Review

Osmosensation in vasopressin neurons: changing actin density to optimize function

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The proportional relation between circulating vasopressin concentration and plasma osmolality is fundamental for body fluid homeostasis. Although changes in the sensitivity of this relation are associated with pathophysiological conditions, central mechanisms modulating osmoregulatory gain are unknown. Here, we review recent data that sheds important light on this process. The cell autonomous osmosensitivity of vasopressin neurons depends on cation channels comprising a variant of the transient receptor potential vanilloid 1 (TRPV1) channel. Hyperosmotic activation is mediated by a mechanical process where sensitivity increases in proportion with actin filament density. Moreover, angiotensin II amplifies osmotic activation by a rapid stimulation of actin polymerization, suggesting that neurotransmitter-induced changes in cytoskeletal organization in osmosensory neurons can mediate central changes in osmoregulatory gain.

Vasopressin and body fluid homeostasis

Mammals have evolved powerful homeostatic mechanisms that work together to maintain plasma osmolality near 300 mOsmol/kg. In healthy individuals, fluctuations larger than ±3 mOsmol/kg away from the species–specific set point (e.g. 286 mOsmol/kg in humans) normally induce appropriate changes in salt and water intake or excretion to achieve homeostasis [1–3]. This *systemic* osmoregulation is vital, because changes in cell volume caused by severe acute hyperosmolality or hypoosmolality can irreversibly damage organs and cause lethal neurological trauma [4–7]. The osmotic control of vasopressin (VP) secretion plays a key role in systemic osmoregulation because this peptide stimulates water reabsorption by the kidney. VP (also known as antidiuretic hormone) is synthesized in the somata of hypothalamic magnocellular neurosecretory cells (MNCs) located in the supraoptic and paraventricular nuclei. The axons of MNCs project to the neurohypophysis, where Ca^{2+}-dependent exocytosis in their nerve terminals causes VP to be secreted into the blood in proportion with the rate at which action potentials are discharged by MNC somata [8] (Figure 1). Experiments *in vivo* [9] have shown that when plasma osmolality falls below the osmotic set point, the resting action potential firing rate of MNCs decreases, thus lowering basal VP release and promoting diuresis (Figure 1). Conversely, when plasma osmolality rises above the set point, MNCs are excited, thereby increasing VP secretion and enhancing water reabsorption.

A stable and approximately linear relation is normally observed between VP concentration and plasma osmolality under resting conditions [10,11]. The slope of this relation reflects the overall sensitivity of this homeostatic mechanism and we henceforth use the term *osmoregulatory gain* when referring to this parameter. Previous studies have shown that osmoregulatory gain can change to further optimize homeostasis under specific conditions. For example, osmoregulatory gain is enhanced at nighttime to minimize dehydration during sleep [12]. Osmoregulatory gain is also increased during hypovolemia (e.g. after hemorrhage) to help maintain arterial pressure and restore blood volume [10,11]. Conversely, osmoregulatory gain is attenuated during hypovolemia to promote homeostasis by favoring diuresis [10,11]. Interestingly, inappropriate changes in osmoregulatory gain appear to contribute to the development of various fluid balance disorders, including hypernatremia or hyponatremia. For example, a recent study has shown that an increase in central osmoreception can cause inappropriate thirst and vasopressin secretion following myocardial infarction, and could thus be a factor in the development of hyponatremia and pulmonary edema in this condition [13]. Exaggerated osmoreception can also be involved in hyponatremic conditions associated with some forms of the syndrome of inappropriate antidiuretic hormone release [14,15]. In contrast, decreases in osmoregulatory gain caused by inadequate osmosensation are a common cause of hypernatremia in the aged [16] and could mediate hyponatremia associated with decreases in osmotically-induced thirst and vasopressin release following surgery for craniopharyngioma [17] and many forms of central diabetes insipidus [18]. It is therefore important to characterize the mechanisms that mediate the osmotic control of VP release, so that we can better understand the etiology of such fluid balance disorders and design new approaches for clinical intervention. The objective of this review is to provide a synthesis of our current understanding of the cellular and molecular mechanisms contributing to the intrinsic osmosensitivity of VP-releasing MNCs and to highlight how modulation of this specific process might mediate central changes in osmoregulatory gain. We will

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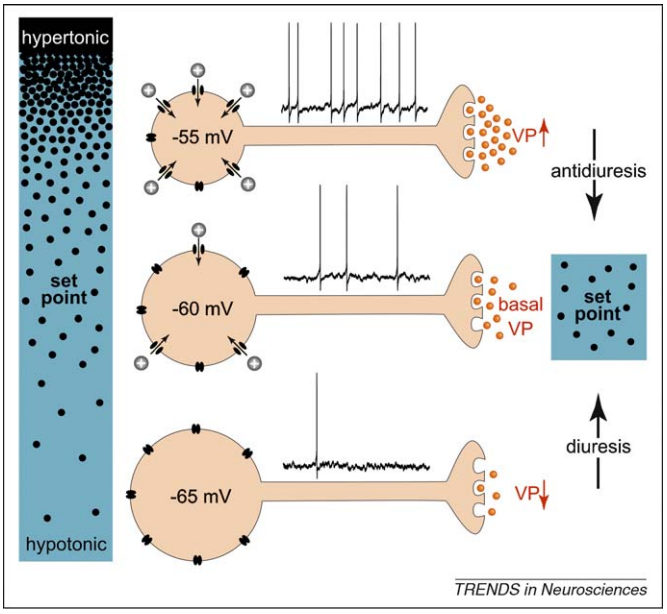

Figure 1. Cell autonomous osmoreception in vasopressin neurons. Changes in osmolality cause inversely proportional changes in soma volume. Shrinkage activates nonselective cation channels (NSCCs) and the ensuing depolarization increases action potential firing rate and vasopressin (VP) release from axon terminals in the neurohypophysis. Increased VP levels in blood enhance water reabsorption by the kidney (antidiuresis) to restore extracellular fluid osmolality toward the set point. Hypotonic stimuli inhibit NSCCs. The resulting hyperpolarization and inhibition of firing reduces VP release and promotes diuresis.

first discuss the various integrated mechanisms that are at play in the osmotic control of vasopressin releasing MNCs, and then explain in detail how the osmotic regulation of a specific ion channel contributes to this process. The final part of the review will highlight new findings that show how the actin cytoskeleton of MNCs plays a determining role in their osmosensitivity, and how regulated changes in actin density could modulate osmoregulatory gain.

Factors contributing to the osmotic control of MNCs

Previous studies have shown that both extrinsic and intrinsic factors contribute to the osmotic control of firing rate and VP release in MNCs [2,19]. Notably, MNCs receive direct excitatory inputs from osmoreceptor neurons in the organum vasculosum lamina terminalis (OVLT) [20], where firing rate increases proportionally with fluid osmolality [21]. The rate at which glutamate-mediated excitatory postsynaptic potentials are generated in MNCs therefore increases in proportion with osmolality [22], and this excitatory synaptic drive plays an essential role in the osmotic control of firing rate and VP release by MNCs [23]. Recent studies have also revealed that the astrocytes surrounding MNCs release the free amino acid taurine as an inverse function of fluid osmolality [24]. Taurine is an effective agonist of inhibitory glycine receptors on MNCs [25]. Therefore, hyperosmotic conditions can promote the excitation of MNCs by removing the hyperpolarizing effect of glycine receptors [25]. Finally, it is now well established that VP-releasing MNCs can act as cell autonomous osmoreceptors [26,27]. Electrophysiological recordings from MNCs from adult rodents have shown that hyperosmotic stimuli depolarize these cells via the activation of nonselective cation channels (NSCCs), and that hypoosmolality causes hyperpolarization by inhibiting NSCCs that are active under resting conditions [28–30]. MNCs have therefore been proposed to encode dynamic changes in osmolality by causing proportional changes in the probability of opening of a single population of NSCCs [28,29]. Although extrinsic influences are necessary for the osmotic control of firing rate and VP release by MNCs, recent studies have indicated that the modulation of NSCCs can be particularly important for the control of osmoregulatory gain [31,32]. Therefore, in the remainder of this review we focus our attention on the osmotic control and modulation of NSCCs in MNCs.

Molecular identity of the osmosensory channel

Recent studies have shown that the NSCCs mediating osmosensory transduction in MNCs are highly permeable to calcium [33] and can be blocked by extracellular application of gadolinium [34] or ruthenium red [35]. These findings are therefore generally consistent with recent reports suggesting that members of the transient receptor potential vanilloid (TRPV) family of cation channels could play an important role in osmosensory transduction [21,35–40]. Indeed, previous studies have shown that expression of mammalian TRPV2 or TRPV4 in heterologous cell lines can generate functional osmotically-gated NSCCs[37,39,41]. However, unlike the native NSCCs expressed in MNCs, recombinant homomultimeric TRPV2 and TRPV4 channels are activated by hypoosmolality and are unaffected by hyperosmolality [37,39]. Nonetheless, it has been proposed that TRPV2 or TRPV4 subunits could participate in the formation of hypertonicity sensors when associated with other proteins in their native environment [42]. Support for this hypothesis was obtained in a well-designed study by Liedtke *et al.* showing that transgenic expression of the mammalian TRPV4 channel can rescue the loss of hyperosmolality avoidance behavior in the *osm-9* mutant of *Caenorhabditis elegans* [36]. Unfortunately, inactivation of the *trpv4* gene in mice has yielded more ambiguous results. In one study, *trpv4* knockout mice were reported to display impaired thirst and weaker VP responses to systemic hypertonicity [38], whereas similar mice generated by another group showed no difference in water intake and seemed to generate an exaggerated VP response to hypertonicity [40]. The reasons for the differences observed in these studies remain to be clarified, and the precise role of TRPV4 channels in osmoregulation need to be established.

More recently, another TRPV channel, TRPV1, was shown to contribute to osmoreception. Rodent MNCs were found to express an N-terminal splice variant of the *trpv1* gene, but not the full-length product (i.e. TRPV1, the capsaicin receptor) [35]. Interestingly, mice lacking expression of the *trpv1* gene were found to be chronically hyperosmolar and to display attenuated VP responses to hyperosmotic stimulation *in vivo*. Strikingly, MNCs acutely isolated from *trpv1* knockout mice were not depolarized or excited by acute hyperosmolality, suggesting that the *trpv1* gene could encode a pore-forming component of the osmotically gated NSCC in MNCs [35]. Although further studies are required to establish the

molecular structure of the native mammalian osmosensory channel, it is interesting to note that NSCCs also mediate osmosensory transduction in OVLT neurons and that the intrinsic osmosensitivity of these cells is also lost in *trpv1* knockout mice [21]. In the next section, we describe how the effects of osmotic stimuli mediate changes in NSCC activity during osmoreception.

Osmosensory transduction is a mechanical process

Studies in isolated MNCs have shown that increased NSCC activity and action potential firing upon exposure to hypertonicity are proportionally linked to an accompanying decrease in cell volume [28]. Unlike many other types of cells, which can partly or completely resist osmotically evoked volume changes through compensatory mechanisms [43,44], MNCs display reversible changes in volume that are inversely proportional to osmolality and can be sustained for many minutes (osmometry) [28,45]. Although these observations suggest that changes in cell volume could be involved in the modulation of NSCCs in MNCs, it is important to recognize that osmotically-induced volume changes are also accompanied by inversely proportional changes in ionic strength and in the concentration of intracellular solutes. Many ion channels are modulated by intracellular ligands or changes in ionic strength [46], and thus it is conceivable that osmotically-induced changes in these parameters could form the basis for the osmotic control of NSCCs. However, studies in isolated MNCs have shown that these channels can be activated when cell volume is reduced in the absence of changes in ionic strength or solute concentration [28]. Notably, it was found that changes in cell volume caused by increasing or decreasing the pressure inside a recording pipette could cause inverse changes in NSCC activity that were quantitatively equivalent to those induced by osmotic stimulation [32]. Moreover, the activation of NSCCs caused by hyperosmolality-induced shrinking could be reversed by restoring cell volume with positive pressure, even though the cell was maintained in the presence of a hypertonic solution (Figure 2a). Similarly, the inhibition of NSCCs caused by hypoosmolality-induced swelling could be reversed by restoring cell volume with negative pressure [32]. These results indicate that osmosensory transduction in MNCs does not depend on changes in solute concentration or ionic strength but on a mechanical effect linked to changes in cell volume.

In agreement with this hypothesis, previous studies have shown that NSCCs in MNCs are inhibited by local membrane stretch during cell-attached single channel recording [28,47]. Although this behavior is consistent with the osmotic modulation of NSCCs during changes in cell volume, it remains unclear if the mechanosensitivity of NSCCs during patch clamp recordings mediates osmosensory transduction at the whole cell level. Although the exact nature of the mechanism by which changes in cell volume cause changes in NSCC activity remains to be determined, recent data reviewed in the following section show that actin filaments can participate in this process.

Mechanical regulation of NSCCs requires actin filaments

Studies in various mechanosensitive systems have indicated that several structural cellular elements can participate in the transmission and detection of forces applied to the cell surface. These include various elements of the cytoskeleton as well as molecular components of the extracellular matrix and cell adhesion complex (for recent reviews see Refs [48–50]). Although many of these elements could play important roles during osmosensory transduction *in situ*, the intracellular cytoskeleton appears to be a strategic component that could relay forces generated during changes in cell volume to the molecular mechanotransduction

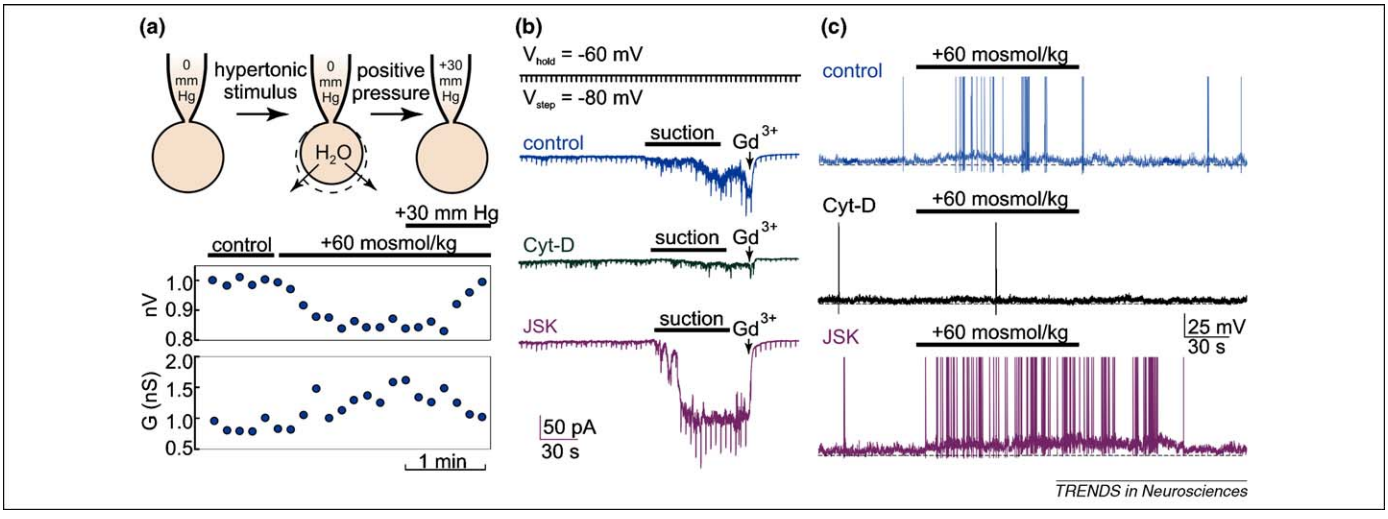

Figure 2. Actin filaments mediate mechanical gating during osmosensory transduction. **(a)** Effects of soma volume changes during whole cell patch clamp recordings from rat magnocellular neurosecretory cells (MNCs). Hyperosmotic stimulation (shaded area) causes a decrease in normalized volume (nV) which can be reversed by applying positive pressure to the pipette. Note that the shrinking-induced increase in membrane conductance (G) is reversed by restoring cell volume with hydrostatic pressure. **(b)** Effects of suction-induced shrinking (~10%) on whole cell current in isolated rat MNCs. Holding voltage (V_{HOLD}) was –60 mV and cells were briefly stepped (V_{STEP}) to –80 mV to assess membrane conductance (upper trace). Gadolinium (Gd^{3+}, 300 μM) is added to the bath (arrow) to confirm that the suction-induced inward current is mediated by nonselective cation channels. Note that compared with control, suction-induced currents are reduced in cells pretreated with cytochalasin D (Cyt-D) and enhanced by Jasplakinolide (JSK), compounds that decrease and increase actin filament density, respectively. **(c)** Effects of hypertonic stimulation on membrane potential and action potential firing. Note that compared with control, the depolarization and firing responses of MNCs are suppressed by Cyt-D and enhanced by JSK. Adapted, with permission, from Ref. [32].

elements that modulate NSCC activity in acutely isolated MNCs (i.e. in the absence of functional cell–cell contacts). Indeed, recent studies have shown that the increase in NSCC current, membrane depolarization and action potential firing evoked by shrinking in isolated MNCs are blocked by cytochalasin D (Figure 2b,c), a compound that causes the depolymerization of actin filaments (F-actin) [31,32]. In contrast, responses to hypertonicity, or suction-induced shrinking, are exaggerated in MNCs treated with jasplakinolide, a drug that promotes actin polymerization [31,32] (Figure 2b,c). These findings indicate that F-actin plays a key role in the osmotic and mechanical regulation of NSCCs and that the sensitivity of the osmosensory transduction complex varies in proportion with F-actin density.

Role of the cytoskeleton in osmosensory transduction

Interestingly, there are widely divergent views on the role of the cytoskeleton in the regulation of mechanosensitive ion channels. Several studies, including those on MNCs and other subsets of neuronal [51,52] and non-neuronal cells [53,54], have reported that disruption of the actin cytoskeleton impairs mechanosensitive gating. Therefore, in these studies it was concluded that F-actin actively participates in the mechanical control of mechanosensitive channels. However, studies using other preparations have indicated that mechanosensitive channel gating can be enhanced by disruption of the cytoskeleton [55–57]. In these cases, it was suggested that mechanosensitive gating is specifically mediated by changes in the physical interaction of the channel protein with the local lipid bilayer, and that the cytoskeleton actually "protects" the channel from mechanical activation by providing physical support for the plasma membrane. Cytoskeleton-dependent mechanoprotection has been reported for several mechanosensitive channels [58], including native stretch-activated cation channels in *Xenopus* oocytes [59], stretch-activated potassium channels in some molluscan neurons [60] and subsets of stretch-activated potassium channels of the TWIK (Tandem of Pore-domains in a Weakly Inward rectifying K^+) family, including TREK (TWIK-1 related K^+) and TRAAK (TWIK-1 related arachidonic acid-stimulated K^+ channel) [61,62]. For some of these channels, additional evidence for bilayer-dependent gating has been provided by the observation that recombinant channels reconstituted in cytoskeleton-free artificial bilayers can still respond to mechanical stimulation or application of amphipathic compounds that cause crenation (small dome-shaped foldings) of the bilayer [59,61,63]. Evidently, the role played by the cytoskeleton in the regulation or modulation of gating in mechanosensitive channels (i.e. as a mediator of gating or as a shield protecting the channels against mechanical activation) must be determined not only by the subtype of ion channel involved but also by the other proteins with which it associates *in situ*.

The finding that osmomechanical transduction gain increases with F-actin density in MNCs indicates that the cytoskeleton actively participates in the mechanical control of NSCCs in these cells. Interestingly, previous research has shown that increases in F-actin density can stiffen a crosslinked network of actin filaments [64,65]. Therefore, if a network of F-actin is used to transmit mechanical forces associated with compression or expansion during changes in cell volume, then the amount of force applied per unit volume change would increase with F-actin density. As a result, the apparent sensitivity of response of the mechanotransducer to a given change in volume would be expected to increase. Although these combined observations support the notion that F-actin mediates the mechanical modulation of NSCCs during osmotic stimulation in MNCs, it remains unclear how the actin cytoskeleton contributes to this process. One possibility is that the cytoskeleton modulates these channels indirectly via the cytoskeleton-dependent mechanical modulation of a putative enzyme that could be involved in the regulation of NSCCs [66]. However, the absence of pronounced washout effects in patch clamp experiments and the tight temporal coupling between cell volume and NSCC activity oppose this type of mechanism. An alternate hypothesis is that NSCC activity might be regulated through a direct tether-type interaction between the channel and the cytoskeleton [63,66,67]. Indeed, the TRPV1 protein comprises intracellular domains that might allow direct or indirect interactions with cytoskeleton elements [68,69]. Finally, we cannot exclude the possibility that NSCCs are not attached to the cytoskeleton but that they are subject to a form of bilayer-dependent gating that relies on specialized plasma membrane microdomains whose structure is determined by the cytoskeleton [70,71]. Although additional research is required to define the molecular basis for osmosensory transduction, recent experiments summarized below have shown that receptor-mediated changes in actin filament density can modulate the mechanical control of NSCCs.

Enhancement of osmosensory gain by angiotensin II

Although the central modulation of osmoregulatory gain plays a key role in the physiology and pathology of fluid balance and in the coordination of cardiovascular and hydromineral homeostasis [10–12,16,18], the basis for such modulation is unknown. Previous studies have shown that neurons in the subfornical organ (SFO) are excited during hypovolemia [72,73] and hypotension [74]. SFO neurons project axons onto MNCs [75,76], where they might contribute to the potentiation of osmotically-evoked action potential firing and VP release in the neurohypophysis [77,78]. A body of evidence indicates that SFO neurons contain angiotensin II (Ang II) [74,76] and that the release of this peptide can lead to excitation of MNCs [78,79], as well as an increase in the osmotic activation of MNCs [47,80,81]. Previous studies have shown that MNCs express the Ang II type 1 receptor (AT1R) [82,83], and mice lacking this receptor show attenuated VP release in response to hyperosmolality compared with wild type mice [84]. It is therefore reasonable to hypothesize that Ang II is released onto MNCs by the axon terminals of SFO neurons during hypovolemia, a condition which is known to enhance osmoregulatory gain [10,11]. We therefore recently examined the effects of Ang II on osmosensory transduction in acutely isolated MNCs. We found that Ang II causes a rapid (within 60 s) potentiation of the relationship between action potential firing frequency and fluid osmolality via an increase in the amplitude of the depolarizing osmoreceptor potentials

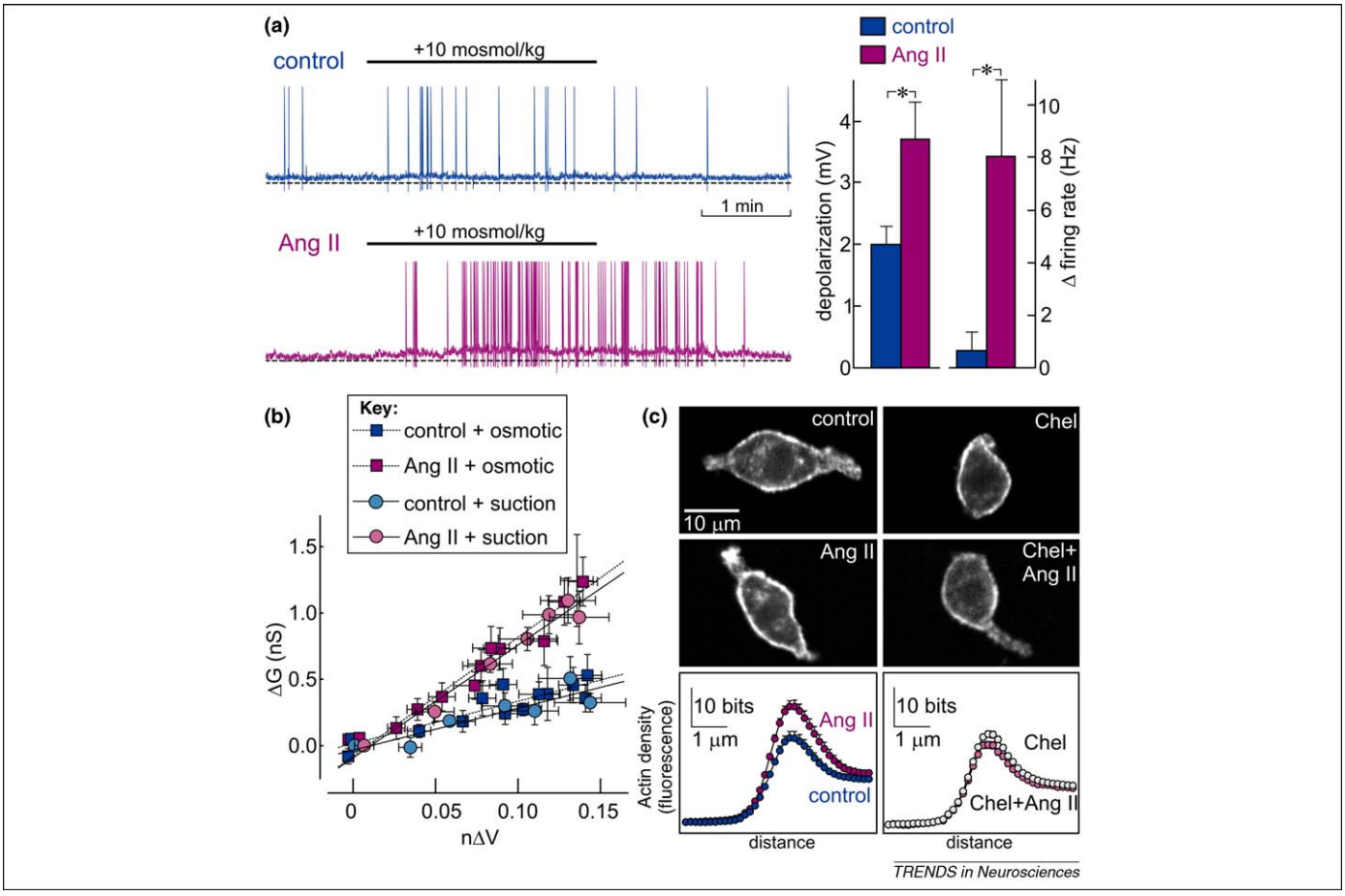

Figure 3. Angiotensin II (Ang II) increases F-actin density and osmosensory gain. **(a)** Whole cell current clamp recordings from isolated MNCs (left) and averaged data from multiple cells (bar graph at right) show that the depolarizing and action potential firing responses induced by a hypertonic stimulus are significantly enhanced in the presence of 100 nM Ang II. **(b)** Mean changes in membrane conductance (ΔG) evoked by osmotic (squares) or mechanical stimuli (suction, circles) are plotted as a function of the normalized volume decrease ($n\Delta V$) in the absence (open symbols) or presence (filled symbols) of Ang II. Note that Ang II increases the slopes of these relations by equivalent amounts. **(c)** Upper panels are confocal images showing F-actin intensity (fluorescently labeled phalloidin) in four isolated MNCs treated in different conditions. The lower panels are line scan plots showing mean (±SEM) values of fluorescence (F-actin density; in bits) at the perimeter of many cells exposed to the corresponding treatments. Note that Ang II increases F-actin density and that this effect can be inhibited by chelerythrine (Chel), a potent selective inhibitor of protein kinase C. Adapted, with permission, from Ref. [31].

generated by hyperosmotic stimuli (Figure 3a) [31,47]. Further analysis revealed that Ang II does not affect the magnitude of volume changes caused by osmotic stimulation but that it enhances osmosensory gain by amplifying mechanosensory transduction (Figure 3b) through a signaling pathway involving phospholipase C (PLC) and a calcium-dependent form of protein kinase C (PKC). Interestingly, exposure to Ang II also caused a rapid PKC-dependent increase in F-actin density (visible within 2 min; Figure 3c). Moreover, prior depolymerization of actin cytoskeleton with cytochalasin D abolished the effect of Ang II on mechanosensitivity, whereas prior enhancement and stabilization of F-actin with jasplakinolide occluded the effects of Ang II on mechanosensitivity [31].

Role of receptor-mediated cytoskeletal plasticity

The findings discussed above suggest that a receptor-mediated increase in cortical actin density can cause a physiologically relevant increase in the mechanosensitivity of MNCs [31]. If this is the case, then neurotransmitters or modulators (e.g. Ang II) released by neural afferents to MNCs (e.g. from the SFO) could mediate the central modulation of osmoregulatory gain. But what might be the basis for such rapid, receptor-mediated, changes in F-actin density? Studies in cultured hippocampal neurons have shown that the activation of PKC can increase F-actin density by stimulating actin polymerization via RhoA and Rac1 [85], members of the Rho family of small GTPases. Moreover, G-protein-coupled receptors can mediate Rho GTPase activation [86–89], and Ang II has been shown to mediate a very rapid (<60 s) Rho-dependent increase in F-actin density in a variety of cell types [90–92]. Thus, a modulation of F-actin density, controlled by RhoA activity, could provide a molecular mechanism by which osmosensory gain is enhanced in MNCs (Figure 4). Interestingly, recent studies have shown that Ang II can enhance the expression of guanine nucleotide exchange factors (GEFs) such as LARG and p115RhoGEF [89,93–96] which can mediate the activation of RhoA. Enhanced RhoA activation due to increased expression of these GEFs could potentially cause chronic increases in osmoregulatory gain under different physiological or pathological conditions *in vivo*. Additional studies will be required to investigate the possible involvement of Rho family GTPases, and their regulatory factors, in mediating the effects of Ang II on MNCs, and to investigate the effects of cytoskeleton remodeling on cell autonomous osmosensory transduction.

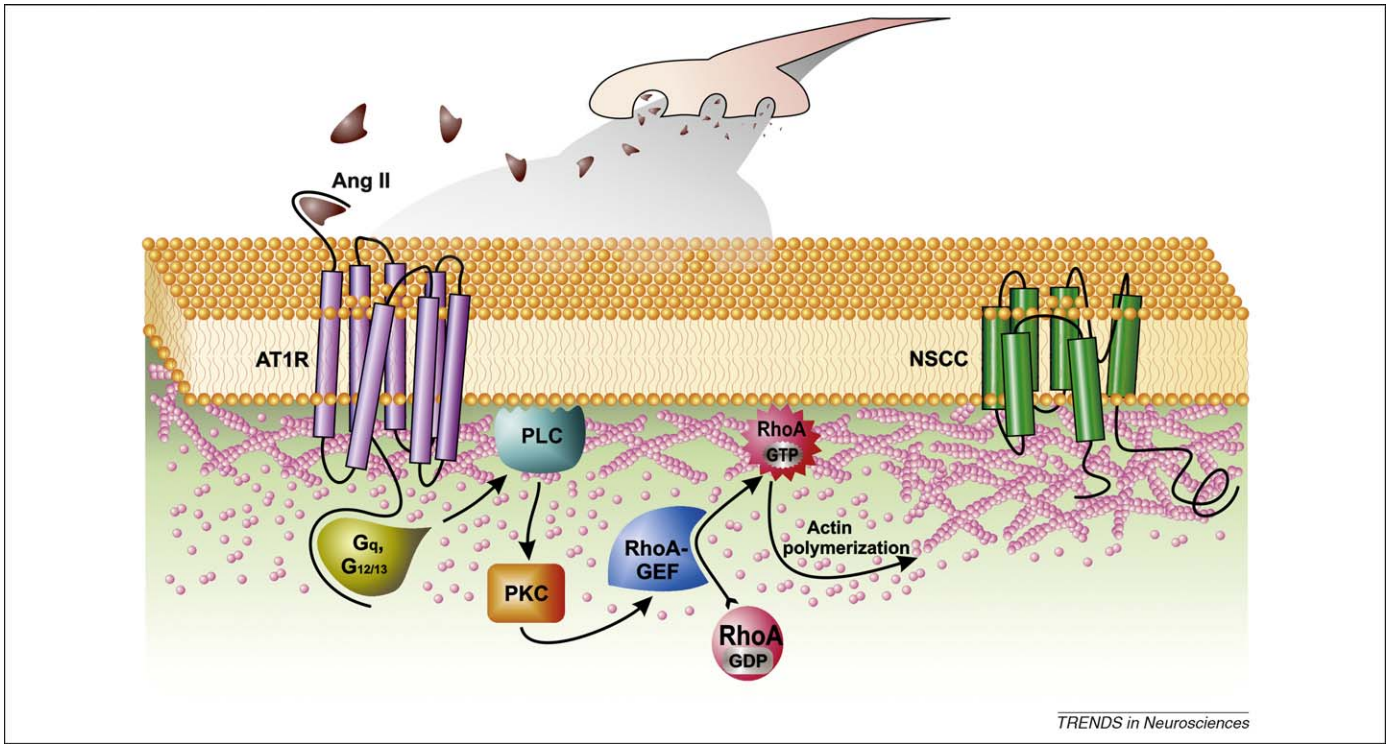

Figure 4. Hypothetical events mediating central Ang II enhancement of osmosensory gain. Ang II released by afferent nerve terminals (e.g. during hypovolemia) binds to AT1 receptor (AT1R) coupled to G proteins such as G_q or/and $G_{12/13}$. Activated G proteins signal through phospholipase C (PLC) and protein kinase C (PKC) to activate a RhoA-specific guanine nucleotide exchange factor (RhoA–GEF), such as p115RhoGEF or LARG (leukemia-associated Rho guanine–nucleotide exchange factor) [93,95]. Activation of RhoA–GEF converts inactive cytosolic RhoA (RhoA–GDP) into active, membrane-associated RhoA–GTP by promoting the exchange of GDP to GTP. Activated RhoA induces actin polymerization and increases submembrane F-actin density to enhance the mechanical gating of NSCCs.

Concluding remarks

Recent experiments have shown that rodent VP-releasing MNCs are cell autonomous osmosensors, and that the intrinsic osmosensitivity of these cells is mediated by the volume-dependent mechanical regulation of NSCCs that may comprise a product of the *trpv1* gene. It has also been shown that F-actin is required for the osmotic and mechanical regulation of NSCCs, and that rapid receptor-mediated changes in F-actin density cause proportional changes in osmosensory gain. Despite these important advances, many aspects of the mechanism that underlies the osmotic control of NSCCs remain unknown. Is channel activity modulated by cytoskeleton-dependent enzymes? Does the actin cytoskeleton contribute to this process by acting as a force-transmitting tether or by providing structural support for microdomains in the plasma membrane? Is F-actin physically associated with the channel? Do other cytoskeletal elements contribute to osmosensory transduction? Indeed, microtubules, myosins, cadherins and extracellular matrix proteins are also regulated by RhoA [97], and these cytoskeletal elements are known to be an integral part of the mechanotransduction complex in other systems [49]. Although questions such as these will continue to focus our attention on molecular aspects of the osmosensory process, we must also elaborate *in vivo* approaches to expand our greater understanding of the modulation of osmoregulatory gain under physiological and pathological conditions.

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