



Published in final edited form as:

Pflugers Arch. 2013 February ; 465(2): 177–186. doi:10.1007/s00424-012-1190-z.

Novel insights into TRPV4 function in the kidney

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Abstract

Kidneys are complex highly organized paired organs of nearly 1 million nephrons each. They rigorously process about 180 liters of plasma daily to keep whole body homeostasis. To effectively perform such a titanic work, kidneys rely on mechanisms able to sense dynamic changes in composition and flow rates of protourine along the renal tubule. It is envisioned that Ca^{2+} permeable transient receptor potential channels (TRPs), and specifically mechanosensitive TRPV4, can serve to interpret these external mechanical cues in the form of elevated intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). This, in turn, initiates multiple cellular responses and adaptation mechanisms. The current review summarizes up-to-date knowledge about the sites of TRPV4 expression in renal tissue as well as discusses the functional role of the channel in cellular responses to hypotonicity and tubular flow. We will also provide insights as to how TRPV4 fits into classical polycystin mechanosensory complex in cilia and will speculate about previously underappreciated clinical implication of pharmacological TRPV4 targeting in treatment of polycystic kidney disease.

Keywords

collecting duct; connecting tubule; intracellular Ca^{2+} elevations; hypotonicity; tubular flow; polycystic kidney disease

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Disclosures

Authors declare that no conflicts of interest exist.

Introduction

Mechanosensitivity is a pivotal interface employed by living organisms to survey the surrounding environment. Many cell membranes are equipped with mechanosensitive ion channels that respond to osmotic gradients, shear stress, touch, vibration and texture. These channels are present in a great variety of life forms: from bacteria to higher plants and vertebrate animals (reviewed in [42,63]). A number of discovered mechanosensitive channels belong to a broad family of transient receptor potential (TRP) channels [13,63,67,80,102,118]. They share the common feature of six transmembrane domains and are repeatedly reported to participate in cellular responses to a vast array of stimuli, including temperature, chemical agents and mechanical forces [13,57,63,66,67,80,102,118]. According to their sequence similarity, TRPs can be subdivided into 7 subfamilies: classical or canonical (TRPC), vanilloid (TRPV), melastatin (TRPM), ankyrin (TRPA), no mechanoreceptor potential (TRPN), polycystin (TRPP) and mucolipidin (TRPML) [102,118]. TRPV4 (initially named VROAC, VRL-2, TRP12, OTRPC4) is the fourth member of TRPV subfamily, which was originally identified as a mammalian homolog of the *Caenorhabditis elegans* osmosensory protein OSM-9 [17,22,47,64,84,115]. Since then TRPV4 is, perhaps, one of the most recognized mammalian mechanosensitive TRP channels, which was routinely showed to be activated by hypoosmotic challenges and mechanical shear stress arising from fluid flow [6,22,27,48,49,53,56,84,91,115,117]. While direct gating of TRPV4 by mechanical stimuli is debatable [11,63,65,66], substantial evidence argues that TRPV4 is indispensable for a wide variety of mechanosensitive processes [1,6,27,47–49,53,56,64,80,84,91,117]. The channel has a tetrameric subunit arrangement and possesses a modest selectivity to Ca^{2+} [22,68,69]. TRPV4 expression is detected in many tissues including lung, heart, brain, endothelial cells, sensory ganglia and kidney [6,17,22,47,84,93,115]. In the present review we will discuss recent advances in our understanding of a role for TRPV4 in conferring mechanosensitive properties to renal epithelial cells with a special emphasis on the distal nephron.

TRPV4 expression pattern in the kidney

Initial studies revealed that TRPV4 transcript is abundantly expressed in the kidney [17,47,84,115]. High levels of TRPV4 mRNA were found in the inner cortex with only punctate distribution in the outer cortex [84]. Early immunolocalization experiments demonstrated that TRPV4 is confined to the apical border of cells lining the distal tubules [17,84]. Further study systematically characterized TRPV4 expression pattern in mouse and rat kidneys. Abundant TRPV4 expression was detected in the cortex, medulla and papilla. TRPV4 immunofluorescent signal indicated that the protein might be restricted to nephron segments constitutively or conditionally impermeant to water [93]. Specifically, strong immunoreactivity was observed in the thin ascending limb, thick ascending limb and distal convoluted tubule, while moderate levels of TRPV4 were detected in collecting duct and papillary epithelium. TRPV4 subcellular distribution in these nephron segments was regarded as predominantly basolateral [93].

Recently our group assessed the functional TRPV4 expression in mouse kidney using a combination of immunohistochemical, fluorescent imaging and genetic tools. We demonstrated that TRPV4 is abundantly expressed along the entire length of collecting duct system throughout the medulla and cortex [6]. TRPV4 immunostaining was confined to aquaporin-2 (AQP2) positive nephron segments: from the connecting tubule to the papillary collecting duct. The most apparent immunoreactivity to TRPV4 was observed near the apical membrane of principal cells, whereas intercalated cells had lower levels of TRPV4 protein with more diffuse subcellular distribution [6].

The observed variations in TRPV4 expression pattern may be attributable to differences in antibody specificity, technical aspects of the employed approaches or animal preconditioning. As far as subcellular distribution of the channel is concerned, it should be noted that so far there is no experimental evidence demonstrating functional activity of TRPV4 on the basolateral membrane of renal epithelium. On the other hand, apical localization of the channel is consistent with previous observations [15,27] and functional assessment of TRPV4 activity in renal cells (see further). Nevertheless, taken together the existing data strongly favor TRPV4 abundance in the distal part of the renal nephron.

Additional evidence for TRPV4 expression in the kidney comes from the cell lines endogenously expressing the channel. Thus, TRPV4 mRNA was detected in various cultured renal cells including smooth muscle like mouse mesangial cells and several epithelial cell lines: M-1 cortical collecting duct (CCD) cells, Madin-Darby canine kidney (MDCK) and inner medullary collecting duct (IMCD-3) cells [20,27,41,117].

TRPV4 as an osmosensor in the distal nephron

Renal micropuncture studies demonstrated that tubular fluid leaving the loop of Henle is typically hypotonic. Indeed, the osmolality of the fluid entering the latter distal convoluted tubule is around 100 mOsm/kg [14,29]. In the absence of antidiuretic hormone vasopressin low osmolality can be maintained along the entire length of the distal nephron from the cortex to the papillary collecting duct [14,29,30]. Thus, depending on systemic hydration status, distal segments of renal nephron are partially or completely exposed to hypotonic luminal milieu. Numerous observations suggest that renal cells respond to hypoosmolality with elevations of intracellular Ca^{2+} [24,25,34,53,55,90,94,95,99,117]. Considering the fact that renal TRPV4 expression is primarily restricted to the distal nephron, this strongly suggests that the channel is perfectly positioned to play an essential role in cellular adaptations to decreased osmolality of the tubular fluid.

Our group convincingly demonstrated that hypotonicity stimulates endogenously expressed TRPV4 in M-1 CCD cells. Activation of TRPV4 with hypotonic medium was abolished by TRP channel inhibitor ruthenium red and after siRNA TRPV4 knockdown [117]. Of interest, hypotonicity is known to induce ATP release from many epithelia, including that of the distal nephron [73,76]. The apically localized connexin 30 hemichannel serves as a conduit for ATP release at this site [81]. Numerous studies suggest that activation of purinergic signaling is a critical component of cellular responses to mechanical stress, such as hypotonicity [73]. We have recently documented a functional link between ATP cascade and TRPV4 in distal nephron cells [53]. We found that locally released ATP via P2Y2 receptors and PLC cascade stimulates TRPV4 for sustained elevation of $[Ca^{2+}]_i$. Genetic ablation of TRPV4 results only in a transient elevation of $[Ca^{2+}]_i$ due to Ca^{2+} release from the endoplasmic reticulum [53]. Similar mechanism was also reported in the perfused thick ascending limbs, where decreased osmolality of tubular fluid resulted in TRPV4-mediated ATP release leading to elevation of $[Ca^{2+}]_i$ [79]. It appears that reciprocal coupling between purinergic signaling and TRPV4 activation is essential for proper mechanosensitive response in kidney cells. Indeed, distal nephrons from P2Y2^{-/-} mice have markedly impaired responses to hypotonicity [53]. Activation of P2Y2 receptors by ATP or adenosine A1 receptors by ATP breakdown product – adenosine, inhibits vasopressin-dependent water transport in the collecting duct [76]. Consistently, P2Y2^{-/-} mice display increased urinary concentrating ability and develop prominent hypertension associated with augmented sodium and water transport in the distal nephron [70,76,83,124]. However, the precise role of TRPV4-mediated $[Ca^{2+}]_i$ elevations in water reabsorption in the distal nephron and urinary concentrating ability remains elusive. Interestingly, in *Caenorhabditis elegans* mutants devoid of the endogenous TRPV isoform – OSM-9, TRPV4 expression restores

avoidance responses to hypertonicity [49], while in mammalian cells the channel is activated by hypotonic solutions [1,9,10,25,38,53,117]. This exciting discrepancy merits further investigation and, perhaps, can be attributed to different pathways regulating TRPV4 functioning in various species.

The significance of TRPV4 osmosensitivity in the collecting duct epithelium is further emphasized by an important role played by the channel in cell volume regulation and maintenance of the intracellular osmotic homeostasis. As mentioned before, the apical membrane of distal nephron cells typically faces a hypotonic tubular fluid, which can be further diluted under conditions of high water intake and subsequent water diuresis [14,29,30]. Exposure to a hypoosmotic environment induces cell swelling as a result of water entry along the osmotic gradient. The latter can be dramatically increased by water channels akin to AQP2 expressed on the apical membrane of principal cells in the collecting duct [24]. Dilution of the cytoplasm significantly alters the functioning of intracellular macro-molecular machinery, while elevated hydrostatic pressure may compromise the integrity of the cell. Regulatory volume decrease (RVD) is a pivotal adaptation mechanism employed to preserve cellular osmotic balance in response to hypotonicity. In most cells osmotic stress induces Ca^{2+} entry, which is followed by the efflux of ions and osmotically active organic solutes (reviewed in [36,43,44,113]). Recent studies proposed that in renal cortical collecting duct cells association of AQP2 and TRPV4 is required for Ca^{2+} entry induced by hypotonicity and subsequent RVD response [25]. Both elevation of $[\text{Ca}^{2+}]_i$ induced by hypotonic solutions and the following RVD response were impeded after depolymerization of tubulin microtubules with colchicine and actin microfilaments with cytochalasine. This points to the importance of cytoskeleton integrity for hypotonicity-induced TRPV4-mediated $[\text{Ca}^{2+}]_i$ increase and volume regulation in collecting duct epithelium [24,25]. Consistently, microtubule-associated protein 7 was shown to interact with TRPV4 both *in vitro* and in renal cortex. This interaction enhances the channel expression on the cellular membrane [87]. A few observations indicate that N-terminal ankyrin repeat domain (ARD) is responsible for correct TRPV4 trafficking to the plasma membrane [22,47]. It might anchor the channel to cytoskeleton or establish/constitute a mechanical link necessary for proper gating. Interestingly, deletion of ARD dramatically impairs TRPV4 mechanosensitivity [22,47]. TRPV4 also significantly improves RVD, when expressed in CHO cells, which normally have a poor response to decreased osmolarity [5]. A functional interaction between TRPV4 and F-actin is critical for sensing hypotonicity and the onset of RVD in this model [4]. Another study demonstrated that functional interplay between TRPV4 and aquaporin-5 observed in salivary gland epithelia is critical for RVD induced by hypotonic swelling in this tissue [52].

The aforementioned experiments resonate with the initial observations in heterologous cellular systems demonstrating that TRPV4 is highly sensitive to changes in extracellular osmolality [47,84]. Cells transfected with TRPV4 responded to hypotonic solution with elevations of $[\text{Ca}^{2+}]_i$. Even minor (as low as 1–10%) fluctuations of extracellular osmolality significantly altered Ca^{2+} influx [47,84]. On the contrary, increased osmolality (above 300 mosmol/l) decreased $[\text{Ca}^{2+}]_i$ levels and currents in the cells exhibiting spontaneous activity [84]. TRPV4-mediated currents in CHO cells were significantly increased by inflation of cell volume, achieved by positive pressure through a patch pipette [88]. The latter closely resembles cell swelling induced by hypotonicity. Subsequently, TRPV4 has been suggested to act as an osmolality sensor in the airway smooth muscle cells [38] and function as an osmo-transducer in primary afferent nociceptive nerve fibers [1].

Consistent with the osmosensitive properties of the channel, TRPV4^{-/-} mice exhibit abnormal osmotic regulation [48,56]. Initially, these abnormalities were attributed to disruption of central TRPV4 signaling, specifically, in the sensory neurons of *organum*

vasculosum lamina terminalis (OVLT) [48]. This hypothalamic brain nucleus, which is located outside the blood-brain barrier, regulates the secretion of antidiuretic hormone and controls the osmolality of extracellular fluid [80]. Subsequently, it was shown that modulation of OVLT neurons by tonicity or mechanical stimulation was unaffected by deletion of TRPV4, but rather requires TRPV1 [12]. The exact mechanism, by which hypertonicity modulates the activity of OVLT neurons, remains to be determined, and it is unclear whether TRPV1 and TRPV4 both contribute to this process. Finally, a recent study demonstrated that TRPV4 expressing thoracic dorsal root ganglia neurons innervating hepatic blood vessels detect physiologically pertinent decreases in blood osmolality. These hepatic sensory neurons respond even to small changes in osmolality (~15 mOsm). The evoked ionic currents have a pharmacological and biophysical profile similar to that of TRPV4 channel [45]. In TRPV4^{-/-} mice hepatic sensory neurons do not exhibit osmosensitive inward currents and activation of peripheral osmoreceptors is abolished [45]. The important osmosensitive role of peripherally expressed TRPV4 is further underscored by the observation that patients with liver transplants, which are, presumably, denervated, have a significantly higher baseline blood osmolality compared to a healthy control cohort [45]. Interestingly, genetic ablation of TRPV4 also significantly attenuates sensitization of nociceptive nerve fibers to mechanical and hypotonic stimuli induced by inflammatory mediators [10]. Endothelial cells in TRPV4 knock-outs show a decreased response to hypotonic solutions as well [106]. Since the existing evidence arguing for the involvement of renal TRPV4 in systemic osmoregulation is scanty, future studies testing this highly probable mechanism are granted.

Extensive studies provide insights into the mechanisms underlying regulation of TRPV4 activity by hypoosmolality. The accrued evidence suggests that hypotonicity does not directly result in channel opening, but rather leads to stimulation of intracellular signaling cascades to activate TRPV4 [69,86,107,111,117]. Production of arachidonic acid metabolites – epoxyeico-satrienoids, through phospholipase A₂ (PLA₂) – cytochrome P450 epoxygenase (CYP450) pathway, appears to be a dominant mechanism inducing hypotonic activation of the channel [107]. Consistently, activation of TRPV4 by osmotic cell swelling is virtually abolished by four structurally unrelated PLA₂ inhibitors [107]. CYP450 blockers also strongly diminish [Ca²⁺]_i elevations induced by hypotonicity [107]. Yet, other studies indicate that phosphorylation by Src-family tyrosine kinase could also be involved in TRPV4-mediated response to anisotonic stress [112,119]. N-terminal proline rich domain of TRPV4 interacts with PACSIN3 protein, which strongly inhibits the basal activity of the channel and its activation by cell swelling [15,16]. The interaction with PACSIN 3 affects both the expression of the channel on the plasma membrane and its gating properties [15,16,22]. TRPV4 sensitivity to hypotonicity is greatly augmented at physiological temperature (+37 °C) [27,107]. However, robust [Ca²⁺]_i elevations in response to hypotonic solution were also observed at room temperature [53,84,107]. Intact intracellular environment and cytoskeleton structure appear to be important prerequisites for TRPV4-mediated responses to reduced osmolarity [4,25,87,109]. Nevertheless, despite this vigorous effort, comprehensive understanding of TRPV4 regulation by hypotonicity is just beginning to emerge.

TRPV4 as a sensor of tubular fluid flow

Another focus in the field of TRPV4 functioning in the kidney is sensitivity of the channel to the tubular fluid flow. Renal tubule and particularly distal nephron is subjected to a highly variable flow of the protourine. The variations can be induced acutely by altered glomerular filtration rate [39,46,122], tubuloglomerular feedback [37], renal pelvic peristalsis [21] or use of diuretics; and chronically during diabetes [71], hypertensive states [3,19] and upon variations of dietary sodium [59], potassium [8] and protein intake [78]. Luminal fluid

mechanics affects the cells of the tubular lining exerting shear stress, transmural pressure and stretch. Extensive support from the literature suggests that $[Ca^{2+}]_i$ elevations mediate cellular responses to mechanical stimuli in the distal nephron [28,50,51,53,61,72,73,116]. Indeed, the distal nephron is a well-recognized flow-sensitive tissue, where luminal flow is regarded as an important determinant of vectorial electrolyte transport [51,58,60,77,116]. Again, taking into consideration predominantly distal nephron TRPV4 expression, this strongly favors the idea that TRPV4 can participate in flow sensing.

Virtually all aspects of electrolyte transport, including K^+ secretion [60,77], Na^+ reabsorption [58] and, importantly, Ca^{2+} influx [51,116] were shown to be flow-sensitive both in native collecting duct and cultured cells. A subsequent study demonstrated that luminal, but not basolateral application of a TRPV4 agonist – 4 α -PDD, significantly enhances flow-dependent Na^+ reabsorption and K^+ secretion in perfused murine collecting ducts [91]. The effects of flow and 4 α -PDD were abolished in TRPV4^{-/-} mice. Interestingly, enhanced urinary K^+ excretion was diminished in TRPV4 knockouts following intravenous injection of a loop diuretic – furosemide [91]. Furthermore, Ca^{2+} influx through TRPV4 was shown to be a critical component of purinergic response in aldosterone sensitive distal nephron [53]. Since ATP release from distal nephron cells is known to be stimulated by mechanical stress [79,81], purinergic signaling serves as an important facilitator of TRPV4-mediated $[Ca^{2+}]_i$ elevations in response to flow. The most compelling evidence for a flow-sensitive nature of TRPV4 was provided by our group using ratiometric Fura 2 Ca^{2+} imaging in individual cells of split-opened distal nephrons. We showed that elevated fluid flow induced a rapid and sustained influx of Ca^{2+} in both collecting duct and connecting tubule cells [6]. Principal cells exhibited larger responses to flow when compared to intercalated cells. This is consistent with higher levels of TRPV4 expression in the former cell type [6]. Elevations of $[Ca^{2+}]_i$ were abolished by the TRP channel inhibitor – ruthenium red, or in tubules isolated from TRPV4 deficient animals [6]. These data are in full agreement with our previous studies, demonstrating that heterologously (in HEK293 and CHO cells) and endogenously (in M-1 CCD cells) expressed TRPV4 is activated by increased flow [27,117]. Transfection of M-1 CCD and TRPV4-expressing HEK cells with siRNA specific to TRPV4 leads to a loss of flow-induced Ca^{2+} influx [117]. Consistently, shear stress produced by increased viscous load activates TRPV4 in hamster oviductal ciliary cells, which natively express the channel, and TRPV4-transfected HeLa cells [2]. Endogenously expressed TRPV4 also exhibits sensitivity to flow in carotid artery endothelial cells [40,54]. Therefore, an emerging body of evidence demonstrates that TRPV4 functions as a sensor/transducer of flow-induced mechanical stimuli in collecting duct and, likely, in other epithelia.

Little is known about the mechanisms underlying TRPV4 activation by flow/shear stress. Slow onset rate of TRPV4 responses to flow [6,20,117] may indicate indirect stimulation. However, direct activation of the channel by flow is not completely ruled out. Similarly to hypotonic stress, flow activation of TRPV4 in ciliated oviductal cells and cerebral arteries also seems to involve PLA₂-dependent pathway [2,54]. Increased temperature is a potent enhancer of TRPV4-mediated response to flow [117], though flow-induced Ca^{2+} influx via TRPV4 was repeatedly demonstrated at room temperature [6,20,40,54]. In addition, channel gating is modulated by tyrosine phosphorylation [112,119], protein kinase C [27], external and internal Ca^{2+} concentrations [85,86,105,110] and PLC activity [23,53]. However, these signaling cascades likely sensitize TRPV4 to mechanical stimuli rather than directly activate the channel.

TRPV4 association with polycystin-2

Recent studies suggest that mechanosensitive nature of $[Ca^{2+}]_i$ elevations in distal nephron cells could be even more complex since TRPV4 is likely a part of a larger flow sensing apparatus. Specifically, it was shown that TRPV4 forms a mechanosensitive complex with another TRP family member – polycystin-2 (TRPP1 according to the current IUPHAR nomenclature, also widely referred to as TRPP2 or PKD2) [20,41,82]. The complex has a putative 2:2 stoichiometry and an alternating subunit arrangement [82]. The data demonstrate that flow-induced Ca^{2+} entry into cultured collecting duct cells is mediated by heteromeric TRPV4-TRPP2 channels localized to the primary cilium [20,41]. This multifunctional apical structure is thought to work as a sensory cellular antenna in the tubular lumen, reporting velocity of the tubular flow [18,74,75]. The expression of dominant negative constructs for TRPV4 and TRPP2 each abolished flow-induced Ca^{2+} influx in M-1 CCD cells [20]. This argues that both proteins are equally important to maintain mechanosensitive responses in collecting duct renal epithelium. Interestingly, only transient $[Ca^{2+}]_i$ responses to flow are observed in HEK293 cells heterologously expressing TRPV4 construct alone [20]. However, simultaneous expression of TRPV4 and TRPP2 results in a sustained flow-induced Ca^{2+} influx [20], similar to that observed by our group in native tissue [6]. This supports the concept that TRPV4 serves as a valuable component of mechanosensitive complex on the primary cilium of renal epithelium. Interestingly, TRPV4 was also shown to be co-expressed with polycystin-2 and localized to an analogous ciliary structure in murine oviduct lining cells [92] and cholangiocytes [31].

TRPV4 in polycystic kidney disease

Polycystin-2 gained its mere scientific renown as a Ca^{2+} -permeable channel and a crucial part of the polycystin mechanosensitive complex, localized to the primary cilium of renal epithelial cells [61]. Defects in polycystin-2 or its partnering protein polycystin-1 result in the development of autosomal dominant polycystic kidney disease (ADPKD) [33,97]. With the frequency of 1:400–1000, ADPKD is the most common in the family polycystic kidney diseases (PKD) [33,89,97]. PKD are characterized by formation of numerous fluid-filled cysts within the kidney [33,89,96,114]. Expanding cysts invade the adjacent parenchyma, causing decline of the kidney function, eventually progressing to the end-stage renal disease (ESRD) [33,89]. Typically diagnosed in adults, ADPKD accounts for 5–9% of the individuals with ESRD requiring dialysis or kidney transplantation. In approximately 85% of cases ADPKD is caused by mutations in *PKD1* gene encoding a large receptor-like protein – polycystin-1. The remaining 15% of ADPKD patients have mutations in *PKD2* gene, encoding polycystin-2 [33,89,97]. Polycystins were reported to interact at their carboxyl termini to form a heteromultimeric Ca^{2+} permeable mechano-sensitive complex [32,100,101]. Perhaps, defects in either of the proteins preclude the binding of polycystins or render the complex inactive.

Another form of PKD closely related to mechanosensitive properties of renal epithelium is autosomal recessive PKD (ARPKD). It has the estimated incidence of 1:20000 and causes morbidity and mortality in utero and neonates [33,89]. The genetic basis of the disease lies in the mutations of *PKHD1* gene, encoding a multidomain integral protein – fibrocystin (or polyductin) [33,89,123]. The function of fibrocystin merits further investigation. However several observations suggest that the protein normally interacts with polycystin-2 as a part of the polycystin multiprotein complex, regulating $[Ca^{2+}]_i$ response to external stimuli [31,61,108], and mutations in the *PKHD1* gene prevent these interactions. Based on the initial studies, polycystin-1 is suggested to be a primary mechanosensor, which triggers a Ca^{2+} influx through polycystin-2 in response to elevated flow, while fibrocystin serves as a regulator of the mechanosensitive polycystin complex [31,61,108]. Nevertheless, the exact

role of both polycystins and fibrocystin in the regulation of $[Ca^{2+}]_i$ levels remains to be determined.

Despite different genetic mutations that underlie the two forms of PKD, cystic epithelia share common phenotypic abnormalities. During the disease onset and progression renal cysts are predominantly (in ADPKD patients) [98,104] or exclusively (individuals with ARPKD) [103,123] derived from the collecting duct. At the cellular level PKD progression is associated with transformation of well-differentiated slowly proliferating reabsorptive epithelia to partially dedifferentiated secretory epithelia with polarization defects and high rates of proliferation and apoptosis [89]. Experimental evidence suggests that enhanced proliferation observed during PKD is related to the inability to sense mechanical stimuli and decreased basal $[Ca^{2+}]_i$ levels [26,33,61,62,120,121]. This is consistent with the function of polycystin/fibrocystin complex as a regulator of $[Ca^{2+}]_i$ in response to environmental stimuli and its suggested role in maintaining the differentiated state of the renal epithelia. However, it is unclear whether the polycystins serve as a route for Ca^{2+} influx *per se* or they trigger other mechanisms to elevate $[Ca^{2+}]_i$. Interestingly, a recent study suggests that polycystin-2 fails to respond to mechanical stimuli on its own and acquires mechanosensitive properties only after heteromerization with TRPV4 [20,41]. Coimmunoprecipitation and double immunostaining studies demonstrate physical interaction and co-localization of polycystin-2 and TRPV4 in the primary cilium [20,41]. Whereas Ca^{2+} imaging in MDCK cells and M-1 CCD cells shows that TRPV4 and polycystin-2 (TRPP2) form a functional complex, which is crucial for flow sensation [20,41]. This disputes the exclusive role of polycystin complex as a mechanosensor in CD and is consistent with cumulative evidence increasingly appreciating TRPV4 involvement in mechanosensitivity and maintenance of $[Ca^{2+}]_i$ homeostasis in CD epithelium. Interestingly, ADPKD patients have significant defects in osmoregulation, affecting the release of vasopressin in response to increased plasma osmolality upon water deprivation [7,35]. This phenotype is similar to that observed in TRPV4^{-/-} mice showing a lower increase in circulating vasopressin upon hypertonic stress and being more hyperosmolar than wild-type littermates [48]. Finally, recent findings point to TRPV4 involvement in PKD progression, demonstrating that systemic stimulation of TRPV4 with a highly selective agonist – GSK1016790A, significantly inhibits cell proliferation in the liver and decreases renal cystic area and kidney to body weight ratio pointing to a possible clinical implication of TRPV4 targeting in PKD treatment [31]. It appears that the relationship between mechanosensitivity and PKD progression is not as straightforward, as it was originally thought. Indeed, TRPV4 deficient zebrafish and mice lack flow-induced $[Ca^{2+}]_i$ response, but do not develop cysts [41]. Therefore, future studies are necessary to determine the extent of TRPV4 involvement in PKD progression.

Concluding remarks

During the last decade our understanding of the role that TRPV4 plays in the kidney has greatly advanced. Multiple studies identified the distal part of the renal nephron as a primary segment expressing TRPV4. A combination of new approaches in knock-out and transgenic animals with numerous techniques probing TRPV4 expression, subcellular localization and function revealed that TRPV4 is paramount for sensitivity of distal nephron epithelium to decreased osmolarity and flow-induced shear stress. Substantial evidence argues that the channel is involved in local regulation of transmural ion transport in the distal nephron by flow and hypotonicity. Recent studies revise the current scientific paradigm and shift it towards the idea that TRPV4 is a vital part of a larger multimolecular mechanosensitive complex, which requires orchestrated interaction of multiple components to maintain a proper mechanosensitive response. Figure 1 summarizes the evidence supporting this idea. It includes, but, perhaps, is not limited to modulation of TRPV4 sensitivity to hypoosmolarity by epoxyeicosatrienoic acids or PACSIN3 protein and reciprocal activation of TRPV4 by

purinergic cascade. Finally, heteromerization of TRPV4 with other proteins akin to polycystin-2 leads to formation of new complexes with a unique set of features. This opens another exciting chapter in the field of renal mechanosensitive signaling. It appears that until now the physiological role of TRPV4 in the kidney was underappreciated as polycystin complex and specifically polycystin-2 was considered to be a major Ca^{2+} -permeable channel, implicated in mechanosensation. While direct experimental evidence demonstrating polycystin-2 mechanosensitive properties is scanty, a mounting body of evidence clearly demonstrates that TRPV4 is pivotal to mechanosensitivity of CD cells and maintenance of $[\text{Ca}^{2+}]_i$ balance under normal physiological conditions. Moreover, stimulation of the channel in the animals with ARPKD drastically attenuates renal manifestations of this devastating disease. Beneficial actions of TRPV4 activation on disease progression make the channel an attractive pharmacological target for development of effective therapeutic approaches in PKD treatment.

Acknowledgments

The research in Pochynyuk's lab is supported by the National Institutes of Health DK095029.

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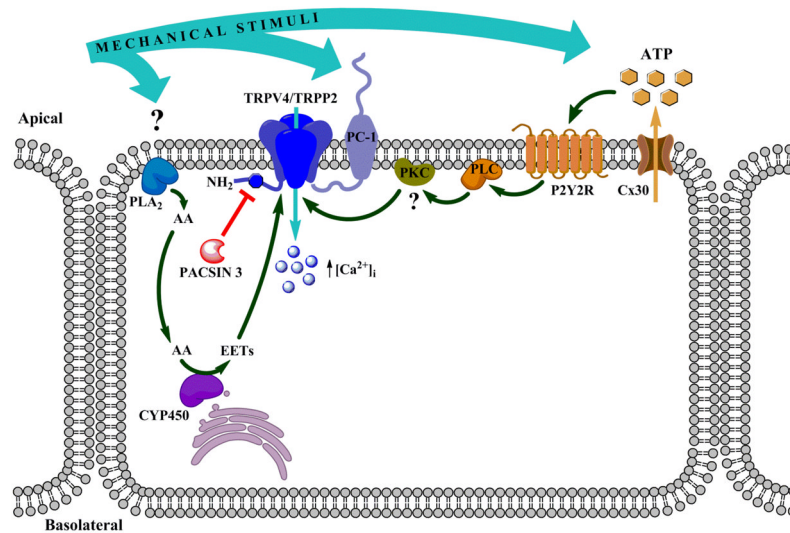


Figure 1.

Proposed scheme, illustrating the involvement of TRPV4 in mechanosensitivity in the distal nephron cells. PLA₂ – phospholipase A₂, AA – arachidonic acid, CYP450 – cytochrome P450 epoxygenase, EETs – epoxyeicosatrienoic acids, ● – proline rich domain, PC-1 – polycystin-1, PKC – protein kinase C, PLC- phospholipase C, P2Y2R – P2Y2 receptor, Cx30 – connexin 30 hemichannel. In the kidney TRPV4 forms a heteromeric channel with polycystin-2 (TRPP2), which is a part of a larger mechanosensitive complex also involving of PC-1. Mechanical stress stimulates PLA₂ – CYP450 pathway, which metabolizes AA to EETs. EETs activate TRPV4 channel to elicit Ca²⁺ influx in response to hypotonicity or elevated flow. This activation can be prevented by interaction of TRPV4 N-terminal proline rich domain with PACSIN 3 protein. On the other hand, mechanical stimuli induce ATP release from distal nephron cells through Cx30 hemichannels. 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.