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Role of Renal TRP Ion Channels in Physiology and Pathology

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Abstract

Kidneys critically contribute to the maintenance of whole-body homeostasis by governing water and electrolyte balance, regulating extracellular fluid volume, plasma osmolality and blood pressure. Renal function is regulated by numerous systemic endocrine and local mechanical stimuli. Kidneys possess a complex network of membrane receptors, transporters and ion channels which allows responding to this wide array of signaling inputs in an integrative manner. Transient receptor potential (TRP) channel family members with diverse modes of activation, varied permeation properties and capability to integrate multiple downstream signals are pivotal molecular determinants of renal function all along the nephron. This review summarizes experimental data on the role of TRP channels in a healthy mammalian kidney and discusses their involvement in renal pathologies.

INTRODUCTION

Kidneys are the master regulators of the internal milieu of the body. In spite of greatly varying dietary intake of water and electrolytes, these paired organs adjust urinary volume and composition in a discrete but synchronous manner to achieve systemic homeostasis. Through a direct interaction with cardiovascular system at the site of numerous glomeruli, around 40x plasma volume (~180 Liters in humans) is filtered on a daily basis, whereas only <1% of the filtrate is excreted with urine. To accomplish this ultimate physiological function, kidneys possess complex and highly structurally organized molecular cascades to perform vectorial movement (i.e. transport) of water and solutes, such as all electrolytes, glucose, amino acids, etc., across epithelial monolayers in renal tubules. The bulk of filtered plasma is reabsorbed in the proximal tubule (PT) and the loop of Henle in a constitutive manner and the distal nephron segments, including the distal convoluted tubule (DCT), the connecting tubule (CNT) and the collecting duct (CD) operate at a much lower capacity and are responsible for fine-tuning of water and electrolyte balance in response to dietary and endocrine inputs [1-4]. In addition, glomeruli serve as a reliable barrier preventing a loss of blood cells and large plasma proteins, such as albumins and immunoglobulins, with urine. Even subtle alterations in kidney function due to genetic defects can have profound pathophysiological consequences resulting in systemic imbalance of electrolytes and blood

pressure distortions [5]. Extensive experimental and clinical effort during the last several decades drastically improved our understanding molecular nature of the transporting and signaling mechanisms underlying the processes of filtration, reabsorption and secretion of water and solutes by the kidney. Serving as conduits for facilitated movement of cations through otherwise virtually impermeable plasma membrane of cells and being activated in response to variety of environmental signals, many transient receptor potential (TRP) channels are now becoming recognized as essential components of both transport and sensory/signaling processes in the kidney tissue.

Superfamily of TRP channels consists of 28 proteins sharing six-transmembrane domain homology with a remarkable diversity of gating properties, selectivity and activation in response to various stimuli, including temperature, chemical agents, and mechanical forces [6]. TRP channels can be further divided into TRPC (canonical; 7 members), TRPV (vanilloid; 6 members), TRPM (melastatin; 8 members), TRPP (polycystin; 3 members), TRPA (ankyrin; 1 member), TRPML (mucolipin; 3 members), and TRPN (no mechanoreceptor potential C; 1 member, not found in mammals) subfamilies [6]. The majority of the TRP channels are abundantly expressed along the renal nephron starting from glomerulus to the inner medullary CD [7, 8]. Of interest, the expression patterns are usually restricted to one (such as TRPM6) or a couple of (as TRPV4, TRPV5 and TRPC6, for instance) nephron segments [7-10]. Such spatial separation and distinct activation mechanisms likely underlie the specific function of a particular TRP channel in the kidney. In this review, we discuss recent experimental evidence revealing physiological roles of TRP channels in the kidney epithelia and emphasize hereditary Mendelian disorders resulting from TRP channels malfunctioning as well as novel insights into TRP functions obtained from genetic animal models.

TRPC6 and glomerular health

A glomerulus is a dense capillary network contained within Bowman's (or glomerular) capsule at the beginning of the tubular component of the nephron (Figure 1A). It serves as the initial basic filtration unit of the kidney. Glomerular filtration barrier is a complex and highly specialized apparatus consisting of three major components: the fenestrated endothelium of the glomerular capillaries; the glomerular basement membrane (GBM) formed by the fused basal laminas of the capillary endothelium and podocytes – visceral epithelial cells enveloping the capillaries, and, finally, by the slit diaphragms of the podocytes. Another specialized cell type of smooth muscle origin – the intraglomerular mesangial cells, provide structural support for the glomerular capillaries and regulate blood flow by their contractile activity. Intrinsically high hydrostatic pressure in the glomerular capillaries drives the process of filtration allowing free movement of water and relatively small solutes to the Bowman capsule, while restricting penetration of blood cells and large macromolecules, such as plasma albumins and immunoglobulins. Podocytes are highly specialized epithelial cells with a limited regenerative ability in the inner part of the Bowman capsule, which form the slit diaphragms between their foot processes attached to the GBM to govern the final step of filtration process [11] (see Figure 1A).

Podocytes are widely recognized to play a key role in normal glomerular function as increased GBM permeability is restricted to the area of podocytes damage [12-17]. Podocytes are known to sense dynamically changing hydrostatic pressure by contracting and elevating intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) [18, 19]. Indeed, real-time multiphoton imaging of the intact rat kidney *in vivo* reveals that $[\text{Ca}^{2+}]_i$ elevations and contractions in podocytes coincide with afferent arteriole contraction when intraglomerular capillary pressure is at its lowest [16]. Several Ca^{2+} -permeable TRP channels, such as TRPC1, TRPC2, TRPC3, TRPC5, and TRPC6; are expressed in either native or immortalized podocytes [7, 20, 21]. As will be detailed below, TRPC6 plays a dominant role in these $[\text{Ca}^{2+}]_i$ elevations in podocytes. The contribution of other TRPC channels is less clear, but recent genetic animal knockout studies also demonstrate a critical role for TRPC5 in $[\text{Ca}^{2+}]_i$ signaling in podocytes [22, 23].

TRPC6 is a heterotetrameric, non-selective (relative permeability of Ca^{2+} to Na^+ as 2:1) cation channel that can be activated in response to G protein signaling specifically via a diacylglycerol (DAG)-dependent pathway [24, 25]. TRPC6 basal activity is very low in podocytes, but can be greatly potentiated in response to post-translational modifications (i.e. glycosylation and phosphorylation) as well as mechanical pressure [26-28]. While the channel itself is not intrinsically mechanosensitive, it was proposed that TRPC6 can interact with podocin, a podocyte-specific protein, to acquire mechanosensitive properties [29, 30]. It is viewed that this interaction might be a part of a large mechanosensitive multiprotein channel complex, also including nephrin [21, 31], NOX2 [32], phospholipase C (PLC)- γ 1 [33], F-actin [18, 34] and probably other proteins, responding to extracellular pressure and activating calcium influx through TRPC6 (see Figure 1B). It has been well described that long-term increases in hydrostatic pressure in the glomerular capillaries can cause podocyte damage, leading to proteinuria [35]. It is logical to assume that $[\text{Ca}^{2+}]_i$ -dependent process and cytoskeleton reorganization contribute to the pathology. Indeed, the field was electrified by the relatively recent discovery that gain-of-function mutations in N- and C-termini of the TRPC6 cause Focal and Segmental Glomerulosclerosis (FSGS) in humans [17, 21]. FSGS symptoms include renal insufficiency, proteinuria, hypoalbuminemia, and hypertension eventually leading to kidney failure. FSGS-causing mutations associated with both increases in surface channel expression and single channel TRPC6 activity have been reported [17, 21, 36]. These mutations result in a slow onset FSGS (16-61 years) and the severity and progression of the disease seem to depend on the magnitude of TRPC6 potentiation (reviewed in [37]). The general mechanism is that sustained hyper-activation of podocyte TRPC6 channels results in $[\text{Ca}^{2+}]_i$ overload, foot process effacement, podocyte detachment, and glomerulosclerosis. Coherent experimental evidence have been also obtained in animal genetic models where podocyte-specific over-expression of wild-type or FSGS causing mutations of TRPC6 was sufficient to induce glomerular lesions and proteinuria similar to that of FSGS, although the phenotype was mild and had a late onset [38]. Furthermore, in cultured podocytes transiently over-expressing TRPC6, cell processes were remarkably reduced in association with the derangement of cytoskeleton demonstrated by the abnormal distribution of intracellular F-actin [34]. However, this phenomenon has not been observed in TRPC6-overexpressing native podocytes [39]. While only a minor portion of the described hereditary FSGS (5 out of 71 families) is caused by TRPC6 malfunction, FSGS

can also be caused by mutations and deletions in multiple genes encoding proteins associated with TRPC6, such as podocin [40]. Of interest, knockdown of podocin markedly increased stretch-evoked activation of TRPC6 in cultured podocytes, thus mimicking gain-of-function state of the channel [41]. Overall, the existing experimental evidence so far support the concept that pharmacological inhibition of TRPC6 or signaling pathways causative for the channel activation has potential to treat various proteinuric pathologies in the clinic.

Elevated blood pressure, particularly associated with increased circulating Angiotensin II (Ang II) levels is detrimental to glomerular health leading to glomerulosclerosis and proteinuria in patients and in numerous experimental animal models (reviewed in [42]). Several research groups documented a marked upregulation of single channel TRPC6 activity and channel expression in response to Ang II leading to elevated $[Ca^{2+}]_i$ [43-46]. TRPC6 ablation drastically attenuated Ang II-induced $[Ca^{2+}]_i$ elevations in native podocytes [43]. The signaling pathway likely involves AT_1R - $G_{q/11}$ -PLC dependent generation of DAG, which can either directly or indirectly via stimulation of NOX2 and production of reactive oxygen species (ROS) stimulate TRPC6 activity [44] (Figure 1B). TRPC6-mediated Ca^{2+} entry into podocytes activates Ca^{2+} -dependent serine/threonine phosphatase, calcineurin to stimulate the nuclear factor NFAT through an unknown mechanism [46]. NFAT is capable to increase TRPC6 mRNA levels leading to a long term increase in TRPC6 expression, Ca^{2+} overload, and apoptosis of podocytes [45]. In addition, Ang II can also promote TRPC6 expression in an ERK1/2-NF- κ B dependent manner [45]. Consistently, inhibition of AT_1R with losartan have a beneficial role on reducing proteinuria and podocyte injury via downregulation of TRPC6 expression in podocytes [47].

Diabetes mellitus commonly leads to diabetic nephropathy associated with podocyte loss and compromised slit diaphragm, causing overt proteinuria and kidney function decline [48, 49]. Elevated glucose levels specifically increase TRPC6 protein and mRNA expression and TRPC6-dependent Ca^{2+} influx in cultured podocytes [50]. Similarly, TRPC6 expression is increased in podocytes from STZ-treated rats, a well-accepted model of Diabetes Mellitus Type I [50]. Interestingly, this upregulation occurs in an Ang II-dependent manner, since AT_1R blockade and inhibition of local Ang II production through angiotensin-converting enzyme inhibition prevents glucose-mediated increases in TRPC6 expression [50]. Exposure to high glucose also upregulates renin mRNA expression in podocytes and increases the relative proportion of renin-positive cells leading to increased Ang I production [51]. Thus, it appears that high glucose activates the intrarenal renin-angiotensin system, promoting local Ang II production, generation of ROS and augmented TRPC6 activity [50-52]. The complete pathway has yet to be established, but a critical role of Syndecan-4 (SDC-4), a member of the type I transmembrane heparan sulfate proteoglycan superfamily has been recently proposed [52] (Figure 1B). Interestingly, high glucose downregulates TRPC6 protein expression and impairs TRPC6-dependent Ca^{2+} entry in response to ANG II in contractile mesangial cells, which are located within glomerular capillary loops to regulate glomerular hemodynamics [53]. The mechanism of this downregulation is unknown but it is proposed that TRPC6 deficiency and impaired contractility of the mesangial cells might be an underlying mechanism for the hyperfiltration seen in the early stages of diabetes.

In addition to Ang II, purinergic signaling cascade also plays a critical role in upregulation of TRPC6-mediated Ca^{2+} influx. ATP is a ubiquitous extracellular signaling molecule and augmented ATP release/secretion in response to mechanical stress have been described for kidney epithelial cells [54]. Sustained ATP signaling via stimulation of P2Y receptors and subsequently TRPC6 activity could contribute to foot process effacement, Ca^{2+} -dependent changes in gene expression, and/or detachment of podocytes [55]. Interestingly, augmented TRPC6 activity in response to ATP also depends on generation of ROS [55]. It is possible that the close association of the channel with NOX cascade (likely NOX2) makes TRPC6 particularly susceptible to local ROS production (see Figure 1B). Indeed, ROS are known to be deleterious for podocyte function and glomerular health [56] and ROS scavengers, such as tempol, have prominent anti-proteinuric effects [57].

While pathophysiological consequences of TRPC6 over-activation in podocytes are beyond doubts, the significance of the channel during the normal physiology is less clear. TRPC6 channels have been suggested to physically and functionally interact with large conductance Ca^{2+} -activated K^+ (BK) channels in podocytes [32] (Figure 1B). This coupling might be important for controlling membrane voltage in podocytes and for fine-tuning mechanosensitive Ca^{2+} influx in these cells. However, no alterations in glomeruli morphology and filtration properties have been reported in TRPC6 knockout mice [58]. One possibility could be that the lack TRPC6 expression can be compensated by other mechanisms, most notably TRPC5 [22]. However, genetic deletion of either pore forming α or Ca^{2+} -modulatory β subunits of BK also do not result in appreciable alterations of glomerular structure/function despite moderate hypertension and hyperaldosteronism [59, 60]. Alternatively, a purinergic P2X4 channel, capable of permeating both Ca^{2+} and K^+ , can exert a mechanotransductive function in podocytes independently of TRPC channels [61]. Apparently, future studies are required to fully delineate the physiological relevance of TRPC6 and other TRPC channels in glomeruli.

Importance of TRPM6 and TRPV5 for controlled renal handling of divalent cations

Divalent cations, such as Mg^{2+} and Ca^{2+} , participate in a variety of physiologically relevant processes ranging from protein and DNA synthesis, intracellular signaling to neurotransmission, hormone secretion, vascular contractility, and bone formation [62, 63]. Both ions are kept within tightly defined limits in the plasma (~1 mM for ionized Ca^{2+} and ~0.5 mM for ionized Mg^{2+}) due to coordinated intestinal absorption and renal reabsorption regulated by several endocrine factors, most notably parathyroid hormone (PTH), vitamin D, and epidermal growth factor (EGF), as will be detailed below. Systemic Mg^{2+} and Ca^{2+} imbalance results in numerous severe pathologies associated with arrhythmias, seizures, etc. [64].

Approximately 90% of the filtered amount of Mg^{2+} and Ca^{2+} ions is reabsorbed in the PT and the thick ascending limb (TAL) in a passive manner through paracellular diffusion secondary to the NaCl transport. The remaining ~10% is reabsorbed exclusively by transcellular route in the DCT and the initial part of the CNT (only for Ca^{2+}). Since no Mg^{2+} and Ca^{2+} can be reabsorbed in the downstream segments, divalent transport in the DCT directly determines urinary excretion of these electrolytes and its disruption alone is

sufficient to elicit profound aberrations at the level of whole organism [65-67]. It appears that TRPM6 and TRPV5 channels serve as conduits for magnesium and calcium influx across the apical membrane, respectively.

DCT, located immediately after the TAL and macula densa, is an important site of controlled transport of Na^+ , Cl^- , K^+ , Mg^{2+} , and Ca^{2+} [68]. Despite being the shortest tubular segment of the renal nephron, DCT can be further divided into two functionally different parts: DCT1 (also often termed as early DCT) and DCT2 (late DCT) based on their responsiveness to mineralocorticosteroid aldosterone. DCT1, being insensitive to aldosterone, is the major place of TRPM6-dependent Mg^{2+} reabsorption by the renal nephron, whereas transcellular Ca^{2+} transport here is minimal [69]. In contrast, DCT2 responds to aldosterone due to expression of the enzyme $11\text{-}\beta$ hydroxycorticosteroid dehydrogenase protecting mineralocorticosteroid receptors from circulating cortisol, and along with the initial part of CNT constitute the site of regulated Ca^{2+} reabsorption via TRPV5 [9]. DCT2 can still reabsorb Mg^{2+} , whilst to a lesser extent due to a lower concentration gradient for the ion (Figure 2).

TRPM6 channel has now been recognized as the dominant pathway for Mg^{2+} entry across the apical membrane in DCT1 and DCT2 [70]. In the kidney, TRPM6 expression is restricted to the DCT, and the channel is also present in intestinal epithelium where its activity is critical for Mg^{2+} transport [10]. Genetic ablation of TRPM6 is embryonically lethal in mice. TRPM6 $\pm$ animals display mild hypomagnesemia but urinary Mg^{2+} excretion was unaffected [65]. Furthermore, the importance of TRPM6 for Mg^{2+} homeostasis was emphasized by the recent discovery that loss-of-function mutations in the channel cause hereditary hypomagnesemia with secondary hypocalcemia (HSH) in humans [71-73]. The pathology is characterized by reduced intestinal Mg^{2+} absorption, increased urinary Mg^{2+} levels, and hypoparathyroidism causing hypocalcemia. Other symptoms usually involve convulsions, seizures, and tetany. Dietary Mg^{2+} supplementation, while cannot completely restore normal plasma Mg^{2+} levels, partially mitigates the symptoms of HSH [74, 75]

Similar to other TRPs, TRPM6 is a voltage-insensitive, cation-permeable channel consisting of four 6-transmembrane domain subunits [76]. The distinctive feature of TRPM6 is that the channel is capable of conducting Mg^{2+} at much higher rate than Ca^{2+} (the ratio is 5:1) [10] enabling TRPM6 to serve as an effective conduit for the apical Mg^{2+} entry in the DCT. The basolateral Mg^{2+} exit could possibly be mediated by the recently discovered magnesium/sodium exchanger SLC41A1 family [77, 78], while other undefined mechanisms may also exist. TRPM6 activity is inhibited when intracellular Mg^{2+} concentration is high [10]. The mechanism likely involves a direct regulation of C-terminal kinase activity of the channel by Mg-ATP [79]. It is possible that this negative feedback protects DCT cells from over-activation of TRPM6. Since TRPM7, another Mg^{2+} -permeable channel, is also expressed in DCT, it remains debatable whether TRPM6 exists primarily as a homotetramer or TRPM6/7 heterotetramer [80, 81]. Embryonic lethality of TRPM7 knockout mice [82] precludes further investigations of the importance of the channel in systemic Mg^{2+} balance. It should be noted though that deletion of *Trpm7* in T cells did not affect acute uptake of Mg^{2+} ,

arguing against a role of TRPM7 [82]. Furthermore, TRPM7-causing pathologies of systemic Mg^{2+} balance have not been described in humans.

Relatively little is known about mechanisms controlling TRPM6 activity and TRPM6-mediated Mg^{2+} transport in the DCT. Only a minimal concentration gradient exists between extracellular and intracellular Mg^{2+} and the transport seems to greatly depend on trans-epithelial voltage existing in the DCT. In support of this concept, a loss-of-function mutation in the apically localized voltage gated $K_v1.1$ channel in the DCT causes autosomal dominant hypomagnesemia in humans [83, 84]. It is proposed that $K_v1.1$ by secreting K^+ into tubular lumen provides a favorable electrochemical driving force for TRPM6-dependent Mg^{2+} influx (Figure 2). Similarly, mutations in $K_{ir4.1}$ (KCNJ10) channel, mediating basolateral K^+ recycling and basolateral membrane voltage, lead to EAST/SESAME syndrome also associated with renal Mg^{2+} wasting and hypomagnesemia [85, 86], at least partially due to disruption of basolateral Mg^{2+} exit in DCT.

TRPM6 activity and expression are under control of several endocrine factors, most notably estrogen, insulin and EGF [87-89]. Estrogen has been proposed to stimulate TRPM6 through a mechanism involving disassociation of the repressive estrogen responsive protein [90]. EGF through its basolaterally localized receptors (EGFR) increases TRPM6 activity and surface expression through both the Src family of tyrosine kinases and the downstream effector Rac1 [88]. Insulin also augments TRPM6 activity via a phosphoinositide 3-kinase and Rac1-mediated elevation of cell surface expression of TRPM6 [89]. Of clinical relevance, renal magnesium wasting is a common side effect during anti-cancer chemotherapy involving inhibitors of EGFR, such as cetuximab [91]. Consistently, cisplatin downregulates the EGF and TRPM6 mRNA levels and increases the fractional excretion Mg^{2+} , suggesting that cisplatin treatment results in a diminished renal Mg^{2+} reabsorption via TRPM6 [92]. Adenylyl Cyclase 3 (AC3)-PKA signaling pathway A also increases TRPM6 abundance at the apical plasma membrane and channel activity, but the upstream extracellular signal was not defined [93]. In addition to hormonal control, renal TRPM6 expression is under control of other systemic factors. Thus, dietary Mg^{2+} restriction upregulates TRPM6 expression, while Mg^{2+} supplementation resulted in a significant decrease in protein levels [94]. TRPM6 activity also depends on pH status. Systemic acidosis decreases TRPM6 abundance and promotes urinary Mg^{2+} wasting [95-97], whereas alkalosis leads to renal magnesium retention [97, 98]

TRPV5 (originally named as ECaC1, the epithelial Ca^{2+} channel) has been identified as the dominant conduit mediating Ca^{2+} influx across the apical plasma membrane in the DCT and CNT [9]. The transcellular Ca^{2+} transport cascade also involves Calbindin-D28k which rapidly captures Ca^{2+} transported through TRPV5 and shuttles it to the basolateral membrane where Ca^{2+} is exported through the Na^+/Ca^{2+} exchanger (NCX1) and the plasma membrane calcium-ATPase PMCA1b [99, 100] (Figure 2). Unlike other TRP channels, TRPV5 is highly selective to Ca^{2+} (permeability Ca^{2+} to Na^+ as 100:1) [101, 102]. TRPV5 is constitutively active, when $[Ca^{2+}]_i$ is low. Calbindin-D28K translocates towards the plasma membrane and directly associates with TRPV5 at low $[Ca^{2+}]_i$ levels to augment TRPV5-dependent Ca^{2+} influx [100]. In contrast, TRPV5 activity rapidly decreases when $[Ca^{2+}]_i$ raises [103], likely protecting the cells from Ca^{2+} poisoning. Structurally and

functionally similar to TRPV5, TRPV6 (originally termed as CaT1 and ECac2) may also play a role in the transcellular Ca^{2+} reabsorption in the distal nephron. In rodents, TRPV6 mRNA can be detected starting from the DCT2 to the medullary collecting ducts [104], whilst to a much lower level than TRPV5 [105, 106]. TRPV5 $-/-$ mice demonstrate drastic urinary Ca^{2+} wasting and exhibit significant disturbances in bone structure, including reduced trabecular and cortical bone thickness [66]. Similarly, TRPV6 $-/-$ are hypercalciuric, and exhibit a mild reduction in bone mineral density [67]. To some disappointment, there are no up-to-date clinical reports for disease-causing mutations of TRPV5 and TRPV6 in patients with hypercalciuria. It has been speculated that higher allele frequencies of TRPV5 and TRPV6 SNPs in African population when compared to Caucasians might contribute to a lower urinary Ca^{2+} excretion, the risk of developing kidney stones, and lower incidence of osteoporosis-related fractures (reviewed in [107]). However, epidemiological studies have yet to be carried out. Overall, despite the unequivocal experimental evidence in animals, the relevance of TRPV5/6 channels in clinic has not been directly established.

Thiazide diuretics, one of the most commonly prescribed antihypertensive drug classes to inhibit sodium reabsorption in the DCT by blocking sodium chloride cotransporter (NCC), are known to cause hypocalciuria (reviewed in [68]). The mechanism is not defined and both increased passive Ca^{2+} reabsorption in the proximal tubule and altered distal nephron TRPV5-dependent Ca^{2+} transport may contribute to calcium retention by the kidneys in response to thiazides [104, 108-110]. The controversies in experimental results may be at least partially explained by the ability of the DCT to become hypertrophic or hypotrophic depending on the extracellular fluid volume status [68].

Distal nephron calcium reabsorption is regulated by parathyroid hormone (PTH), which is released in response to decreased extracellular Ca^{2+} levels [64]. Upon binding to its receptor (PTH1R) in the DCT, PTH stimulates active Ca^{2+} reabsorption via the adenylyl cyclase–cAMP–protein kinase A (PKA) pathway to directly phosphorylate TRPV5 (but not TRPV6), thereby increasing single channel open probability [111]. The same signaling pathway has been recently reported to play a role in regulation of TRPV5 activity by β 1-adrenergic receptor signaling [112], however, anti-calciuretic effects of adrenergic signaling in the kidney are not well described in clinic. In addition, PTH has been reported to affect TRPV5 function via other molecular mechanisms likely involving phosphorylation of the channel by PKC [113-115]. Activation of PKC was demonstrated to diminish the constitutive caveolae-mediated endocytosis of TRPV5, resulting in an increased channel abundance on the plasma membrane [115].

The vitamin D and specifically its most active form, 1,25-Dihydroxyvitamin D₃, is known to play a key role in controlling systemic Ca^{2+} homeostasis [64]. In the kidney, vitamin D receptor (VDR) expression is most prominent in the DCT and CNT, where it stimulates TRPV5-dependent Ca^{2+} reabsorption [9, 70, 99, 116]. Mice deficient for VDR have increased urinary Ca^{2+} levels and reduced TRPV5 expression [106]. Impaired vitamin D production and loss-of-function mutations in VDR lead to vitamin D-deficient rickets associated with compromised Ca^{2+} balance and hypocalcemia [117, 118]. Ca^{2+} supplementation has been proved to be beneficial in not only restoring Ca^{2+} balance but also

in treating rickets [117]. Thus, it is quite possible that pharmacological potentiation of TRPV5 (as well as TRPV6) might be of clinical importance to manage this pathology.

Significant experimental effort was devoted to investigation of the anti-aging single transmembrane glycoprotein Klotho in the renal calcium handling [119, 120]. Of interest, the pattern of Klotho expression in the kidney coincides with the expression of TRPV5 [121]. Klotho exerts complex upregulation of TRPV5 activity from both the inside and outside of cells [122]. The intracellular action of Klotho is likely due to enhanced forward trafficking of channel proteins, whereas the extracellular action is due to inhibition of endocytosis by hydrolyzing extracellular sugar residues on TRPV5, entrapping the channel in the plasma membrane [121, 122]. Klotho $-/-$ mice exhibit renal Ca^{2+} wasting due a failure to absorb Ca^{2+} in the DCT/CNT via TRPV5 [123].

TRPP2/TRPV4 complexes and mechanosensitivity of the renal tubule

Dynamic alterations in tubular flow produce mechanical pressure (also termed the shear stress) on the apical membrane [124]. The ability of cells to recognize and respond to these mechanical stimuli plays an important role in a variety of processes ranging from transport of water and solutes to cellular growth and differentiation [124, 125]. Epithelial cells respond to mechanical stimuli, in part, by elevating $[\text{Ca}^{2+}]_i$ [124, 126-133]. Recent studies identified a critical role for a special cellular organelle, the primary cilium in mechanosensitivity in the renal tubule cells. The cilium protrudes into the tubular lumen and bends when tubular flow gets high [134-136]. This mechanical distortion activates the G-protein coupled receptor polycystin 1 (PC1) and the non-selective Ca^{2+} -permeable channel from TRP family, TRPP2 (also known as polycystin 2; PC2). This induces Ca^{2+} influx at the base of the primary cilium triggering subsequent release of Ca^{2+} from the endoplasmic reticulum intracellular stores [134]. It should be noted though that intercalated cells in the CD, known to have no cilium [137], respond to elevated flow with a similar rise in $[\text{Ca}^{2+}]_i$ [8, 126, 138], suggesting that the primary cilium is not an absolute requirement for mechanosensitivity.

Polycystic kidney disease (PKD) encompasses a broad group of hereditary renal pathologies which are characterized by development and progressive growth of cysts filled with fluid (reviewed in [139]). PKD often progresses to the end-stage renal disease and sharp decline in GFR [139]. The most two common forms: autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD), are caused by genetic mutations in genes encoding PC1/TRPP2 and fibrocystin, respectively [140, 141]. In both cases, renal cysts predominantly (in ADPKD patients) [142, 143] or exclusively (individuals with ARPKD) [144, 145] develop in the distal renal tubule, notably the CD. Multiple studies support the concept that de-differentiation and augmented cAMP-driven 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, [134, 139, 146-153]. However, a direct mechanical activation of polycystins has never been demonstrated. Instead, TRPP2 heteromerizes with another mechanosensitive channel from TRP family, TRPV4, to gain mechanosensitive properties [154-156]. Indeed, disrupted activity of either TRPP2 [134] or TRPV4 [8] leads to inability of the renal tubule cells to respond to elevated flow with an increased $[\text{Ca}^{2+}]_i$. PC1 and

fibrocystin also directly interact with TRPP2, [157-160], thereby forming a multiprotein mechanosensitive complex (Figure 3). It is interesting that TRPP2 mutations lead to ADPKD, whereas TRPV4 $-/-$ mice and zebra fish have normal kidneys [155, 161]. This suggests that the relation between cystogenesis and disruption of mechanosensitivity is not straightforward. Recent effort of our group demonstrated that the genetic defect in fibrocystin in a rat model of ARPKD does not lead to immediate disruption of TRPV4-dependent mechanosensitive $[Ca^{2+}]_i$ elevations in non-transformed tubules and loss of mechanosensitivity occurs just prior to or as an early event during cyst development [133]. We further demonstrated that prolonged systemic pharmacological stimulation of TRPV4 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 treatment [133]. However, the efficiency of this strategy in other forms of PKD (most notably ADPKD) has not been tested yet.

It is well-established that increases in tubular flow, for example during treatment with diuretics, such as furosemide, produces marked kaliuresis [162, 163]. Inability of the kidney to exert a so-called flow-dependent potassium secretion leads to hyperkalemia and aldosteronism [60]. The activity of the Ca^{2+} -dependent large conductance K^+ (BK) channel in the CNT and CD plays a critical role in this process [162, 164-167]. The modulatory $\beta 1$ subunit greatly potentiates sensitivity of the channel to increases in $[Ca^{2+}]_i$, with its ablation leading to compromised flow-induced K^+ secretion [60]. In addition to BK, we have recently documented that the Ca^{2+} -dependent small conductance SK3 (KCa2.3) channel [168] is also expressed at the apical border of both the CNT and CD [169]. Importantly, it has much higher affinity for Ca^{2+} over that observed for the BK channel [170, 171] and, hence, is well posed to play a key role in flow-dependent K^+ secretion. Future studies are required to probe interplay and supremacy between these channels. Ca^{2+} -permeable mechanosensitive TRPP2/TRPV4 complexes are ideally suited to regulate and control the process of flow-induced K^+ secretion serving as a source of elevated $[Ca^{2+}]_i$ (Figure 3). Genetic ablation of TRPV4 prevents flow-dependent elevations in K^+ secretion in perfused CDs and blunts furosemide-induced kaliuresis, whereas pharmacological stimulation of TRPV4 recapitulates the effect of flow on potassium transport in wild type animals [165]. However, the physiological relevance of the regulation of flow-dependent potassium secretion by mechanosensitive TRPP2/TRPV4 complexes on systemic K^+ balance has not been directly demonstrated.

Concluding remarks

TRP channels are abundantly expressed in the kidney where they play a critical role in many physiological processes, including maintenance of glomerular barrier and regulation of electrolyte transport by renal epithelium. High propensity to heteromerize allows TRP channels to form macromolecular associations with unique set of features and functions, such as mechanosensitive complex of PC1, TRPP2 and TRPV4. Genetic defects in TRPC6, TRPM6 and TRPP2 channels underlie clinically relevant channelopathies having renal origin. TRP channels are becoming increasingly appreciated as converging points and end-effectors of numerous endocrine pathways. This provides a strong impetus for the development of novel therapeutic approaches targeting TRP family members. At the same time, existing experimental evidence suggests that many more TRP channels with unknown

or much less understood function exist in the renal tissue. While they do not play a role in kidney function at the baseline, these channels might be critical for adaptation to a particular stress condition. Future studies are granted to reveal their yet undefined physiological and pathophysiological relevance.

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Figure 1A

Figure 1B

Figure 1. TRPC6 function in glomerulus

(A) Principal scheme of glomerular structure. 1 - Bowman's capsule; 2 - Bowman's space; 3 - Podocyte; 3A - Foot processes; 3B - Slit diaphragm; 4 - Glomerulus capillaries; 5 - Mesangial cells; 6 - Afferent arteriole; 7 - Efferent arteriole. (B) Regulation of TRPC6 activity in podocytes. Ang II – Angiotensin II; AT1R – Angiotensin II receptor type 1; ATP – Adenosine triphosphate; P2Y – purinergic receptor type Y; ERK1/2 – extracellular-signal-regulated kinases (also known as MAPK kinases) PLC –phospholipase C; DAG – diacylglycerol; NOX2 – NADPH oxidase isoform 2; ROS – reactive oxygen species; BK – Ca^{2+} -activated big conductance K^{+} channel; NFAT – nuclear factor of activated T-cells; NF- κB – nuclear factor kappa-light-chain-enhancer of activated B cells; Glu – glucose; iRAS – intrarenal Renin Angiotensin System.

Figure 2 left panel

Figure 2 right panel

Figure 2. Divalent cation transport in the distal convoluted tubule (DCT)

Left and right panels represent regulation of TRPM6-dependent Mg^{2+} reabsorption in the DCT1 (early DCT) and TRPV5-dependent Ca^{2+} reabsorption in the DCT2 (late DCT), respectively. Left panel: Kv1.1 - Potassium voltage-gated channel subfamily A member 1; PKA – protein kinase A; Rac1 - Ras-related C3 botulinum toxin substrate 1; cAMP – cyclic adenosine monophosphate; PI3K - Phosphatidylinositol-4,5-bisphosphate 3-kinase; Src - proto-oncogene encoding tyrosine kinase; SLC41A1 – solute carrier Mg^{2+} transporter family 41 member 1; InsR – insulin receptor; EGF - epidermal growth factor; EGFR - epidermal growth factor receptor. Right panel: NCC – sodium chloride cotransporter; PKA – protein kinase A; PKC – protein kinase C; cAMP - cyclic adenosine monophosphate; PTH – parathyroid hormone; PTH1R – parathyroid hormone receptor type 1; VDR – vitamin D

receptor; NCX – $\text{Na}^+/\text{Ca}^{2+}$ exchanger; PMCA1b – plasma membrane Ca^{2+} ATPase type 1b; ATP - Adenosine triphosphate.

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Figure 3. Functional coupling between mechanosensitivity and flow-dependent potassium secretion

PC1 – polycystin 1; FPC – fibrocystin; SK3 - small conductance calcium-activated potassium channel 3; BK – Ca^{2+} -activated big conductance K^+ channel; cAMP - cyclic adenosine monophosphate.