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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 discuss 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 response.[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, several important moieties have been identified, including Rho family G proteins,[31,32] cofilin,[33] the ezrin/radixin/moesin (ERM) proteins ezrin[34] 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)P_2 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 Ca^{2+} 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]

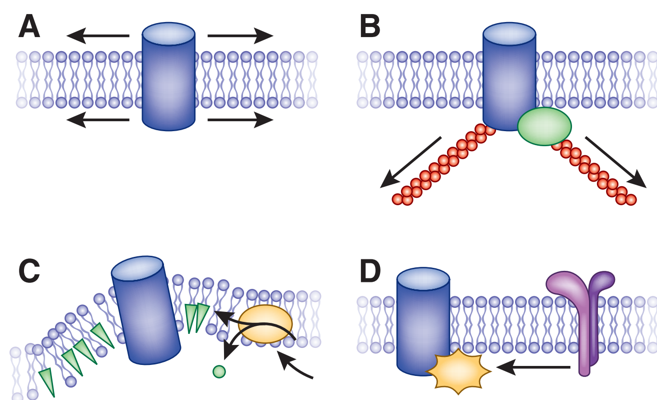

Figure 1. Mechanisms through which a membrane protein may sense cell volume perturbations. (A) Direct stretch-sensitivity. (B) Direct or indirect tethering to the cytoskeleton. (C) Changes in membrane curvature, either mechanical or through changes in membrane composition. (D) Via interaction with integrins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

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

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 β1-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 β1 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 Tyr^{397} in the N-terminal domain.[63] This recruits Src,[64] phosphatidyl-inositol 3-kinase (PI-3K), and phospholipase Cγ (PLC-γ), and subsequent phosphorylation of Tyr^{925} 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 Tyr^{576} and Tyr^{577} in the kinase domain,[66] and Tyr^{861} between the kinase domain and the Focal adhesion Targeting (FAT) domain.[67] *Hypo*tonic 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 Tyr^{861} phosphorylation is stimulated by hyperosmotic 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 Tyr^{576} and Tyr,[861] and independent phosphorylation at Tyr^{397} (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 (PDGFRβ), 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 volume 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*$^{-/-}$ mice[95] (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 neurons[97] (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 A_2,[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 stress[117] and by osmotic swelling, and knockdown of TRPM7[118] 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 PLCγ-1,[124] PtdIns(4,5)P_2, 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 ECM *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

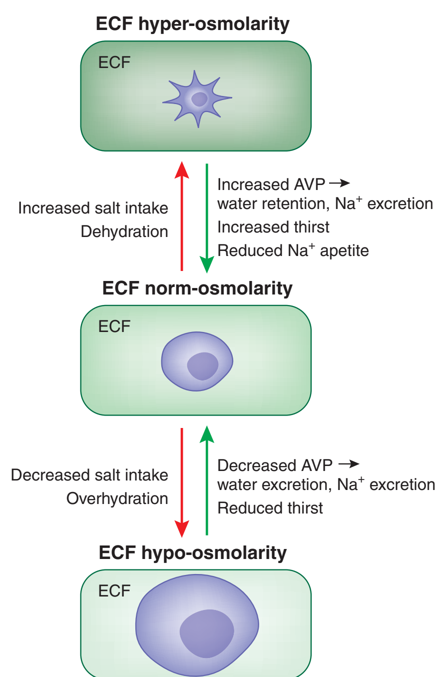

Figure 2. Overview of the processes of extracellular fluid (ECF) osmolarity disturbance and regulation. ECF hyper-osmolarity causes osmotic shrinkage of osmosensory cells (likely both central and peripheral). This stimulates AVP release, increased water retention and Na^+ excretion, increased thirst 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 laminaee 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 ECM 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 phase[97] 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^+ current[152] and L-type Ca^{2+} currents[153] 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-angiotesin 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 several 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, Ca^{2+}-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 in 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 eneuresis,[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 Ca^{2+} signal.[20,207] PKD mutations impair mechanically-evoked Ca^{2+} 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 Ca^{2+}-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.

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REFERENCES

1. Hoffmann EK, Lambert IH, Pedersen SF: Physiology of cell volume regulation in vertebrates. *Physiol Rev* 89: 193–277, 2009
2. Koivusalo M, Kapus A, Grinstein S: Sensors, transducers, and effectors that regulate cell size and shape. *J Biol Chem* 284: 6595–6599, 2009
3. Wehner F, Olsen H, Tinel H, Kinne-Saffran E, Kinne RKH: Cell volume regulation: Osmolytes, osmolyte transport, and signal transduction. *Reviews of Physiology, Biochemistry and Pharmacology* 148: 1–80, 2003
4. Lang F, Busch GL, Ritter M, Volkl H, Waldegger S, Gulbins E, Haussinger D: Functional significance of cell volume regulatory mechanisms. *Physiol Rev* 78: 247–306, 1998
5. Burg MB: Macromolecular crowding as a cell volume sensor. *Cell Physiol Biochem* 10: 251–256, 2000
6. Minton AP: Macromolecular crowding. *Curr Biol* 16: R269–R271, 2006
7. Pedersen SF, Nilius B: Transient receptor potential channels in mechanosensing and cell volume regulation. *Methods Enzymol* 428: 183–207, 2007
8. Zhang Z, Bourque CW: Osmometry in osmosensory neurons. *Nat Neurosci* 6: 1021–1022, 2003
9. Levitan I, Christian AE, Tulenko TN, Rothblat GH: Membrane cholesterol content modulates activation of volume-regulated

anion current in bovine endothelial cells. *J Gen Physiol* 115: 405–416, 2000
10. Volonte D, Galbiati F, Pestell RG, Lisanti MP: Cellular stress induces the tyrosine phosphorylation of caveolin-1 (Tyr(14)) via activation of p38 mitogen-activated protein kinase and c-Src kinase. Evidence for caveolae, the actin cytoskeleton, and focal adhesions as mechanical sensors of osmotic stress. *J Biol Chem* 276: 8094–8103, 2001
11. Trouet D, Hermans D, Droogmans G, Nilius B, Eggermont J: Inhibition of volume-regulated anion channels by dominant-negative caveolin-1. *Biochem Biophys Res Commun* 284: 461–465, 2001
12. Klausen TK, Hougaard C, Hoffmann EK, Pedersen SF: Cholesterol modulates the volume-regulated anion current in Ehrlich-Lettre ascites cells via effects on Rho and F-actin. *Am J Physiol Cell Physiol* 291: C757–C771, 2006
13. Alexander RT, Malevanets A, Durkan AM, Kocinsky HS, Aronson PS, Orlowski J, Grinstein S: Membrane curvature alters the activation kinetics of the epithelial Na+/H+ exchanger, *NHE3 J Biol Chem* 282: 7376–7384, 2007
14. Fuster D, Moe OW, Hilgemann DW: Lipid- and mechanosensitivities of sodium/hydrogen exchangers analyzed by electrical methods. *Proc Natl Acad Sci U S A* 101: 10482–10487, 2004
15. Patel AJ, Lazdunski M, Honore E: Lipid and mechano-gated 2P domain K(+) channels. *Curr Opin Cell Biol* 13: 422–428, 2001
16. Dedman A, Sharif-Naeini R, Folgering JH, Duprat F, Patel A, Honore E: The mechano-gated K(2P) channel TREK-1. *Eur Biophys J* 38: 293–303, 2009
17. Pazour GJ, Witman GB: The vertebrate primary cilium is a sensory organelle. *Curr Opin Cell Biol* 15: 105–110, 2003
18. Christensen ST, Pedersen SF, Satir P, Veland IR, Schneider L: The primary cilium coordinates signaling pathways in cell cycle control and migration during development and tissue repair. *Curr Top Dev Biol* 85: 261–301, 2008
19. Praetorius HA, Spring KR: Removal of the MDCK cell primary cilium abolishes flow sensing. *J Membr Biol* 191: 69–76, 2003
20. Praetorius HA, Spring KR: The renal cell primary cilium functions as a flow sensor. *Curr Opin Nephrol Hypertens* 12: 517–520, 2003
21. Patel A, Honore E: Polycystins and renovascular mechanosensory transduction. *Nat Rev Nephrol* 6: 530–538, 2010
22. Kottgen M, Buchholz B, Garcia-Gonzalez MA, Kotsis F, Fu X, Doerken M, Boehlke C, Steffl D, Tauber R, Wegierski T, Nitschke R, Suzuki M, Kramer-Zucker A, Germino GG, Watnick T, Prenen J, Nilius B, Kuehn EW, Walz G: TRPP2 and TRPV4 form a polymodal sensory channel complex. *J Cell Biol* 182: 437–447, 2008
23. Bahn YS, Xue C, Idnurm A, Rutherford JC, Heitman J, Cardenas ME: Sensing the environment: Lessons from fungi. *Nat Rev Microbiol* 5: 57–69, 2007
24. Levitan I, Almonte C, Mollard P, Garber SS: Modulation of a volume-regulated chloride current by F-actin. *J Membr Biol* 147: 283–294, 1995
25. Hallows KR, Law FY, Packman CH, Knauf PA: Changes in cytoskeletal actin content, F-actin distribution, and surface morphology during HL-60 cell volume regulation. *J Cell Physiol* 167: 60–71, 1996
26. Pedersen SF, Mills JW, Hoffmann EK: Role of the F-actin cytoskeleton in the RVD and RVI processes in Ehrlich ascites tumor cells. *Exp Cell Res* 252: 63–74, 1999
27. Pedersen SF, Beisner KH, Hougaard C, Willumsen BM, Lambert IH, Hoffmann EK: Rho family GTP binding proteins are involved in the regulatory volume decrease process in NIH3T3 mouse fibroblasts. *J Physiol* 541: 779–796, 2002
28. Di Ciano C, Nie Z, Szaszi K, Lewis A, Uruno T, Zhan X, Rotstein OD, Mak A, Kapus A: Osmotic stress-induced remodeling of the cortical cytoskeleton. *Am J Physiol Cell Physiol* 283: C850–C865, 2002
29. Ciano-Oliveira C, Thirone AC, Szaszi K, Kapus A: Osmotic stress and the cytoskeleton: The R(h)ole of Rho GTPases. *Acta Physiol (Oxf)* 187: 257–272, 2006
30. Pedersen SF, Hoffmann EK, Mills JW: The cytoskeleton and cell volume regulation. *Comp Biochem Physiol A Mol Integr Physiol* 130: 385–399, 2001
31. Lewis A, Di Ciano C, Rotstein OD, Kapus A: Osmotic stress activates Rac and Cdc42 in neutrophils: role in hypertonicity-induced actin polymerization. *Am J Physiol Cell Physiol* 282: C271–C279, 2002
32. Ciano-Oliveira C, Sirokmany G, Szaszi K, Arthur WT, Masszi A, Peterson M, Rotstein OD, Kapus A: Hyperosmotic stress activates Rho: Differential involvement in Rho kinase-dependent MLC phosphorylation and NKCC activation. *Am J Physiol Cell Physiol* 285: C555–C566, 2003
33. Thirone AC, Speight P, Zulys M, Rotstein OD, Szaszi K, Pedersen SF, Kapus A: Hyperosmotic stress induces Rho/Rho kinase/LIM kinase-mediated cofilin phosphorylation in tubular cells: Key role in the osmotically triggered F-actin response. *Am J Physiol Cell Physiol* 296: C463–C475, 2009
34. Rasmussen M, Alexander RT, Darborg BV, Mobjerg N, Hoffmann EK, Kapus A, Pedersen SF: Osmotic cell shrinkage activates ezrin/radixin/moesin (ERM) proteins: Activation mechanisms and physiological implications. *Am J Physiol Cell Physiol* 294: C197–C212, 2008
35. Tamma G, Procino G, Svelto M, Valenti G: Hypotonicity causes actin reorganization and recruitment of the actin-binding ERM protein moesin in membrane protrusions in collecting duct principal cells. *Am J Physiol Cell Physiol* 292: C1476–C1484, 2007
36. Pedersen SF, Hoffmann EK: Possible interrelationship between changes in F-actin and myosin II, protein phosphorylation, and cell volume regulation in Ehrlich ascites tumor cells. *Exp Cell Res* 277: 57–73, 2002
37. Kapus A, Szaszi K, Sun J, Rizoli S, Rotstein OD: Cell shrinkage regulates Src kinases and induces tyrosine phosphorylation of cortactin, independent of the osmotic regulation of Na+/H+ exchangers. *J Biol Chem* 274: 8093–8102, 1999
38. Fernandez P, Pullarkat PA: The role of the cytoskeleton in volume regulation and beading transitions in PC12 neurites. *Biophys J* 99: 3571–3579, 2010
39. Jensen BH, Schröder JM, Pedersen LB, and Pedersen, SF: Osmotic stress induces rapid cytoskeletal reorganization in human retinal pigment epithelial cells in a manner dependent on the microtubule plus-end tracking proteins EB1 and EB3. *Acta Physiol (Oxf)* 198[Suppl 677], 212, 2010
40. Nielsen DK, Jensen AK, Harbak H, Christensen SC, Simonsen LO: Cell content of phosphatidylinositol (4,5)bisphosphate in Ehrlich mouse ascites tumour cells in response to cell volume perturbations in anisotonic and in isosmotic media. *J Physiol* 582: 1027–1036, 2007
41. Lim Y, Lim ST, Tomar A, Gardel M, Bernard-Trifilo JA, Chen XL, Uryu SA, Canete-Soler R, Zhai J, Lin H, Schlaepfer WW, Nalbant P, Bokoch G, Ilic D, Waterman-Storer C, Schlaepfer DD: PyK2 and FAK connections to p190Rho guanine nucleotide exchange factor regulate RhoA activity, focal adhesion formation, and cell motility. *J Cell Biol* 180: 187–203, 2008
42. Hoffmann EK, Pedersen SF: Shrinkage insensitivity of NKCC1 in myosin II-depleted cytoplasts from Ehrlich ascites tumor cells. *Am J Physiol Cell Physiol* 292: C1854–C1866, 2007
43. Matthews JB, Smith JA, Mun EC, Sicklick JK: Osmotic regulation of intestinal epithelial Na(+)-K(+)-Cl- cotransport: Role of Cl- and F-actin. *Am J Physiol* 274: C697–C706, 1998
44. Posern G, Treisman R: Actin' together: Serum response factor, its cofactors and the link to signal transduction. *Trends Cell Biol* 16: 588–596, 2006
45. Gorbatenko A, Wiwel M, Klingberg H, Nielsen AB, Kapus A, and Pedersen, SF: Hyperosmotic stress strongly potentiates Serum Response Factor (SRF) dependent transcriptional activity in Ehrlich Lettré Ascites cells through a mechanism involving p38 mitogen-activated protein kinase. *J Cell Physiol* 2011, doi: 10.1002/jcp.22628
46. Dubash AD, Menold MM, Samson T, Boulter E, Garcia-Mata R, Doughman R,

Burridge K: Chapter 1. Focal adhesions: New angles on an old structure. *Int Rev Cell Mol Biol* 277: 1–65, 2009
47. Browe DM, Baumgarten CM: Stretch of beta 1 integrin activates an outwardly rectifying chloride current via FAK and Src in rabbit ventricular myocytes. *J Gen Physiol* 122: 689–702, 2003
48. Browe DM, Baumgarten CM: Angiotensin II (AT1) receptors and NADPH oxidase regulate Cl- current elicited by beta1 integrin stretch in rabbit ventricular myocytes. *J Gen Physiol* 124: 273–287, 2004
49. Browe DM, Baumgarten CM: EGFR kinase regulates volume-sensitive chloride current elicited by integrin stretch via PI-3K and NADPH oxidase in ventricular myocytes. *J Gen Physiol* 127: 237–251, 2006
50. vom DS, Schliess F, Reissmann R, Gorg B, Weiergraber O, Kocalkova M, Dombrowski F, Haussinger D: Involvement of integrins in osmosensing and signaling toward autophagic proteolysis in rat liver. *J Biol Chem* 278: 27088–27095, 2003
51. Haussinger D, Reinehr R, Schliess F: The hepatocyte integrin system and cell volume sensing. *Acta Physiol (Oxf)* 187: 249–255, 2006
52. Kajimura M, O'Donnell ME, Curry FE: Effect of cell shrinkage on permeability of cultured bovine aortic endothelia and frog mesenteric capillaries. *J Physiol* 503(Pt 2):413–425, 1997
53. Sheikh-Hamad D, Suki WN, Zhao W: Hypertonic induction of the cell adhesion molecule beta 1-integrin in MDCK cells. *Am J Physiol* 273: C902–C908, 1997
54. Sheikh-Hamad D, Youker K, Truong LD, Nielsen S, Entman ML: Osmotically relevant membrane signaling complex: Association between HB-EGF, beta(1)-integrin, and CD9 in mTAL. *Am J Physiol Cell Physiol* 279: C136–C146, 2000
55. Moeckel GW, Zhang L, Chen X, Rossini M, Zent R, Pozzi A: Role of integrin alpha1beta1 in the regulation of renal medullary osmolyte concentration. *Am J Physiol Renal Physiol* 290: F223–F231, 2006
56. Schliess F, Reissmann R, Reinehr R, vom DS, Haussinger D: Involvement of integrins and Src in insulin signaling toward autophagic proteolysis in rat liver. *J Biol Chem* 279: 21294–21301, 2004
57. Becchetti A, Pillozzi S, Morini R, Nesti E, Arcangeli A: New insights into the regulation of ion channels by integrins. *Int Rev Cell Mol Biol* 279: 135–190, 2010
58. Arcangeli A, Becchetti A: Complex functional interaction between integrin receptors and ion channels. *Trends Cell Biol* 16: 631–639, 2006
59. Levite M, Cahalon L, Peretz A, Hershkoviz R, Sobko A, Ariel A, Desai R, Attali B, Lider O: Extracellular K(+) and opening of voltage-gated potassium channels activate T cell integrin function: Physical and functional association between Kv1.3 channels and beta1 integrins. *J Exp Med* 191: 1167–1176, 2000
60. Artym VV, Petty HR: Molecular proximity of Kv1.3 voltage-gated potassium channels and beta(1)-integrins on the plasma membrane of melanoma cells: Effects of cell adherence and channel blockers. *J Gen Physiol* 120: 29–37, 2002
61. Cherubini A, Hofmann G, Pillozzi S, Guasti L, Crociani O, Cilia E, Di SP, Degani S, Balzi M, Olivotto M, Wanke E, Becchetti A, Defilippi P, Wymore R, Arcangeli A: Human ether-a-go-go-related gene 1 channels are physically linked to beta1 integrins and modulate adhesion-dependent signaling. *Mol Biol Cell* 16: 2972–2983, 2005
62. Cahalan MD, Chandy KG: The functional network of ion channels in T lymphocytes. *Immunol Rev* 231: 59–87, 2009
63. Hamadi A, Bouali M, Dontenwill M, Stoeckel H, Takeda K, Ronde P: Regulation of focal adhesion dynamics and disassembly by phosphorylation of FAK at tyrosine 397. *J Cell Sci* 118: 4415–4425, 2005
64. Hanks SK, Polte TR: Signaling through focal adhesion kinase. *Bioessays* 19: 137–145, 1997
65. Parsons JT: Focal adhesion kinase: The first ten years. *J Cell Sci* 116: 1409–1416, 2003
66. Corsi JM, Rouer E, Girault JA, Enslen H: Organization and post-transcriptional processing of focal adhesion kinase gene. *BMC Genomics* 7: 198, 2006
67. Cohen LA, Guan JL: Mechanisms of focal adhesion kinase regulation. *Curr Cancer Drug Targets* 5: 629–643, 2005
68. Hirakawa M, Oike M, Karashima Y, Ito Y: Sequential activation of RhoA and FAK/paxillin leads to ATP release and actin reorganization in human endothelium. *J Physiol* 558: 479–488, 2004
69. Kim RD, Darling CE, Roth TP, Ricciardi R, Chari RS: Activator protein 1 activation following hypoosmotic stress in HepG2 cells is actin cytoskeleton dependent. *J Surg Res* 100: 176–182, 2001
70. de La Paz LD, Lezama R, Torres-Marquez ME, Pasantes-Morales H: Tyrosine kinases and amino acid efflux under hyposmotic and ischaemic conditions in the chicken retina. *Pflugers Arch* 445: 87–96, 2002
71. Lezama R, Ortega A, Ordaz B, Pasantes-Morales H: Hyposmolarity-induced ErbB4 phosphorylation and its influence on the non-receptor tyrosine kinase network response in cultured cerebellar granule neurons. *J Neurochem* 93: 1189–1198, 2005
72. Tilly BC, Edixhoven MJ, Tertoolen LG, Morii N, Saitoh Y, Narumiya S, de Jonge HR: Activation of the osmo-sensitive chloride conductance involves P21rho and is accompanied by a transient reorganization of the F-actin cytoskeleton. *Mol Biol Cell* 7: 1419–1427, 1996
73. Szucs G, Buyse G, Eggermont J, Droogmans G, Nilius B: Characterization of volume-activated chloride currents in endothelial cells from bovine pulmonary artery. *J Membr Biol* 149: 189–197, 1996
74. Derkinderen P, Siciliano J, Toutant M, Girault JA: Differential regulation of FAK+ and PYK2/Cakbeta, two related tyrosine kinases, in rat hippocampal slices: Effects of LPA, carbachol, depolarization and hyperosmolarity. *Eur J Neurosci* 10: 1667–1675, 1998
75. Lunn JA, Rozengurt E: Hyperosmotic stress induces rapid focal adhesion kinase phosphorylation at tyrosines 397 and 577. Role of Src family kinases and Rho family GTPases. *J Biol Chem* 279: 45266–45278, 2004
76. Lunn JA, Jacamo R, Rozengurt E: Preferential phosphorylation of focal adhesion kinase tyrosine 861 is critical for mediating an anti-apoptotic response to hyperosmotic stress. *J Biol Chem* 282: 10370–10379, 2007
77. Zhang Z, Avraham H, Cohen DM: Urea and NaCl differentially regulate FAK and RAFTK/PYK2 in mIMCD3 renal medullary cells. *Am J Physiol* 275: F447–F451, 1998
78. Franco R, Lezama R, Ordaz B, Pasantes-Morales H: Epidermal growth factor receptor is activated by hyposmolarity and is an early signal modulating osmolyte efflux pathways in Swiss 3T3 fibroblasts. *Pflugers Arch* 447: 830–839, 2004
79. Kippenberger S, Loitsch S, Guschel M, Muller J, Kaufmann R, Bernd A: Hypotonic stress induces E-cadherin expression in cultured human keratinocytes. *FEBS Lett* 579: 207–214, 2005
80. Lezama R, Diaz-Tellez A, Ramos-Mandujano G, Oropeza L, Pasantes-Morales H: Epidermal growth factor receptor is a common element in the signaling pathways activated by cell volume changes in isosmotic, hyposmotic or hyperosmotic conditions. *Neurochem Res* 30: 1589–1597, 2005
81. Pasantes-Morales H, Lezama RA, Ramos-Mandujano G: Tyrosine kinases and osmolyte fluxes during hyposmotic swelling. *Acta Physiol* 187: 93–102, 2006
82. Rosette C, Karin M: Ultraviolet light and osmotic stress: Activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* 274: 1194–1197, 1996
83. Copp J, Wiley S, Ward MW, van der Geer P: Hypertonic shock inhibits growth factor receptor signaling, induces caspase-3 activation, and causes reversible fragmentation of the mitochondrial network. *Am J Physiol Cell Physiol* 288: C403–C415, 2005
84. Nielsen MB, Christensen ST, Hoffmann EK: Effects of osmotic stress on the activity of MAP kinases and PDGFRβ-mediated signal transduction in NIH3T3-fibroblasts. *Am J Physiol Cell Physiol* 294: C1046–C1055, 2008
85. Reinehr R, Becker S, Hongen A, Haussinger

D: The Src family kinase Yes triggers hyperosmotic activation of the epidermal growth factor receptor and CD95. *J Biol Chem* 279: 23977–23987, 2004
86. Moro L, Venturino M, Bozzo C, Silengo L, Altruda F, Beguinot L, Tarone G, Defilippi P: Integrins induce activation of EGF receptor: Role in MAP kinase induction and adhesion-dependent cell survival. *EMBO J* 17: 6622–6632, 1998
87. Knies Y, Bernd A, Kaufmann R, Bereiter-Hahn J, Kippenberger S: Mechanical stretch induces clustering of beta1-integrins and facilitates adhesion. *Exp Dermatol* 15: 347–355, 2006
88. Pedersen SF, Owsianik G, Nilius B: TRP channels: An overview. *Cell Calcium* 38: 233–252, 2005
89. Ramsey IS, Delling M, Clapham DE: An introduction to TRP channels. *Annu Rev Physiol* 68: 619–647, 2006
90. Voets T, Talavera K, Owsianik G, Nilius B: Sensing with TRP channels. *Nat Chem Biol* 1: 85–92, 2005
91. Nilius B, Owsianik G, Voets T, Peters JA: Transient receptor potential cation channels in disease. *Physiol Rev* 87: 165–217, 2007
92. Christensen AP, Corey DP: TRP channels in mechanosensation: Direct or indirect activation? *Nat Rev Neurosci* 8: 510–521, 2007
93. Yin J, Kuebler WM: Mechanotransduction by TRP channels: General concepts and specific role in the vasculature. *Cell Biochem Biophys* 56: 1–18, 2010
94. Kung C: A possible unifying principle for mechanosensation. *Nature* 436: 647–654, 2005
95. Liedtke W: Transient receptor potential vanilloid channels functioning in transduction of osmotic stimuli. *J Endocrinol* 191: 515–523, 2006
96. Loukin SH, Su Z, Kung C: Hypotonic shocks activate rat TRPV4 in yeast in the absence of polyunsaturated fatty acids. *FEBS Lett* 583: 754–758, 2009
97. Sharif NR, Witty MF, Seguela P, Bourque CW: An N-terminal variant of Trpv1 channel is required for osmosensory transduction. *Nat Neurosci* 9: 93–98, 2006
98. Liedtke W, Choe Y, Marti-Renom MA, Bell AM, Denis CS, Sali A, Hudspeth AJ, Friedman JM, Heller S: Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell* 103: 525–535, 2000
99. Liedtke W, Kim C: Functionality of the TRPV subfamily of TRP ion channels: Add mechano-TRP and osmo-TRP to the lexicon! *Cell Mol Life Sci* 62: 2985–3001, 2005
100. Liedtke W: Role of TRPV ion channels in sensory transduction of osmotic stimuli in mammals. *Exp Physiol* 92: 507–512, 2007
101. Cohen DM: TRPV4 and the mammalian kidney. *Pflugers Arch* 451: 168–175, 2005
102. O'Neil RG, Heller S: The mechanosensitive nature of TRPV channels. *Pflugers Arch* 451: 193–203, 2005
103. Liedtke W, Tobin DM, Bargmann CI, Friedman JM: Mammalian TRPV4 (VR-OAC) directs behavioral responses to osmotic and mechanical stimuli in Caenorhabditis elegans. *Proc Natl Acad Sci U S A* 100[Suppl 2]: 14531–14536, 2003
104. Pedersen S, Lambert IH, Thoroed SM, Hoffmann EK: Hypotonic cell swelling induces translocation of the alpha isoform of cytosolic phospholipase A2 but not the gamma isoform in Ehrlich ascites tumor cells. *Eur J Biochem* 267: 5531–5539, 2000
105. Nilius B, Vriens J, Prenen J, Droogmans G, Voets T: TRPV4 calcium entry channel: A paradigm for gating diversity. *Am J Physiol Cell Physiol* 286: C195–C205, 2004
106. Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B: Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* 424: 434–438, 2003
107. Kohler R, Heyken WT, Heinau P, Schubert R, Si H, Kacik M, Busch C, Grgic I, Maier T, Hoyer J: Evidence for a functional role of endothelial transient receptor potential V4 in shear stress-induced vasodilatation. *Arterioscler Thromb Vasc Biol* 26: 1495–1502, 2006
108. Hartmannsgruber V, Heyken WT, Kacik M, Kaistha A, Grgic I, Harteneck C, Liedtke W, Hoyer J, Kohler R: Arterial response to shear stress critically depends on endothelial TRPV4 expression. *PLoS ONE* 2: e827, 2007
109. Strotmann R, Harteneck C, Nunnenmacher K, Schultz G, Plant TD: OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nat Cell Biol* 2: 695–702, 2000
110. Su Z, Zhou X, Loukin SH, Haynes WJ, Saimi Y, Kung C: The use of yeast to understand TRP-channel mechanosensitivity. *Pflugers Arch* 458: 861–867, 2009
111. Becker D, Blase C, Bereiter-Hahn J, Jendrach M: TRPV4 exhibits a functional role in cell-volume regulation. *J Cell Sci* 118: 2435–2440, 2005
112. Goswami C, Kuhn J, Heppenstall PA, Hucho T: Importance of non-selective cation channel TRPV4 interaction with cytoskeleton and their reciprocal regulations in cultured cells. *PLoS ONE* 5: e11654, 2010
113. Matthews BD, Thodeti CK, Tytell JD, Mammoto A, Overby DR, Ingber DE: Ultra-rapid activation of TRPV4 ion channels by mechanical forces applied to cell surface beta1 integrins. *Integr Biol (Camb)* 2: 435–442, 2010
114. Fu Y, Subramanya A, Rozansky D, Cohen DM: WNK kinases influence TRPV4 channel function and localization. *Am J Physiol Renal Physiol* 290: F1305–F1314, 2006
115. Liu X, Bandyopadhyay BB, Nakamoto T, Singh BB, Liedtke W, Melvin JE, Ambudkar IS: A role for AQP5 in activation of TRPV4 by hypotonicity: Concerted involvement of AQP5 and TRPV4 in regulation of cell volume recovery. *J Biol Chem* 281: 15485–15495, 2006
116. Sidhaye VK, Guler AD, Schweitzer KS, D'Alessio F, Caterina MJ, King LS: Transient receptor potential vanilloid 4 regulates aquaporin-5 abundance under hypotonic conditions. *Proc Natl Acad Sci U S A* 103: 4747–4752, 2006
117. Numata T, Shimizu T, Okada Y: Direct mechano-stress sensitivity of TRPM7 channel. *Cell Physiol Biochem* 19: 1–8, 2007
118. Numata T, Shimizu T, Okada Y: TRPM7 is a stretch- and swelling-activated cation channel involved in volume regulation in human epithelial cells. *Am J Physiol Cell Physiol* 292(1): C460–C467, 2006
119. Maroto R, Raso A, Wood TG, Kurosky A, Martinac B, Hamill OP: TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat Cell Biol* 7: 179–185, 2005
120. Spassova MA, Hewavitharana T, Xu W, Soboloff J, Gill DL: A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. *Proc Natl Acad Sci U S A* 103: 16586–16591, 2006
121. Dietrich A, Kalwa H, Storch U, Mederos YS, Salanova B, Pinkenburg O, Dubrovska G, Essin K, Gollasch M, Birnbaumer L, Gudermann T: Pressure-induced and store-operated cation influx in vascular smooth muscle cells is independent of TRPC1. *Pflugers Arch* 455: 465–477, 2007
122. Gottlieb P, Folgering J, Maroto R, Raso A, Wood TG, Kurosky A, Bowman C, Bichet D, Patel A, Sachs F, Martinac B, Hamill OP, Honore E: Revisiting TRPC1 and TRPC6 mechanosensitivity. *Pflugers Arch* 455: 1097–1103, 2008
123. Patel A, Sharif-Naeini R, Folgering JR, Bichet D, Duprat F, Honore E: Canonical TRP channels and mechanotransduction: From physiology to disease states. *Pflugers Arch* 460: 571–581, 2010
124. van Rossum DB, Patterson RL, Sharma S, Barrow RK, Kornberg M, Gill DL, Snyder SH: Phospholipase Cgamma1 controls surface expression of TRPC3 through an intermolecular PH domain. *Nature* 434: 99–104, 2005
125. Sharif-Naeini R, Folgering JH, Bichet D, Duprat F, Delmas P, Patel A, Honore E: Sensing pressure in the cardiovascular system: Gq-coupled mechanoreceptors and TRP channels. *J Mol Cell Cardiol* 48: 83–89, 2010
126. Sharif-Naeini R, Dedman A, Folgering JH, Duprat F, Patel A, Nilius B, Honore E: TRP channels and mechanosensory transduction: Insights into the arterial myogenic response. *Pflugers Arch* 456: 529–540, 2008
127. Kang L, Gao J, Schafer WR, Xie Z, Xu XZ: C. elegans TRP family protein TRP-4 is a pore-forming subunit of a native mechanotrans-

duction channel. *Neuron* 67: 381–391, 2010
128. Kindt KS, Viswanath V, Macpherson L, Quast K, Hu H, Patapoutian A, Schafer WR: Caenorhabditis elegans TRPA-1 functions in mechanosensation. *Nat Neurosci* 10: 568–577, 2007
129. Walker RG, Willingham AT, Zuker CS: A Drosophila mechanosensory transduction channel. *Science* 287: 2229–2234, 2000
130. Corry B, Hurst AC, Pal P, Nomura T, Rigby P, Martinac B: An improved open-channel structure of MscL determined from FRET confocal microscopy and simulation. *J Gen Physiol* 136: 483–494, 2010
131. Hamill OP, Martinac B: Molecular basis of mechanotransduction in living cells. *Physiol Rev* 81: 685–740, 2001
132. Christensen O, Hoffmann EK: Cell swelling activates K+ and Cl- channels as well as nonselective, stretch-activated cation channels in Ehrlich ascites tumor cells. *J Membr Biol* 129: 13–36, 1992
133. Clemo HF, Baumgarten CM: Swelling-activated Gd3+-sensitive cation current and cell volume regulation in rabbit ventricular myocytes. *J Gen Physiol* 110: 297–312, 1997
134. Popp R, Hoyer J, Meyer J, Galla HJ, Gogelein H: Stretch-activated non-selective cation channels in the antiluminal membrane of porcine cerebral capillaries. *J Physiol* 454: 435–449, 1992
135. Sachs F: Stretch-activated ion channels: what are they? *Physiology (Bethesda)* 25: 50–56, 2010
136. Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A: Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* 330: 55–60, 2010
137. Renneboog B, Musch W, Vandemergel X, Manto MU, Decaux G: Mild chronic hyponatremia is associated with falls, unsteadiness, and attention deficits. *Am J Med* 119: 71–78, 2006
138. Bourque CW: Central mechanisms of osmosensation and systemic osmoregulation. *Nat Rev Neurosci* 9: 519–531, 2008
139. Stocker SD, Osborn JL, Carmichael SP: Forebrain osmotic regulation of the sympathetic nervous system. *Clin Exp Pharmacol Physiol* 35: 695–700, 2008
140. Qiu DL, Shirasaka T, Chu CP, Watanabe S, Yu NS, Katoh T, Kannan H: Effect of hypertonic saline on rat hypothalamic paraventricular nucleus magnocellular neurons in vitro. *Neurosci Lett* 355: 117–120, 2004
141. Verney EB: The antidiuretic hormone and the factors which determine its release. *Proc R Soc Lond B Biol Sci* 135: 25–106, 1947
142. Bourque CW: Ionic basis for the intrinsic activation of rat supraoptic neurones by hyperosmotic stimuli. *J Physiol* 417: 263–277, 1989
143. Oliet SH, Bourque CW: Mechanosensitive channels transduce osmosensitivity in supraoptic neurons. *Nature* 364: 341–343, 1993
144. Zhang Z, Kindrat AN, Sharif-Naeini R, Bourque CW: Actin filaments mediate mechanical gating during osmosensory transduction in rat supraoptic nucleus neurons. *J Neurosci* 27: 4008–4013, 2007
145. Toney GM, Stocker SD: Hyperosmotic activation of CNS sympathetic drive: Implications for cardiovascular disease. *J Physiol* 588: 3375–3384, 2010
146. Taylor AC, McCarthy JJ, Stocker SD: Mice lacking the transient receptor vanilloid potential 1 channel display normal thirst responses and central Fos activation to hypernatremia. *Am J Physiol Regul Integr Comp Physiol* 294: R1285–R1293, 2008
147. Mizuno A, Matsumoto N, Imai M, Suzuki M: Impaired osmotic sensation in mice lacking TRPV4. *Am J Physiol Cell Physiol* 285: C96–C101, 2003
148. Liedtke W, Friedman JM: Abnormal osmotic regulation in trpv4-/- mice. *Proc Natl Acad Sci U S A* 100: 13698–13703, 2003
149. Carreno FR, Ji LL, Cunningham JT: Altered central TRPV4 expression and lipid raft association related to inappropriate vasopressin secretion in cirrhotic rats. *Am J Physiol Regul Integr Comp Physiol* 296: R454–R466, 2009
150. Han J, Gnatenco C, Sladek CD, Kim D: Background and tandem-pore potassium channels in magnocellular neurosecretory cells of the rat supraoptic nucleus. *J Physiol* 546: 625–639, 2003
151. Honore E: The neuronal background K2P channels: Focus on TREK1. *Nat Rev Neurosci* 8: 251–261, 2007
152. Zhang W, Wang D, Liu XH, Kosala WR, Rajapaksha JS, Fisher TE: An osmosensitive voltage-gated K+ current in rat supraoptic neurons. *Eur J Neurosci* 29: 2335–2346, 2009
153. Zhang W, Star B, Rajapaksha WR, Fisher TE: Dehydration increases L-type Ca(2+) current in rat supraoptic neurons. *J Physiol* 580: 181–193, 2007
154. Deleuze C, Alonso G, Lefevre IA, Duvoid-Guillou A, Hussy N: Extrasynaptic localization of glycine receptors in the rat supraoptic nucleus: Further evidence for their involvement in glia-to-neuron communication. *Neuroscience* 133: 175–183, 2005
155. Stricker EM, Hoffmann ML: Presystemic signals in the control of thirst, salt appetite, and vasopressin secretion. *Physiol Behav* 91: 404–412, 2007
156. Egan G, Silk T, Zamarripa F, Williams J, Federico P, Cunnington R, Carabott L, Blair-West J, Shade R, McKinley M, Farrell M, Lancaster J, Jackson G, Fox P, Denton D: Neural correlates of the emergence of consciousness of thirst. *Proc Natl Acad Sci U S A* 100: 15241–15246, 2003
157. Geelen G, Keil LC, Kravik SE, Wade CE, Thrasher TN, Barnes PR, Pyka G, Nesvig C, Greenleaf JE: Inhibition of plasma vasopressin after drinking in dehydrated humans. *Am J Physiol* 247: R968–R971, 1984
158. Hoffmann ML, DenBleyker M, Smith JC, Stricker EM: Inhibition of thirst when dehydrated rats drink water or saline. *Am J Physiol Regul Integr Comp Physiol* 290: R1199–R1207, 2006
159. Carlson SH, Beitz A, Osborn JW: Intragastric hypertonic saline increases vasopressin and central Fos immunoreactivity in conscious rats. *Am J Physiol* 272: R750–R758, 1997
160. Bykowski MR, Smith JC, Stricker EM: Regulation of NaCl solution intake and gastric emptying in adrenalectomized rats. *Physiol Behav* 92: 781–789, 2007
161. Stricker EM, Hoffmann ML: Control of thirst and salt appetite in rats: Early inhibition of water and NaCl ingestion. *Appetite* 46: 234–237, 2006
162. McHugh J, Keller NR, Appalsamy M, Thomas SA, Raj SR, Diedrich A, Biaggioni I, Jordan J, Robertson D: Portal osmopressor mechanism linked to transient receptor potential vanilloid 4 and blood pressure control. *Hypertension* 55: 1438–1443, 2010
163. Kuramochi G, Kobayashi I: Regulation of the urine concentration mechanism by the oropharyngeal afferent pathway in man. *Am J Nephrol* 20: 42–47, 2000
164. Andersen LJ, Jensen TU, Bestle MH, Bie P: Gastrointestinal osmoreceptors and renal sodium excretion in humans. *Am J Physiol Regul Integr Comp Physiol* 278: R287–R294, 2000
165. Dooley CP, Valenzuela JE: Duodenal volume and osmoreceptors in the stimulation of human pancreatic secretion. *Gastroenterology* 86: 23–27, 1984
166. Adachi A, Niijima A, Jacobs HL: An hepatic osmoreceptor mechanism in the rat: electrophysiological and behavioral studies. *Am J Physiol* 231: 1043–1049, 1976
167. Baertschi AJ, Vallet PG: Osmosensitivity of the hepatic portal vein area and vasopressin release in rats. *J Physiol* 315: 217–230, 1981
168. Haberich FJ: Osmoreception in the portal circulation. *Fed Proc* 27: 1137–1141, 1968
169. Choi-Kwon S, Baertschi AJ: Splanchnic osmosensation and vasopressin: Mechanisms and neural pathways. *Am J Physiol* 261: E18–E25, 1991
170. May M, Jordan J: The osmopressor response to water drinking. *Am J Physiol Regul Integr Comp Physiol* 300(1): R40–R46, 2011
171. Lechner SG, Markworth S, Poole K, Smith ES, Lapatsina L, Frahm S, May M, Pischke S, Suzuki M, Ibañez-Tallon I, Luft FC, Jordan J, Lewin GR: The molecular and cellular identity of peripheral osmoreceptors. *Neuron* 69: 332–344, 2011
172. Niijima A: Afferent discharges from osmoreceptors in the liver of the guinea pig. *Science* 166: 1519–1520, 1969
173. Chwalbinska-Moneta J: Role of hepatic por-

tal osmoreception in the control of ADH release. *Am J Physiol* 236: E603–E609, 1979

174. Adachi A: Thermosensitive and osmoreceptive afferent fibers in the hepatic branch of the vagus nerve. *J Auton Nerv Syst* 10: 269–273, 1984
175. Vallet PG, Baertschi AJ: Spinal afferents for peripheral osmoreceptors in the rat. *Brain Res* 239: 271–274, 1982
176. Craig AD: Interoception: The sense of the physiological condition of the body. *Curr Opin Neurobiol* 13: 500–505, 2003
177. Grundy D: Neuroanatomy of visceral nociception: Vagal and splanchnic afferent. *Gut* 51[Suppl 1]: i2–i5, 2002
178. Duggan KA, Ye VZ: Stimulation of the gastric sodium monitor reduces hepatic angiotensin-converting enzyme activity. *Clin Exp Pharmacol Physiol* 24: 449–450, 1997
179. Woudenberg-Vrenken TE, Bindels RJ, Hoenderop JG: The role of transient receptor potential channels in kidney disease. *Nat Rev Nephrol* 5: 441–449, 2009
180. Cohen DM: The transient receptor potential vanilloid-responsive 1 and 4 cation channels: Role in neuronal osmosensing and renal physiology. *Curr Opin Nephrol Hypertens* 16: 451–458, 2007
181. Silva GB, Garvin JL: TRPV4 mediates hypotonicity-induced ATP release by the thick ascending limb. *Am J Physiol Renal Physiol* 295: F1090–F1095, 2008
182. Bell PD, Lapointe JY, Sabirov R, Hayashi S, Peti-Peterdi J, Manabe K, Kovacs G, Okada Y: Macula densa cell signaling involves ATP release through a maxi anion channel. *Proc Natl Acad Sci U S A* 100: 4322–4327, 2003
183. Praetorius HA, Leipziger J: Intrarenal purinergic signaling in the control of renal tubular transport. *Annu Rev Physiol* 72: 377–393, 2010
184. Taniguchi J, Tsuruoka S, Mizuno A, Sato J, Fujimura A, Suzuki M: TRPV4 as a flow sensor in flow-dependent K+ secretion from the cortical collecting duct. *Am J Physiol Renal Physiol* 292: F667–F673, 2007
185. Wu L, Gao X, Brown RC, Heller S, O'Neil RG: Dual role of the TRPV4 channel as a sensor of flow and osmolality in renal epithelial cells. *Am J Physiol Renal Physiol* 293: F1699–F1713, 2007
186. Heer M, Baisch F, Kropp J, Gerzer R, Drummer C: High dietary sodium chloride consumption may not induce body fluid retention in humans. *Am J Physiol Renal Physiol* 278: F585–F595, 2000
187. Titze J, Maillet A, Lang R, Gunga HC, Johannes B, Gauquelin-Koch G, Kihm E, Larina I, Gharib C, Kirsch KA: Long-term sodium balance in humans in a terrestrial space station simulation study. *Am J Kidney Dis* 40: 508–516, 2002
188. Haljamae H: Sampling of nanoliter volumes of mammalian subcutaneous tissue fluid and ultra-micro flame photometric analyses of the K and Na concentrations. *Acta Physiol Scand* 78: 1–10, 1970
189. Go WY, Liu X, Roti MA, Liu F, Ho SN: NFAT5/TonEBP mutant mice define osmotic stress as a critical feature of the lymphoid microenvironment. *Proc Natl Acad Sci U S A* 101: 10673–10678, 2004
190. Machnik A, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K, Park JK, Beck FX, Muller DN, Derer W, Goss J, Ziomber A, Dietsch P, Wagner H, van Rooijen N, Kurtz A, Hilgers KF, Alitalo K, Eckardt KU, Luft FC, Kerjaschki D, Titze J: Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med* 15: 545–552, 2009
191. Machnik A, Dahlmann A, Kopp C, Goss J, Wagner H, van Rooijen N, Eckardt KU, Muller DN, Park JK, Luft FC, Kerjaschki D, Titze J: Mononuclear phagocyte system depletion blocks interstitial tonicity-responsive enhancer binding protein/vascular endothelial growth factor C expression and induces salt-sensitive hypertension in rats. *Hypertension* 55: 755–761, 2010
192. Titze J, Machnik A: Sodium sensing in the interstitium and relationship to hypertension. *Curr Opin Nephrol Hypertens* 19: 385–392, 2010
193. Ferraris JD, Burg MB: Tonicity-dependent regulation of osmoprotective genes in mammalian cells. *Contrib Nephrol* 152: 125–141, 2006
194. Kwon MS, Lim SW, Kwon HM: Hypertonic stress in the kidney: A necessary evil. *Physiology (Bethesda)* 24: 186–191, 2009
195. Tong EH, Guo JJ, Huang AL, Liu H, Hu CD, Chung SS, Ko BC: Regulation of nucleocytoplasmic trafficking of transcription factor OREBP/TonEBP/NFAT5. *J Biol Chem* 281: 23870–23879, 2006
196. Kwon MS, Lee SD, Kim JA, Colla E, Choi YJ, Suh PG, Kwon HM: Novel nuclear localization signal regulated by ambient tonicity in vertebrates. *J Biol Chem* 283: 22400–22409, 2008
197. Rodgaard T, Schou K, Friis MB, Hoffmann EK: Does the intracellular ionic concentration or the cell water content (cell volume) determine the activity of TonEBP in NIH3T3 cells? *Am J Physiol Cell Physiol* 295: C1528–C1534, 2008
198. Norgaard JP, Djurhuus JC, Watanabe H, Stenberg A, Lettgen B: Experience and current status of research into the pathophysiology of nocturnal enuresis. *Br J Urol* 79: 825–835, 1997
199. Kujubu DA, Aboseif SR: An overview of nocturia and the syndrome of nocturnal polyuria in the elderly. *Nat Clin Pract Nephrol* 4: 426–435, 2008
200. Majzoub JA, Srivatsa A: Diabetes insipidus: Clinical and basic aspects. *Pediatr Endocrinol Rev* 4[Suppl 1]: 60–65, 2006
201. Boone M, Deen PM: Physiology and pathophysiology of the vasopressin-regulated renal water reabsorption. *Pflugers Arch* 456: 1005–1024, 2008
202. Boone M, Deen PM: Congenital nephrogenic diabetes insipidus: What can we learn from mouse models? *Exp Physiol* 94: 186–190, 2009
203. Noda Y, Sohara E, Ohta E, Sasaki S: Aquaporins in kidney pathophysiology. *Nat Rev Nephrol* 6: 168–178, 2010
204. Veland IR, Awan A, Pedersen LB, Yoder BK, and Christensen, ST: Primary cilia and signaling in human health and disease. *Nephron Physiol* 111(3): 39–53, 2009
205. Cardenas-Rodriguez M, Badano JL: Ciliary biology: Understanding the cellular and genetic basis of human ciliopathies. *Am J Med Genet C Semin Med Genet* 151C: 263–280, 2009
206. Hurd TW, Hildebrandt F: Mechanisms of nephronophthisis and related ciliopathies. *Nephron Exp Nephrol* 118: e9–14, 2011
207. Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AE, Lu W, Brown EM, Quinn SJ, Ingber DE, Zhou J: Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* 33: 129–137, 2003
208. Nauli SM, Rossetti S, Kolb RJ, Alenghat FJ, Consugar MB, Harris PC, Ingber DE, Loghman-Adham M, Zhou J: Loss of polycystin-1 in human cyst-lining epithelia leads to ciliary dysfunction. *J Am Soc Nephrol* 17: 1015–1025, 2006
209. Harris PC, Torres VE: Polycystic kidney disease. *Annu Rev Med* 60: 321–337, 2009
210. Tian W, Fu Y, Garcia-Elias A, Fernandez-Fernandez JM, Vicente R, Kramer PL, Klein RF, Hitzemann R, Orwoll ES, Wilmot B, McWeeney S, Valverde MA, Cohen DM: A loss-of-function nonsynonymous polymorphism in the osmoregulatory TRPV4 gene is associated with human hyponatremia. *Proc Natl Acad Sci U S A* 106: 14034–14039, 2009