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

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Mamenko M, Zaika O, Boukelmoune N, O’Neil RG, Pochynyuk O. Deciphering physiological role of the mechanosensitive TRPV4 channel in the distal nephron. Am J Physiol Renal Physiol 308: F275–F286, 2015. First published December 10, 2014; doi:10.1152/ajprenal.00485.2014.—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 Ca2+ concentration. 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 the development of polycystic kidney disease. The molecular mechanisms conferring mechanosensitive properties to epithelial tubular cells involve activation of transient receptor potential (TRP) channels, such as TRPV4, allowing direct Ca2+ influx to increase intracellular Ca2+ concentration. In this review, 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 the progression of polycystic kidney disease. We also review the physiological relevance of TRPV4-based mechanosensitivity in controlling flow-dependent K+ secretion in the distal renal tubule.

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 (~180 l/day) via a 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 that allows epithelial cells to properly adjust transport rates of water and solutes (see Refs. 90 and 127). Recent experimental evidence suggests a pivotal role of mechanosensitive transient receptor potential (TRP)V4 channels 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 collecting duct (CD) system. Quantitatively, the distal nephron processes only ~10% of glomerular filtrate. However, this region is not autoregulated via tubuloglomerular feedback so that local control of 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. These include 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. Principal cells (PCs) comprise more than two-thirds of the total cell population. PCs reabsorb Na+ [mediated mainly by the epithelial Na+ channel (ENaC)] and water [through aquaporin (AQP)2-dependent osmotic water reabsorption] and are responsible for K+ secretion, mainly via renal outer medullary K+ channel-dependent pathways (74, 98). The remaining one-third of the cells are intercalated cells (ICs), which are critical for maintaining the acid/base balance. These can be further subdivided into H+-secreting A-type, HCO3−-secreting B-type, and intermediate non-A-non-B type cells (8). ICs also substantially contribute to large-conductance K+ (BK) channel-dependent (40) and possibly small-conductance KCa2.3 (SK3) channel-dependent (4) K+ secretion in response to elevated K+ intake and loop/thiazide diuretic treatments.

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Current Understanding of Molecular Mechanisms of Mechanosensitivity in Distal Nephron Cells

Increases in the delivery of glomerular ultrafiltrate to the distal nephron, for example, in response to Na\(^{+}\) overload/volume expansion and elevated K\(^{+}\) intake (40), greatly potentiate tubular flow at this site, exerting shear stress to the apical (also referred as luminal) membrane. Multiple studies have demonstrated that distal nephron epithelial cells respond to mechanical stimuli, in part, by elevating intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) (32, 54, 56, 58, 81, 95, 127, 133, 143). However, the exact molecular mechanism of these [Ca\(^{2+}\)]\(_i\) responses remains a matter of debate and revision.

The most common cellular model of mechanosensitive [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 the detection of changes in tubular flow (65, 82, 83). This bending causes activation of the proteins associated with the cilium: the G protein-coupled receptor polycystin 1 and the nonselective Ca\(^{2+}\)-permeable channel polycystin 2 (also known as TRPP2). This induces Ca\(^{2+}\) influx at the base of the primary cilium, triggering the subsequent release of Ca\(^{2+}\) from endoplasmic reticulum intracellular stores (65). However, studies in both perfused and split-opened murine distal nephrons have revealed that cilium-lacking ICs (86) respond to high flow over the apical surface with [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 microplacae of 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 (for a review, see Ref. 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 microvilli serve to augment transport function in ICs (in this particular case, proton secretion). In contrast, no direct transport function for the primary cilium, built up from microtubules (128), has ever been reported. Furthermore, it is doubtful that polycystins (particularly polycystin 1) are present in the microvilli and microplacae, since deletion of polycystin 1 in PCs causes the rapid development of cysts in the distal nephron, whereas deletion of polycystin 1 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 a coordinated [Ca\(^{2+}\)]\(_i\) response to flow in both cell types (for a review, see Ref. 79). It has long been recognized that various types of mechanical stress, including cell swelling, application of hydrostatic pressure, or simply touching cells with a 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 Na\(^{+}\) retention, hypertension, hypokalemia, and increased urinary osmolality (87, 145). Interestingly, P2Y2\(^{-/-}\) mice have prominently reduced [Ca\(^{2+}\)]\(_i\) 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 manners to activate P2Y2 receptors and to increase [Ca\(^{2+}\)]. Consistently, elevated Na\(^{+}\) 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 net ATP secretion during various physiological states are currently not known. It has been further demonstrated that the primary cilium is required for ATP release in Madin-Darby canine kidney (MDCK) cells (80, 81). However, it is unclear whether cilium-dependent ATP release can underlie flow-induced [Ca\(^{2+}\)]\(_i\) in ICs, since genetic ablation of P2Y2 receptor, while substantially blunting, fails to abolish mechanosensitive responses in distal nephron cells (56). Furthermore, residual mechanosensitivity can be also detected in CD cells from connexin 30\(^{-/-}\) mice (105).

Other studies of distal nephron function have also pointed to purinergic signaling as a key component in mechanical sensing. Primary cultured human cyst cells from patients with polycystic kidney disease (PKD) 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 (ARPKD) cysts, harvested from multiple donor kidneys, contain up to 10 \(\mu\)M 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 (42). Indeed, inhibition of purinergic P2X7 receptors has been reported to reduce PKD progression (9). At the same time, cystic cells from both autosomal dominant PKD (ADPKD) and ARPKD fail to increase [Ca\(^{2+}\)]\(_i\) in response to luminal shear stress (65, 143). Furthermore, using pheochromocytoma PC12 cells as a ATP biosensor, it was detected that flow-induced ATP release was approximately threefold 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). As described above, ICs have no primary cilium. Another interesting anatomic feature of the distal nephron is that ICs of the distal nephron protrude \(\approx 1-3\) \(\mu\)m further into the lumen than PCs. This may suggest that direct mechanical distortion of the apical plasma membrane will lead to activation of a membrane-localized mechanosensitive complex, which is not necessarily limited to the primary cilium. This, in turn, leads to Ca\(^{2+}\) influx through Ca\(^{2+}\)-permeable channels and concomitant ATP release to facilitate/potentiate the mechanosensitive [Ca\(^{2+}\)]\(_i\) response (56).

Ca\(^{2+}\)-permeable TRP channels serve as sensors of a variety of environmental stimuli, including changes in temperature, various chemical compounds, mechanical forces, and so on (for a review, see Ref. 114). Based on sequence homology, TRP channels have been categorized into seven subfamilies:
The functional TRPV4 channel consists of four subunits (TRPV1–7), each having six transmembrane domains and intracellular NH2- and COOH-termini, thus sharing a general amino acid sequence. Recent experimental efforts have provided compelling evidence that the activity of TRPV4 is mandatory for flow-mediated [Ca2+]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 PCs (17, 48, 146). Furthermore, a direct link exists between TRPV4 and purinergic signaling in distal nephron segments (56, 143). As detailed below, 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). The functional TRPV4 channel consists of four subunits (~870 amino acids), each having six transmembrane domains and intracellular NH2- and COOH-termini, thus sharing a general structure with other TRP channels (18, 43, 69, 70, 115). TRPV4 exists as a homotetramer or can heteromerize with other members of the TRP family, most notably with TRPP2 (48, 146). The single channel conductance of TRPV4 is 90–100 pS for outward currents and 50–60 pS for inward currents (124–126). While generally considered nonselective, the channel has a modest preference for Ca2+ with a Ca2+/Na+ permeability of 6–10 (119). Thus, TRPV4 stimulation results in significant Ca2+ influx and activation of intracellular Ca2+-dependent pathways. Recent advances in development of specific TRPV4 agonists (GSK-1016790A and RN-1747), antagonists (HC-067047 and RN-1734) (19, 46, 118), and TRPV4 knockout mouse strains (52, 63) have provided powerful experimental tools to decipher TRPV4 functions at the cellular and systemic levels. Originally, TRPV4 was cloned as a mammalian homolog of the Caenorhabditis elegans osmosensory protein osmotic avoidance abnormal family member 9 and was activated by hypotonicity-induced cell swelling (15, 51, 131). Since then, TRPV4 has also been 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 polyunsaturated fatty acids, 4α-phorbol ester derivatives, and warm temperatures above 25°C (18, 35, 72).

Mechanosensitive properties of TRPV4 have been reported not only in cells natively expressing the channel (28, 134) but also in overexpression 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 s 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 shows the existing experimental evidence about molecular determinants of TRPV4 activation by mechanical stimuli. It has been proposed that activation of a phospholipase A2-dependent pathway and synthesis of epoxyeicosatrienoic acids confers mechanosensitivity to the channel (18, 120). The NH2-terminus of TRPV4 seems to be critical for activation of the channel by mechanical stress. Thus, deletion of the ankyrin repeat domain dramatically impairs TRPV4 mechanosensitivity by disrupting its association with the cytoskeleton, which possibly provides a mechanical link for gating (51). In addition, the proline-rich domain, located just before the ankyrin repeat domain, interacts with PKC and casein kinase substrate in neurons 3 protein, which strongly inhibits TRPV4 activation by cell swelling (13, 14). Interestingly, the proline-rich domain 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 used to activate the channel in response to different types of mechanical and chemical stimulation. Inhibition of phospholipase A2-related pathways disrupts activation of TRPV4 by osmotic cell swelling but not by heat and 4α-phorbol 12,13-didecanoate (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-phorbol 12,13-didecanoate (120).

TRPV4 is expressed in many epithelial tissues (including the kidney, lung, spleen, skin, and sweat glands) as well as in endothelia, the heart, skeletal muscles, some sensory neurons (such as dorsal root ganglia, circumventricular organs, and organum vasculosum laminae terminalis), 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). Furthermore, 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 central nervous system did not hold true (11). Interestingly, a recent study (50) has demonstrated that TRPV4-expressing thoracic dorsal root ganglion neurons innervating hepatic blood vessels are able to sense physiologically relevant small changes in plasma osmolality and that this mechanism is abrogated upon TRPV4 deletion. 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 CDs (CDDs) (107). TRPV4 has also a prominent role in endothelial cells, therefore contributing to control of vascular tone by promoting vasodilatation (21, 97, 103). While a direct contribution of TRPV4 to establishing systemic blood pressure is not clear, it has been proposed that channel activity coun-

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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 present review, mutations in TRPV4 underlie an impressive but puzzling set of skeletal diseases and neuropathies in humans (for a review, see Ref. 69).

**TRPV4 Activity Mediates Flow-Dependent \( \text{[Ca}^{2+}\text{]}_i \)**

**Elevations in the Distal Nephron**

Since the identification of TRPV4, it is widely acknowledged that the channel is abundant in kidney tissue (15, 51, 131). Immunofluorescent studies have demonstrated 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, CNT, and 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 the 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-phorbol 12,13-didecanoate augmented K+ secretion in the perfused CCD. Furthermore, increases in tubular flow similarly stimulated K+ and Na+ transport in CDs from wild-type but not TRPV4−/− mice (107). In concert with these observations, our group recently demonstrated that TRPV4 is expressed along the distal nephron, including the CNT and 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 antibodies used 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 \( \text{[Ca}^{2+}\text{]}_i \) 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 \( \text{Ca}^{2+} \) influx in both PCs and ICs (3). The magnitude of the 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 \( \text{[Ca}^{2+}\text{]}_i \) in distal nephron cells (3, 58, 143). Consistently, transfection of cultured M-1 cells (a model for PCs in the CCD) with small interfering RNA specific for TRPV4 led to a time-dependent decrease in TRPV4 expression and loss of flow-dependent \( \text{Ca}^{2+} \) influx (134). A similar role for TRPV4...
has also been reported in perfused thick ascending limbs, where activation of the channel in response to flow mediates nitric oxide 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 distal nephron cells. Transfection of M-1 cells with small interfering RNA for TRPV4 (134) or pharmacological inhibition with HC-067047 (44) virtually abolished elevations in [Ca2+]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. The regulatory volume decrease is a pivotal adaptation mechanism used to preserve cellular osmotic balance in response to hypotonicity. Recent studies have revealed that associations and interactions between the water channel AQP2 and TRPV4 are required for Ca2+ entry induced by hypotonicity and the subsequent regulatory volume decrease response in cultured renal CCD cells (24). Both elevation of [Ca2+]i, induced by hypotonic solutions and the after the regulatory volume decrease response were dependent on an intact cytoskeleton (23, 24). It is not clear, though, what is the mechanism and relevance of hypotonicity-induced [Ca2+]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 CD to exhibit the regulatory volume decrease, 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 Fig. 1). As described above, 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 [Ca2+]i, where the transient peak is followed by a sustained Ca2+ plateau in both PCs and ICs (56, 133). Whereas the initial phase is chiefly mediated by phospholipase C-dependent Ca2+ release from intracellular endoplasmic reticulum stores, the sustained phase requires Ca2+ influx from the extracellular milieu (56). Genetic deletion of TRPV4 nearly abolishes the ATP-induced Ca2+ plateau, pointing to a critical contribution of TRPV4 in this process (56). The exact mechanism of TRPV4 activation by purinergic cascade may involve a direct interaction of inositol triphosphate, the second messenger formed in response to phospholipase C activation from the membrane phospholipid phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2], with the COOH-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 [Ca2+]i elevations in distal nephron cells (56). This strongly supports the view that activation of purinergic signaling by mechanical stimuli reciprocally contributes to the mechanosensitivity by activating Ca2+-permeable TRPV4 channels. It is also possible that the initial TRPV4-driven Ca2+ influx is necessary for augmenting tubular ATP levels, especially assuming vesicular model of ATP release from the distal nephron epithelium (5). A similar mechanism has been reported in the thick ascending limb, where intraluminal decreases in osmolarity led to ATP release, and this process was depended on TRPV4 activation (94). Moreover, activation of TRPV4 in response to hypotonicity triggers hemichannel-mediated ATP release in the porcine lens epithelium (93). However, the relation between TRPV4 activation and ATP release from distal nephron cells requires further validation in TRPV4−/− animals.

TRPV4-Based Mechanosensitivity in the Pathogenesis of PKD

PKD encompasses a broad group of hereditary renal and hepatic pathologies that are characterized by the development and progressive growth of cysts filled with fluid (for a review, see Ref. 37). PKD can progress to end-stage renal disease, requiring renal replacement therapy (for a review, see Ref. 37). The most two common forms, ADPKD and ARPKD, are caused by genetic mutations in genes encoding polycystin 1/polycystin 2 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 have suggested that the partial dedifferentiation and augmented cellular proliferation observed during PKD are related to an inability to sense mechanical stimuli, such as elevated flow, and decreased basal [Ca2+]i, levels (25, 37, 41, 42, 65, 66, 96, 138, 141). In addition, the reduced [Ca2+]i levels likely lead to increased levels of cAMP, as has been found in several PKD animal models (30, 113, 140). A vasopressin V2 receptor (V2R) antagonist decreased cAMP levels, as a result of a decreased activity of Ca2+-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 decline in kidney function over a 3-yr period (112). Of interest, pharmacologically increased [Ca2+]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 Ca2+ (for reviews, see Refs. 37, 62, and 85). However, a direct mechanical activation of polycystins has never been demonstrated. Instead, it was found that polycystin 2 (also known as TRPP2), a Ca2+-permeable channel originally thought to be a conduit for flow-induced Ca2+ influx in ciliated cells (65), interacts and heteromerizes with TRPV4, presumably with a 2:2 stoichiometry of subunits (99), to form a functional 23-pS channel at the apical membrane of PCs (146). This heterotetrameric channel can be activated by mechanical stimuli, producing an even higher Ca2+ influx than the homomeric TRPV4 channel (17, 48). Interestingly, overexpression of TRPV4 in human embryonic kidney (HEK)-293 cells results predominantly in a transient elevation of [Ca2+]i in response to increased flow, whereas coexpression of polycystin 2 and TRPV4 leads to a sustained flow-induced [Ca2+]i elevation (17), recapitulating the pattern observed in native distal nephron cells (3, 58, 143). Furthermore, overexpression of
dominant negative/inactive TRPV4 and polycystin 2 abolishes Ca:\(^{2+}\) responses to flow in M-1 cells (17). Thus, the existing experimental evidence suggests that the heterotetramer prevails in distal nephron cells. This allows a consolidation of previous observations demonstrating a loss of flow sensitivity upon disruption of polycystin 2 (65) and TRPV4 (3). Other major proteins involved in the pathology of ADPKD and ARPKD, namely, polycystin 1 and fibrocystin, have also been shown to be directly associated with polycystin 2 (60, 121, 123, 142), and it is reasonable to propose that their dysfunction can compromise functional status of the TRPV4/polycystin 2 channel. A schematic representation of our working model of the mechanosensory complex in distal nephron cells is shown in Fig. 2A. However, the precise molecular structure of the complex remains enigmatic.

While it is apparent that the TRPV4/polycystin 2 complex is important in mechanotransduction, the function of individual subunits in related processes can be highly divergent. Indeed, the most striking difference is that polycystin 2 dysfunction leads to PKD development, whereas TRPV4\(^{-/-}\) mice and zebrafish have cyst-free kidneys (48, 52). This suggests that the disruption of mechanosensitivity per se is not sufficient to

![Fig. 2. Role of TRPV4 in the multiprotein mechanosensory complex in the collecting duct and cystic epithelia. PC-1, polycystin 1; FPC, fibrocystin (polyductin). A: elevated flow causes activation of the multiprotein mechanosensory complex, where the 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 the purinergic cascade, as described in Fig. 1. Elevated \([Ca^{2+}]_i\) decreases cAMP levels, likely in a phosphodiesterase-dependent manner, thus limiting cellular division and favoring differentiation. B: loss-of-function mutations in FPC during autosomal recessive polycystic kidney disease 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.](http://ajprenal.org)
trigger cystogenesis and that additional factors, probably related to development or injury, are necessary. Tamoxifen-induced polycystin 1 inactivation in the kidney does not initiate sufficient autonomous cell proliferation leading to cyst formation when performed in 1-mo-old mice (49). Thus, a possibility exists that TRPV4−/− animals may develop cystic kidneys after an appropriate but yet undefined stimulus/treatment. This, though, does not disqualify a possible important role of dysfunctional flow-dependent [Ca²⁺]i signaling in the pathogenesis of PKD, as was articulated previously.

Only a minor portion of renal tubules (<1%) undergo 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 nondilated CDs in kidneys from a rat model of ARPKD, PCK453 (143). We identified that a genetic defect in fibrocystin (which causes the progression of ARPKD in PCK 453 rats) does not lead to immediate disruption of mechanosensitive [Ca²⁺]i elevations in nontransformed CDs. In contrast, greatly diminished TRPV4 function, loss of flow-dependent Ca²⁺ responses, and decreased basal [Ca²⁺]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-PKA-dependent pathways (58). Consistent with observations in the 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 PKC-mediated pathways (58). Specific dysfunction of TRPV4 and loss of mechanosensitivity in cyst cells argue that mechanosensitive [Ca²⁺]i signaling might have a permissive role for normal tubular function and that its disruption occurs just before or as an early event during cyst development, likely serving as a facilitator of cystogenesis. Importantly, prolonged systemic pharmacological stimulation of TRPV4 with GSK-1016790A markedly blunted renal ARPKD manifestations by reducing cyst size and decreasing the kidney weight-to-total body weight ratio, pointing to potential therapeutic and clinical relevance of this strategy (143).

At the cellular level, this led to a partial restoration of mechanosensitivity and decreased intracellular cAMP levels, as 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 (for a review, see Ref. 16). While it was not accurately measured, we did not observe an obvious increase of urinary production in GSK-1016790A-treated animals compared with that in control animals. This indicates that pharmacological stimulation of TRPV4 could be effective in the 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 (33) has identified an antiproliferative role for TRPV4 in cholangiocytes from ARPKD rats, where TRPV4 activation tended to decrease liver cysts. Furthermore, PKD is often accompanied by substantial vascular abnormalities and the development of intracranial aneurysms (for a review, see Ref. 37). TRPV4 is also abundantly expressed in the endothelium, where it has been proposed to modulate vascular relaxation in response to plasma flow (21, 97, 103). Overall, recent findings have convincingly demonstrated a 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 polycystin 1 or polycystin 2.

We have 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 the normal CD (Fig. 2A) (79). However, paradoxical upregulation of purinergic signaling in PKD pathology does not improve mechanosensitive properties of cyst cells (42). Our group found that the ATP-triggered [Ca²⁺]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 the purinergic cascade in ARPKD has a minimal effect on [Ca²⁺]i levels (Fig. 2B). The absence of the plateau in cyst cells is consistent with TRPV4 dysfunction and impaired mechanical perception. In contrast, systemic pharmacological stimulation of TRPV4 causes restoration of the ATP-induced Ca²⁺ plateau (143). Therefore, this allows consolidation between the upregulation of purinergic signaling inside the cyst lumen and the loss of mechanosensitivity in ARPKD.

Role of TRPV4 in Controlling Flow-Dependent K⁺ Secretion

It has long been recognized that K⁺ excretion by the kidney exhibits a flow-dependent pattern (47, 132). An animal model with deficient flow-dependent K⁺ secretion demonstrates profound blood pressure abnormalities, aldosteronism, hyperkalemia, and reduced urinary K⁺ clearance, pointing to altered K⁺ homeostasis (34). Furthermore, elevated K⁺ intake, which is known to increase renal tubular flow, exacerbates these symptoms, pointing to a compromised ability of the kidney to eliminate the excess of dietary K⁺. 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 K⁺ transport is predominantly related to mechanical control of K⁺ secretion by the CNT and CD, but it is unrelated to the renal outer medullary K⁺ channel. Instead, the activity of the BK channel underlies, at least in part, flow-dependent K⁺ secretion, and this process depends on Ca²⁺ 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 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 β₁-subunit, which bestows increased Ca²⁺-sensitivity to BK channel activation, is predominantly expressed in the...
CNT, and animals lacking the β1-subunit exhibit markedly blunted flow-induced K⁺ secretion (34). In contrast, the β2-subunit, which does not confer increased Ca²⁺ sensitivity of the BK channel, is expressed in IC cells of the CD, whereas expression of the β1-subunit is not apparent at this site (75). Moreover, ICs have low levels of Na⁺-K⁺-ATPase activity, which argues against a major K⁺ secretory flux.

It has recently become apparent that K⁺ transporting systems other than BK channels may contribute to the phenomenon of flow-dependent K⁺ secretion (4). For this, we screened for other K⁺ channels in M-1 cells and identified Ca²⁺-dependent SK3 channels (44), 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 Ca²⁺ over that observed for the BK channel (submicromolar vs. micromolar, respectively) (136, 137) and, hence, is well posed to play a key role in Ca²⁺-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 Ca²⁺ levels.

Recent evidence suggests that the activity of the Ca²⁺-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 ICs and PCs (3, 58). Furthermore, genetic ablation of TRPV4 prevents flow-dependent elevations in K⁺ secretion in perfused CDs, whereas pharmacological stimulation of TRPV4 recapitulated the effect of flow on K⁺ transport in wild-type animals (107). Finally, urinary K⁺ excretion was significantly lower in TRPV4⁻/⁻ mice than in TRPV4⁺/⁺ mice when urine production was stimulated by a venous application of furosemide (107). Thus, TRPV4 appears to be the key link in regulating flow-induced K⁺ secretion (Fig. 3). However, whether TRPV4 dysfunction results in alterations 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 channels are predominantly expressed in endothelial cells, whereas BK channels are preferentially located in surrounding smooth muscle cells (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

Fig. 3. Contribution of TRPV4 to flow-mediated K⁺ secretion in distal nephron cells. A: elevated tubular flow increases [Ca²⁺], via the TRPV4/TRPP2 route to stimulate high-affinity Ca²⁺-sensitive small-conductance Kᵥ2.3 (SK3) channels and low-affinity Ca²⁺-sensitive large-conductance K⁺ (BK) channels to promote K⁺ secretion. B: genetic deletion of TRPV4 precludes flow-mediated increase in [Ca²⁺], therefore abolishing stimulation of distal nephron K⁺ secretion in TRPV4⁻/⁻ mice.
chemical agonist, leading to the activation of SK3 channels in 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 channel-induced hyperpolarization subsequently inhibits the voltage-activated Ca\(^{2+}\) channel, causing smooth muscle relaxation and vessel dilation. In this case, TRPV4 and SK3 channels 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 andCNT or whether TRPV4 regulates SK3 and BK channels 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 fact that the channel was cloned as a potential osmosensor, TRPV4 activity underlies flow-induced [Ca\(^{2+}\)] elevation in 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 countering cyst progression during PKD and may possibly lack some adverse effects of the existing pharmacological strategies in the clinic. Furthermore, experimental evidence indicates that the functional status of TRPV4 determines the rate 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 toward a complete understanding of the function of this versatile and remarkable channel in the kidney.

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DISCLOSURES

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