Deciphering physiological role of the mechanosensitive TRPV4 channel in the distal nephron

M. Mamenko, O. Zaika, N. Boukelmoune, R. G. O'Neil, and O. Pochynyuk

Department of Integrative Biology and Pharmacology; The University of Texas Health Science Center at Houston, USA

Please correspond with Oleh Pochynyuk at:

University of Texas Health Science Center at Houston,
Department of Integrative Biology and Pharmacology
6431 Fannin, Houston TX, 77030;
Ph. (713) 500-7466; Fx. (713) 500-7455;
Oleh.M.Pochynyuk@uth.tmc.edu.

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ABSTRACT

Long-standing experimental evidence suggests that epithelial cells in the renal tubule are able to sense osmotic and pressure gradients caused by alterations in ultrafiltrate flow by elevating intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)). These responses are viewed as critical regulators of a variety of processes ranging from transport of water and solutes to cellular growth and differentiation. A loss in the ability to sense mechanical stimuli has been implicated in numerous pathologies associated with systemic imbalance of electrolytes and to development of the polycystic kidney disease (PKD). The molecular mechanisms conferring mechanosensitive properties to the epithelial tubular cells involve activation of transient receptor potential (TRP) channels, such as TRPV4, allowing direct Ca\(^{2+}\) influx to increase [Ca\(^{2+}\)]\(_i\). In this paper, we critically analyze the current evidence about signaling determinants of TRPV4 activation by luminal flow in the distal nephron, and discuss how dysfunction of this mechanism contributes to PKD progression. We also review the physiological relevance of TRPV4-based mechanosensitivity in controlling flow-dependent K\(^+\) secretion in the distal renal tubule.

Key words: flow-sensitivity, mechanosensitive [Ca\(^{2+}\)]\(_i\) signaling, polycystic kidney disease, renal potassium excretion.
INTRODUCTION

The strategic function of the kidneys is to maintain homeostasis of the internal body milieu via control of urinary production. Kidneys filter enormous quantities of plasma (approximately 180 L/day) via the process called glomerular ultrafiltration. This enables efficient correction of the circulating plasma volume and elimination of metabolite wastes. In addition, the kidneys possess powerful solute and water reabsorptive machinery which leads to the conservation useful substances, virtually preventing their excretion with urine as necessary. This important function gives “freedom” to have dietary intake with greatly varying amounts of water and electrolytes from day to day. Daily alterations in dietary regimen induce substantial changes in the ultrafiltrate flow and osmotic pressure gradients along the nephron with the greatest values occurring in the distal tubular segments (134). Mechanical stress, arising from these alterations in ultrafiltrate delivery is viewed as an important signal, which allows the epithelial cells to properly adjust transport rates of water and solutes (discussed in (90, 127)). Recent experimental evidence suggests a pivotal role of mechanosensitive TRPV4 channel in mediating cellular responses to these stimuli (see below).

In this review, we refer to the distal nephron as the site comprising the connecting tubule (CNT) and the collecting duct (CD) system. Quantitatively, the distal nephron processes only approximately 10% of glomerular filtrate. However, this region is not autoregulated via tubuloglomerular feedback (TGF) so that local control of the distal nephron transport rates shapes the final urine volume and composition (74, 98). Dysfunction of the transporting systems in this site is linked to a number of disease states associated with disturbances in the circulating plasma volume and imbalance of electrolytes. This includes
blood pressure abnormalities, nephrogenic diabetes insipidus, and Cushing syndrome, to name a few (2, 67, 91). The distal nephron contains two different cell types with clearly distinct functions. The principal cells (PCs) comprise more than 2/3 of the total cell population. PCs reabsorb sodium (mediated mainly by the epithelial Na⁺ channel or ENaC) and water (through aquaporin type 2 or AQP2-dependent osmotic water reabsorption), and are responsible for K⁺ secretion, mainly via the renal outer medullary K⁺ channel (ROMK)-dependent pathways (74, 98). The remaining 1/3 of the cells are intercalated cells (ICs), which are critical for maintaining acid/base balance. These can be further subdivided into H⁺ secreting A-type, HCO₃⁻ secreting B-type, and intermediate non-A-non-B type (8). ICs also substantially contribute to the large conductance K⁺ channel (BK)-dependent (40) and possibly the small-conductance (SK3) KCa2.3-dependent (4) potassium secretion in response to elevated potassium intake and loop/thiazide diuretic treatments.

CURRENT UNDERSTANDING OF MOLECULAR MECHANISMS OF MECHANOSENSITIVITY IN THE DISTAL NEPHRON CELLS

Increases in the delivery of glomerular ultrafiltrate to the distal nephron, for example in response to sodium overloading/volume expansion and elevated potassium intake (40), greatly potentiate tubular flow at this site exerting shear stress to the apical (also referred as luminal) membrane. Multiple studies demonstrate that distal nephron epithelial cells respond to mechanical stimuli, in part, by elevating intracellular Ca²⁺ concentration ([Ca²⁺]i) (32, 54, 56, 58, 81, 95, 127, 133, 143). However, the exact molecular mechanism of these [Ca²⁺]i responses remains a matter of debate and revision.
The most common cellular model of the mechanosensitive \([\text{Ca}^{2+}]_i\) elevations is based on shear stress-induced bending of a special cellular organelle, the primary cilium. The cilium protrudes into the tubular lumen and is thought to act as an antenna for detection of changes in tubular flow (65, 82, 83). This bending causes activation of the proteins associated with cilium: the G-protein coupled receptor polycystin 1 (PC1) and the non-selective \(\text{Ca}^{2+}\)-permeable channel polycystin 2 (PC2, also known as TRPP2). This induces \(\text{Ca}^{2+}\) influx at the base of the primary cilium triggering subsequent release of \(\text{Ca}^{2+}\) from the endoplasmic reticulum intracellular stores (65). However, studies in both perfused and split-opened murine distal nephrons revealed that cilium-lacking ICs (86) respond to high flow over the apical surface with \([\text{Ca}^{2+}]_i\) elevations comparable to those in PCs, which possess the primary cilium (3, 54, 57). It was proposed that increased luminal flow over the numerous microvilli and microplicae of the ICs may result in mechanical bending of the cumulative torque even to a higher extent than that occurring for the primary cilium in PCs (127). However, microvilli/brush border structures, made of actin filaments (12), are mainly designed to modulate reabsorptive function as it occurs, for instance, in proximal tubule cells (reviewed in (61)). Consistently, stimulation of cAMP-dependent cascades caused prominent microvilli elongation with respective accumulation of the proton pump, V-ATPase, in A-type ICs (73). This argues that the microvilli serve to augment transport function in the ICs (in this particular case, proton secretion). In contrast, no direct transport function for the primary cilium, built up from microtubules (128), has been ever reported. Furthermore, it is doubtful that polycystins (particularly PC1) are present in the microvilli and microplicae, since deletion of PC1 in PCs causes rapid development of cysts in the distal nephron, whereas deletion of PC1 in ICs results in virtually cyst-free kidney development in mice (84).
Another model suggests that PCs and ICs have a system for communication that allows coordinated \([\text{Ca}^{2+}]\) response to flow in both cell types (reviewed in (79)). It has been long recognized that various types of mechanical stress, including cell swelling, application of hydrostatic pressure, or simply touching cells with micropipette, leads to ATP release from many epithelia, including that of the distal nephron (5, 6, 95, 105). Purinergic signaling substantially modulates electrolyte transport in this tubular segment by inhibiting ENaC (76, 77, 108), and diminishing AQP2-dependent water reabsorption (87, 129). The physiological relevance of this regulation has been demonstrated using mice lacking P2Y2, the major purinergic receptor in the distal nephron. These mice exhibit altered homeostasis due to extensive renal sodium retention and hypertension, hypokalemia, and increased urinary osmolarity (87, 145). Interestingly, P2Y2 -/- mice have prominently reduced \([\text{Ca}^{2+}]\) responses to elevated flow and hypotonicity in the distal nephron (56). This suggests that mechanical stimuli result in ATP release acting in autocrine and paracrine manner to activate P2Y2 receptors and to increase \([\text{Ca}^{2+}]\). Consistently, elevated sodium intake, which is known to cause augmented ultrafiltrate delivery to the distal nephron, also increases tubular ATP levels (100). Both vesicular (5) and hemichannel-mediated via connexin 30 (95) or pannexin 1 (36) mechanisms of ATP release have been proposed to account for increased extracellular ATP levels. The relative contribution and supremacy of these processes for the net ATP secretion during various physiological states are currently not known. It was further demonstrated that primary cilium is required for ATP release in MDCK cells (80, 81). However, it is unclear whether cilium-dependent ATP release can underlie flow-induced \([\text{Ca}^{2+}]\), in ICs, since genetic ablation of P2Y2-/-, while substantially blunting, fails to abolish mechanosensitive responses in distal nephron cells (56). Further, residual mechanosensitivity can be also detected in collecting duct cells from connexin 30 -/- mice (105).
Other studies of distal nephron function also point to purinergic signaling as a key component in mechanical sensing. Primary cultured human cyst cells from PKD patients release ATP across the apical membrane as much or more readily than normal kidney cell monolayers (92, 130). Indeed, flash-frozen cyst fluids from autosomal dominant PKD cysts, harvested from multiple donor kidneys contain up to 10 µM ATP (130). This exacerbated purinergic signaling has been proposed to play a detrimental role in cyst expansion by stimulating intraluminal Cl⁻ secretion via the cystic fibrosis transmembrane conductance regulator (CFTR) (42). Indeed, inhibition of the purinergic P2X7 receptors has been reported to reduce on PKD progression (9). At the same time, cystic cells from both autosomal dominant and autosomal recessive PKD fail to increase [Ca²⁺], in response to luminal shear stress (65, 143). Furthermore, using pheochromocytoma PC12 cells as ATP biosensor, it was detected that flow-induced ATP release was approximately three-fold greater when the sensor was positioned next to ICs than next to PCs (95). Cultured MDCK C11 cells (a model of A-type ICs) release higher ATP levels than MDCK C7 cells (a model of PCs) in response to flow/shear stress stimulation (39). Recall, ICs have no primary cilium. Another interesting anatomic feature of the distal nephron is that ICs of the distal nephron protrude ~1-3 µm further into the lumen than PCs. This may suggest that direct mechanical distortion of the apical plasma membrane will lead to activation of membrane localized mechanosensitive complex, which is not necessarily limited to the primary cilium. This, in turn, leads to Ca²⁺ influx through Ca²⁺-permeable channels and concomitant ATP release to facilitate/potentiate the mechanosensitive [Ca²⁺], response (56).

Ca²⁺-permeable transient receptor potential (TRP) channels serve as sensors of a variety of environmental stimuli, including changes in temperature, various chemical compounds, mechanical forces, and so on (reviewed in (114)). Based on sequence homology,
TRP channels are categorized into seven subfamilies: classical or canonical (TRPC1-7), vanilloid (TRPV1-6), melastatin (TRPM1-8), ankyrin (TRPA1), no mechanoreceptor potential (TRPN, not present in mammals), polycystin (TRPP1-3), and mucolipidin (TRPML1-3) (114, 135). Recent experimental effort provided compelling evidence that the activity of the TRP subfamily V member 4 (TRPV4) channel is mandatory for flow-mediated \([\text{Ca}^{2+}]_i\) responses in both PCs and ICs of the distal nephron (3, 56-58, 78, 134, 143). TRPV4 can be also found in the primary cilium where it directly interacts and heteromerizes with polycystins (17, 48, 146). Furthermore, a direct link exists between TRPV4 and purinergic signaling in the distal nephron segments (56, 143). As will be further detailed, unraveling this important role of TRPV4 substantially improves our understanding of the molecular mechanisms of mechanosensitivity in tubular epithelial cells and allows consolidation of many seemingly contradictory observations.

STRUCTURE, FUNCTION, AND PHYSIOLOGICAL RELEVANCE OF TRPV4

TRPV4 is probably one of the most recognized mechanosensitive channels and is typically activated by various physical forces (3, 18, 28, 51, 52, 56-58, 101, 102, 107, 134, 143). Functional TRPV4 channel consists of four subunits (approximately 870 amino acids), each having six transmembrane domains and intracellular amino and carboxyl termini, thus, sharing general structure with other TRP channels (18, 43, 69, 70, 115). TRPV4 exists as a homotetramer or can heteromerize with other members of TRP family, most notably with TRPP2 (48, 146). A single channel conductance of TRPV4 is 90-100 pS for outward currents and 50-60 pS for inward currents (124-126). While generally considered non-selective, the
channel has modest preference for \( \text{Ca}^{2+} \) with \( P_{\text{Ca}}/P_{\text{Na}} \) of 6-10 (119). Thus, TRPV4 stimulation results in significant \( \text{Ca}^{2+} \) influx and activation of intracellular \( \text{Ca}^{2+} \)-dependent pathways. Recent advances in development of specific TRPV4 agonists (GSK1016790A, RN-1747), antagonists (HC 067047 and RN-1734) (19, 46, 118), and TRPV4 knockout mouse strains (52, 63) provided powerful experimental tools to decipher TRPV4 functions at the cellular and systemic levels. Originally, TRPV4 was cloned as a mammalian homolog of the \text{Caenorhabditis elegans} osmosensory protein OSM-9 and was activated by hypotonicity-induced cell swelling (15, 51, 131). Since then, TRPV4 was also reported to be sensitive to other types of mechanical stress, such as touch and elevated flow over the plasma membrane (18, 134). In addition, the channel can be activated by poly-saturated fatty acids, 4\( \alpha \)-phorbol ester derivatives, and warm temperatures above 25 °C (18, 35, 72).

Mechanosensitive properties of TRPV4 were reported not only in cells natively expressing the channel (28, 134), but also in over-expression systems originally lacking TRPV4 (134). Despite this, the direct effect of mechanical stimuli on TRPV4 gating remains questionable (10, 68). TRPV4 fails to fulfill the criterion of rapid kinetics of activation by mechanical stress (68). Activation of TRPV4 by hypotonicity (cell swelling) and shear stress (caused by elevated flow) is relatively slow and occurs within 10-30 sec after application of the stimuli (3, 19, 58, 70, 134). In contrast, directly mechanosensitive channels (such as Piezo, TREK, and TRAAK) are expected to respond to the stimuli within milliseconds (68). Figure 1 summarizes the existing experimental evidence about molecular determinants of TRPV4 activation by mechanical stimuli. It is proposed that activation of a phospholipase A2 (PLA2)-dependent pathway and synthesis of epoxyeicosatrienoic acids (EETs) confers mechanosensitivity to the channel (18, 120). The N-terminus of TRPV4 seems to be critical for
activation of the channel by mechanical stress. Thus, deletion of the ankyrin repeat domain (ARD) dramatically impairs TRPV4 mechanosensitivity by disrupting its association with cytoskeleton, which possibly provides a mechanical link for gating (51). In addition, proline-rich domain (PRD), located just prior to ARD, interacts with PACSIN3 protein strongly inhibiting TRPV4 activation by cell swelling (13, 14). Interestingly, PRD is not present in other TRPV channels (14) and, therefore, may contribute to the specific mechanosensitive properties of TRPV4. However, it is currently unclear whether the same molecular mechanism is employed to activate the channel in response to different types of mechanical and chemical stimulation.

Inhibition of PLA2-related pathways disrupts activation of TRPV4 by osmotic cell swelling but not by heat and 4α 12,13-phorbol didecanoate (4α-PDD) (120). Conversely, point mutation Y555A in the third transmembrane domain has little effect on TRPV4 activation by osmolarity but abolishes stimulatory effects of temperature and 4α-PDD (120).

TRPV4 is expressed in many epithelial tissues, including kidney, lung, spleen, skin, sweat glands; as well as in endothelia, heart, skeletal muscles, some sensory neurons, such as dorsal root ganglia (DRG), circumventricular organs, organum vasculosum laminae terminalis (OVLT); osteoblasts and chondrocytes (51, 52, 59, 64, 114). Owing its most prominent property of being activated by hypotonic stimuli, TRPV4 is thought to play an essential role in whole body osmoregulation. Indeed, TRPV4 knockout (TRPV4 -/-) mice, while able to maintain normal osmolarity in unstressed conditions, have impaired responses to both hyper- and hypotonic stimuli (52, 63). Further, in humans certain TRPV4 polymorphisms appear to be associated with modest hyponatremia which may reflect subtle underlying alterations in water balance (110). The exact mechanism of this pathology is currently unclear, because the original hypothesis that genetic deletion of TRPV4 compromises osmotic sensing in the CNS did not hold true (11). Interestingly, a recent study demonstrated that TRPV4-
expressing thoracic DRG neurons innervating hepatic blood vessels are able to sense physiologically relevant small changes in plasma osmolality and this mechanism is abrogated upon TRPV4 deletion (50). However, the relevance of this mechanism in osmoregulation at the level of whole organism requires further investigation. In addition, TRPV4 -/- mice also exhibit several other phenotypes related to abnormal mechanosensitivity. This includes a reduced sensitivity to application of harmful pressure to the tail and hind paw (52, 63), disrupted voiding behavior due to diminished sensitivity to bladder distension (31), and absence of flow-mediated K⁺ secretion in the cortical collecting ducts (107). TRPV4 has also a prominent role in endothelial cells therefore contributing to control of vascular tone by promoting vasodilation (21, 97, 103). While direct contribution of TRPV4 to establishing systemic blood pressure is not clear, it is proposed that channel activity counteracts the development of salt-sensitive hypertension (26), and this mechanism is impaired in Dahl salt-sensitive rats (27). In contrast, TRPV4 -/- mice are essentially normotensive (71) and TRPV4 antagonisms did not affect blood pressure and heart rate (109). Finally, while not in the scope of the current review, mutations in TRPV4 underlie an impressive but puzzling set of skeletal diseases and neuropathies in humans (reviewed in (69)).

TRPV4 ACTIVITY MEDIATES FLOW-DEPENDENT [Ca²⁺]ᵢ ELEVATIONS IN THE DISTAL NEPHRON

Since identification of TRPV4, it is widely acknowledged that the channel is abundant in the kidney tissue (15, 51, 131). Immunofluorescent studies demonstrate TRPV4 expression in the cortex, medulla and papilla regions of the renal tubule (3, 111). Originally, TRPV4 was
found in the loop of Henle, the distal convoluted tubule (DCT), the CNT, and in the ICs of the CD (111). In all segments, TRPV4 expression was predominantly localized to the basolateral membrane (111). Based on these studies, it was hypothesized that TRPV4 may serve as an osmosensor, since its expression is restricted to water impermeable segments, where generation of substantial osmotic gradients occurs. However, osmotic defects observed in TRPV4 -/- mice are independent of kidney function and related to defects in the central nervous system (52) or more likely to dysfunction of hepatic sensory neurons (50). In contrast, genetic ablation of TRPV4 leads to diminished furosemide-induced urinary K⁺ excretion (107), the process in the distal nephron that is known to be dependent on tubular flow. Luminal but not basolateral stimulation of TRPV4 with 4α-PDD augmented potassium secretion in perfused cortical CD. Furthermore, increases in tubular flow similarly stimulated K⁺ and Na⁺ transport in the collecting ducts from wild type but not in TRPV4 -/- mice (107). In concert with these observations, our group recently demonstrated that TRPV4 is expressed along the distal nephron, including the CNT and the CD and the most apparent fluorescent signal was observed near the apical membrane of PCs, whereas ICs have lower levels of TRPV4 expression with more diffuse subcellular localization (3). Notably, specificity of the employed antibodies was verified by the absence of staining in kidney sections from TRPV4 -/- mice (3). Altogether, this suggests that TRPV4 functions rather as a flow sensor/transducer in the renal tubule. Indeed, using direct monitoring of [Ca²⁺]i dynamics in the split-opened murine distal nephron, we reported that elevations in flow over the apical membrane, producing physiologically relevant shear stress, elicit TRPV4-dependent Ca²⁺ influx in both PCs and ICs (3). The magnitude of response to flow positively correlated with the level of TRPV4 expression and was modestly higher in PCs than in ICs. Importantly, genetic deletion or pharmacological inhibition of TRPV4 precluded flow-dependent increases in [Ca²⁺]i in distal
nephron cells (3, 58, 143). Consistently, transfection of cultured M-1 cells (a model for cortical collecting duct PCs) with siRNA specific for TRPV4 led to a time-dependent decrease in TRPV4 expression and loss of flow-dependent Ca\(^{2+}\) influx (134). A similar role for TRPV4 was also reported in perfused thick ascending limbs (TAL), where activation of the channel in response to flow mediates nitric oxide (NO) production (7).

Despite the fact that TRPV4 -/- mice have intact renal water handling, experimental evidence suggests that the channel also participates in adaptations to altered extracellular osmolarity in the distal nephron cells. Transfection of M-1 cells with siRNA for TRPV4 (134) or pharmacological inhibition with HC 067047 (44) virtually abolish elevations in [Ca\(^{2+}\)]\(_i\) induced by hypotonicity. However, the precise contribution of TRPV4 in response to hypotonicity in native distal nephron cells requires further careful examination. In general, decreases in osmolarity induce initial swelling of cells as a result of water entry along the osmotic gradient. Regulatory volume decrease (RVD) is a pivotal adaptation mechanism employed to preserve cellular osmotic balance in response to hypotonicity. Recent studies revealed that association and interaction between water channel AQP2 and TRPV4 are required for Ca\(^{2+}\) entry induced by hypotonicity and subsequent RVD response in cultured renal cortical collecting duct cells (24). Both elevation of [Ca\(^{2+}\)]\(_i\) induced by hypotonic solutions and the following RVD response were dependent on intact cytoskeleton (23, 24). It is not clear, though, what is the mechanism and relevance of hypotonic-induced [Ca\(^{2+}\)]\(_i\) elevations in ICs lacking AQP2 expression. Of interest, a loss of TRPV4 expression in response to high glucose attenuates the ability of the cultured human collecting duct to exhibit RVD, an effect that may contribute to the pathology of fluid and electrolyte imbalance, as observed in diabetic nephropathy (38).

Recent experimental evidence established a direct functional link between TRPV4-based mechanosensitivity and purinergic signaling in the distal nephron (see Figure 1). As
was mentioned earlier, mechanical stimuli lead to augmented ATP release from the distal tubular epithelium (32, 79, 95). Acting predominantly on P2Y2 receptors, ATP triggers a biphasic increase in \([\text{Ca}^{2+}]_i\), where the transient peak is followed by the sustained \(\text{Ca}^{2+}\) plateau in both PCs and ICs (56, 133). While the initial phase is chiefly mediated by the phospholipase C (PLC)-dependent \(\text{Ca}^{2+}\) release from the intracellular endoplasmic reticulum stores, the sustained phase requires \(\text{Ca}^{2+}\) influx from the extracellular milieu (56). Genetic deletion of TRPV4 nearly abolishes the ATP-induced \(\text{Ca}^{2+}\) plateau pointing to a critical contribution of TRPV4 in this process (56). The exact mechanism of TRPV4 activation by purinergic cascade may involve direct interaction of inositol triphosphate (IP3), the second messenger formed in response to PLC activation from the membrane phospholipid PI(4,5)P2, with carboxyl terminus of TRPV4 at the calmodulin-binding site. This interaction was shown to further contribute to TRPV4 stimulation in response to mechanical and osmotic stimuli in epithelial cells (20, 29). Importantly, genetic ablation of P2Y2 receptors markedly blunts flow- and hypotonic-induced \([\text{Ca}^{2+}]_i\) elevations in the distal nephron cells (56). This strongly supports the view that activation of purinergic signaling by mechanical stimuli reciprocally contributes to the mechanosensitivity by activating \(\text{Ca}^{2+}\)-permeable TRPV4 channel. It is also possible that initial TRPV4-driven \(\text{Ca}^{2+}\) influx is necessary for augmenting tubular ATP levels, especially assuming vesicular model of ATP release from the distal nephron epithelia (5). A similar mechanism was reported in the TAL where intraluminal decreases in osmolarity led to ATP release and this process depended on TRPV4 activation (94). Moreover, activation of TRPV4 in response to hypotonicity triggers hemichannel-mediated ATP release in porcine lens epithelium (93). However, relation between TRPV4 activation and ATP release from the distal nephron cells requires further validation in TRPV4 -/- animals.
Polycystic kidney disease (PKD) encompasses a broad group of hereditary renal and hepatic pathologies which are characterized by development and progressive growth of cysts filled with fluid (reviewed in (37)). PKD can progress to the end-stage renal disease (ESRD) requiring renal replacement therapy (reviewed in (37)). The most two common forms: autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD), are caused by genetic mutations in genes encoding PC1/PC2 and fibrocystin (also known as polyductin), respectively (89, 122). In both cases, renal cysts predominantly (in ADPKD patients) (113, 117) or exclusively (individuals with ARPKD) (116, 144) develop in the CD. Multiple studies using primary cultured or immortalized cystic cells suggest that partial dedifferentiation and augmented cellular proliferation observed during PKD is related to the inability to sense mechanical stimuli, such as elevated flow, and decreased basal \([\text{Ca}^2+]_i\) levels (25, 37, 41, 42, 65, 66, 96, 138, 139, 141). In addition, the reduced \([\text{Ca}^2+]_i\) levels likely lead to increased levels of cyclic adenosine monophosphate (cAMP) as has been found in several PKD animal models (30, 113, 140). Vasopressin V2 receptor antagonist decreased cAMP levels, as a result of a decreased activity of \(\text{Ca}^{2+}\)-dependent phosphodiesterases, and greatly diminished disease progression in both animal models of ADPKD and ARPKD (30, 113). In ADPKD patients clinical trials demonstrated that the V2R inhibitor, tolvaptan, was effective in slowing the increase in total kidney volume and the decline in kidney function over a 3-year period (112). Of interest, pharmacologically increased \([\text{Ca}^2+]_i\) levels also reduced detrimentally high cAMP and slowed cyst growth in culture (139). Notably, all proteins involved in PKD progression are found in the primary cilium where they physically interact to form a multiprotein complex (65, 121). Experimental evidence suggests that a major role of this complex may be to regulate
levels of intracellular Ca\textsuperscript{2+} (reviewed in (37, 62, 85)). However, a direct mechanical activation of polycystins has never been demonstrated. Instead, it was found that PC2 (also known as TRPP2), a Ca\textsuperscript{2+} permeable channel originally thought to be a conduit for flow-induced Ca\textsuperscript{2+} influx in ciliated cells (65), interacts and heteromerizes with TRPV4, presumable with a 2:2 stoichiometry of subunits (99), to form a functional 23 pS channel at the apical membrane of principal cells (146). This heterotetrameric channel can be activated by mechanical stimuli producing even a higher Ca\textsuperscript{2+} influx than the homomeric TRPV4 channel (17, 48). Interestingly, overexpression of TRPV4 in HEK293 cells results predominantly in a transient elevation of [Ca\textsuperscript{2+}]\text{\textsubscript{i}} in response to increased flow, whereas co-expression of PC2 and TRPV4 leads to a sustained flow-induced [Ca\textsuperscript{2+}]\text{\textsubscript{i}} elevation (17) recapitulating the pattern observed in native distal nephron cells (3, 58, 143). Furthermore, overexpression of dominant negative/inactive TRPV4 and PC2 abolishes Ca\textsuperscript{2+} responses to flow in M-1 cells (17). Thus, the existing experimental evidence suggests that the heterotetramer prevails in the distal nephron cells. This allows consolidation of previous observations demonstrating loss of flow-sensitivity upon disruption of PC2 (65) and TRPV4 (3). Other major proteins involved in the pathology of ADPKD and ARPKD, namely PC1 and fibrocystin, were also shown to be directly associated with PC2 (60, 121, 123, 142), and it is reasonable to propose that their dysfunction can compromise functional status of the TRPV4/PC2 channel. A schematic representation of our working model of the mechanosensory complex in the distal nephron cells is presented in Figure 2A. However, the precise molecular structure of the complex remains enigmatic.

While it is apparent that the TRPV4/PC2 complex is important in mechanotransduction, the function of individual subunits in related processes can be highly divergent. Indeed, the most striking difference is that PC2 dysfunction leads to PKD development, whereas TRPV4 -/- mice and zebra fish have cyst-free kidneys (48, 52). This suggests that disruption of
mechanosensitivity per se is not sufficient to trigger cystogenesis and additional factors, probably related to development or injury, are necessary. Tamoxifen-induced PC1 inactivation in the kidney does not initiate sufficient autonomous cell proliferation leading to cyst formation when performed in 1 month old mice (49). Thus, a possibility exists that TRPV4 -/- animals may develop cystic kidneys after appropriate, but yet undefined stimulus/treatment. This, though, does not disqualify a possible important role of dysfunctional flow-dependent $[\text{Ca}^2+]_i$ signaling in pathogenesis of PKD, as was articulated previously.

Only a minor portion of renal tubules (less than 1%) undergoes the transformation into cystic epithelium and it is unclear whether differences exist in mechanosensitive properties of normal and cystic cells in the same kidney. To address this important question, our group has succeeded in mechanical isolation of cystic monolayers, as well as of non-dilated collecting ducts in kidneys from a rat model of ARPKD, PCK453 (143). We identified that the genetic defect in fibrocystin (which causes progression of ARPKD in PCK 453 rats) does not lead to immediate disruption of mechanosensitive $[\text{Ca}^2+]_i$ elevations in non-transformed CD. In contrast, greatly diminished TRPV4 function, loss of flow-dependent $\text{Ca}^{2+}$ responses and decreased basal $[\text{Ca}^{2+}]_i$ levels were observed exclusively in CD-derived cystic monolayers (143). While almost dysfunctional, TRPV4 was predominantly localized to the apical plasma membrane of cyst cells which appeared to be a consequence of increased intracellular cAMP levels. Indeed, we were able to recapitulate apical translocation of TRPV4 in response to stimulation of cAMP-protein kinase A (PKA)-dependent pathways (58). Consistent with the observations in cystic epithelium, cAMP-PKA induced TRPV4 translocation did not result in augmented cellular responses to elevated flow suggesting that the channel needs to be further activated probably via protein kinase C (PKC)-mediated pathways (58). Specific dysfunction of TRPV4 and loss of mechanosensitivity in cyst cells argues that mechanosensitive $[\text{Ca}^{2+}]_i$
signaling might have a permissive role for normal tubular function and its disruption occurs just prior to or as an early event during cyst development likely serving as a facilitator of cystogenesis. Importantly, prolonged systemic pharmacological stimulation of TRPV4 with GSK1016790A markedly blunted renal ARPKD manifestations by reducing cyst size and decreasing kidney/total body weight ratio, pointing to potential therapeutic and clinical relevance of this strategy (143). At the cellular level, this led to partial restoration of mechanosensitivity and decreased intracellular cAMP levels, as was indicated by cytosolic retrieval of AQP2 channels from the apical membrane (143). Of interest, polyuria, thirst and related adverse events may impact the ability of some patients to tolerate effective doses of the V2R antagonist, tolvaptan, to reduce cAMP levels and to counteract cystogenesis in clinical trials (reviewed in (16)). While it was not accurately measured, we did not observe an obvious increase of urinary production in GSK1016790A-treated animals compared to that in the control group. This indicates that pharmacological stimulation of TRPV4 could be effective in PKD setting and importantly might not lead to adverse effects related to polyuria, as was observed after V2R inhibition.

The beneficial effects of TRPV4 activation during ARPKD are not limited to the kidney. A recent study identifies an anti-proliferative role for TRPV4 in cholangiocytes from ARPKD rats where TRPV4 activation tended to decrease liver cysts (33). Furthermore, PKD is often accompanied with substantial vascular abnormalities and development of intracranial aneurysms (reviewed in (37)). TRPV4 is also abundantly expressed in endothelium where it is proposed to modulate vascular relaxation in response to plasma flow (21, 97, 103). Overall, recent findings convincingly demonstrate temporal correlation between loss of TRPV4-based mechanosensitivity and cyst development during ARPKD. The efficiency of TRPV4 stimulation
to interfere with cystogenesis requires further careful experimental examination for the more clinically relevant ADPKD which is caused by dysfunction of either PC1 or PC2.

We recently gained new insights into the relation between purinergic signaling and TRPV4-based mechanosensitivity in ARPKD. Mechanically-induced ATP release is viewed as an important component of mechanosensitivity in normal CD (79) (see also Figure 2A). However, paradoxical upregulation of purinergic signaling in PKD pathology does not improve mechanosensitive properties of cyst cells (42). Our group found that ATP-triggered $[\text{Ca}^{2+}]_i$ response virtually lacks the sustained plateau phase in cyst cells (143) recapitulating the pattern observed upon genetic TRPV4 deletion (56). This suggests that chronic potentiation of purinergic cascade in ARPKD has a minimal effect on $[\text{Ca}^{2+}]_i$ levels (Figure 2B). The absence of the plateau in cyst cells is consistent with TRPV4 dysfunction and impaired mechanical perception. On the contrary, systemic pharmacological stimulation of TRPV4 causes restoration of ATP-induced $\text{Ca}^{2+}$ plateau (143). Therefore, this allows consolidation between the upregulation of purinergic signaling inside the cyst lumen and the loss of mechanosensitivity in ARPKD.

THE ROLE OF TRPV4 IN CONTROLLING FLOW-DEPENDENT $\text{K}^+$ SECRETION

It has long been recognized that $\text{K}^+$ excretion by the kidney exhibits a flow-dependent pattern (47, 132). An animal model with deficient flow-dependent potassium secretion demonstrates profound blood pressure abnormalities, aldosteronism, hyperkalemia and reduced urinary $\text{K}^+$ clearance pointing to altered potassium homeostasis (34). Furthermore, elevated potassium intake, which is known to increase renal tubular flow, exacerbates these
symptoms pointing to compromised ability of the kidney to eliminate excess of dietary potassium. In recent years the molecular players and mechanisms of flow-dependent $K^+$ excretion have started to be uncovered. It is now recognized that the flow-dependency of renal potassium transport is predominantly related to mechanical control of $K^+$ secretion by the CNT and CD, but it is unrelated to the ROMK channel. Instead, the activity of BK channel underlies, at least in part, flow-dependent $K^+$ secretion and this process depends on $\text{Ca}^{2+}$ influx (53, 106, 107, 132, 134). Not only has the BK channel been shown to be expressed in the CNT and CD, but several laboratories have demonstrated that pharmacological blockade or genetic ablation of the BK channel markedly blunts flow-induced $K^+$ secretion (1, 75, 88). The BK channel has been shown to be functionally expressed in both the ICs and PCs of the CNT/CD and the existing experimental evidence indicates that a dominant site for BK channel expression is at the apical border especially within the CNT (22, 75). Furthermore, the $\beta_1$ subunit which bestows increased $\text{Ca}^{2+}$-sensitivity to BK activation, is predominantly expressed in the CNT and animals lacking $\beta_1$ exhibit markedly blunted flow-induced $K^+$ secretion (34). In contrast, the $\beta_4$ subunit, which does not confer increased $\text{Ca}^{2+}$-sensitivity of the BK channel, is expressed in IC cells of the CD, whereas expression of $\beta_1$ is not apparent at this site (75). Moreover, the IC cells have low levels of $\text{Na}^+-K^+$-ATPase activity which argues against a major $K^+$ secretory flux.

It has recently become apparent that $K^+$ transporting systems other than BK may contribute to the phenomenon of the flow-dependent potassium secretion (4). For this, we screened for other $K^+$ channels in M-1 cells and identified the $\text{Ca}^{2+}$-dependent small conductance SK3 (KCa2.3) channel (45), which we have now shown to be also present in the mouse nephron with most prominent expression at the apical border of both the CNT and CD (4). The channel is particularly intriguing since it has much higher affinity for $\text{Ca}^{2+}$ over that
observed for the BK channel (submicromolar versus micromolar, respectively) (136, 137) and, hence, is well posed to play a key role in Ca\(^{2+}\)-dependent and, by extension, flow-dependent K\(^+\) secretory states. It remains to be tested whether this channel acts in concert with the BK channel and/or whether it acts somewhat independently through the common link of changes in intracellular Ca\(^{2+}\) levels.

Recent evidence suggests that the activity of the Ca\(^{2+}\)-permeable mechanosensitive TRPV4 channel is essential for control of flow-induced K\(^+\) secretion. We have clearly demonstrated localization and function of this channel along the luminal border of the CNT and CCD of both IC and PC (3, 58). Furthermore, genetic ablation of TRPV4 prevents flow-dependent elevations in K\(^+\) secretion in perfused collecting ducts, whereas pharmacological stimulation of TRPV4 recapitulated the effect of flow on potassium transport in wild type animals (107). Finally, urinary K\(^+\) excretion was significantly lower in TRPV4 -/- than in TRPV4 +/+ mice when urine production was stimulated by a venous application of furosemide (107). So TRPV4 appears to be the key link in regulating flow-induced K\(^+\) secretion (Figure 3). However, whether TRPV4 dysfunction results in alteration in whole body K\(^+\) handling has yet to be addressed. A similar functional interplay among TRPV4 and SK3/BK channels has been revealed in vascular resistive vessels where TRPV4 and SK3 are predominantly expressed in the endothelial cells while BK is preferentially located in the surrounding smooth muscle cell (55, 97, 104). Communication between the cell types appears to occur via myoendothelial gap junctions between the two cell types. Here, again, TRPV4 can be activated by mechanical stress or chemical agonist leading to activation of SK3 in the endothelial cells. The associated hyperpolarization of the endothelial cell membrane is transmitted to the smooth muscle cell to activate voltage-stimulated Ca\(^{2+}\) channels and, in turn, activation of BK in the smooth muscle cell. The BK-induced hyperpolarization subsequently inhibits the voltage-activated Ca\(^{2+}\)
channel causing smooth muscle relaxation and vessel dilation. In this case TRPV4 and SK3
appear to function as one complex unit which, in turn, controls BK activation as the terminal
effector to control vessel relaxation. It will be interesting to determine whether such a
functional division of channels is at play in the CCD and CNT or whether TRPV4 regulates the
SK3 and BK independently of each other.

CONCLUDING REMARKS

During recent years, we have substantially improved our understanding about the role
and physiological relevance of TRPV4 function in the renal tubule. Despite the channel was
cloned as a potential osmosensor, TRPV4 activity underlies flow-induced [Ca^{2+}]_i elevation in
the distal nephron cells. Because of this important finding, TRPV4, along with polycystin
proteins, is viewed as a crucial component of a larger multimolecular mechanosensitive
complex orchestrating many important physiological processes in kidney cells, including [Ca^{2+}],
signaling, transport of ions and water, cell proliferation, etc. Manipulation of TRPV4 activity
may be a novel target for counteracting cyst progression during PKD possibly lacking some
adverse effects of the existing pharmacological strategies in clinic. Furthermore, experimental
evidence indicate that functional status of TRPV4 determines the rates of K^{+} excretion by the
kidney which can be potentially used to treat various hyperkalemic states. Despite this rapid
progress, we are only at the beginning of the long way towards complete understanding of the
function of this versatile and remarkable channel in the kidney.
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**FIGURE LEGENDS**

**Figure 1 TRPV4 involvement in sensing mechanical stress in epithelial cells.** PLA2 phospholipase A2, AA arachidonic acid, CYP450 cytochrome P450 epoxygenase, EETs epoxyeicosatrienoic acids, PLC phospholipase C, P2Y2R P2Y2 receptor. The green octagon represents the proline-rich domain. The green and red arrows indicate stimulatory and inhibitory actions, respectively. Elevated flow either directly activates TRPV4 channel to increase [Ca$^{2+}$], or acts on an unknown upstream effector to stimulate PLA2–CYP450 pathway, which metabolizes AA to EETs leading to TRPV4 activation. Stimulation of TRPV4 by
mechanical stress can be prevented by interaction of TRPV4 N-terminal proline-rich domain with PACSIN3 protein. On the other hand, mechanical stimuli induce ATP release from distal nephron cells through mechanism possibly involving activation of Connexin 30, Pannexin 1 hemichannels, or vesicular trafficking. Locally released ATP binds to purinergic P2Y2 receptors on the apical membrane of renal epithelium. This leads to G_{q/11}-dependent activation of PLC and, likely, PKC and further augmenting TRPV4 activity.

Figure 2. A role of TRPV4 in multiprotein mechanosensory complex in collecting duct and cystic epithelia. PC-1 polycystin 1, FPC fibrocystin (polyductin), PLC phospholipase C, P2Y2R P2Y2 receptor. (A) Elevated flow causes activation of multiprotein mechanosensory complex, where TRPV4/TRPP2 heteromeric forms a permeability pore for Ca^{2+} and PC-1 and FPC exert a modulatory role. In addition, TRPV4 function can be augmented via activation of purinergic cascade as described in Figure 1. Elevated [Ca^{2+}], decreases cAMP levels likely in a phosphodiesterase-dependent manner, thus limiting cellular division and favoring differentiation. (B) Loss-of-function mutations in fibrocystin during autosomal recessive PKD compromises function of the TRPV4/TRPP2 heteromer making it also resistant to purinergic stimulation. This, in turn, results in augmented cAMP levels promoting partial dedifferentiation and cystic growth.

Figure 3. Contribution of TRPV4 to flow-mediated K^+ secretion in the distal nephron cells. (A) Elevated tubular flow increases [Ca^{2+}] via TRPV4/TRPP2 route to stimulate high affinity Ca^{2+}-sensitive SK3 (KCa2.3) and low affinity Ca^{2+}-sensitive BK channels to promote K^+ secretion. (B) Genetic deletion of TRPV4 precludes flow-mediated increases in [Ca^{2+}], therefore abolishing stimulation of the distal nephron K^+ secretion in TRPV4 -/- mice.