Calcium-permeable ion channels in the kidney

Yiming Zhou¹ and Anna Greka¹,²

¹Department of Medicine and Glom-NExT Center for Glomerular Kidney Disease and Novel Experimental Therapeutics, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts; and ²The Broad Institute of MIT and Harvard, Cambridge, Massachusetts

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Zhou Y, Greka A. Calcium-permeable ion channels in the kidney. Am J Physiol Renal Physiol 310: F1157–F1167, 2016.—Calcium ions (Ca²⁺) are crucial for a variety of cellular functions. The extracellular and intracellular Ca²⁺ concentrations are thus tightly regulated to maintain Ca²⁺ homeostasis. The kidney, one of the major organs of the excretory system, regulates Ca²⁺ homeostasis by filtration and reabsorption. Approximately 60% of the Ca²⁺ in plasma is filtered, and 99% of that is reabsorbed by the kidney tubules. Ca²⁺ is also a critical signaling molecule in kidney development, in all kidney cellular functions, and in the emergence of kidney diseases. Recently, studies using genetic and molecular biological approaches have identified several Ca²⁺-permeable ion channel families as important regulators of Ca²⁺ homeostasis in kidney. These ion channel families include transient receptor potential channels (TRP), voltage-gated calcium channels, and others. In this review, we provide a brief and systematic summary of the expression, function, and pathological contribution for each of these Ca²⁺-permeable ion channels. Moreover, we discuss their potential as future therapeutic targets.

transient receptor potential canonical; transient receptor potential vaniloid; voltage-gated calcium channels; transient receptor potential polycystin; actin cytoskeleton; glomerulus; tubule; renal

Calcium Homeostasis

INTRACELLULAR CALCIUM ([Ca²⁺]ᵢ) plays an important role in various cellular events and orchestrates diverse signaling. It shapes action potentials as a divalent cation, regulates enzyme activity as a cofactor, and bridges extracellular and intracellular signal transduction as a second messenger (25). Therefore, total body calcium levels are tightly regulated, with 99% of the calcium stored in bones in the form of calcium phosphate. The extracellular fluid (ECF) contains ~2.2–2.6 mmol/l (9–10.5 mg/dl) total calcium and 1.3–1.5 mmol/l (4.5–5.6 mg/dl) free calcium ions (11). The concentration of calcium in the intracellular fluid (ICF) is 10,000 times less that in the ECF. Depending on the cell type, the calcium concentration in ICF is ~50–200 nmol/l (138).

Intracellular Ca²⁺ rises by various mechanisms, either entry from outside the cell through Ca²⁺-permeable ion channels, such as transient receptor potential (TRP) and voltage-gated Ca²⁺ channels, or by release from intracellular Ca²⁺ stores, such as the ER and mitochondria (Fig. 1) (80). The intracellular Ca²⁺ concentration is in turn quickly reduced by many types of Ca²⁺ pumps, such as the plasma membrane Ca²⁺-ATPase and Na⁺/Ca²⁺ exchanger pumping Ca²⁺ out of the cell and sarcoplasmic reticulum Ca²⁺ pump (SERCA) and the mitochondria calcium uniporter (MCU) pumping Ca²⁺ back into the ER and mitochondria, respectively (Fig. 1) (79, 91). The timing and amount of intracellular Ca²⁺ increases are regulated precisely by these ion channels and pumps to achieve intracellular Ca²⁺ homeostasis, which is indispensible for all cellular physiological functions (25).

Ca²⁺ filtration and reabsorption in the kidney, together with intestinal absorption, bone resorption, and deposition, determine total body Ca²⁺ balance. The ionized Ca²⁺ in the blood and extracellular fluids is filtered every 2 h, and any change in the capacity to reabsorb Ca²⁺, even small changes, can significantly affect Ca²⁺ balance. The reabsorptive capacity of the kidney involves two pathways: the paracellular and the transcellular pathway (134). Movement of Ca²⁺ through the tight junctions between epithelial cells proceeds through the paracellular pathway, whereas the transcellular pathway involves the transport of Ca²⁺ through epithelial cells. As the filtrate runs along the nephron the tubular Ca²⁺ concentration decreases, thus decreasing the drive for the paracellular pathway, which triggers the transcellular component. The transcellular pathway includes Ca²⁺ entry across the apical membrane via Ca²⁺-permeable ion channels, followed by intracellular diffusion of Ca²⁺ from the apical to the basolateral membrane mediated by calcium-binding proteins and buffers (such as calbindins) and, finally, exit through the basolateral membrane via the Ca²⁺ pump or the Na⁺/Ca²⁺ exchanger. Although the majority of Ca²⁺ is reabsorbed via the energy-saving paracellular route, the transcellular route is important in fine-tuning the total body Ca²⁺ homeostasis, a process tightly regulated by vitamin D and parathyroid hormone (10, 125).
The adult human kidneys process ~180 liters of plasma each day, in which roughly 10 g of calcium is filtered (39, 60). However, the excretion amount of calcium in the urine normally ranges from 100 to 200 mg/24 h. To secure that reabsorption proceeds through the paracellular pathway, and/or loss-of-function mutations in TRPC6 cause focal segmental sclerosis, mutations in polycystin 1/polycystin 2 (PKD1/PKD2) genes are linked to polycystic kidney disease, and mutants in TRPM6 are associated with hypomagnesemia with secondary hypocalcemia. The involvement of these Ca\(^{2+}\)-permeable ion channels in hereditary kidney diseases offers a novel understanding of the contribution of Ca\(^{2+}\) signaling in the kidney and may reveal new therapeutic targets. In this review, we give a brief and systematic summary of Ca\(^{2+}\) signaling contributed by several Ca\(^{2+}\)-permeable ion channels in the kidney in both health and disease. Specifically, we will review previous and recent studies on how they contribute to calcium homeostasis at the molecular, cellular, and tissue levels in kidney.

**TRP Channels**

The transient receptor potential (TRP) superfamily consists of 28 members in mammals. They are known to function as a tetrameric channel where each subunit contains intracellular NH\(_2\) and COOH termini, six transmembrane domains (S1–S6), and a pore loop between the S5 and S6 segments. Most TRP channels are nonselective cation channels with high Ca\(^{2+}\) permeability (26, 95). The TRP superfamily is divided into six subfamilies in mammals based on their homologous sequences and functions: TRPC (canonical), TRPV (vanilloid), TRPA1 (ankyrin), TRPM (melastatin), TRPML (mucolipin), and TRPP (polycystin). The TRPC subfamily is most closely related to other TRP subfamilies are more distantly related. To date, numerous studies have shown that the TRP superfamily is expressed in various cell types and tissues and participates in a wide range of physiological and pathological events from signal transduction to cell proliferation, migration, and death (102). Of particular interest to this review, a large number of studies have shown that several TRP channels are expressed in mammalian kidney, including members of the TRPC, TRPV, TRPM, and TRPP subfamilies (33, 108, 144, 155).

**Ca\(^{2+}\) and Kidney Disease**

Recently, studies have shown that disruption of Ca\(^{2+}\) signaling in the kidney leads to kidney disease. For example, gain and/or loss-of-function mutations in TRPC6 cause focal segmental sclerosis, mutations in polycystin 1/polycystin 2 (PKD1/PKD2) genes are linked to polycystic kidney disease, and mutants in TRPM6 are associated with hypomagnesemia...
TRPC1 is activated by the activation of the PLC pathway (102). Some reports have also identified intracellular Ca\(^{2+}\) store depletion as an activating event for TRPC1, suggesting that it plays a role in store-operated calcium entry (151, 157), perhaps through interactions with the inositol triphosphate receptor type 3 (12, 137, 165) or STIM1/Orai1 (23, 84, 85, 98, 106). However, most of these conclusions have been drawn from calcium imaging studies, and therefore, due to the inability to control the transmembrane voltage in these studies, there is significant doubt that there is any definitive evidence that TRPC1 or other TRPC channels are store operated (26, 102). Moreover, the discovery of the STIM and Orai families as the molecular components of store-operated channels supports the fundamental and now widely accepted notion that TRPC channels are receptor-operated channels.

In the kidney, TRPC1 is expressed in mesangial cells and regulates contractility, cell proliferation, and extracellular matrix proteins (37, 152). Glomerular mesangial cells (123) provide structural support for glomerular capillaries and regulate blood flow through their contractile activity. The contraction of mesangial cells decreases the surface area of the basement membrane of the capillary endothelial cells and results in a decreased glomerular filtration rate (GFR) (131). Interestingly, the contraction mechanism of mesangial cells is similar to that of smooth muscle. Considering the proposed physiological roles of TRPC1 in smooth muscle, including muscle contraction and proliferation (34, 49), it is possible that TRPC1 plays a similar role in mesangial cells. Studies also confirm that mesangial cell proliferation and mesangial matrix expansion play significant roles in a wide range of glomerular diseases, particularly in diabetic nephropathy (2, 28, 56). Reduced expression of TRPC1 in mesangial cells has been associated with diabetic nephropathy in several diabetic animal models, such as Zucker diabetic rats, streptozotocin-induced diabetic rats, and db/db mice, as well as in patients with diabetic nephropathy (63, 99). Intriguingly, a recent study shows that TRPC1 polymorphisms are closely associated with type 2 diabetes and diabetic nephropathy in a Han Chinese population, although a previous study found no association of TRPC1 polymorphisms with diabetic nephropathy in the GoKinD and an African-American population (22, 168). In summary, TRPC1 dysfunction may play a role in the pathogenesis and development of diabetic nephropathy, although its specific contribution still needs to be defined.

TRPC3. Both in native and heterologous expression systems, TRPC3 can form both a homotetrameric channel and a heterotetrameric channel with its close relatives TRPC6 and TRPC7, likely because of their sequence and structural similarities (145). Using in situ hybridization, TRPC3 has been shown to be abundant in endothelial cells from cerebral and coronary arteries (161). TRPC3 is activated by the PLC pathway to participate in a wide range of G protein-coupled receptor (GPCR)-modulated, Ca\(^{2+}\)-dependent functions, including nitric oxide production, cell proliferation, and death (101, 160).

TRPC3 is expressed abundantly in various kidney cells, including renal fibroblasts, podocytes, distal convoluted tubules, and cortical and medullary collecting ducts (46, 47, 83, 121). Expression of TRPC3 in renal fibroblasts is associated with increased Ca\(^{2+}\) entry, ERK1/2 phosphorylation, and fibroblast proliferation (121). In distal convoluted tubules, TRPC3 physically interacts with aquaporin-2 and is responsible for Ca\(^{2+}\) reabsorption in principal cells (47). Although TRPC3 and TRPC6 can form a heteromeric channel, this TRPC3/TRPC6 channel may be less functionally relevant in the kidney. Several studies have shown that although both channels are expressed in kidney, their contribution and involvement are not always parallel and consistent. Detailed electrophysiology studies at the single-channel level revealed TRPC6 homomeric channels, and no heteromeric channels, in podocytes (142). TRPC3, but not TRPC6, is associated with large-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel (BKCa) trafficking and activation in podocytes (78). Moreover, an increased expression of TRPC3 and a decreased expression of TRPC6 in kidney cortex from Munich Wistar Frontier rats has been observed (90). These studies argue that TRPC3 and TRPC6 may play complementary or contrary roles in the kidney as functional homomeric channels rather than as an integrative heteromeric complex. This hypothesis is supported by the fact that there is differential trafficking of TRPC3 and TRPC6 in the renal collecting duct, where TRPC3 is localized primarily to the apical membrane, whereas TRPC6 localizes to both the apical and basolateral membranes, and the two channels are sorted in separate vesicular populations (46, 47).

TRPC5. TRPC5 was initially cloned from mouse brain, where TRPC5 is activated by GPCR and receptor tyrosine kinase activation (104, 110). In addition, TRPC5 is modulated by intracellular Ca\(^{2+}\), oxidative stress, cold temperature, lysophospholipids, and mechanical stress (42, 48, 52, 162, 171). In brain, TRPC5 regulates Ca\(^{2+}\) influx in neurons and regulates neuronal growth cone motility (50). Elimination of TRPC5 impairs innate fear behavior in mice (114). In smooth muscle, activation of TRPC5 regulates muscle motility (158). Another tissue with high expression of TRPC5 is kidney (104). Inhibition or genetic deletion of TRPC5 in kidney podocytes prevents glomerular filtration barrier damage in lipopolysaccharide (LPS)- and protamine sulfate (PS)-induced kidney injury mouse models (122). This study provides a possible molecular mechanism of how LPS and PS damage the kidney filter and lead to proteinuria by activating TRPC5 in podocytes. LPS has been shown to directly activate TRPC5 (6). TRPC5 activation is coupled to Rac1 activation and synaptopodin degradation, leading to cytoskeleton remodeling (142). More than 30 years ago, studies found that polycations such as PS can induce podocyte injury and damage to the kidney filter (76). Yet the molecular mechanism of this effect was largely unknown. Interestingly, subsequent work showed that polycations can increase intracellular Ca\(^{2+}\), which was attenuated by reducing extracellular Ca\(^{2+}\) (118), indicating a Ca\(^{2+}\)-permeable ion channel was involved in the PS effect. The fact that inhibition of genetic deletion of TRPC5 reduces LPS- and PS-induced podocyte injury and proteinuria provides evidence that TRPC5 may be the Ca\(^{2+}\)-permeable ion channel involved in podocyte injury. Therefore, TRPC5 may be a novel therapeutic target to prevent podocyte injury and kidney filter damage.

TRPC6. Mutations in TRPC6 have been associated with hereditary focal segmental glomerulosclerosis (FSGS) of an autosomal dominant pattern (113, 153), providing genetic evidence that TRPC6 is an important regulator of podocyte calcium signaling. Since then, a large number of studies have confirmed the expression of TRPC6 in podocytes and as a component of the glomerular slit diaphragm (24, 38, 93, 149).
FSGS is characterized by impaired glomerular structure and function, severe proteinuria, and nephrotic syndrome. It accounts for the majority of treatment-resistant nephrotic syndrome in children and adults and progresses to kidney failure if left untreated (112). The finding that excessive Ca$^{2+}$ influx mediated by gain-of-function TRPC6 mutations is the cause of disease in some FSGS patients highlights the central concept that abnormal Ca$^{2+}$ signaling in podocytes is a cause of FSGS. Mutants of TRPC6, causing either increased current amplitude or prolonged channel opening time, significantly increase Ca$^{2+}$ influx in podocytes (64, 92, 113), leading to injury. In some cases, excess TRPC6-mediated Ca$^{2+}$ influx in podocytes activates downstream nuclear factor of activated T cells (NFAT), a transcription factor triggered by Ca$^{2+}$-mediated calcineurin activity (81, 124). Activation of NFAT leads to podocyte hypertrophy, similar to NFAT function in cardiac myocytes, and makes podocytes more vulnerable to damage (35). Interestingly, NFAT was also recently found to increase TRPC6 expression (81, 100, 156). In addition, activation of TRPC6 activates RhoA and leads to cytoskeleton remodeling (73, 89, 127, 142), which also affects podocyte function.

It is important to note, however, that TRPC6/RhoA signaling is also required for normal podocyte cytoskeletal homeostasis (142). Notably, a recent study shows that loss-of-function mutations in TRPC6 (TRPC6 G757D) are also associated with FSGS, acting in a dominant negative manner to inhibit TRPC6 channel activity (115). Taken together, the data showing that both loss-of-function and gain-of-function mutations in TRPC6 result in the same human disease further bolster the notion that TRPC6 activity plays an important homeostatic role in podocytes.

Diabetic nephropathy, one of most common complications of both insulin-dependent and -independent diabetes mellitus, is closely associated with podocyte loss and proteinuria (51, 139). Increased expression of TRPC6 was observed in cultured podocytes stimulated by high glucose in an angiotensin II-dependent manner. In vivo, protein expression of TRPC6 was increased in the streptozotocin-induced diabetic rats with high proteinuria, and an angiotensin II receptor blocker (ARB), losartan, blocked high-glucose-induced TRPC6 activity (129), although the detailed mechanism of how the increased expression of TRPC6 contributes to diabetic nephropathy is not well understood. Another study found that high glucose modifies TRPC6 via increased oxidative stress by syndecan-4 in human podocytes (140). The effect of high glucose on TRPC6 expression is attenuated by a superoxide dismutase mimetic, TEMPO, a membrane-permeable free radical scavenger. Given the fact that AGRT1 activation can induce the production of reactive oxygen species in many tissues, including kidney (119), AGRT1-mediated increases in TRPC6 expression may be through a ROS-dependent pathway. A recent study found that constitutive activation of a Goα subunit (Gq$^{209L}$) upregulates TRPC6 in mouse podocytes and leads to podocyte death in puromycin aminonucleoside-induced nephrosis and in Akita diabetic mouse models (148). Upregulation of TRPC6 was through the activation of calcineurin by Goα. Furthermore, increased expression of TRPC6 may be abolished by administration of calcitriol in streptozotocin-induced diabetic rats (169). Although these results are presently hard to reconcile into coherent signaling pathways, taken together, these studies provide evidence that disrupted TRPC6 activity may be involved in a number of glomerular kidney diseases.

TRPV4. TRPV4 is highly expressed in kidney, liver, and heart (86, 132). It can be activated by mechanical stimulation, warm temperature, and phorbol derivatives (53, 141, 150). Although it was initially proposed as a mechanosensitive cation channel, critical data for direct gating by mechanical stimulation are still elusive. Existing evidence indicates that TRPV4 may be indirectly gated via lipids during mechanical stimulation (147), thereby supporting a role for TRPV4 in mechanosensing (111). In support of this concept, studies in TRPV4 knockout mice show an aberrant reaction to osmotic or mechanical stimuli (87, 136). In kidney, TRPV4 is expressed predominantly in renal tubular epithelial cells, where it senses osmolarity changes and regulates water reabsorption in kidney tubules (143). TRPV4 expression is noted from the ascending thin loop throughout the rest of the tubule all the way to the collecting duct, except for the macula densa, a highly specialized segment of the tubule. In other words, TRPV4 is expressed in tubules lacking constitutive apical water permeability and an existing transcellular osmotic gradient. However, controversy still exists about the specific localization of TRPV4 at the cellular level, with different studies showing it at the apical or the basolateral membrane (27, 135, 143). Nonetheless, taken together, these studies confirm an important role for TRPV4 in tubular physiology. In the thick ascending limb, activation of TRPV4 increases intracellular Ca$^{2+}$ and subsequently induces ATP release in a Ca$^{2+}$-dependent manner (126). The released ATP in turn inhibits ion reabsorption via purinergic (P2X) receptors (82). In the cortical collecting duct, TRPV4 interacts with aquaporin 2 and responds to hypotonicity with a rapid regulatory decrease in volume (7, 43). In addition, activation of TRPV4 by hypotonicity activates Ca$^{2+}$-dependent K$^+$ channels (BK and SK3), which regulate K$^+$ secretion (74).

TRPV5. TRPV5 and TRPV6 are distinct from the rest of the TRPV family members (TRPV1–TRPV4) because they are highly Ca$^{2+}$ permeable. TRPV5 is expressed predominantly in the apical membrane of the renal tubular epithelial cells, whereas TRPV6 is expressed mainly in the apical membrane of intestinal enterocytes, where it regulates transcellular Ca$^{2+}$ reabsorption (68, 109, 146, 166). The transcellular Ca$^{2+}$ transport pathway involves several biological events: first, activation of TRPV5 mediates Ca$^{2+}$ entry across the apical membrane of epithelial cells; second, incoming Ca$^{2+}$ binds to calcium-binding proteins (such as calbindin) and is transported to the basolateral membrane; and finally, the Na$^+/Ca^{2+}$ exchanger protein (NCX1) and a Ca$^{2+}$-ATPase (PMCA1b) at the basolateral membrane extrude Ca$^{2+}$ from the cells. TRPV5 is constitutively open under physiological conditions and is inhibited by increasing intracellular Ca$^{2+}$, probably as a safeguard against cell death due to Ca$^{2+}$ toxicity (68). TRPV5 knockout mice exhibit excess Ca$^{2+}$ in the urine (severe hypercalcemia) despite an enhanced vitamin D level (69). This demonstrates the key function of TRPV5 in active Ca$^{2+}$ reabsorption and Ca$^{2+}$ homeostasis in the kidney.

In addition, TRPV5 interacts with several well-characterized Ca$^{2+}$ homeostasis regulators, including the parathyroid hormone (PTH), 1,25 dihydroxyvitamin D [1,25(OH)$_2$D$_3$ or calcitriol], Klotho, and fibroblast growth factor 23 (5, 16, 30, 154). Low extracellular Ca$^{2+}$ levels trigger the release of PTH,
which acts on PTH G protein coupled receptors at target organs (i.e., bone) and regulates calcium homeostasis. In the distal nephron, PTH is found to directly activate TRPV5 via PKA-dependent phosphorylation, thus increasing Ca\(^{2+}\) reabsorption (30). Phosphorylation of TRPV5 at Thr\(^{509}\) significantly increases TRPV5 open channel probability in the context of low intracellular Ca\(^{2+}\) (30). Another study showed that calmodulin, which is activated by intracellular Ca\(^{2+}\), binds physically to TRPV5 at COOH-terminal residues 696 to 729 and inhibits channel opening. Phosphorylation of Thr\(^{399}\) residues by PKA abolishes the inhibitory effect of calmodulin (29). In addition, PTH can also increase TRPV5 expression via PKC activation by inhibiting caveolae-mediated endocytosis of TRPV5 (17, 67). Deletion of the vitamin D receptor (VDR) disrupts calcium homeostasis in mice (88). VDR-knockout mice display hypercalcemia and, interestingly, a decreased expression of TRPV5, suggesting that VDR regulates TRPV5 expression in the kidney (128). Klotho, a type 1 transmembrane enzyme with glucuronidase activity and predominantly expressed in kidney, is coexpressed with TRPV5 and hydrolyzes the extracellular sugar residues of the channel. This modification activates TRPV5 and increases Ca\(^{2+}\) reabsorption in tubular epithelial cells (18). Moreover, fibroblast growth factor 23, a growth factor responsible for phosphate homeostasis, promotes renal Ca\(^{2+}\) reabsorption through TRPV5 activation via an ERK1/2-PKA-dependent pathway (5). Recently, a study found that a TRPV5 polymorphism (rs4236480) is associated with calcium-containing calculi (kidney stones or nephrolithiasis) (77). Although this study provides clinical evidence that TRPV5 may play an important role in nephrolithiasis, further mechanistic studies will reveal whether this TRPV5 polymorphism causes aberrant regulation of channel activation or inhibition, leading to disease, and thus whether TRPV5 may be targeted for future therapeutics.

**TRPP (PKD1/PKD2).** The polycystin or TRPP subfamily is divided into two distinct groups, TRPP1 (PKD1-like) and TRPP2 (PKD2-like), mainly on the basis of structural similarities. The nomenclature can be confusing, where the PKD1-like group consists of PKD1, PKDREJ, PKD1L1, PKD1L2, and PKD1L3. On the other hand, the PKD2-like group consists of PKD2, PKD2L1, and PKD2L2.

PKD1 contains 11 putative transmembrane domains with an intracellular COOH terminus, and a very large extracellular NH\(_2\) terminus. In contrast, PKD2 contains six transmembrane domains, with intracellular NH\(_2\) and COOH termini, similar to most typical TRP channels. It has been proposed that PKD1-like proteins form functional heteromeric complexes with PKD2-like proteins through coiled-coil domains localized at the COOH terminus (15, 163, 170). Interestingly, PKD1 and PKD2 form a heteromeric receptor/channel with a 1:3 subunit stoichiometry (163). A similar phenomenon was also described in PKD1L3 and PKD2L2 complexes (164). In the kidney, PKD1 and PKD2 complexes are localized in the primary cilia of renal epithelial cells, where they have been proposed to function as flow sensors or mechanosensors (55, 96). PKD1 is not observed in the plasma membrane without PKD2 coexpression, and PKD2 homomeric channels do not respond to mechanical stimulation (19, 45, 163). This has led to the notion that PKD2 is necessary for PKD1 translocation to the plasma membrane, whereas PKD1 functions as a “sensor” for PKD2 activity. Based on this notion, loss-of-function mutations in either protein could disrupt their partner’s function and lead to polycystic kidney disease. Loss-of-function mutations in PKD1 account for 85% cases of autosomal dominant polycystic kidney disease, whereas loss-of-function mutations in PKD2 account for the remaining 15% of cases.

The detailed mechanism of cyst formation in PKD patients is not well understood, although several hypotheses and models have been proposed. Studies have shown elevated levels of cAMP and upregulated PKA activity in animal models of PKD (44, 71, 159). Dysregulation of intracellular Ca\(^{2+}\) homeostasis as well as gene expression profiles by cAMP and PKA may lead to impaired tubulogenesis, increased fluid secretion, interstitial inflammation, and cell proliferation (21, 105). Recent work illuminating Ca\(^{2+}\) dynamics within cilia may hold the key to understanding the pathogenesis of PKD (31, 32).

**Voltage-Gated Calcium Channels**

The existence of voltage-gated calcium channels (VGCC) was proposed in 1975, using the egg cell membrane of starfish (54). These classical studies showed there are distinct Ca\(^{2+}\)-permeable ion channels with different voltage thresholds and kinetics. VGCC were initially divided into two classes: high-voltage-activated (HVA) and low-voltage-activated (LVA) Ca\(^{2+}\) channels. The activation thresholds for HVA Ca\(^{2+}\) channels are around −30 to −20 mV, whereas the thresholds for LVA Ca\(^{2+}\) channels are around −60 to −50 mV. Further studies have confirmed that VGCC consist of many subunits, including α1-, α2-, β-, δ-, and γ-subunits. The α-subunit is the main component as the pore-forming subunit. Based on the properties of the α-subunit, VGCC are further classified into T-, L-, P/Q-, N-, and R-type channels (36). Among them, only T-type VGCC correspond to the LVA Ca\(^{2+}\) channels. Decades ago, classical studies in renal physiology showed that Ca\(^{2+}\) channel blockers increase renal blood flow and GFR significantly (1, 65, 117), indicating the important contribution of calcium channels to renal function.

**T-Type Ca\(^{2+}\) Channels (Ca\(_{a.3.1}\) and Ca\(_{a.3.2}\))**

T-type Ca\(^{2+}\) channels are LVA calcium channels that mediate Ca\(^{2+}\) influx after weak depolarization. The activation threshold for T-type Ca\(^{2+}\) channels is about −60 to −50 mV, which is similar to that of voltage-gated sodium channels. A transient calcium increase is observed when T-type Ca\(^{2+}\) channels open. Activation of T-type Ca\(^{2+}\) channels initiates muscle contraction in cardiac and vascular smooth muscle cells caused by increases in the cytosolic Ca\(^{2+}\) concentration (14). Two members of the T-type Ca\(^{2+}\) channel family, Ca\(_{a.3.1}\) and Ca\(_{a.3.2}\), are expressed in the kidney as well as in the cardiovascular system (4, 107). Both Ca\(_{a.3.1}\) and Ca\(_{a.3.2}\) are expressed at afferent and efferent arterioles. Activation of T-type Ca\(^{2+}\) channels regulates renal function by controlling the contraction of the renal vasculature. Specifically, activation of these channels causes membrane depolarization and afferent arteriole constriction (70, 97). In the efferent arteriole, however, involvement of these channels is more complicated. Studies have found that T-type Ca\(^{2+}\) channel blockers such as mibebradil and Ni\(^{2+}\) affect efferent arteriole constriction induced by angiotensin II in rat single-isolated perfused nephrons (61, 107). Activation of Ca\(_{a.3.1}\) leads to efferent arteriole constriction, and blocking of Ca\(_{a.3.1}\) increases renal blood flow.
but does not alter GFR, since its function is similar in both afferent and efferent arterioles (70). In contrast, activation of Ca\textsubscript{3,2} induces vasodilation in a nitric oxide (NO)-dependent way in the efferent arteriole, and therefore, blockage or deletion of Ca\textsubscript{3,2} causes increased vascular resistance and an increased filtration fraction (70). In a clinical study, bendipine (a combined L- and T-type antagonist) reduces blood pressure and proteinuria, whereas the L-type-specific antagonist amlopidine does not (103). Studies have also found T-type Ca\textsuperscript{2+} channels expressed in the distal nephron and the collecting duct, yet their functions remain unclear (4, 13).

### L-/P-/Q-Type Ca\textsuperscript{2+} Channels (Ca\textsubscript{1,2} and Ca\textsubscript{2,1})

L-/P-/Q-type Ca\textsuperscript{2+} channels are HVA calcium channels, which require strong depolarization for activation. Studies have shown that preglomerular vascular smooth muscle cells express Ca\textsubscript{1,2} (L-type) and Ca\textsubscript{2,1} (P-/Q-type) channels (58, 59). Ca\textsubscript{1,2} is expressed in efferent arterioles from the juxtamedullary glomeruli and vasa rectae, whereas no calcium channels have been detected in cortical efferent arterioles from the rat. In isolated human renal and intrarenal arteries, L- and P-/Q-type calcium channels have been confirmed by immunohistochemical labeling of human kidney sections (59). Depolarization of both L-type and T-type calcium channels are involved in the vasoconstriction of the preglomerular vasculature (58, 62, 97). A cooperative action of both L- and T-type channels is required to elicit full contraction in response to depolarization (58); however, there are no additive effects on dilation using L- and T-type channel blockers (41). Therefore, T-type channels may be reciprocally dependent on L-type channel activity, whereby T-type channels are involved in the initiation of the calcium

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### Table 1. Channels and proteins contributing to Ca\textsuperscript{2+} signaling in kidney

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Human Gene Name</th>
<th>Expression in the Kidney</th>
<th>Agonists and Activators</th>
<th>Function in the Kidney</th>
<th>Relevant Kidney Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPC1</td>
<td>TRPC1, TRP1</td>
<td>MC</td>
<td>PLC (1, 2)</td>
<td>Regulates mesangial cell contractility (3, 4)</td>
<td>DN</td>
</tr>
<tr>
<td>TRPC3</td>
<td>TRPC3, TRP3</td>
<td>P, DCT, CD</td>
<td>DAG, PLC (5–7)</td>
<td>Regulates SOCE in podocytes, Ca\textsuperscript{2+} reabsorption in DCT and CD (8, 9)</td>
<td>Williams-Beuren syndrome hypercalcemia, renal fibrosis</td>
</tr>
<tr>
<td>TRPC5</td>
<td>TRPC5, TRP5</td>
<td>P, JGC</td>
<td>Intracellular Ca\textsuperscript{2+}, lysophospholipids, oxidative stress, rosiglitazone, riluzole, PLC (10–17)</td>
<td>Dysregulates podocyte actin cytoskeleton, degrades synaptopodin, and activates Rac1 (18, 19)</td>
<td>Podocyte injury, glomerular disease</td>
</tr>
<tr>
<td>TRPC6</td>
<td>TRPC6, TRP6, FSGS2</td>
<td>P, CD</td>
<td>PLC, DAG, hyperforin, lysophosphatidylcholine, 20-HETE (6, 20–23)</td>
<td>Regulates podocyte slit diaphragm (24, 25)</td>
<td>FSGS, DN</td>
</tr>
<tr>
<td>TRPV4</td>
<td>TRPV4, VR-OAC, OTRPC4</td>
<td>ATL, TAL, DCT, CNT</td>
<td>Mechanical stress, warm (&lt;33°C), 4α-PDD, GSK1016790A (26–29)</td>
<td>Regulates renal osmolality and water reabsorption (30)</td>
<td>Ca\textsuperscript{2+} reabsorption</td>
</tr>
<tr>
<td>TRPV5</td>
<td>TRPV5, CAT2, ECaC1</td>
<td>DCT, CNT</td>
<td>Constitutively active, PKA-dependent phosphorylation, shear stress, PIP\textsubscript{2} (31–35)</td>
<td></td>
<td></td>
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<tr>
<td>TRPP</td>
<td>PKD1/PKD2 complex</td>
<td>Epithelial cells of TAL, DCT</td>
<td>Mechanical stress, intracellular Ca\textsuperscript{2+} (36–38)</td>
<td>Activates G protein signaling cascades, mechanosensor (36, 39)</td>
<td>ADPKD</td>
</tr>
<tr>
<td>T-type VGCC</td>
<td>CACNAI/G</td>
<td>Afferent and efferent arterioles, MC, DCT, CD</td>
<td>Low voltage (40)</td>
<td>Regulates blood flow (41, 42)</td>
<td>DN, fibrosis, glomerular hypertension</td>
</tr>
<tr>
<td>L-type VGCC</td>
<td>CACNAIH</td>
<td>Afferent and efferent arterioles, MC, DCT</td>
<td>Low voltage (40)</td>
<td>Regulates glomerular filtration rate (41)</td>
<td></td>
</tr>
<tr>
<td>P-Q-type VGCC</td>
<td>CACNA1C</td>
<td>Afferent and efferent arterioles, MC, DCT</td>
<td>High voltage, 1,4-dihydropyridines, FPL-64176 (43–45)</td>
<td>Vasocostriction, modifies the formation of kidney cysts (46, 47)</td>
<td>Glomerular hypertension, PKD (?)</td>
</tr>
<tr>
<td>Ca\textsubscript{2,1}</td>
<td>CACNA1A</td>
<td>Afferent arterioles, MC</td>
<td>High voltage (40)</td>
<td>Depolarization-mediated contraction in renal afferent arterioles (48, 49)</td>
<td></td>
</tr>
</tbody>
</table>

TRP, transient receptor potential; VGCC, voltage-gated calcium channels; P, podocyte; MC, mesangial cell; PCT, proximal convoluted tubule; ATL, ascending thin limb; TAL, thick ascending limb; DCT, distal convoluted tubule; CNT, connecting tubule; CD, collecting duct; SOCE, store-operated Ca\textsuperscript{2+} entry; PIP\textsubscript{2}, phosphatidylinositol 4,5-bisphosphate; JGC, juxtaglomerular cell; ADPKD, autosomal dominant polycystic kidney disease; DN, diabetic nephropathy; NDI, nephrogenic diabetes insipidus; FSGS, focal segmental glomerulosclerosis.
transients, whereas L-type channels work to maintain a high intracellular Ca\(^{2+}\) concentration.

P/Q-type channels are also found to be involved in rodent preglomerular arterioles (57). P/Q-type calcium channels exhibit slow calcium currents, and they can be modulated by G proteins (167). In human, P/Q-type-mediated currents have been observed in human arteries and rat preglomerular vascular smooth muscle cells (3, 120). The expression and activity of P/Q-type channels in human renal vasculature suggests that these channels may contribute to the contraction mechanism in renal arteries. This illuminates a new understanding for putative hormonal effects on intrarenal vascular reactivity, with implications for novel therapeutic approaches.

**Other Calcium-Permeable Channels**

Although no review can be exhaustive, this review would not be complete without making mention of P2X receptors in the kidney. Indeed, a number of studies have involved them in virtually every segment of the kidney, with roles ranging from vasoconstriction to sodium and water reabsorption (8, 72). Because of the extensive nature of this topic, we have elected not to review this here in detail.

Finally, store operated channels (SOC) involving STIM and Orai channels should be briefly mentioned, although there is limited evidence for their involvement in the kidney per se. In one study (20), Orai/STIM channels were shown to play a role in glomerular mesangial cells in the setting of diabetic kidney disease. More work is needed to clearly delineate the role of these channels in the kidney.

**Conclusion**

A single ion channel allows more than 10 million ions per second to cross the plasma membrane (66). Calcium (Ca\(^{2+}\)) ions in particular mediate a host of fundamental cellular functions (Fig. 1) (25). In virtually every cell, Ca\(^{2+}\) permeates through the plasma membrane to modulate vital processes such as vesicle secretion, muscle contraction, gene transcription, and cytoskeletal structure. The timing and entry of Ca\(^{2+}\) ions passing into the cell is precisely controlled, and cellular homeostatic mechanisms modulate the direction and compartmentalization of all intracellular Ca\(^{2+}\) (Fig. 1) (25). Here, we have reviewed the emerging role of Ca\(^{2+}\)-regulated channels and Ca\(^{2+}\) signaling in kidney health and disease (Table 1). Since ion channels and the GPCRs that regulate them comprise 60% of all druggable targets to date (75), further exploration of the ion channels discussed in this review may indeed pave the way toward a new generation of kidney disease therapeutics.

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We apologize to our colleagues whose work we were not able to cite in this review due to space limitations. Reviews were often quoted at the expense of original work. We thank Drs. Peter Mundel and Joseph Bonventre for helpful discussions.

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**AUTHOR CONTRIBUTIONS**

Y.Z. and A.G. prepared figures; Y.Z. and A.G. drafted manuscript; Y.Z. and A.G. edited and revised manuscript; Y.Z. and A.G. approved final version of manuscript.

**REFERENCES**

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