependent.¹ Another is the Na⁺/K⁺/2Cl⁻ cotransporter, NKCC1, an important player in RVI, which is dependent on F-actin both for quiescence in the unstimulated state and for activation by cell shrinkage.^{32,42,43}

Integrins as Cell Volume Sensors

Integrin receptor heterodimers (α , β subunits) associate with a large array of cytoskeletal and signaling proteins to form Focal Adhesions (FAs). FAs are major cellular signaling hubs that link the actin cytoskeleton to integrins, and thus the ECM, playing an essential role in the regulation of cell survival, migration, and proliferation.⁴⁶ In

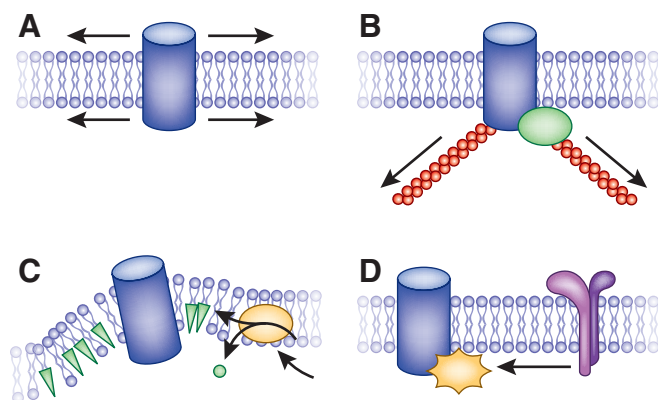


Figure 1. Mechanisms through which a membrane protein may sense cell volume perturbations. (A) Direct stretch-sensitivity. (B) Direct or indirect tethering to the cytoskeleton. (C) Changes in membrane curvature, either mechanical or through changes in membrane composition. (D) Via interaction with integrins activated by the cell volume perturbation. See text for examples and details.

our view, it has yet to be proven whether integrins can act as primary osmosensors.¹ However, integrins are clearly activated rapidly after cell volume perturbations in many cell types and have been proposed to serve as volume sensors both after swelling^{47–51} and after shrinkage.^{52–55}

For instance, in the rat liver, cell swelling increases the plasma membrane level of activated β 1-integrin.⁵⁶ Interestingly, integrins form macromolecular complexes with certain ion channels, constituting a platform for downstream signals depending on both integrin signaling and channel activity.^{57,58} Several volume-sensitive K^+ channels, including $K_v1.3$ ^{59,60} and $K_v11.1$,^{57,61} are regulated by β 1 integrins and, in turn, couple to other signaling molecules, and, for $K_v11.1$,⁶² also to growth factor and chemokine receptors.⁵⁷ In myocytes, integrin stretch activates the volume-regulated anion channel (VRAC) through activation of focal adhesion kinase (FAK), Src, and the receptor tyrosine kinase (RTK) EGF receptor (EGFR, also known as ErbB1R).^{47–49} Finally, integrins are involved in the hypertonicity-induced increase in expression of tonicity-responsive enhancer-binding protein (TonEBP).⁵⁵

Upon integrin activation, the nonreceptor tyrosine kinase, FAK, which is a central component of the FA, is recruited and undergoes phosphorylation on Tyr³⁹⁷ in the N-terminal domain.⁶³ This recruits Src,⁶⁴ phosphatidylinositol 3-kinase (PI-3K), and phospholipase C γ (PLC- γ), and subsequent phosphorylation of Tyr⁹²⁵ promotes binding to the RTK-bound adaptor, Grb2.⁶⁵ Thus, FAK is an important coordinator of integrin and RTK-mediated cellular signaling events. Other important phosphorylation sites on FAK are Tyr⁵⁷⁶ and Tyr⁵⁷⁷ in the kinase domain,⁶⁶ and Tyr⁸⁶¹ between the kinase domain and the Focal adhesion Targeting (FAT) domain.⁶⁷ Hypotonic stress stimulates phosphorylation of tyrosine residues on FAK in several cell types,^{68–71} in a manner reported to depend on Rho family G proteins,⁶⁸ or on the RTK, ErbB4.⁷¹ As noted above, in myocytes, FAK modulates VRAC after integrin stretch, yet this mechanism is distinct from that of swelling-activation of VRAC.^{47,72} Moreover, in endothelial cells, there seems to be no involvement of FAK

in activation of VRAC.⁷³ Hyper-osmotic stress likewise stimulates FAK phosphorylation in some cell types.^{74–77} FAK Tyr⁸⁶¹ phosphorylation is stimulated by hyper-osmotic stress in many cell types, including epithelial cell lines, in which the phosphorylation is inhibited by Src inhibitors but insensitive to F-actin disrupting agents.⁷⁶ Finally, we find that in NIH 3T3 fibroblasts, hyper-osmotic stress induces a rapid, transient increase in Src-dependent FAK phosphorylation at Tyr⁵⁷⁶ and Tyr,⁸⁶¹ and independent phosphorylation at Tyr³⁹⁷ (B. Jørgensen, SFP, EKH, unpublished).

Receptor Tyrosine Kinases (RTKs)

Hypotonic swelling activates EGFR in several cell types,^{78–81} and interplay between integrins, FAK, Src and EGFR in swelling-activation of VRAC has been proposed.⁴⁹ Hypertonic shrinkage phosphorylates both EGFR and the insulin receptor in some cell types, and shrinkage-induced receptor clustering has been proposed as a mechanism of osmosensing.⁸² On the other hand, cell shrinkage inhibits some RTKs, including PDGF receptor β (PDGFR β), resulting in reduced Akt and extracellular signal regulated kinase (ERK) activity.^{83,84} Moreover, in hypertonically stressed rat hepatocytes, EGFR activation is downstream of a Src family kinase,⁸⁵ and in Vero kidney cells, shrinkage-inhibition of EGFR signaling is downstream of Ras.⁸³ Thus, EGFR is clearly not a primary osmosensor in all cell types. However, the role of RTK regulation as an early signal in the osmotransduction process is well established. Interaction between RTKs and integrins probably contributes to the complexity, both because of the many converging signaling pathways, as noted above, and because integrin activation is known to transactivate RTKs.^{49,86,87}

Transient Receptor Potential Channels (TRPs) and Mechanosensitive Channels

The mammalian TRP channels are divided into six subfamilies: the canonical (TRPC), vanilloid (TRPV), melastatin (TRPM), mucolipin (TRPML), ankyrin (TRPA1), and polycystin (TRPP) families.^{88–91} The selectivity of TRPs range from cation nonselective to highly Ca^{2+} -selective.^{88,91} Several TRPs are sensitive to cell vol-

ume and/or to membrane expansion or stretch.^{1,7,92,93} Some of these, such as TRPC1, TRPC6, TRPV4, and TRPM7, are ubiquitously expressed and might be volume sensors.^{88,89,91} While a detailed discussion of this is outside the scope of this review, it is important to note that experimental separation of osmosensitivity and mechanosensitivity is challenging, as is determination of the precise stimulus that triggers activation of a channel in response to cell swelling or membrane stretch.^{7,94} Hence, whether swelling or stretch regulates TRP activity is not always clear. The TRPV family is, by far, the best studied in the context of volume sensing. TRPV1 is unequivocally shown to be involved in osmosensing, as indicated from studies of *TRPV1*^{-/-} mice⁹⁵ (see below). When expressed in yeast, however, TRPV1 responds to heat but not to hypotonicity, leading to the suggestion that TRPV1 may not be directly osmosensitive or mechanosensitive.⁹⁶ It is, however, shown that an N-terminal variant of TRPV1 is essential for osmosensory transduction in mouse SON neurons⁹⁷ (see also below). TRPV4, the mammalian homologue of *C. elegans* OSM-9, is clearly a swelling-activated channel.^{95,98–100} Thus, TRPV4 mediates swelling-activated Ca^{2+} influx,^{101,102} cells from *TRPV4*^{-/-} mice have reduced RVD rates,⁹⁵ and mammalian TRPV4 rescues mechanosensitive and osmosensitive defects in OSM-9 mutants.¹⁰³ In mammalian cells, hypotonic swelling activates phospholipase A₂,¹⁰⁴ releasing arachidonic acid (AA), and swelling-activation of TRPV4 is dependent on the AA metabolite 5', 6'-epoxyeicosatrienoic acid (EET).^{105–108} Interestingly, although yeast cannot synthesize EET, TRPV4 also responds to hypotonicity when expressed in yeast.⁹⁶ This may suggest that direct mechanosensitivity of TRPV4 is possible under some conditions. The mechanism is unclear, as it was previously shown that swelling-activation of TRPV4 was independent of direct membrane stretch.^{98,109,110} TRPV4 activity is central to actin cytoskeleton-dependent RVD,¹¹¹ and forms a supramolecular complex containing regulatory kinases and cytoskeletal proteins.¹¹² Furthermore, TRPV4 is activated after integrin activation by stretch.¹¹³ While a detailed discussion of this

will not be given here, a number of other protein-protein interactions appear to be involved in the osmosensitivity of TRPV4, including With No Lysine kinase (WNK4)¹¹⁴ and aquaporin 5.^{115,116}

TRPM7 is also proposed to be activated both by mechanical stress¹¹⁷ and by osmotic swelling, and knockdown of TRPM7¹¹⁸ attenuates RVD. Finally, TRPC1 and TRPC6 are reported to be mechanosensitive^{119,120}; however, this has been disputed recently.^{121,122} It is possible that stretch sensitivity of these TRPs is indirect and requires components that are differentially expressed in different cell types.¹²³ In accordance with this suggestion, TRPs interacts with many molecules of relevance to osmosensing, such as PLC γ -1,¹²⁴ PtdIns(4,5)P₂, aquaporin 5, various protein kinases, and cytoskeletal proteins.^{88,91,124,125} For instance, for TRPC6/3, the angiotensin II receptor (AT1R) acts as the primary mechanosensor upstream of the channel.¹²⁵ Nonetheless, it is a contentious issue whether mammalian TRPs are directly mechanosensitive.^{7,126} Genetic screens in *C. elegans*^{127,128} and *D. melanogaster*¹²⁹ have identified mechanosensitive TRPs.¹³⁰ In bacteria, stretch-activated, nonselective cation channels (SACs) have been cloned and analyzed in detail.^{94,131} Nonselective cation currents activated by cell swelling and stretch has also been demonstrated in cells from vertebrate organisms.^{132–134} Recently, two proteins, Piezo1 and Piezo2, were identified, overexpression of which results in mechanically activated currents very similar to SACs.¹³⁶ Whether Piezos contain the pore region of SAC or, rather, are components necessary for channel function is unclear.

CENTRAL OSMOSENSORS

Even very minor changes in systemic osmolarity are associated with severe symptoms,¹³⁷ and extracellular fluid (ECF) osmolarity is, accordingly, very tightly regulated. ECF *hyper*-osmolarity (generally caused by increased salt intake relative to water) stimulates central osmoreceptor cells, which transmit this signal to orchestrate a series of signaling and ion transport events in other central and peripheral cells. Collectively, this results in increased thirst

and in vasopressin (AVP) release, the latter, in turn, leading to increased free water retention through antidiuresis and natriuresis. Conversely, when ECF *hypo*-osmolarity is detected by osmoreceptor neurons, the net result is reduced AVP release and diuresis, and thus reduced water retention.¹³⁸ Figure 2 outlines the overall mechanisms of systemic osmoregulation.

The precise location of the central osmosensory mechanisms responding to changes in systemic osmolarity has only recently been elucidated, and much is still obscure regarding these essential homeostatic mechanisms.^{138,139} A major part of the systemic response to perturbations in

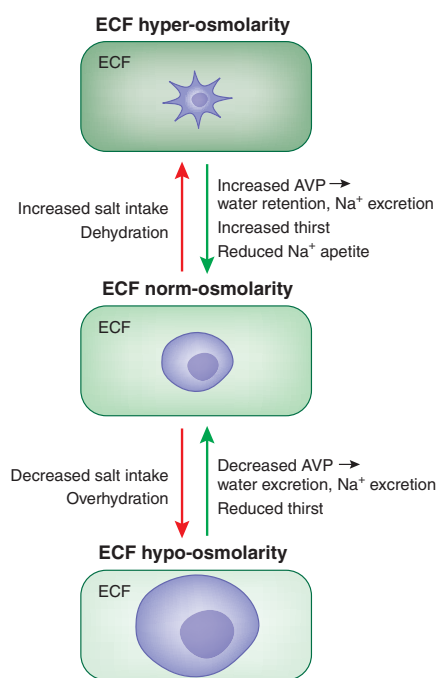


Figure 2. Overview of the processes of extracellular fluid (ECF) osmolarity disturbance and regulation. ECF hyper-osmolarity causes osmotic shrinkage of osmosensory cells (likely both central and peripheral). This stimulates AVP release, increased water retention and Na⁺ excretion, increased thirst and increased Na⁺ appetite, collectively leading to normalization of ECF osmolarity and osmosensory cell volume. Conversely, ECF hypo-osmolarity results in osmosensory cell swelling, leading to reduced AVP release and, in turn, water excretion, salt uptake, and reduced thirst, and, hence, normalization of ECF osmolarity and osmosensory cell volume. See text for details.

ECF osmolarity is mediated by osmoreceptor neurons found in two locations in the central nervous system (CNS). Available evidence suggests that the most influential intrinsically osmosensitive neurons in the brain reside within the forebrain lamina terminalis, specifically in the organum vasculosum laminae terminalis (OVLT) and the subfornical organ (SFO).¹³⁹ Intrinsically osmosensitive neurons are also found in magnocellular neurosecretory cells (MNCs) in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) in the hypothalamus.¹⁴⁰

As first described by Verney,¹⁴¹ these osmoreceptor neurons perceive ECF volume perturbations and translate them into electrical activity that is transmitted to other neurons as changes in action potential firing, eventually resulting in altered AVP release from the pituitary gland. Which, if any, of the volume sensory mechanisms outlined above are employed by the central osmosensory neurons? In general, it seems that osmoreceptor neurons respond to hyper-osmotic stimuli by activation of a nonselective cation conductance, resulting in plasma membrane depolarization and thus increased action potential firing rate, whereas the reverse is true for hypo-osmotic stimuli.^{142,143} The available evidence indicates that of the three main types of volume signals outlined above, the central osmosensors react to the mechanical stimulus elicited by cell volume change.^{138,144}

Biophysical characteristics and inhibitor profiles indicated, a number of years ago, that at least some of these nonselective cation channels responsible for central osmosensing belong to the TRP family. Specifically, an N-terminal variant of TRPV1 appears to be activated by cell shrinkage and is involved in central osmosensing of ECF hyper-osmolarity, at least in the acute phase⁹⁷ but possibly not in the chronic phase.^{145,146} In contrast, TRPV4 seems to be essential in systemic sensing of hypo-osmolarity. Thus, *TRPV4*^{-/-} mice have aberrant systemic osmosensitivity,^{147,148} and TRPV4 staining in the brain reveals high expression in the OVLT and SON.¹⁴⁸ The general mechanisms through which TRPV4 is activated by osmotic cell swelling are outlined above. In the context of cen-

tral osmosensing, it is interesting to note that the central response to perturbations of ECF osmolarity is strongly dependent on the actin cytoskeleton.¹⁴⁴ Moreover, in a rat model of liver cirrhosis, TRPV4 expression is increased, and its lipid raft association decreased, in the SON, correlating with elevated AVP release.¹⁴⁹ This suggests that lipid rafts may regulate TRPV4 function and that altered TRPV4 expression and localization may contribute to deranged AVP release in cirrhosis.

In addition to neuronal TRPV1 and TRPV4, a number of other mechanisms are likely to contribute to systemic osmosensing. Thus, several stretch-activated two-pore K⁺ channels were identified in MNG neurons,^{150,151} and, recently, hyperosmotic dehydration-induced activation of a voltage-dependent K⁺ current¹⁵² and L-type Ca²⁺ currents¹⁵³ was reported in these cells. Finally, swelling-activated glial taurine release seems to contribute to AVP release through activation of neuronal glycine receptors.¹⁵⁴

PERIPHERAL OSMOSENSORS

While surprisingly little is known about peripheral sensing of systemic osmotic perturbations, the existence of peripheral osmoreceptors has been suggested by two sets of intriguing observations. The first is the presence of anticipatory mechanisms: osmotically induced hormonal or behavioral responses occur much faster than any other change detected in the ECF, that is, before the information can reach the central osmosensors. Thus, water drinking quenches thirst,^{155–158} and changes in gastric osmotic load due to water or food intake modulate AVP secretion^{159–161} before any alteration in ECF osmolarity. Second, local changes in osmotic concentration (for example, due to oral or intragastric sodium or water load) elicit larger regulatory responses than what the same load provokes when administered intravenously.¹⁶² Based on these findings, peripheral osmoreceptors have been proposed to localize to the oropharyngeal area,¹⁶³ along the gastrointestinal tract (stomach, duodenum),^{159,164,165} in the liver,¹⁶⁶ portal vein,^{167,168} and splanchnic mesentery.¹⁶⁹

These receptors might sense changes in osmolarity, cell volume, or salt concentration (sodium monitor).¹³⁸ So far, neither the sensed parameters, nor the identity of the receptors, nor the sensing mechanisms have been established with absolute certainty. Nonetheless, osmotic activation of TRPV4 channels (see above) emerges as an important mechanism.^{162,170} Accordingly, TRPV4^{-/-} mice exhibit impaired adaptive responses to changes in gastroduodenal osmolarity.¹⁷¹ The osmosensors may be local cells or sensory neurons such as vagal afferents or—as increasing evidence suggests—neurons of the dorsal root ganglia.^{172–177} Once stimulated, they send signals to the CNS, where they modulate thirst and satiety and AVP secretion and/or to peripheral effector organs, particularly to the kidney. Regarding the latter, increased gastric osmolarity reduces sympathetic renal nerve activity, which, in turn, suppresses the intrarenal renin-angiotensin system, resulting in enhanced natriuresis.¹⁷⁸ Clearly, the characterization of peripheral systemic osmosensors is a topic of primary importance, which lags behind our more in-depth understanding of the central mechanisms and warrants more attention in the future.

KIDNEY CELLS AS POTENTIAL PERIPHERAL OSMOSENSORS?

While the mechanisms through which renal epithelial cells sense and regulate their own volume have been well characterized,^{1,2,29} much less is known regarding the possible role of kidney cells as peripheral sensors of systemic osmolarity. Interestingly, TRPV4 is abundantly expressed along the nephron,^{179,180} particularly enriched along in the collecting duct. This raises the possibility that tubular cells might themselves act as systemic osmosensors. Accordingly, hypotonicity was shown to provoke TRPV4-triggered ATP release from the thick ascending limb.¹⁸¹ This phenomenon is of particular interest, because swelling-induced ATP release in the macula densa may be a key mediator of tubuloglomerular feedback.¹⁸² Besides its effects on renal hemodynamics, extracellular ATP also controls salt reabsorption in sev-

eral nephron segments.¹⁸³ Moreover, TRPV4 and polycystin-2 (TRPP2) were shown to form a polymodal sensory complex in tubular cells, which acts as a flow-activated, Ca²⁺-permeable channel in the primary cilium.²² Finally, increased flow alters tubular K⁺ secretion and Na⁺ reabsorption in a TRPV4-dependent manner.¹⁸⁴ This scenario then raises a multitude of interesting questions. What are the effectors through which renal epithelial cells relay information about systemic osmolarity to other cell groups—does this involve ATP and/or other paracrine factors? How do different modalities (changes in osmolarity *versus* flow) acting through the same sensor get integrated into the final response?¹⁸⁵ What is the contribution of TRPV4 *versus* other renal TRP channels, such as TRPM3,¹⁷⁹ to the overall responses of renal cells to aniso-osmolarity? How are the sensory inputs through TRP channels integrated with signals emanating from other cellular osmosensors such as integrins or growth factors? Future research should address these intriguing questions, using genetically manipulated tubular cells and transgenic animals with tubule-specific deletion of TRPV4 or other osmosensors or volume sensors.

ARE MACROPHAGES MOBILE PERIPHERAL OSMOSENSORS?

It has been repeatedly shown that, during salt loading, Na⁺ can accumulate in the skin without commensurate water,^{186,187} presumably due to negatively charged glycosaminoglycans. This Na⁺ is in dynamic equilibrium with the viscous connective tissue and, in this sense, it is not osmotically inactive. Indeed, the skin and the lymphatic tissue have higher osmolarity than the blood plasma.^{188,189} According to a new paradigm, this local hypertonicity activates TonEPB signaling in mononuclear phagocytes, which, in turn, secrete vascular endothelial growth factor-C (VEGF-C).^{190–192} VEGF-C induces lymphatic vessel formation and endothelial nitric oxide synthase expression, thereby modulating BP, volume homeostasis, and, presumably, kidney function. Notably, deletion of macrophages augments the Na⁺ load-induced

rise in BP.¹⁹⁰ These findings are especially intriguing, since, originally, TonEPB was shown to be essential for the induction of osmoprotective genes in highly hyper-osmotic environments, such as the kidney medulla.^{193,194} However, as the above-mentioned data show, in addition to these renoprotective functions exerted in a robustly hyper-osmotic milieu, TonEBP may play an important regulatory role in non-renal cells under (nearly) iso-osmotic conditions.¹⁹² Future studies should address whether TonEBP, which may be considered as a self-contained tonicity- or ionic-strength sensor/effector system,^{195–197} plays additional roles in central or peripheral osmosensing during health and disease.

DISTURBANCES IN OSMOSENSING: A RENAL VIEWPOINT

The kidney is involved in osmosensory pathophysiology in two conceptually different aspects. First, it is the main *effector organ* of systemic osmoregulation responsible for water and salt homeostasis, and, as such, it is controlled by central osmosensing machinery through both hormonal and neuronal pathways. Accordingly, insufficiency of AVP production or secretion causes a plethora of diseases, including enuresis,¹⁹⁸ nocturia,¹⁹⁹ and central diabetes insipidus. Conversely, incapability of the kidney to respond to AVP results in nephrogenic diabetes insipidus, either acquired or congenital, the latter most frequently caused by various mutations affecting AVP Receptor-2 (90%) or aquaporin-2 (10%). This field has been extensively covered by excellent reviews.^{200–203} Second, since the kidney itself, as discussed above, emerges as a systemic osmosensing and mechanosensing organ, pathologies can be associated with anomalies of the molecular apparatus responsible for these functions in the renal cells. As noted above, such molecular sensors and the related signaling systems are highly enriched in the primary cilium. Recently, numerous so-called ciliopathies have been identified in which impaired ciliary function underlies severe mechanosensory, developmental, or degenerative

disorders.^{204–206} Of these, the prototypic example is autosomal dominant polycystic kidney disease, which is caused by mutations in polycystin-1 (PKD1, TRPP1) or polycystin-2 (PKD2, TRPP2). Under normal conditions, these proteins form a mechanosensitive cation channel in the primary cilium, which opens upon the flow-induced ciliary bending, generating a Ca²⁺ signal.^{20,207} PKD mutations impair mechanically-evoked Ca²⁺ transients and lead to the formation of fluid-filled cysts and enhanced cell proliferation, implying that intact mechanotransduction is essential for normal tubular function and structure.^{208,209} As mentioned above, PKD2 can form a Ca²⁺-conducting complex with the osmo-/volume-sensitive TRPV4,²² raising the possibility that osmotic stimuli might also contribute to the preservation of normal kidney architecture and prevent cyst formation. On the other hand, it should be stressed that *TRPV4*^{-/-} mice and zebrafish lack renal cysts, arguing against a role for at least TRPV4 in preventing cyst formation.²² Finally, although, so far, no TRPV4 or TRPM3 mutations have been directly associated with known renal disorders (see, however,²¹⁰) it is noteworthy that an array of other TRP channel malfunctions has been shown to play pathogenic roles in various kidney diseases, including diabetic nephropathy (TRPC1), focal segmental glomerulosclerosis (TRPC6), and hypomagnesemia (TRPM6).¹⁷⁹

CONCLUSIONS

An interesting point, often forgotten in the discussion of cellular volume regulation, is that a cell that regulates its own volume in response to an ECM perturbation is, in effect, compromising the common good of the organism it is part of, by negatively impacting on systemic volume regulation. Thus the evolutionary development of systemic osmoregulation must have modified the elementary volume-regulating responses of specialized cells. What determines whether cellular or systemic volume regulation is prioritized? Notably and logically, osmoreceptor neurons seem to lack functional volume-regulatory machinery and thus act as osmometers, swelling and

shrinking in a manner correlating with the difference in tonicity between the cell and the ECF to which they are exposed.⁸ In our view, essential areas of research needed to be explored in future studies include the extended molecular identification of osmosensors and volume sensors and their subcellular locations, the clarification of the nature of the upstream signals of volume change, and the mechanisms whereby they interact with the primary sensors in health and disease.

DISCLOSURES

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